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ABSTRACT

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## Abstracts

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## S1. Molecular biophysics. Structure and dynamics of biopolymers and biomacromolecular systems

### S1.1. Molecular dynamics of α-helical poly-l-glutamic acid in water solution

Chirgadze Yu.N.[1], Likhachev I.V.[2], Balabaev N.K.[2], Brazhnikov E.V.[1]*
[1]*Institute of Protein Research of RAS, Pushchino, Russia;*
[2]*Institute of Mathematical Problems of Biology, Branch of Keldysh Institute of Applied Mathematics, Russian Academy of Sciences, Pushchino, Russia ;*
* tefg@vega.protres.ru

α-Helix is a basic element of secondary structure from which the globular proteins are built. Since true native protein exists in water solution the structural behavior of protein is determined essentially by their dynamic properties. However, the problem is rather complicated because a majority of protein structures has been obtained in the crystal state. Here we have studied the dynamic properties of poly-L-glutamic acid model in a helical conformation in water solution. It includes 16 Glu residues placed in 4.5 turns of right-handed α-helix structure built with the data of Pauling & Corey (1951). In acidic water solution at pH about 3.5 poly-L-glutamic acid undergoes the helical conformation. Thus, our model has non-ionized side carbonyl Glu groups, as COOH, and ionized terminal groups, as NH3+ and COO-. An analysis of all the atomic groups makes no special sense. So, we have concentrated solely on dynamic study of peptide skeleton from Cα-atoms. Computational system included helical fragment, water solution molecules, and ions of sodium and chlorine. There were introduced 11 Na and 9 Cl ions which supply zero total charge of the sysytem. Numerical simulations were performed on the hybrid supercomputing system K-60 at the Keldysh Institute of Applied Mathematics, Russian Academy of Sciences. The initial part of trajectories, from 0 to 500 psec, corresponds to the refinement and relaxation of the model. A dynamic trajectory of α-helical poly-L-glutamic acid has been calculated from 0.0 to 25.0 nsec. We have inspected fluctuations of the Cα-chain at each integer numbers of time, in nanoseconds. That has been done by calculating the absolute shift values of Cα-atom positions at the next 1.0 nanosec intervals. The model has displayed several fluctuation modes along the dynamic trajectory. The most interesting modes show the distinctive shifts of Cα-atoms. These modes include two adjacent in the turns clusters of Cα-atoms which are placed approximately at one side of the helix. The observed modes are intrinsically dynamic feature of a single fragment of α-helix structure. And they suggest playing a key role in dynamics of protein molecules.

### S1.2. Multiscale modelling of DNA repair by photoenzymes

Domratcheva T.[1]*
[1]*MV Lomonosov Moscow State University;*
* t.domratcheva@lcc.chem.msu.ru

Photolyase photoenzymes, binding to damaged DNA sites, repair the main DNA photoproducts formed under the action of UV radiation. The functioning of photolyases is based on the reaction of photoinduced intermolecular electron transfer. Especially interesting from the point of view of the chemical mechanism is (6-4) photolyase, which repairs the most cytotoxic (6-4) pyrimidine-pyrimidone photoproducts of DNA. Despite the extensive study of the (6-4) photolyase mechanism using the high-end experimental and computational methods, the chemical details of the repair reaction have not been definitively established. Multiscale modeling, combining classical molecular dynamics and quantum chemical calculations of photoexcited states and reaction coordinate, is able to resolve some of the contradictions existing today in understanding the (6-4) photolyase mechanism.
The present study considers the main stages of the (6-4) photoprodroduct repair by (6-4) photolyase including photoinduced electron transfer leading to the formation of a photoprodroduct radical, breaking and formation of covalent bonds in the photoprodroduct radical and back electron transfer. Using density functional theory calculations, optimized geometries were obtained for modeling the repair reaction involving various forms of the critically important amino acid residue His365, whose role in the repair has been extensively discussed in the literature. In the case of neutral His365, the photoproduct radical rearranges by the OH-group transfer, for which the enzyme reduces the reaction energy barrier. In the presence of protonated His365, electron transfer coupled to proton transfer takes place leading to the formation of a protonated (neutral) photoproduct radical. In order for the repair reaction to proceed along this path, it is necessary to adjust electron affinity of the photoproduct. Estimates of the effect of the macromolecular environment on electronic energies were carried by computing excited electronic states for structures comprising the repair rection coordinate using the multiconfiguration quantum chemical method XMCQDPT2-CASSCF. Within the framework of these calculations, the electronic coupling matrix elements were also evaluated. The influence of the macromolecular environment on electron transfer energies was evaluated using classical molecular dynamics. To assess the electron transfer reaction rate, the results of the quantum chemical and molecular dynamics calculations were combined. The estimated electron-transfer rates indicated that the rapid recombination of the radical pair takes place in the presence of neutral His365. The presence of protonated His365, acting as a proton donor for the photoproduct radical, may substantially slow down back electron transfer. Thus, the

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overall rate and quantum yield of the (6-4) photoproduct repair by the photolyase should critically depend on the protonation state of His365. The work was carried out with the support of the RNF 22-23-00418.

### S1.3. A model of monolayer-monolayer membrane fusion using methods of molecular dynamics simulation

Minkevich M.M.[1]*, Molotkovsky R.J.[1], Batishchev O.V.[1]
[1]*Frumkin Institute of Physical Chemistry and Electrochemistry;*
* maria.minkevich16@gmail.com

Membrane fusion is involved in a plethora of crucial biological processes, such as secretion, signal transduction, exocytosis, etc. The process of membrane fusion is coupled with high kinetic barriers as it requires energy to overcome hydration repulsion and considerable local deformations in membranes. Fusion is initiated in 2-3 nm distance between membranes, followed by point-like protrusions of acyl lipid chains. The attraction between them leads to the formation of a hemifusion stalk, and then a fusion pore [1]. The stalk is a key intermediate structure, because the energy barrier of the stalk formation determines the speed of the fusion process.

Kalutsky et al., 2022 studied the process of monolayer-bilayer membrane fusion between peroxisomes and lipid droplets and estimated the energy profile for stalk formation [2]. The results had shown that the stalk formed by monolayer-bilayer fusion was energetically more stable than in case of bilayer-bilayer fusion. Notably, the stability of the stalk increased due to two main reasons: 1) the addition of different lipid components to membranes, such as DOPE; 2) shortening the distance between membranes.

The goal of this project is to reproduce the protocol from Kalutsky et al., 2022 for monolayer-monolayer fusion using molecular dynamics simulations. We investigated how the energy barrier of stalk formation in monolayer-monolayer fusion changed compared to monolayer-bilayer and bilayer-bilayer fusion. We studied the effect of different lipid components and the distance between membranes on the stability of the stalk. For this purpose we utilized a modified version of GROMACS 2018.8 with specifically designed reaction coordinate ξch, called "chain coordinate" [3]. This chain coordinate describes the interaction of two monolayers in a specific cylindrical volume. Using chain coordinate allowed us to reproduce the previously obtained results, as well as to calculate the energy barrier pf the stalk fomation for monolayer-monolayer fusion.

The work was carried out with the financial support of the Russian Science Foundation, grant No. 22-23-00551.

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### S1.4. AFM study of the effect of chromium (III) salts on the conformation of collagen molecules

Dubrovin E.V.[1]*, Sergeeva I.A.[1]
[1]*Lomonosov Moscow State University;*
* dubrovin@polly.phys.msu.ru

Collagens represent the family of proteins, which present in many cells, organs and tissues of living organisms and perform important biological functions, including mechanical, structural and protective. Due to the high degree of biocompatibility, collagen is a popular protein that is a component of biomaterials. A common structural element of collagens is a triple-stranded right-twisted helix from three polypeptide chains consisting of approximately 1000 amino acid residues (tropocollagen). In this work, the effect of various chromium (III) salts, which are widely used for processing collagen fibers, on the conformation and mechanical rigidity of tropocollagen molecules was studied using atomic force microscopy (AFM). A significant decrease in the persistent length (increase in flexibility) of tropocollagen was revealed upon treatment with chromium (III) salts, and a mechanism explaining this effect is proposed. This study is supported by the Russian Science Foundation grant 22-23-00395.

### S1.5. Absorption properties and photosensitizing effect of molecular oxygen

Krasnovsky A.A.[1]*, Benditkis A.S.[1], Kozlov A.S.[1]
[1]*FRC of Biotechnology RAS;*
* phoal@mail.ru

In view of the exceptional importance of oxygen in the biosphere, any properties of its molecules are of great interest for study. As a result of many-year research of our group, using activation of oxygen by laser radiation we for the first time, succeeded in measurement of the oxygen absorption spectra in aerated organic solvents and water under natural conditions. Approximately the same absorption properties of oxygen one can expect in the cells of living organisms. Success was achieved due to measuring the rates of chemical trapping of singlet oxygen and detection of the intensity of its own phosphorescence at 1270 nm under laser irradiation in the wavelength range of 500-1300 nm. Two main maxima of the oxygen activation action spectrum were found at 765 and 1273 nm. The relative intensity of these maxima depended on the nature of the solvents. In non-polar hydrophobic media, the 765 nm band is 6 times smaller than the long wavelength band. In water and alcohols, the 765 nm band is 1.5-2 times smaller than the IR band. In addition to these main bands, much weaker maxima were found at 690 and 1070 nm. The amplitude of the first maximum is about 15 times weaker than that at 765 nm, the amplitude of the second maximum in all media is about 100 times weaker than that at 1273 nm. Under the action of red light at 630 nm, which corresponds to the absorption maximum of dimeric oxygen, the generation of singlet oxygen could not be detected. Thus, it has been established that the absorption spectrum of dissolved oxygen contains the same four main maxima as the absorption spectrum of gaseous oxygen in the Earth's atmosphere. However, the main absorption maximum of atmospheric oxygen corresponds to the Fraunhofer line at 762 nm, and its integral absorption coefficient is 300 times greater than that of the band in the 1270 nm region. For dissolved oxygen, the strongest band is in the region of 1270 nm, which is 6 times larger in amplitude than the band at 762 nm. Bands of dimeric oxygen in visible region were not revealed in our experiments. The significance of these facts for understanding photodynamic activity (light-oxygen effect) and the function of oxygen in cells is discussed. Detailed information is provided in the publications cited below and references therein. This work was supported in part by RFBR grant No. 19-04-00331 A and the state assignment of the FRC Biotechnology RAS.

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### S1.6. Adsorption of polyadenines of double-stranded DNA onto the surface of gold nanoparticles

Sokolov P.A.[1]*, Kasyanenko N.A.[1], Ramazanov R.R.[1]
[1] *St.-Petersburg University, Russia;*
* p.a.sokolov@spbu.ru

The spatial organization of gold nanoparticles (NPs) using deoxyribonucleic acids (DNA) has a wide range of applications in the field of nanotechnology to create objects with unique physical properties. In particular, DNA can be used to control the shape and size of NPs during their synthesis or to create biosensors, since it can carry target-specific sequences (DNA aptamers). The stability of the DNA duplex plays a crucial role, since it determines the final geometry of these nanostructures and, accordingly, their useful properties. In this regard, the adsorption of DNA on a gold surface deserves close attention. This process can lead to degradation of the formed nanostructures, changing their geometry or making DNA aptamers inaccessible for the target. In the general case, the adsorption of double-stranded DNA on the gold surface of NPs does not take place. Adsorption of one of the strands of double-stranded DNA is determined by a number of factors. Among them, one can distinguish, firstly, the stability of the duplex itself, which determines the probability of local unwinding and, thus, the availability of the strand to interact with the surface. Secondly, there is the rate of the adsorption reaction, which depends on the properties of a particular single-stranded DNA sequence and the surface itself. In our work, we investigated the effect of magnesium ions on the stability of a DNA duplex conjugated with 5 nm nanoparticles, which were used to synthesize larger dumbbell-shaped nanostructures [1]. Experimental results show that magnesium not only stabilizes DNA, but also recharges the negatively charged NP surface. This may contribute to the partial wrapping of DNA around NPs, which is accompanied by damage to the secondary structure of DNA. This effect was especially clearly demonstrated by us when studying the interaction of single-stranded polyadenine sequences with NPs [2]. We also showed that monovalent sodium ions, in contrast to magnesium, screen the NP surface and DNA charge without leading to an increase in the number of bound oligonucleotides. Polyadenine regions of DNA have the property of specific adsorption on gold and are widely used for immobilization of sequences linked to them. The proposed scheme of adsorption of single-stranded polyadenine DNA regions on gold NPs deserves special attention. We have shown that the ionic strength also plays a key role. We hope that this approach can be used to increase the stability of DNA-organized NPs and also applied in a number of biosensor schemes to increase their detection limit. The research was supported by RSF grants No. 19-73-00113 and No. 22-25-00302.

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### S1.7. Affinity degree of ethidium bromide and methylene blue to single-stranded polyadenilic acid

Vardevanyan P.O.[1]*, Antonyan A.P.[1], Parsadanyan M.A.[1], Movsesyan Z.H.[1]
[1] *Yerevan State University, Armenia;*
* p.vardevanyan@ysu.am

The interaction peculiarities of phenothiazine dye methylene blue (MB) and phenantridine dye ethidium bromide (EtBr) with synthetic single-stranded polynucleotide poly(rA) have been studied at the solution ionic strength 100 mM and ratio change 1.0<r<20 (r=D/P – ligand/phosphate), by the methods of absorption and fluorescence spectroscopies. Based on the obtained spectra the binding curves of MB and EtBr with ss-poly(rA) were constructed in Scatchard's coordinates (dependence of r/Cf on r). Scatchard's curve reflects the cooperatve binding of MB to ss-poly(rA), since at the sufficiently low values of r the curve is bell-shaped, at high values of r the curve sharply decreases, then insignificantly alters at higher values of r. Analogous analysis of the absorption spectra at the interaction of EtBr with ss-poly(rA) gives non-linear Scatchard's curve, which sharply decreases at low values of r, but at higher values of r the curve changes a little. Non-linear curves in Scatchard's coordinates indicate that the interaction is either anti-cooperative, or is implemented according to the principle of excluded binding sites. It can also be the result of interaction by more, than one mode of binding with different binding constants. The obtained curves were analyzed, proceeding from the fact that both ligands can form al least two types of complexes with ss-polynucleotides [1]. From these curves the parameters for two modes of the binding of the mentioned ligands with ss-poly(rA) were determined – association constant values K and number of bases n per binding site.
Linear region on the mentioned binding curves, corresponding to low values of r, has a high slope and characterizes the strong binding of both ligands to ss-polynucleotide. Analysis of this region gives high values of K (105x106 M-1). On the other hand, MB and EtBr in solution are in cationic form, poly(rA) is a polyanion, due to which these ligands may bind to this polynucleotide by electrostatic mechanism as well. This mode is characterized by binding constant value K (103x104 M-1). Quantitative analysis of the values of the obtained parameters revealed that the binding constant of MB to ss-poly(rA) by strong mode is lower by an order, than that of EtBr binding to this polynucleotide. Sufficiently high value is received for n at the strong binding mode of Mb to the mentioned polynucleotide, while for EtBr analogous value is in correspondence to those, obtained for the complexes of EtBr with ss- and ds- DNA [1]. High value of K (for the strong mode) at the binding of MB to ss-poly(rA) reflects the high affinity of this ligand to polyadenilic acid in ss-state. For EtBr the binding constant values by the strong mode indicate that the affinity of this ligand to ss-poly(rA) is less pronounced, than for MB. This fact in maintained on the basis on the values of n as well: for MB there are more limited number of binding sites on this polynucleotide with high affinity to him, while for EtBr the number of binding sites is less limited. In actual, the structure of polynucleotide is more available for semi-intercalation of EtBr, than for MB, despite the fact that the latter binds with higher affinity to these centers. The obtained data in in good accordance to the literature results [2,3].
Based on the aforementioned, we conclude that semi-intercalation is the main mode for the binding of both MB and EtBr to ss-poly(rA).

Another important conclusion is that MB exhibits high affinity to ss-poly(rA) with binding constant value K is about 106 M-1. Though, the interaction of MB to this polynucleotide is cooperative, which is due to the high values of K and n. For EtBr the cooperativity is absent, which indicates that the structure of polynucleotide is more available for this ligand and more number of binding centers exist on this polynucleotide, than for MB.

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### S1.8. All-D-enantiomeric peptide designed for Alzheimer´s disease treatment dynamically interacts with amyloidogenic region of amyloid-β precursor

Okhrimenko I.S.[1,2], Volynsky P.E.[1,2], Efremov R.G.[1,2], Bocharov E.V.[1,2]*
[1]*Moscow Institute of Physics and Technology ;*
[2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS;*
* edvbon@mail.ru

Alzheimer's disease is a devastating neurodegenerative disease resulting in severe dementia. Detailed information on the structure, dynamics, and various intermolecular interactions for biomolecules directly involved in the development of Alzheimer's disease is required for the rational development of new biologically active compounds and screening of existing ones to obtain the most effective candidates for medicines [1]. Genetic evidence strongly suggests that aberrant generation and/or clearance of the neurotoxic amyloid-β peptide (Aβ), being the products of sequential cleavage of amyloid precursor protein (APP), triggers the disease. Aβ accumulates at the contact points of neurons as ordered strands and fibrils, forming the so-called senile plaques. At the same time, Aβ isoforms of various lengths are found in the brain of healthy people regardless of age and, apparently, play a role in signaling pathways in the brain and have neuroprotective properties at low concentrations.

All-D-enantiomeric peptide D3 and its derivatives were recently selected with the aid of phage display to directly destroy cytotoxic Aβ aggregates [2]. Currently, one of the D3-like compounds is about to undergo a phase II clinical trial, however, high resolution molecular details of its disease preventing or pharmacological action are not completely clear. To solve the problem, we used complex approach based on biochemical and biophysical methods such as protein engineering, microscopic thermophoresis, fluorescence confocal microscopy, fluorescence polarization, microfluidic diffusion calibration, circular dichroism, high resolution nuclear magnetic resonance spectroscopy (NMR) and computer simulation. We present experimental evidence showing that D3-peptide, being an intrinsically disordered peptide (IDP), can dynamically and specifically bind in IDP/IDP-like manner to the extracellular juxtamembrane (JM) region of membrane-bound Aβ precursor, CTFβ transmembrane fragment of amyloid precursor protein (APP672-726, Aβ1-55 in amyloid-β numeration). Namely, heteronuclear NMR spectroscopy showed that D3-peptide directly binds to the amphiphilic near-membrane JM region of Aβ17-26, which, depending on external conditions, is capable of undergoing a conformational transition from the α-helical conformation to the β-chain, which is involved in folding of β-amyloid into fibrils. The obtained structural data in agreement with ELISA and Western-blot analyzes, which reveal that D3-peptide dynamically binds in the vicinity of α-secretase recognition site situated between the extracellular cation-binding domain and JM helix of APP. The influence of a number of pathogenic mutations located in different structural and functional parts of APP on the peptide binding was also tested.

The data suggest that D-enantiomeric peptide D3 recognizes the amyloidogenic region of APP also before its processing, restricting conformational diversity not compromising its α-helicity and preventing intermolecular hydrogen bond formation, which would create prerequisites for inhibition of early steps of Aβ conversion into β-conformation and its toxic oligomerization associated with early stages of Alzheimer's disease development [3]. The achieved progress in understanding the molecular mechanism of D3-peptide action is an important step towards development of an effective treatment and prevention strategy of Alzheimer's disease.

The study is supported by Russian Science Foundation (project #23-74-00024).

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### S1.9. Amyloid aggregation of human SAA

Trunilina M.V.[1]*, Lapteva Y.S.[1], Marchenkov V.V.[2], Glukhov A.S.[2], Ryabova N.A.[2], Katina N.S.[2]
[1]*Institute of biological instrumentation RAS, Pushchino, Russia;*
[2]*Institute of protein research RAS, Pushchino, Russia;*
* masha.trunilina@mail.ru

SAA is an α-helical protein, one of its functions is immune response. In chronic inflammatory diseases, the concentration of SAA in the blood increases, leading to its aggregation and the amyloids formation in various organs and tissues. To date, there are no effective treatments for SAA amyloidosis, and therapy is aimed only at relieving symptoms. Therefore, the study of amyloid aggregation of this protein is an important direction in molecular biophysics and medicine.

Fibrils consisting of protein fragments are found in the tissues of patients with SAA-amyloidosis. Therefore, to date, there is no answer to the question of whether the full-length SAA forms amyloids, or before aggregation it undergoes proteolysis. The lack of information is because of in most studies, investigation of SAA amyloid formation was carried out on a model of a mouse protein or a human protein with additional methionine at the N-terminus due to expression in bacterial cells. These proteins were characterized by reduces amyloidogenicity or its absence. In contrast, in this work, for the first time, a genetic construct for the expression of SAA in the ubiquitin system was obtained, allowing the purification of the full-length protein without additional amino acid residues.

Data on protein crosslinking with glutaraldehyde indicate that SAA at pH 8.3 is present in solution as a monomer. After 6 hours of incubation at a temperature of 37°C, the protein forms aggregates, which, according to electron microscopy, are fibrils to 150 nm long. Analysis of the circular dichroism spectra in the fat UV region indicates a change in the secondary structure of the aggregates compared to the monomer and decrease in α-helices content in them. The resulting fibrils bind

the fluorescence dye thioflavin T, which is specific for the amyloid cross-β-structure. Thus, the results obtained conclude that under chosen conditions SAA forms amyloids.
To determine the protein fragments involved in the formation of intermolecular interactions in amyloids, limited proteolysis of the monomeric protein and fibrils was carried out using nonspecific proteinase K. It was shown that under conditions where complete cleavage of the monomeric protein is observed, the length of fragments resistant to proteolysis is amyloids correspond to full-length SAA. These data conclude that the SAA full-chain is involved in the formation of intermolecular interactions in amyloids. The results of this work provide new information about the formation of SAA amyloids and indicate the need to use a full-length protein for further studies of its aggregation, as well as the search for approaches to its inhibition.

**S1.10. Analysis of transcription through nucleosome using FRET-based techniques reveals the variability of spatial structures in elongation complexes with the same RNA product length**

Gerasimova N.S.[1,2], Feofanov A.V.[1,2,4], Studitsky V.M.[1,3]*
[1]*Biological Faculty, Lomonosov Moscow State University, Russia;*
[2]*Institute of Gene Biology, Russian Academy of Sciences, Russia;*
[3]*Fox Chase Cancer Center, Philadelphia, USA;*
[4]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Russia;*
* Vasily.Studitsky@fccc.edu

RNA polymerase 2 (Pol 2) is a DNA-dependent RNA polymerase that is responsible for transcription of messenger RNA (mRNA), the majority of small nuclear RNAs (snRNA) and microRNAs in the eukaryotic cells. Moderate transcription through chromatin by Pol 2 is accompanied by survival of the histone proteins on the DNA due to specific Pol 2 type mechanism of elongation conserved from yeasts to human [1]. This process is accompanied by formation of different structural intermediates – elongation complexes (ECs). Spatial features of the ECs are investigated using wide range of experimental approaches including electron microscopy, cryoEM, DNA footprinting, Förster resonance energy transfer (FRET) based techniques etc. FRET-based approaches using fluorescent probes introduced into the nucleosomal DNA allow to determine structural features of the ECs in chromatin in vitro in conditions close to the physiological.
Here we address the structure of EC formed in a close proximity of Pol 2 to the nucleosome (5 bp before the promoter-proximal nucleosomal boundary, EC-5). We used an approach based on the uniquely positioned fluorescently labelled mononucleosomes formed on the high-affinity DNA sequence 603 transcribed in vitro [2, 3]. Positioned nucleosomes present a polar barrier to transcription by Pol 2 type mechanism [4]. Here 603 nucleosomes in permissive transcriptional orientation were used because they better recapitulate the properties of nucleosomes transcribed in vivo [1, 5]. Fluorescent labels were introduced into the adjacent gyres of nucleosomal DNA and reproduced a FRET-pair (donor and acceptor).
Stalled ECs with the enzyme active site in -5 position were obtained during transcription in the presence of a limited set of nucleoside triphosphates. The ECs were divided by electrophoresis in polyacrylamide gel (PAGE) and analyzed using FRET techniques. Measurements were conducted using Amersham Typhoon RGB Imager (Cityva, Sweden) in case of bulk FRET-in-gel. Comparative analysis of FRET efficiency for free nucleosomes and ECs with RNAPs revealed changes in the structure of nucleosomes in ECs. Two types of structures were identified for EC-5. It was found that ECs with the same RNA length could exist in different conformational states.
This work was supported by the Russian Science Foundation (Grant No. 21-64-00001). The study was carried out using equipment of the Interdisciplinary Research and Educational School "Molecular Technologies of Living Systems and Synthetic Biology" of Moscow State University".
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**S1.11. Application of IdeS and IdeZ endopeptidases for peptide mass fingerprinting of immunoglobulins, as well as for medicine and veterinary**

Konstantinova S.V.[1], Ustenko E.V.[1], Boksha I.S.[1]*, Polyakov N.B.[1], Lunin V.G.[1]
[1]*N. F. Gamaleya National Research Center for Epidemiology and Microbiology;*
* boksha_irina@mail.ru

IdeS and IdeZ endopeptidases are secreted by pathogenic Streptococcus pyogenes and S. zooepidemicus, respectively, and possess exceptional substrate specificity. These cysteine proteases cleave IgG heavy chains at a single site in the hinge region in two steps: first cleaving one of the two heavy chains to form a single cleaved IgG molecule (scIgG) keeping intact one of the heavy chains. The scIgG molecule is less susceptible to cleavage than the whole IgG molecule, and the cleavage of the scIgG molecule to F(ab')2 and Fc is 100 times slower than that of the native IgG molecule. The exceptionally high substrate specificity has served to the usage of IdeS and IdeZ as biopharmaceutical agents for the temporary removal of pathogenic IgG in human autoimmune conditions and the prevention of rejection in sensitized patients after kidney transplantation (IdeS), and as a constituent antigen comprising subunit veterinary vaccine against streptococcal infection (IdeZ). According to the literature, the use of IdeS hydrolysis simplifies the procedure for deciphering the amino acid sequence of monoclonal antibodies. The ideS and ideZ genes are cloned (in the case of ideS, the gene is cloned from a collection strain; in the case of ideZ, the gene is synthetic) and expressed in a heterologous expression system in Escherichia coli. The 6-His-tag affinity domain was introduced into the amino acid sequence of each endopeptidase. The enzymes IdeS and IdeZ are isolated and purified by metal affinity chromatography. The identity of the primary structure of the obtained recombinant IdeS and IdeZ to natural enzymes is confirmed by mass spectrometry. The produced recombinant IdeS and IdeZ are homogeneous on polyacrylamide gel electrophoresis (PAGE) and are active against human and various animal IgG. The specificity of the human IgG cleavage by endopeptidases IdeS and IdeZ

is confirmed by PAG electrophoresis and mass spectrometry. We have demonstrated the advantage of using hydrolysis with recombinant IdeS and IdeZ in addition to the standard set of proteases in determining the structure (amino acid sequence) of a monoclonal antibody - human immunoglobulin – using peptide mass fingerprinting. Also, for the first time, a system of enzyme-linked immunosorbent assay (ELISA) was developed, and the use of recombinant IdeZ was demonstrated for diagnosing and determining the titer of specific antibodies in the blood of horses that had contact with pathogenic streptococci and recovered from Horse Strangles. The use of recombinant IdeS and IdeZ is promising from biotechnological, medical, and veterinary points of view.

### S1.12. Application of entropy and information concepts to the study of biosystems: from molecules to organisms

Aristov V.V.[1], Buchelnikov A.S.[2], Karnaukhov A.V.[3], Nechipurenko Y.D.[2,4]*
[1]*Federal Research Center "Computer Science and Control", Russian Academy of Sciences, Moscow, Russia;*
[2]*Sevastopol State University, Sevastopol, Russia;*
[3]*Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, Russia;*
[4]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences;*
* nech99@mail.ru

The concept of entropy, have long been known in thermodynamics, has not yet been widely used in biophysics. Were, we analyze the possibility of using the concepts of entropy (negentropy) and information as tools capable of describing the essence of biosystems. Statistical entropy can be applied to describe local non-equilibrium distributions of a system as a necessary condition of its living state. The application of statistical methods, associated with the name of Blumenfeld, is reinterpreted in the discussion of representations of nonequilibrium processes. The possibilities of applying different definitions of entropy to describe a biosystem are explored. Model problems, for the solution of which methods of statistical physics and kinetic theory are applied, are considered.
Cooperative interactions between molecules, their aggregates and cells lead to a more complex structure and a decrease in statistical entropy of the system. For a multicellular organism entropy is much less than entropy for the same mass of a colony of unicellular organisms. In the limit, the system of organism parts is realized in a single way, which corresponds to entropy minimum. In other cases, entropy is greater. Cooperativity reduces entropy of the system; we have shown this numerically using a simple example of ligand binding to a macromolecule, which has two reaction centers. We considered the qualitative and quantitative relationship between the entropy of the system and the cooperativity of ligand binding to macromolecules. The observed presence of minima and maxima in the entropy dependence on the binding parameters can be interpreted as the ability of the adsorption system to carry more or less information, respectively [1, 2].
The inhomogeneous relaxation problem for the kinetic equation reproduces important features of metabolic processes. Schrödinger's notions of the feeding of a living system by negentropy gain certainty. The nonequilibrium local distribution has less entropy than the equilibrium one, which allows interpreting the nonequilibrium local entropy as inherent to the living state and comparing it with the local equilibrium entropy inherent to nonliving matter.
Non-stationary processes for biosystems are also being studied. The concept of entropy may play a role in the development of theories of aging, bearing in mind the degradation as a peculiar manifestation of the second principle of thermodynamics for open non-equilibrium systems. A kinetic equation is proposed, based on the introduced notion of structural distribution function and the notion of two characteristic scales for a biological system: the time of metabolic processes and the life cycle time. Slow changes in the structural distribution function are interpreted as aging of the biosystem. Aging (degradation) of the living system at different levels, from cell to organism, as a whole is interpreted as a loss of information and an increase in entropy. The connection with the informational theory of aging in different variants and the concept of cooperon is discussed [3-7].
In order to study the processes occurring during aging, it is suggested to consider tissue structures of the skin as a model. Moving from the depth of the skin to its surface, we cross the boundaries of living and dead cell states. Statistical combinatorial approach allows to make an estimation for entropy increase within this model. Also, the Kullback—Leibler entropy (divergence) gives an increase of this value.
The problem of aging is considered from the viewpoint of the possibility to maintain a biosystem in steady state in time. For this purpose, it is supposed to create conditions for 'opening' the system on a large time scale: for example, replacing cell structures with polypotent cells at a certain rate.
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### S1.13. Application of the Brownian dynamics method to describe the sensitivity of the dynamics of tubulin microtubules to temperature

Eltsov I.A.[1,2]*, Ulyanov E.V.[1], Vinogradov D.S.[3], Gudimchuk N.B.[1,3]
[1]*Lomonosov Moscow State University, Moscow, Russia;*
[2]*Moscow Institute of Physics and Technology, Moscow, Russia;*
[3]*Center for Theoretical Problems of Physico-Chemical Pharmacology of RAS, Moscow, Russia;*
* eltsov.ia@phystech.edu

Microtubules are dynamic tubulin polymers, which perform numerous critical functions throughout the life cycle of every eukaryotic cell. Microtubules exhibit a remarkable ability to elongate and shorten for several micrometers with abrupt switches between the phases of assembly and disassembly. This process is highly sensitive to temperature: microtubules are easily depolymerized when they are cooled below the temperatures of about 20 degrees Celsius (at physiological tubulin concentration). The effects of temperature on microtubule stability is poorly understood, but it is exploited in cell biology as a way to quickly probe microtubule stability by subjecting cells to cooling. Recently, our laboratory has developed a computational model, describing microtubule dynamics, using a combined Brownian dynamics and Monte-Carlo approach (Gudimchuk et al., Nat. Commun. 2020). This model has provided accurate descriptions of microtubule tip structures

during assembly and disassembly, and it brought new insights into the mechanisms of generation of pushing and pulling forces by dynamic microtubules. Here we extend the model to describe the sensitivity of microtubule assembly and disassembly to temperature. Temperature plays a complex role in the dynamics of molecules. We consider the general problem of the transition of particles through the energy barrier in order to take into account the temperature effect on the probability of tubulin attachment to the end of the microtubule. Applying the obtained analytical dependence, when modeling the assembly of a microtubule, it is possible to avoid excplicit consideration of all the tubulin molecules floating in solution. Thus, we concentrate on describing the interaction of tubulin monomers inside the microtubule. These interactions in our model are represented by three types of energy functions: the lateral potential, the potential of the longitudinal connection between tubulins and the bending potential. The proposed method allows us to consider the temperature dependence of the dynamics of tubulin molecules outside the microtubule (implicitly), as well as inside the microtubule lattice (explicitly). Applying the created model, we analyzed the process of microtubule transition from assembly to disassembly and formulated two main hypothetical mechanisms of microtubule stability loss during cooling. Collectively, our analysis provides estimates of activation barriers of tubulin-tubulin bonds and it sheds new light onto the old problem of the thermostability of tubulin cytoskeleton.

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### S1.14. Brownian dynamics model of the encounter complex formation of cytochrome c and dimer III respiratory complex in the mitochondrial crista lumenaral space

Abaturova A.M.[1]*, Riznichenko G.Yu.[1]
[1] *Faculty of Biology, Moscow State University;*
* abaturova@list.ru

Expansion of mitochondrial cristae lumen is observed in many diseases and aging, is accompanied by a decrease in ATP production [1, 2]. These phenomena are associated with disruption of the mitochondrial electron transport chain in the region from III to IV of the respiratory complex, where the electron carrier is the only mobile carrier in the lumen, cytochrome c. A small (about 12 kDa) protein, cytochrome c (cytC), diffuses in the intermembrane and luminal space of mitochondria and carries out electron transfer from the cytochrome c1 subunit of complex III dimer (III2) to complex IV. This reaction can be divided into 2 stages: binding of freely diffusible cytC protein to cytC1 of complex III, and electronic transport between cytochromes. This paper presents a model of the Brownian dynamics of the first stage of this process, i.e., the formation of an encounter complex of the water-soluble cytC protein with the cytC1 cofactor of complex III fixed in the membrane. The model was used to study the influence of the initial position of cytC relative to complex III2 and the role of electrostatic interactions in the formation of the encounter complex.

Using the ProKSim program [3], we constructed computer models of diffusion, electrostatic interaction, and binding of oxidized cytC (PDB ID 3O1Y) with reduced cytC1 III2 (PDB ID 1BGY) in solution and in the lumen of the crista. The Poisson-Boltzmann formalism was used to calculate the electrostatic potential around protein molecules. The charges on the protein atoms were arranged in accordance with the CHARMM27 force field, supplemented with parameters for the heme and iron-sulfur cluster. Protein molecules and electrostatic potentials were visualized using PyMol (http://pymol.org/). The estimation of the parameters of the model for the formation of the cytC1-cytC complex was preliminarily performed for the reaction in solution. The values of the model parameters were estimated from the experimental data [4] using the condition that the model and experimental dependences of the binding constant on the ionic strength coincide [5].

Respiratory complexes III and IV can form a supercomplex, a respirasome [6], which creates a kind of "electrostatic channel" that directs the movement of oxidized cytC to complex III and the movement of reduced cytC to complex IV, providing fast electron transfer between complexes III and IV. Swelling of cristae in diseases leads to the destruction of respirasomes [7]. Using the model, we studied the influence of the initial positions and orientation of CytC relative to the respiratory complex III on the rate of formation of the CytC1(III2)-CytC complex. The membranes in the model were taken into account as geometric constraints. Dimensions of the reaction volume: crista lumen width (CLW) 12 and 16 nm, membrane length 26 and 30 nm. Kinetic curves for the formation of preliminary complexes were obtained at an electrostatic interaction energy of proteins of -3.7kT and a distance between the Fe atoms of cytC and the cytC1 P subunit III2 of less than 3.5 nm for an ionic strength of 130 mM, pH 7. The kinetic curve of protein binding for each initial position consisted of 20000 points. From the curves, the half-life of the formation of the encounter complex of proteins t1/2 was determined. 60000 numerical experiments were carried out on the diffusion of cytC and the formation of a encounter complex with III2.

We studied 7 initial positions of CytC, in which the distance from the Fe atom to the Fe of the heme of the active monomer III was 5.6 nm, 0.5 nm away from the membrane surface. In one series of numerical experiments, the orientation of cytC was random with respect to a fixed position of the center of mass of cytC; in others, it was fixed and corresponded to the average structure of 10000 positions of cytC obtained in preliminary calculations of the diffusion of cytC. CytC acquires such orientations if it has been diffusing for a long time in the electrostatic field of the respirasome before binding with cytC1.

For 4 initial positions of cyt C, which are closer to complex IV in the 5GPN respirasome, t1/2 is 0.5–0.9 mks, for the remaining 3 positions t1/2 is 1.2–1.7 mks. With an increase of CLW from 12 to 16 нм, an increase in the probability of finding cytC in the region of the inactive cytC1 D III2 subunit and an increase in the time t1/2 by 4–18% is observed. The greatest difference in t1/2 was obtained for cytC not previously oriented in the field of proteins and from the position closest to IV in the 5GPN respirasome. The resulting effect may contribute to a decrease in the rate of CytC electron transport during the expansion of the cristae and a simultaneous increase in the distance between the respiratory complexes [7], when cytC can't obtain a preliminary orientation in the resprasome electrostatic field. Further consideration of a detailed model that takes into account the conformation of the formed complexes and the structure of respirasomes in cristae will reveal their effect on electron transfer in the respiratory chain.

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### S1.15. C-B-A test of DNA force fields

Strelnikov I.A.[1], Kovaleva N.A.[1], Klinov A.P.[1], Zubova E.A.[1]*
[1]*N.N. Semenov Federal Research Center for Chemical Physics of RAS;*
* elena.0.zubova@gmail.com

The DNA duplex may be locally strongly bent in complexes with proteins, for example, with polymerases or in a nucleosome. At such bends, the DNA helix is locally in the non-canonical forms A (with a narrow major groove and a large amount of north sugars) or C (with a narrow minor groove and a large share of BII phosphates). To model the formation of such complexes by molecular dynamics methods, the force field is required to reproduce these conformational transitions for a naked DNA. We analyzed the available experimental data on the B-C and B-A transitions under the conditions easily implemented in modeling: in an aqueous NaCl solution. We selected six DNA duplexes which conformations at different salt concentrations are known reliably enough. At low salt concentrations, poly(GC) and poly(A) are in the B-form, classical and slightly shifted to the A-form, respectively. The duplexes ATAT and GGT ATACC have a strong and salt concentration dependent bias toward the A-form. The polymers poly(AC) and poly(G) take the C- and A-forms, respectively, at high salt concentrations. The reproduction of the behavior of these oligomers can serve as a test for the balance of interactions between the base stacking and the conformational flexibility of the sugar-phosphate backbone in a DNA force field. We tested [1] the AMBER bsc1 and CHARMM36 force fields and their hybrids, and we failed to reproduce the experiment. In all the force fields, the salt concentration dependence is very weak. The known B-philicity of the AMBER force field proved to result from the B-philicity of its excessively strong base stacking. In the CHARMM force field, the B-form is a result of a fragile balance between the A-philic base stacking (especially for G:C pairs) and the C-philic backbone. Finally, we analyzed some recent simulations of the LacI-, SOX-4-, and Sac7d-DNA complex formation in the framework of the AMBER force field.

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### S1.16. Characterisation of the Src kinase:Na,K-ATPase complex and its role in pathogenesis of Alzheimer's disease

Strelkova M.A.[1]*, Tolstova A.P.[1], Adzhubei A.A.[1,2], Barykin E.P.[1], Tverskoi A.M.[1], Petrovskaya A.V.[1], Leonova O.G.[1], Anashkina A.A.[1], Bogdanova A.Yu.[3], Makarov A.A.[1], Mitkevich V.A.[1], Petrushanko I.Yu.[1]
[1]*Engelhardt Institute of Molecular Biology;*
[2]*Washington University School of Medicine and Health Sciences;*
[3]*University of Zurich;*
* maria7873878@gmail.com

Alzheimer's disease (AD) is nowadays the most widespread neurodegenerative disease in the world, which causes progressive cognitive decline and memory loss. There are different hypotheses regarding the development of this disease. The most common is amyloid hypothesis, according to which neurodegeneration observed in AD pathogenesis is caused by accumulation of amyloid beta (Aβ) protein, a proteolytic byproduct of amyloid-beta precursor protein (APP) normally present in human body at low concentrations. Aβ peptide varies in length from 37 to 43 amino acids and is thought to be involved in synaptic activity regulation, a number of signalling pathways, protection from brain infections and posttraumatic brain recovery. The metabolic disorder of Aβ, especially of Aβ(1-42) which is known to be the most neurotoxic type, observed in AD pathogenesis can be caused by such factors as APP mutations and β-secretase dysregulation, the enzyme involved in the abnormal amyloidogenic proteolysis of APP. In this way, the accumulation of Aβ leads to generation of toxic oligomers of Aβ and its aggregation and deposition in the extracellular space forming distinctive senile plaques.

Monomers and oligomers of Aβ effect on the functionality of various enzymes in the cells. One of the affected enzymes is Na,K-ATPase, the enzyme that maintains the resting membrane potential essential for normal functionality of neurons, thus inhibition of its activity leads to the neuronal electrogenic malfunction observed in patients with AD [1]. Apart from ion transport, Na,K-ATPase is known for its receptor function, which involves numerous signalling pathways and, in particular, Src kinase activation.

The activity of unbound cellular Src is mainly regulated by its two modifications: the inhibiting tyrosine-527 (Y527) phosphorylation and activating tyrosine-416 (Y416) autophosphorylation. But besides that, Src kinase can form a complex with Na,K-ATPase involving two Src domains: regulatory SH2-domain and kinase domain [2]. Binding to Na,K-ATPase prevents the kinase domain from autophosphorylation, however, when the ligand, such as cardiotonic steroid ouabain, is bound to Na,K-ATPase, the kinase domain of Src is released, autophosphorylates by Y416 and, as a result, Src kinase is activated.

We have found that binding Aβ(1-42) to Na,K-ATPase in vitro also results in Src activation [3]. The specificity of Aβ-induced Src activation was proved by the reverse peptide Aβ(42-1) which did not show any activating effect. Of note, Aβ(1-42) in the absence of Na,K-ATPase did not activate Src. This data, together with data obtained from cell experiments, show that Na,K-ATPase appears to be a receptor to Aβ(1-42) triggering Src activation [3]. As Src plays an important role in numerous cellular processes, including neurogenesis, regulation of expression and activity of receptors, the neurotoxic effects of Aβ and demential processes in AD may be associated with the long-term activation of Src through Na,K-ATPase:Aβ interaction, thus making the interaction between Src and Na,K-ATPase the potential therapeutic target.

The full investigation of complex Src:Na,K-ATPase is required for the development the ways of searching for the inhibitors of their interaction and understanding of how this complex works.

We obtained the dissociation constant for dephosphorylated Src:Na,K-ATPase complex equal to $0.21 \pm 0.04$ μM using MicroScale thermophoresis [3]. Further, we measured the dissociation constants for phosphorylated by Y416 and Y527 Src:Na,K-ATPase.

In order to investigate which modifications of Src are bound by Na,K-ATPase in cells, the neuroblastoma culture SH-SY5Y was used. The cells were lysed, and Src was co-immunopreciputated with Na,K-ATPase α1-subunit. Then, the levels of Src phosphorylation by Y416 and Y527 were measured and compared to those in total cell lysates. The obtained data show, that Src with inhibiting phosphotyrosine-527 cannot be detected after the co-immunoprecipitation, yet co-immunoprecipitated Src with activating phosphotyrosine-416 is detected in the presence of ouabain.

The interaction interfaces of Na,K-ATPase and Src kinase in the dephosphorylated and phosphorylated by Y527 states were predicted using molecular docking. In this modeling, the existing structures obtained from PDB were used. These structures do not include N-terminal SH4-unique domain (SH4UD), which has not been crystallized yet due to its unstable conformation. However, this domain has been shown to play regulatory role in Src kinase activity. Therefore, we obtained equilibrium conformations of Src kinase with SH4UD using REHT MD and predicted the interaction interfaces of full-length Src

with Na,K-ATPase. The comparison of predicted interaction interfaces for full-length Src and Src without SH4UD may help understand the role of SH4UD in Src:Na,K-ATPase interaction.
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### S1.17. Characteristics of DNA Hydration Shells in Solutions Obtained Based on the Analysis of Their Complex Permittivity in the Terahertz Region

Penkov N.V.[1]*
[1]*Institute of Cell Biophysics of RAS, Federal Research Center "Pushchino Scientific Center for Biological Research of RAS";*
* nvpenkov@rambler.ru

The study of DNA hydration has been conducted for a long time using a variety of experimental and theoretical approaches. The vast majority of them are aimed at studying hydrate structures, including 10-20 water molecules (per nucleotide) with a strongly changed energy. In this range of hydrate numbers, fundamental DNA transitions occur between conformations B↔A, B↔Z [1]. Even if DNA molecules are in the native state, with such a small hydration, one cannot be sure of the "native" state of their hydration shells, as they are in living nature. Therefore, it is also necessary to study DNA hydration in systems with a higher water content, that is, in dilute aqueous solutions.
One of the informative methods for studying hydration in solutions is dielectric spectroscopy, which allows determining the spectra of complex permittivity (CP). A special kind of dielectric spectroscopy is terahertz time-domain spectroscopy (THz-TDS). The terahertz range lies between the gigahertz region, where the collective dynamics of the molecules of matter is manifested, and the infrared region, reflecting the intramolecular vibrations of small molecular groups. The terahertz range (on energies and frequencies) just corresponds to the intermolecular structure and dynamics of water, therefore it carries rich information about hydration.
An approach based on THz-TDS for the study of DNA hydration is proposed, the essence of which is as follows. The CP spectra of a DNA solution, a pure solvent (a similar aqueous solution without DNA) and a dry DNA sample in the range of 10-110 см^(-1) are recorded. The dielectric contribution of DNA molecules is excluded from the CP spectra of the DNA solution using the effective medium model for two-phase dielectrics with filamentary inclusions developed by us [2]. This makes it possible to obtain CP spectra of the aqueous phase of the DNA solution. The CP spectra of the aqueous phases of the DNA solution and the solvent are subjected to the fitting procedure, according to the model CP, which takes into account three main, well-known, water dispersion regions in the terahertz range:
$\varepsilon^* = (\Delta\varepsilon_1)/(1-i\omega\tau_1) + (\Delta\varepsilon_2)/(1-i\omega\tau_2) + A/(\omega_0^2-\omega^2-i\omega\gamma) + \varepsilon_\infty + i\,\sigma_0/(\varepsilon_0\,\omega)$, (1)
where $\tau_1$ and $\Delta\varepsilon_1$ – relaxation time and strength of bound water molecules (Debye relaxation); $\tau_2$ and $\Delta\varepsilon_2$ – time and strength of orientation relaxation of free water molecules; A, $\omega_0$, $\gamma$ – amplitude, resonant frequency and damping parameter of intermolecular stretching vibrations of water molecules bound by hydrogen bonds; $\varepsilon_\infty$ – high-frequency permittivity, $\sigma_0$ – dc-conductivity, $\varepsilon_0$ – vacuum permittivity, $\omega$ – frequency, i – imaginary unit. The parameters of the aqueous phases of the DNA solution and the solvent calculated on the basis of the fitting were compared with each other to identify differences. Since the aqueous phase of the DNA solution differs from the aqueous phase of the solvent by the presence of hydration shells of DNA, information about these hydration shells can be extracted from comparing the values of their parameters. At the same time, all parameters have a certain physical meaning in terms of the intermolecular structure and dynamics of water, established in the theory of dielectric spectroscopy.
The described method was used to analyze DNA hydration in three solvents: water, aqueous solutions of 40mM MgCl_2 and 150mM KCl. Solutions of pET-11c plasmid DNA in the circular form with a concentration of 25 mg/ml were used. The circular form is preferable (compared to the supercoiled form), since it achieves the maximum contact area of the DNA surface with water, which maximizes the observed effects of hydration.
Statistically significant differences were found for all DNA solutions: a decrease in $\Delta\varepsilon_1$, an increase in $\Delta\varepsilon_2$ and $A/\omega_0^2$ compared to the corresponding solvents. A decrease in $\Delta\varepsilon_1$ indicates the presence of more strongly bound water molecules in the hydration shell. As is known, such molecules are located in the primary hydration shell of DNA, mainly on phosphates. An increase in $\Delta\varepsilon_2$ indicates an increase in the number of free water molecules, apparently in the transition layer between primary hydration and undisturbed water. An increase in $A/\omega_0^2$ in the absence of a change in $\omega_0$ indicates the presence of a hydration region with an increased number of hydrogen bonds. This is most likely realized in the grooves of DNA, since a large number of hydrogen bonds with water molecules are formed there. As a result, it is concluded that there are three different DNA hydration regions with the specified features.
The presence of 40mM MgCl_2 in solution has almost no effect on hydration, whereas the presence of 150mM KCl significantly weakens it, judging by all the parameters discussed. These effects do not correlate with the ionic strength of solutions, so they are ion-specific. Mg^(2+) ions are known to bind strongly to DNA phosphate groups, which leads to a change in the charge sign of these groups from –1 to +1. Therefore, the effect of Mg^(2+) on DNA hydration essentially consists in changing the orientation of water molecules in the primary hydration layer of phosphates and should not affect the rest of the hydration shell in any way, which we see from the results obtained. For K^+ ions, on the contrary, DNA hasn't strong binding sites, which means that they are distributed throughout the hydration shell. At the same time, K^+ is an ion with negative hydration, that is, it has a destructive effect on the water structure. This explains the observed weakening of DNA hydration in the KCl solution. The described effect of K^+ ions with intracellular concentration on DNA hydration may have a biological meaning: it may be one of the factors influencing the functioning of DNA through its hydration shell.
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### S1.18. Characteristics of hemoglobin after glutathionylation and formation of a non-covalent complex with glutathione

Kuleshova Iu.D.[1]*, Poluektov Yu.M.[1], Kalyuzhny D.N.[1], Zaripov P.I.[1], Makarov A.A.[1], Petrushanko I.Yu.[1]
[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Science, Moscow, Russian Federation;*
* julia_kul2000@mail.ru

Hemoglobin is a major protein of erythrocytes. The main function of hemoglobin is to supply oxygen to tissues. Hemoglobin can undergo

various post-translational modifications depending on different conditions. One of these modifications is glutathionylation. This is the addition of a glutathione to the thiol group of a protein with the formation of a disulfide bond. The hemoglobin contains six cysteine residues (β-Cys 112, β-Cys 93 and α–Cys 102). β-Cys 93 is the main target for glutathionylation. Glutathionylation increases the affinity of hemoglobin to oxygen [1]. This can play an important role in the adaptation of erythrocytes to stressful conditions. However, it has not been established yet how glutathionylation affects the conformation of the hemoglobin.

Besides covalent binding to hemoglobin, the intracellular glutathione in reduced state (GSH) forms a non-covalent complex with hemoglobin [2]. As we have shown, the ability of binding GSH decreases during the transition from the oxy to the deoxyform of hemoglobin. The affinity of the oxyform to GSH is higher, so hemoglobin binds four GSH molecules, while the deoxyform is only two. This leads to an increase in the GSH level in erythrocytes under significant deoxygenation [2]. This is important for the adaptation of cells to hypoxia. It should be mentioned that the formation of a non-covalent complex with GSH also leads to an increase in affinity for oxygen [2].

To establish how glutathionylation and the formation of a non-covalent complex with glutathione affect the conformation of the hemoglobin molecule, we characterized the influence of these modifications on the secondary structure of hemoglobin, on the heme and heme environment and on the environment of tryptophans. For this purpose, we analyzed the circular dichroism (CD) spectra in the ultraviolet region and in the Soret band and tryptophan fluorescence spectra.

We found that glutathionylation of hemoglobin does not lead to alterations in CD in the Soret band, which indicates that there are no changes in the heme and heme environment of the protein. The absorption spectrum of glutathionylated hemoglobin also does not change. At the same time, the CDNN program [3] for analysis the circular dichroism spectra revealed differences in the secondary structure of hemoglobin due to glutathionylation. In particular, glutathionylation reduces the percentage of alpha helix by 10 percent. This indicates that glutathionylation makes the secondary structure of the protein less ordered. However, the tertiary structure of the protein does not change significantly. Thus, the melting point of the protein after glutathionylation does not change. It means that this modification does not affect the thermal stability of the protein. Glutathionylation does not affect the tryptophan fluorescence spectrum, which demonstrates the absence of changes in the tryptophan environment in the glutathionylated hemoglobin. Using in-silico modeling, we found that glutathionylation of β-Cys93 and β-112 does not spatially interfere with Trp residues. Minimization of the glutathionylated structure (by β-Cys93 and β-112) in the AMBER99 forcefield also does not revealed any significant changes in Trp positioning.

The formation of a non-covalent complex of glutathione with hemoglobin, on the contrary, does not lead to any significant changes in the secondary structure of the protein, while the tertiary structure changes. The formation of a non-covalent complex leads to a shift of the CD of Soret peak to a shorter wavelength region, its amplitude decreases and peak broadened. Similar changes were observed in the absorption spectrum of the Sore band. Thus, we can suggest changes in the heme and heme environment during the formation of a non-covalent complex.

At the same time, the formation of a non–covalent complex leads to a change in the tryptophan fluorescence spectrum – the signal amplitude increased. The intrinsic fluorescence of hemoglobin is mainly due to the presence of Trp residues in the polypeptide chain. The hemoglobin tetramer contains six Trp residues. The β-Trp 37 residue locates on the surface of α1β2. It is considered to be the main amino acid residue contributing to the intrinsic fluorescence of Hb [4]. In addition, a decrease in the melting point of hemoglobin also indicates an alteration in the tertiary structure of the protein. In-silico modelling revealed that β-Trp37 is located on the surface of the inner cavity of hemoglobin molecule and is involved in the interaction with the GSH, two other Trp residues are located superfacially and does not interact with GSH molecules. Our data reveal that tryptophan environment near GSH binding site changed after the formation of a non-covalent complex Hb with GSH. In addition, a decrease in the melting point of hemoglobin also indicates a change in the tertiary structure of the protein. It means that thermal stability of hemoglobin in complex with GSH has become lower. Crude molecular modelling (structure minimization in AMBER99 forcefield after GSH-hemoglobin docking) showed some structural changes of the β-Trp37 and nearest residues, which are involved in the interaction with GSH.

Also, we have found that the positioning of Trp residues does not differ significantly between the different hemoglobin conformations.

Thus, glutathionylation reduces the alpha helix percentage of hemoglobin, but practically does not affect its tertiary structure, while the formation of a non-covalent complex does not affect the secondary structure of the protein, but affects the heme environment and the environment of tryptophans, and also reduces the thermal stability of the protein.

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### S1.19. Comparative analysis of freshwater fish and molluscan lens: composition and structure

Kapitunova A.I.[1]*, Dominova I.N.[1], Zhukov V.V.[1], Kundalevich A.A.[1], Samusev I.G.[1]

[1]*Immanuel Kant Baltic Federal University, Kaliningrad, Kaliningrad region, Russia;*

* AIKapitunova@mail.ru

It is assumed that the optical properties of the lenses of the camera-type eye are determined mainly by the content of crystallins in them. At the same time, in aquatic animals with camera-type eyes, the spherical lens plays a major role in refraction of light and the creation of an image on the retina. It is known that α-, β- and γ-crystallins are present in the fish lens, among which γM-crystallins quantitatively predominate, being almost absent in other animals. Their presence and the type of folding probably determine the high value of the radial gradient of the refractive index of the fish lens, which improves spherical aberration. The lens of camera-type eyes of invertebrate hydrobionts presumably possess similar properties, however, due to a different protein composition. These eyes are mainly found in molluscs, mainly in gastropods and cephalopods. At the same time, the complete and exact composition of lens-forming crystallins is known only for a limited number of animals, including some mammals and Danio rerio, while information for other fish and molluscs is either completely absent or only individual proteins are known as part of the lens.

In the NCBI Protein database, we found 7 groups of crystallins for molluscs and determined for them the Grand average of hydropathicity index (GRAVY), the percentage of secondary protein structures and refractive index increment values (dn/dc). For comparison, analogous parameters were determined for D. rerio fish lens crystallins. Some

molluscs crystallins are characterized by taxon-specificity. Most of them, with the exception of μ- and ζ-crystallins, are hydrophilic proteins with the predominance of α-helices and coils as secondary structures. alculation of dn/dc values showed that their highest values are characteristic of D. rerio γM-crystallins and the only S-crystallin of Pomacea canaliculata. In general, comparison of the above-mentioned properties of mollusc crystallins with those of D. rerio crystallin proteins allowed us to propose α- and S-crystallins as lens crystallins of molluscs.

Further comparison of aquatic mollusc and fish lens proteins, the Raman spectra of carp Cyprinus carpio, pikeperch Sander lucioperca, freshwater whitefish Coregonus lavaretus, eel Anguilla anguilla, and molluscs Lymnaea stagnalis and Pomacea canaliculata were obtained on a Renishaw Virsa spectrometer. We also obtained a Raman spectrum of the mouse Mus musculus lens as an example of mammalian crystallins. For large C. carpio, S. lucioperca, and M. musculus lens we used a laser with wavelength $\lambda = 532$ nm, and for smaller ones (C. lavaretus, D. rerio, A. anguilla, L. stagnalis, and P. canaliculata) a laser with wavelength $\lambda = 785$ nm, since it has lower energy of radiation and does not change the object structure. The lenses were placed on a quartz substrate and spectra were taken in the region of 250 - 4000 cm-1. The main differences between the spectra of fish and molluscs were found in the range of 1000 - 2000 cm-1. In this case, the spectrum of a mouse lens is almost identical to that of a fish ones that may indicate a similar composition of the lenses of vertebrates, namely, the presence of γ-crystallins in them. At the same time, the amino acid sequences of crystallins of these animals have differences, which is confirmed by the presence of a peak at about 700 cm-1 on fish spectra corresponding to the C-S region of two methionine residues and absent in mouse spectrum. However, there are no principal differences in protein folding, and it is represented mainly by antiparallel beta-sheets and coils, which are reflected in the similar shape of peaks in the ranges of amide- I from 1230 to 1280 cm-1 and amide-III from 1650 to 1680 cm-1.

The Raman spectrum of L. stagnalis and P. canaliculata lens differed significantly from that of fish and mouse, which can be explained by the presence of taxon-specific crystallins the exact belonging of which to a particular group is not yet known. Thus, differences in the spectrum between molluscs, fish and mouse were observed in the region of 1500 - 1700 cm-1. Moreover, the most intense peak was observed in the area of 1550 cm-1 and had a greater intensity than the peak of amide-III - 1650 - 1680 cm-1, which is characteristic of proteins with a large number of α-helices or coils and a complete absence or a smaller number of β-sheets. In addition, another broad peak in the range of about 1240 - 1245 cm-l was observed, characteristic of the coils of the protein. It is also noteworthy that in the spectrum of molluscs and mouse, a peak at 750 cm-l was present, indicating the presence of tryptophan.

The fine structure of S. lucioperca, L. stagnalis and P. canaliculata lenses was analyzed using phase-contrast (Olympus IX51) and scanning electron (Hitachi TM4000Plus) microscopies. Analysis of the structure of slices and surfaces of sections allows us to conclude that the material of S. lucioperca lens has a pronounced layered structure, in contrast to the homogeneous lens of gastropod molluscs.

Altogether, the obtained results allow us to conclude that similar optical tasks facing the vision of hydrobionts are probably solved in fish and molluscs by different structural components of the lens, which require further detailed study.

### S1.20. Comparative analysis of the three-dimensional structure of nonapeptide and octapeptide molecules

Ismailova L.I.[1]*, Abbasli R.M.[1], Akhmedov N.A.[1]
[1]*Baku State University, Institute for Physical Problems;*
* lara.ismailova.52@mail.ru

Currently, the role of regulatory peptides in the life and activity of organisms is being actively studied. Regulatory peptides belong to the group of neuromodulators, which are a key link in the mechanism of regulation of the functions of the human body. Modern medicine and biotechnology set themselves the important task of creating effective drugs that would have a wide spectrum of action and a minimum number of side effects. When creating new drugs, researchers turn to the use of the human body's own resources. To determine the functions they perform, it is necessary to study the spatial structure and structure-functional organization of these molecules.

Computer modeling based on the use of the method of theoretical conformational analysis and programs that allow obtaining a graphical representation of the spatial structures of the molecule was performed for two neuropeptides: the nonapeptide molecule Leu-Pro-Pro-Gly-Pro-Leu-Pro-Arg-Pro-NH2 (Antho -RPamide) and an octapeptide molecule Pro-Pro-Gly-Leu-Gly-Pro-Leu-Arg-NH2. Both molecules are characterized by a high content of proline, the presence of two residues of leucine and one arginine. It is known that the nonapeptide was isolated from a sea anemone neuron and had a high biological activity. As for the octapeptide molecule, its activity is completely absent. This is a purely experimental fact. The aim of our research was to determine the spatial organization of nona- and octapeptide molecules, because the spatial structure of peptide molecule completely determines its functional features. The relevance of studying the spatial structure of neuropeptide molecules is due to the fact that the primary structure of a large number of peptide molecules isolated from mollusk neurons, molecules with inhibitory properties, has now been determined. By inhibiting some processes in the cell, these peptides regulate the biochemical processes occurring in it. Neuropeptide molecules are a class of prominent signaling peptide molecules.

To find the spatial structure of peptides, we used a theoretical approach that allows us to calculate the three-dimensional structure of biomolecules based on a known amino acid sequence and a computer program developed by us. This approach makes it possible to quantitatively describe the geometry of the molecule with sufficient accuracy, determine the dihedral angles of the main chain and side chains of the molecule, and also find the energy interactions of atoms in this molecule. The calculation was carried out within the framework of the mechanical model of molecules, taking into account non-valence, electrostatic and torsion interactions and the energy of hydrogen bonds. When calculating the spatial structure of peptide molecules, the method of theoretical conformational analysis was used. For each of these molecules, low-energy conformations, dihedral angles of the main and side chains of the amino acids included in it were found. In this case, the energy of intra- and interresidual interactions in each low-energy structure was estimated. The calculation of neuropeptide molecules was carried out fragmentarily.

The calculation revealed the presence of a sharp energy differentiation of conformations, forms of the main chain and shapes for both molecules. The energy interval 0–42 kJ/mol for a nonapeptide molecule includes 8 low-energy conformations, and for an octapeptide molecule 11 low-energy structures. The calculation showed that the unfolded forms of the main chain are low-energy for the nonapeptide, and the folded and semi-folded forms of the main chain are for the octapeptide. In the global conformation B2122BRBRB2122RB3122 B (0,0 kJ/mol) of the nonapeptide molecule, the contribution of non-valent interactions is (-150,4) kJ/mol, electrostatic interactions (-11,8) kJ/mol, and torsion interactions 21,0 kJ/mol. The constructed conformational maps for the Arg8 side chain showed that it is directed into the solvent and has conformational freedom, and therefore can interact with the receptor. The molecule may be active.

Octapeptide. the molecule has the global conformation BBBB3222PRR2122B3222. Its C-terminus is folded, which creates conditions for the Arg8 amino acid to interact with all other residues. The side chain of Arg8 has no conformational freedom and, therefore, no possibility to interact with the receptor. Therefore, the octapeptide molecule is inactive.

Thus, the spatial structure of each Leu-Pro-Pro-Gly-Pro-Leu-Pro-Arg-Pro-NH2 molecule and the Pro-Pro-Gly-Leu-Gly-Pro-Leu-Arg-NH2 molecule. can be represented by a limited set of low-energy conformations in which nona- and octapeptide molecules perform their functions. The performed calculation of neuropeptides has led to such structural organizations of molecules that do not exclude the implementation of functions that require interactions with various receptors.

### S1.21. Computational structural biophysics of thermosensitive ion channels of the TRPV family

Trofimov Yu.A.[1,4], Krylov N.A.[1], Efremov R.G.[1,2,3]*
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry;*
[2]*National Research University Higher School of Economics, Moscow, Russia;*
[3]*Moscow Institute of Physics and Technology ;*
[4]*National Research Nuclear University "MEPhI";*
* r-efremov@yandex.ru

Understanding at the molecular level the mechanism of ion channels is important both from a fundamental and applied point of view - for the development of new drugs and therapies for a number of socially significant diseases. Of particular interest in recent years has been the study of proteins of the TRPV (Transient Receptor Potential, Vanilloid type) family – cation channels expressed mainly in sensory neurons and reacting on changes in temperature, pH, mechanical stress and a number of chemical agents. This is due to the presence of experimentally determined models of the spatial structure of a number of representatives of the TRPV family in various functional states – open, sensitized, closed, etc. Computer modeling methods provide important information about the features of the TRPV channels, allowing, on the basis of experimentally obtained models, to evaluate the fine details of their conformational dynamics, analyze the evolution of the physicochemical properties of their pore domains, the role of individual molecules of bound lipids, etc. The results of calculations contribute to the identification at the atomic level of the relationship of these characteristics of channels of different types with the parameters of their operation in the cell. The report presents the results of in silico modeling of a number of proteins from the TRPV family that are in several functional states and embedded in hydrated lipid bilayers that mimic the cell membrane. Calculations of molecular dynamics in the full-atomic representation, as well as original computer technologies for mapping and visualization of physicochemical properties of protein-membrane systems were used as the main methods of computational experiment. The results obtained significantly supplement the information obtained on the basis of structural models deciphered using experimental approaches. The work was supported by the Russian Science Foundation (grant 19-74-30014).

### S1.22. Conformational Flexibility of Anticancer Pentapeptide AAP-H, Determined by Molecular Modeling Methods

Agaeva G.A.[1]*, Agaeva U.T.[1], Godjaev N.M.[1]
[1]*Baku State University, Institute for Physical Problems;*
* gulshen@mail.ru

In recent years, biologically active substances isolated from marine organisms have played an important role in the development of innovative drugs. As is known, prostate cancer (PCa) is one of the most common malignant neoplasms of the male urinary system, as well as the main cause of death from cancer in men [1]. Cancer is a disease characterized by excessive proliferation, including transformation, disorders of apoptosis, proliferation, and metastasis, and is one of the deadliest diseases [1]. Currently, conservative chemotherapy is used to treat cancer due to a lack of effective drugs. In 2018 ,Wu et al. [2] found that a pentapeptide (AAP-H) isolated from the sea anemone Anthopleura anjunae with the amino acid sequence Tyr-Val-Pro-Gly-Pro exhibited some cytotoxicity against prostate cancer DU-145 cells of human, and its mechanism of action may be related to mitochondrial-regulated apoptosis. In addition, Li et al. [3] found that this molecule induces cellular phase S arrest in DU-145 cells. The antitumor mechanism of AAP-H in prostate cancer DU-145 cells was investigated in vitro and in vivo. The results showed that AAP-H was non-toxic and exhibited antitumor activity.

Thus, the hydrophobic AAP-H oligopeptide can be developed as an adjuvant for the prevention or treatment of prostate cancer in the future. To determine the mechanism of action of this pentapeptide and study its structural and functional relationships, it is necessary to know the conformational specificity and flexibility of the main and side chains of the molecule, which makes it possible to rationally construct functional groups that act selectively at their receptor level. The main purpose of this work was to study the conformational dynamics of the backbone and side chains of the AAP-H peptide in vacuum and in a polar medium. To determine the structure-activity relationship of the AAP-H peptide, in addition to studying its spatial structure, it is necessary to elucidate the role of functionally active residues in stabilizing the preferred conformation of the AAP-H oligopeptide.

First, the conformational properties of the pentapeptide were studied by the method of molecular mechanics (MM), which makes it possible to determine a whole set of energetically preferable conformations of the pentapeptide molecule. A sequential method was used, combining all low-energy conformations of constitutive residues. The conformational potential energy of a molecule was set as the sum of independent contributions from nonbonded, electrostatic, torsion interactions, and hydrogen bond energies. A detailed analysis of the conformational flexibility of the AAP-H peptide revealed a limited number of stable conformers. The results obtained showed that the stable conformers of the pentapeptide tend to take the form of a beta-turn, due to the presence of two proline residues in the sequence.

At the second stage of this study, the conformational dynamics of the peptide backbone and the mobility of pentapeptide side chains in the preferred conformational state were studied. The obtained values of the dihedral angles of the low-energy conformations of the pentapeptide were used as initial parameters for modeling by the molecular dynamics (MD) method. The MD method was used to study the behavior of side chains within the energetically preferred conformation of the pentapeptide in vacuum and in the environment of water molecules.

After modeling, other low-energy structures were obtained showing the dynamics of the main and side chains of the preferred conformations. Thus, molecular dynamics modeling revealed a structural reorganization of the global conformational state of the pentapeptide under various environmental conditions.

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### S1.23. Conformational Particuliarities of Beta-Amiloid Peptide 25-35

Agaeva G.A.[1]*, Najafova G.Z.[2]
[1]*Institute for Physical Problems, Baku State University;*
[2]*French-Azerbaijan University , UFAZ;*
* gulshen@mail.ru

It is known, Alzheimer's disease (AD) is the most common neurodegenerative disease and causes dementia. This disease is the pathological development of aggregation of two proteins in brain tissues, namely beta-amyloid (Aβ) and tau protein, characteristic of the brain. There is accumulated evidence that Aβ peptides self-form in soluble oligomers and insoluble fibrils [1]. A small fragment of Aβ (25-35) (with the sequence GSNKGAIIGLLM) is functional domain of the amyloid peptide Aβ responsible for its neurotoxic properties [2] and the biological active region of Aβ [3]. The Aβ(25-35) peptide has many of the characteristics of full-length Aβ(1-40/42), including its amphiphilic nature and tendency to aggregate. It is presence in vivo has only recently been proven, but the toxicity of fibrillar Aβ (25–35) to neuronal cells in vitro has been shown previously [3]. There is also evidence that the monomeric form of this peptide itself can be cytotoxic [3]. The mechanism of amyloid peptide toxicity remains unclear. However, many important details about the soluble Aβ peptide, including its spatial structure, are missing or contradictory. Therefore, the study of the conformational properties of the Aβ(25-35) peptide in its soluble monomeric form can play a significant role in determining the nature of its earlier species before oligomeration. It can be assumed that the conformations adopted by the Aβ(25-35) peptide in solution are extremely sensitive to the methods used and experimental conditions.
In our study, we used the methods of molecular modeling (MM) - methods of molecular mechanics (MM) and molecular dynamics (MD) to obtain information about the spatial organization of the Aβ peptide (25-35) and its conformational dynamics. The advantage of MM molecular modeling over other theoretical approaches is that it takes into account the energy contributions of all types of intra- and intermolecular contacts in stable conformations of the peptide and its fragments, which makes it possible to identify more stable conformations of the peptide. The conformational properties of the amyloid-β peptide (25-35) were studied using the method of molecular mechanics, which makes it possible to successfully solve problems on the structural characteristics of peptide molecules. The calculation program was based on the matrix method for determining the coordinates of atoms and potential functions with the appropriate parametrization. In the current version of this program, the energy is calculated as the sum of independent contributions from the energies of non-valence, electrostatic interactions, energies of torsion barriers and hydrogen bonds. Conformational analysis of each peptide segment of the C-terminal part revealed a limited number of the most probable conformations and quite clearly defined the forces stabilizing the structures. It was also shown that the possibility of the formation of unfolded structures compared to the α-helical structure is higher at the N-terminus and lower at the C-terminus. Our results show that the Aβ(25-35) peptide energetically adopts an α-helical conformation at the C-terminal octapeptide segment. Among the stable structures with a common α-helical conformation at the C-terminus, there is a significant variety of different conformations at the N-terminal tetrapeptide. The molecular dynamics method was used to simulate the pattern of intramolecular mobility of the Aβ peptide (25-35) molecule. The stable conformational states of the molecule were used as initial approximations. It was shown that the flexible structures in the N-terminal region of Aβ (25-35) are differently oriented with respect to the structures in the C-terminal part in low energy conformation. These results are consistent with the hypothesis that the helix-containing conformer is an important intermediate in the assembly of Aβ fibrils [4]. In what follows, the possible role of the α-helical structure in the initiation of Aβ peptide aggregation will be discussed.

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### S1.24. Conformational and redox changes in hemoporphyrins during the redox reaction between neuroglobin and cytochrome c

Semenova M.A.[1]*, Bochkova Z.V.[2,1], Smirnova O.M.[1], Ignatova A.A.[1], Parshina E.Yu.[2], Ziganshin R.H.[1], Bocharov E.V.[1], Brazhe N.A.[2], Maksimov G.V.[2], Kirpichnikov M.P.[1,3], Dolgikh D.A.[1,3], Chertkova R.V.[1]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS (IBCh RAS);*
[2]*Biophysics Department, Biological faculty, Lomonosov Moscow State University;*
[3]*Biological faculty, Lomonosov Moscow State University;*
* marinaapbch@mail.ru

Cell death associated with mitochondrial dysfunction is common in acute neurological disorders, neurodegenerative diseases, hypoxia, and ischemia. Neuronal apoptosis is regulated by a variety of proteins, including neuroglobin (Ngb), a small heme-containing protein from the globin family. Ngb is found in high concentration in neurons and its overexpression promotes neuronal survival, thus reducing brain damage in both stroke and Alzheimer's disease in vivo. One of the most popular hypotheses for the implementation of the neuroprotective function of Ngb is based on the interaction of Ngb with mitochondrial cytochrome c (Cyt c), which prevents the initiation of apoptosis by the Cyt c. However, the molecular basis of the mechanism of interaction between Ngb and Cyt c has not yet been studied, and the nature of their interaction has not been established. In this regard, it seems relevant to study the mechanisms of interaction between Ngb and Cyt c, the understanding of which will allow us to proceed to the rational design of new therapeutic agents for inhibiting the neuronal cells' death under conditions of ischemia and hypoxia of various origins.
We have developed an effective system for the production of recombinant human Ngb in its functionally active holoform: a plasmid vector pET-17b-Ngb with the Ngb gene has been constructed, a bacterial strain of E. coli SHuffleT7-Ngb has been obtained, system for the biosynthesis, isolation and purification of recombinant Ngb has been developed and optimized. Using spectral methods of analysis, the physicochemical characteristics of recombinant Ngb were obtained: according to UV-visible, IR, CD, and NMR spectroscopy, recombinant Ngb is a structured protein in the holoform state. The data of chromatomass-spectrometric analysis allowed us to conclude that the structure of the oxidized form of Ngb contains a correctly formed disulfide bond.

Using Raman and surface-enhanced Raman spectroscopy (RS) with laser excitation at 532 nm, it was shown that the characteristic spectra of Ngb heme in the reduced (Fe2+) and oxidized (Fe3+) forms have bands corresponding to B-type heme, and the iron atom is six-coordinated. Using RS with laser excitation at 633 nm, it was found that in Ngb(Fe2+) the cysteine residues of the protein part are in a free state, while in Ngb(Fe3+) they form a disulfide bond. Differences in the vibration of C-N bonds in the protein part of reduced and oxidized Ngb were also detected, which may be associated with a change in the mutual arrangement of α-helices during the transition of Ngb between redox forms.

We have shown that the Raman spectra of Cyt c(Fe2+) have characteristic peaks in the region of 500-700 cm-1 and 1313 cm-1, which are absent in the Raman spectra of Ngb(Fe2+), while the spectra of Ngb differ in the presence of peaks at 1306 and 1342 cm-1. These spectral features made it possible to determine these proteins when recording the spectrum of their mixture.

RS was used to develop a technique for recording conformational and redox changes in proteins accompanying the redox (RO) reaction between Ngb(Fe2+) and Cyt c(Fe3+). To eliminate the influence of excess reducing agent on the introduced Cyt c(Fe3+), we obtained difference spectra by subtracting from the spectrum of mixture (1): Ngb(Fe2+)+Cyt c(Fe3+) spectrum of mixture (2): Ngb(Fe2+)+buffer+Cyt c(Fe3+). Since the reoxidation of Ngb(Fe2+) occurred in mixture (2), the reduction of the introduced Cyt c(Fe3+) is possible due to the excess of the reducing agent in the solution. Thus, the bands of the difference spectra show the effects caused by the RO-reaction between hemes.

The difference spectra show intense peaks in the region of 600-700 cm-1 associated with vibrations of C-S bonds between the heme and Cys14 and Cys17 Cyt c residues. An intense peak at 1330 cm-1 is characteristic of C-type hemes. Peaks were also observed in the region of 1140 cm-1 (vibrations of methyl radicals) and 1570-1610 cm-1 associated with vibrations of methine bridges. It can be assumed that the observed changes are associated with the adjustment of the heme Cyt c in the heme cavity for RO-interaction with the Ngb heme. Thus, the results obtained indicate that an electron transfer occurred between Ngb(Fe2+) and Cyt c(Fe3+).

In addition, a comparative analysis of the one-dimensional 1H-NMR spectrum of an equimolar mixture of Ngb/Cyt c and the sum of the 1H-NMR spectra of Ngb and Cyt c accumulated separately was carried out. A shift in the number of 1H-NMR signals in the protein mixture was detected, indicating that the recombinant Ngb and Cyt c proteins form a complex when mixed in solution.

Based on the literature data, panels of Ngb(with E60K, E87K, K67E, and K95E mutations) and Cyt c (with K25E, K72E, and K25E/K72E mutations) mutants were constructed and obtained with substitutions of residues in the putative interaction interface of these proteins. These mutations are able to influence electrostatic interactions between proteins due to a change in the charge of the amino acid residue to the opposite.

The method described above was used to study the conformational and redox changes in hemoporphyrins accompanying the RO-interaction of Ngb mutants with wild-type Cyt c and vice versa. It has been shown that the interaction of NgbK67E, NgbE60K, NgbE87K with wild-type Cyt c is accompanied by an RO-reaction with electron transfer, while the interaction of NgbK95E with wild-type Cyt c is probably disturbed, and reduction of Cyt c occurs only due to an excess of reducing agent in solution. It was also suggested that the interaction of Cyt cK25E with wild-type Ngb is attenuated, while no such attenuation was found for the interaction between Cyt cK72E and wild-type Ngb.

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**S1.25. Coumarin-based inhibitors of microtubule dynamic instability targeting the colchicine site of tubulin**

Anisimov M.N.[1,2], Romanov A.N.[3], Borzunova I.N.[1,2], Janibekova M.O.[4], Kechko O.I.[5], Mitkevich V.A.[5], Vorobyev I.A.[1,4], Gudimchuk N.B.[1,2]*

[1]*Lomonosov Moscow State University, Moscow, Russia;*

[2]*Center for theoretical problems of physicochemical pharmacology, Russian Academy of Sciences, Moscow, Russia;*

[3]*N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia;*

[4]*Nazarbayev University, Astana, Kazakhstan;*

[5]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia;*

* nikita_gb@mail.ru

Among cytoskeleton components of eukaryotic cells tubulin polymers, microtubules, play a special role. Microtubules are involved in many processes, including intracellular transport, cell motility, and cell division. Due to the ability to spontaneously switch between polymerization and depolymerization phases, called dynamic instability, microtubules distribute chromosomes between daughter cells. Suppression of dynamic instability with tubulin inhibitors makes it possible to stop the uncontrolled division of malignant cells. This strategy is one of the foundations for the successful anticancer drugs action, the constant search for which is an urgent applied problem.

One of the best known tubulin inhibitors is colchicine. Despite the effective suppression of malignant cell proliferation through mitotic arrest, this substance has not found wide application in tumor therapy due to its high overall toxicity. We attempted to find new colchicine-like tubulin inhibitors based on the following criteria: interaction with the colchicine site of tubulin, suppression of microtubule dynamic instability, and blocking of cell division. The search for potential new ligands was carried out in the US National Cancer Institute database using the COMPARE clustering algorithm based on a comparison of the antitumor activity profiles of substances in relation to various human malignant cell lines with the antitumor activity profile of colchicine. One compound, coumarin-30, which has pronounced fluorescent properties, was chosen for further study. Using its fluorescence, we determined the binding of coumarin-30 to the colchicine site of tubulin with the microscale thermophoresis method. Light microscopy allowed us to establish a concentration-dependent suppression of the microtubule polymerization rates at substoichiometric concentrations of coumarin-30 and, as a result, a delay in mitosis of human lung carcinoma cells. The mechanism of interaction of coumarin-30 with the colchicine site of tubulin was studied experimentally using structural analogues, coumarin-6 and -7, and molecular docking. It was found that, despite only minor differences in their structures, the effects of these compounds on the microtubule dynamics were significantly different. This information illuminates the mechanism of binding of coumarin-30 to the colchicine site of tubulin. A model of the most probable structure of the complex of coumarin-30 and tubulin was created. This model may be useful for possible ways of further modifications of coumarin-30 to improve its antitumor properties.

Thus, we have found a new colchicine-like inhibitor of tubulin, and examined its mechanism of action, both theoretically and experimentally. The search for tubulin inhibitors was supported by the Russian Science Foundation grant #21-74-20035. Anisimov M.N. is a scholarship holder of the Theoretical Physics and Mathematics Advancement Foundation “BASIS”.

### S1.26. DNA complexes with metal coordination compounds containing phenanthroline ligands

Shatitsa M.[1]*, Kasyanenko N.A.[1]
[1]*Saint Petersburg State University, Saint Petersburg, Russia;*
* st067901@student.spbu.ru

Platinum coordination compounds, such as cisplatin, carboplatin and oxaliplatin, are widely used for treatment of oncological diseases. The mechanism of action of platinum coordination compounds based on their interaction with the nitrogenous bases of DNA with the formation of intra- and inter- strand crosslinks, that block DNA synthesis and cell division. Nevertheless, the non-selectivity of action and high toxicity of existing platinum-based drugs in addition to the platinum-resistant tumors development are the main reasons why safer and more effective analogues are searched. In this work we studied the molecular mechanism of complex formation of the DNA molecule (which is the main target of drugs based on metal coordination compounds) with new potential antitumor compounds - palladium and manganese coordination compounds containing phenanthroline ligands.
The presence of phenanthroline ligands in compounds can promote the formation of aggregates of such compounds in an aqueous solution, which will also affect the nature of the interaction of these compounds with DNA. For comparison, the effect of the presence of free phenanthroline in solution on the conformation of a DNA molecule is considered.
The influence of pH and the ionic strength of the solution on the considered complex formation are analyzed. It is important, since the action of the compounds used is aimed to block the division of tumor cells that exist in a slightly acidic environment.
In course of the research studies a commercial formulation of high molecular weight calf thymus DNA (Sigma Aldrich) was used. The molecular weight of DNA samples was obtained with viscosity method, by the intrinsic viscosity of DNA in 0.15 M NaCl. The study of complex formation was carried out in an aqueous solution with the addition of a low molecular weight salt - 0.005 M NaCl. The following experimental methods were used: spectrophotometry, low-gradient viscometry and flow birefringence. In addition, DNA melting and spectrophotometric titration were studied.
The UV spectra of the compounds have two well-resolved absorption bands, which, however, intersect with the absorption region of DNA. The paper proposes a method for analyzing the spectral characteristics of complexes using such systems.
All compounds used were shown to bind to DNA. A molecular mechanism of complex formation has been proposed.

### S1.27. DNA condensation in bacteria

Krupyanskii Y.K.[1]*
[1]*ICP RAS;*
* yuriifkru@gmail.com

DNA is organized in the nucleoid of an actively growing cell hierarchically with three levels of DNA compaction: The lower level (small scale $\geq$ 1 kb bp) is provided by interaction with histone-like NAP proteins. Actively growing cells maintain a dynamic, far from equilibrium order through metabolism. When cells enter a dormant state (practically complete absence of metabolism), the usual biochemical methods of protecting DNA cease to work, and cells, adapting to new conditions, are forced to use the physical mechanisms of DNA protection. The structure of DNA in the nucleoid of dormant cells formed under starvation stress was studied using synchrotron radiation diffraction and transmission electron microscopy (TEM). The experimental results made it possible to visualize the structures of the lower hierarchical level of DNA compaction in the nucleoid of dormant cells. A series of diffraction experiments performed for the first time indicates the presence of a periodic ordered organization of DNA in all studied bacteria. TEM made it possible to extract fine visual information about the type of DNA condensation in the nucleoid of the bacterium Escherichia coli. Intracellular nanocrystalline, liquid-crystalline and folded nucleosome-like structures of DNA have been found. The folded nucleosome-like structure was observed for the first time and is the result of multiple folding of long DNA molecules around the Dps protein and its associates. The different types of DNA condensed state found by us in the studied dormant E. coli cells (DNA condensation heterogeneity) provide additional arguments in favor of the concept that considers a microbial population as a multicellular organism. The study of changes in DNA architecture under the influence of the chemical analogue of the autoinducer of anabiosis 4-hexylresorcinol (4HR) was studied. An increase in the 4GR concentration induces the transition of a part of the cells of the population to an anabiotic dormant state, and then to a mummified state. The studies of the DNA structure in the anabiotic and mummified states show the spectroscopic identity of the DNA structure in the dormant anabiotic state and in the dormant state formed during starvation stress. Studies of the structure of DNA in the mummified state show a strong difference from the structure of DNA in the anabiotic state.

### S1.28. Deoxygenation of globins in the presence of mitochondria. Electrostatic interaction of globins with phospholipid membranes

Postnikova G.B.[1]*, Shekhovtsova E.A.[1], Sivozhelezov V.S.[1]
[1]*Institute of cell biophysics of RAS;*
* shekhovtsova.ekaterina@mail.ru

The detachment of O2 from sperm whale MbO2, as we showed earlier, occurs only when it interacts with mitochondria (MCh) and is completely determined by the intensity of their respiration. Since deoxygenation of other oxyglobins from different organisms proceeds similary, one can assume a similar mechanism of their interaction with phospholipid membranes of MCh. In this work, it was analyzed the role of electrostatic interactions in stabilization of the native conformation of the heme cavity in nine Mb-like globins: monomeric myoglobins (sperm whale, horse, gastropod mollusk Aplysia limacina) and hemoglobins (chironomid larvae Chironomus thummi thummi, fruit fly Drosophila melanogaster, bivalve mollusk Lucina pectinata and soybean leghemoglobin), as well as dimeric hemoglobins (Lucina pectinata and botfly Gasterophilus intestinalis). Having homologous 3D-structures and the proximal His as the fifth heme ligand, these globins differ in the distal part of the heme cavity and in their affinity for oxygen (in ~100 times). It has been shown that the structure of the heme cavity is maintained by a network of hydrogen and ionic bonds, involving the proximal His, the distal protein residue, both A- and D- heme propionates, and neighboring to them Arg and Lys residues. On the proximal side of the heme, this network controls the position of the Fe atom outside or in the protoporphyrin plane, which affects the affinity for the ligand, and in the distal part – the position of the distal protein residue (His, Gln or Arg), in which it is able to form the H-bond with the oxygen ligand, preventing its fast dissociation. When binding to MCh, the found network of ionic and H-bonds should be disturbed due to influence of the negative field of MCh membrane and contacts of phospholipid heads, competing with the A- and D-heme propionates for binding to the membrane-exposed Arg and Lys of the protein. This leads to a decreasing the globin affinity for O2, facilitating its elimination at pO2 values of ~15 mmHg under hypoxic conditions in the cell.

### S1.29. Dependence of the strength of mutant promoters of the rrnB ribosomal RNA gene of E.coli on the parameters of the electrostatic up-element

Korchagina V.M.[1], Osypov A.A.[1]*
[1]*ITEB RAS;*
* v.korchagina98@yandex.ru

The aim of this work was to study the dependence of the strength of mutant promoters of the rrnB gene of E.coli ribosomal RNA on the parameters of the electrostatic up-element.
Objectives:
To calculate the dependence of promoter strength on the maximum potential value in the 21 base pair long window at -59 to -38 positions relative to the transcription start point in accordance with the coordinates of the mutant up-element set.
Calculate the dependence of promoter strength on the total potential value in the same window.
Methods:
The magnitude of the electrostatic up-element potential for the sequences was calculated using DEPPDB database tools [1, 2]. Mutant promoter strengths were taken from the articles by Estrem, Ross et. al [3, 4].
A program was written in R in the RStudio software to carry out the study. Data analysis was performed by plotting the dependence of promoter strength on up-element potential parameters, and their correlation was investigated using the Pearson statistical test.
Results:
The study showed that as the absolute value of the up-element potential increased, so did the promoter strength.
When testing the dependence of the total potential around its absolute minimum of 135 A° also shows an increase in promoter strength with an increase in the absolute value of the potential.
There is also an increase in promoter strength with an increase in the absolute sum of the potential in these coordinates
The correlation between the minima of total up-element potentials and promoter strength was 0.5435669 with p-value = 0.001078
the correlation between the sum of potentials in the vicinity of the minimum of the full up-element potential and the strength of promoters was 0.3935843 with reliability p-value = 0.02344
the correlation between the minima of proximal up-element potential and promoter strength was 0.8396514 with reliability p-value = 0.00463
the correlation between the sum of potentials in the vicinity of proximal up-element minima and promoter strength was 0.8301612 with reliability p-value = 0.005606
the correlation between up-element distal minima and promoter strength was 0.560953 with reliability p-value = 0.006608
the correlation between the sum of potentials in the vicinity of distal up-element minima and promoter strength was 0.5853439 with reliability p-value = 0.00421
the correlation between the minimum potential values and their sum in the vicinity of the minimum was 0.8173967 with reliability p-value = 3.026e-16
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**S1.30. Determination of bioequivalence to the wild type of recombinant SARS-CoV-2 S-protein by assessing the humoral immune response of guinea pigs and the binding specificity of human ACE2**

Kosenko M.N.[1]*, Onkhonova G.S.[1], Gudymo A.S.[1], Danilchenko N.V.[1], Taranov O.S.[1], Olkin S.E.[1], Imatdinov A.R.[1], Prudnikova E.Yu.[1], Danilenko E.I.[1], Zaikovskaya A.V.[1], Evseenko V.A.[1], Pyankov S.A.[1], Ryzhikov A.B.[1]
[1] *State Research Centre of Virology and Biotechnology "Vector", Rospotrebnadzor;*
* kosenko_mn@vector.nsc.ru

The range of tasks in which recombinant proteins are used covers many areas from fundamental biological research to such applied areas as the food industry. Technologies based on recombinant proteins have shown their effectiveness in creating new vaccines against infections circulating in the human population. At the same time, the determination of the bioequivalence of the obtained recombinant proteins with "natural" ones is an important part of both structural and functional studies.
As a result of the study, a method for the expression of the recombinant SARS-CoV-2 S protein in HEK293 cells was developed, followed by purification using affinity and ion-exchange liquid chromatography. Conducting step-by-step control with the involvement of modern means of processing the obtained data made it possible to achieve high purity of the resulting product.
The kinetic method of biolayer interferometry showed the functional activity of the target protein. Also, to assess the biological activity, a double intramuscular immunization of guinea pigs with a complex of the obtained recombinant protein with ISCOM adjuvant "Matrix-B" was carried out. The specificity of the immune response to the recombinant protein was confirmed by ELISA; cross-reactivity with the antigen of the Delta variant of the SARS-CoV-2 virus was 1:3200. In the neutralization reaction of the Delta variant of the SARS-CoV-2 virus, the geometric mean titer of antibodies in immune sera was 1:63.

**S1.31. Development of a method for optimizing the production of microcrystalline samples for protein serial crystallography**

Samygina V.R.[1,2]*, Dubova K.M.[1], Peters G.S.[1], Konarev P.V.[1,2]
[1]*NRC Kurchatov Institute;*
[2]*FSRC "Crystallography and Photonics" RAS;*
* lera@crys.ras.ru

Protein crystallography is the most common method for determining the structure of biological macromolecules. The use of frozen samples can shift the structural ensembles in protein crystals and limit our ability to study protein motions during functioning. Protein serial microcrystallography has opened new perspectives for time-resolved structural determination of reaction intermediates and characterization of conformational states of macromolecules under nearly-physiological conditions. This method requires samples containing a large number of highly ordered nano- or microscale crystals. However, the growth and the characterization of high-quality nanocrystals remain a bottleneck.
Microcrystals are obtained by mixing a protein with precipitant solutions in a batch method, for which a microcrystallization phase diagram is needed to produce massive nucleation. Crystal growth must be controlled and can be stopped by rapid dilution in a high precipitant buffer at the desired crystal size [1].
Here, we demonstrate application of small-angle X-ray scattering (SAXS) for monitoring microcrystallization process of lysozyme in X-ray capillaries using different crystallization solutions. The experiment was performed at the "BioMUR" beamline of the Kurchatov synchrotron radiation source (Moscow, Russia). SAXS allowed us to

determine the time when the first crystals appear after mixing the protein solution with the precipitant buffer and the moment of inhibition of crystal growth. The appearance of Bragg peaks on the recorded SAXS scattering profiles of lysozyme indicated the initial time point of the first crystals formation. The technique can be useful for the preparation of microcrystal suspensions for time-resolved serial crystallography.
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**S1.32. Dimerization of transmembrane domains of receptors of the insulin receptor subfamily**

Bershatsky Ya.V.[1,2]*, Nadezdin K.D.[1], Bocharova O.B.[1], Arseniev A.S.[1], Bocharov E.V.[1,2]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences;*
[2]*Moscow Institute of Physics and Technology (National Research University);*
* yaroslav.bershatskiy@phystech.edu

Receptor tyrosine kinases (RTKs), grouped into 20 subfamilies, play a key role in a variety of cellular activities, participating in various processes, signaling pathways, and are responsible for key events in the body. The insulin receptor subfamily, one of the RTK subfamilies, contains three receptors: the insulin receptor (IR), the insulin-like growth factor-1 receptor (IGF1R), and the insulin-like receptor (IRR). Receptor dysfunction is associated with the development of diseases such as diabetes, cancer and Alzheimer's disease. Despite the high mutual sequence and structure homology, the localization, expression, and functions of the subfamily receptors differ greatly.
Three domains are distinguished in the RTK structure: a ligand-binding extracellular domain, a transmembrane domain (TM), and a catalytic intracellular domain. The structure of the insulin receptor family stands out among other RTKs. The subfamily receptor monomers consist of two polypeptide chains linked by a disulfide bond. In the cell membrane, receptors exist exclusively in the form of covalently bound dimers.
In this work, we studied the dimerization of the TM domains of InsR, IGF1R, and IRR using NMR spectroscopy. We obtained the structures of all TM domains in detergent micelles in the dimeric form, as well as data on the dynamics of TM domains in a membrane-like medium. Despite the high homology of receptor sequences, the resulting structures of TM domains differ not only structurally but also dynamically, which may indicate characteristic differences in signal transduction.
NMR research and biotechnological work were supported by the Russian Foundation for Basic Research (project no. 23-44-10021).

**S1.33. Dismutation of hydroperoxides by the class G immunoglobulins in vitro**

Smirnova L.[1]*, Kazantseva D.[1], Kamaeva D.[1], Voronina V.[2], Krotenko N.[2], Ivanova S.[1]
[1]*Mental Health Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences;*
[2]*Siberian State Medical University;*
* lpsmirnova2016@gmail.com

Catalytically active antibodies or abzymes are immunoglobulins of various classes that have the ability not only to bind an antigen, but also to catalyze chemical reactions. It is shown that natural mammalian IgG, IgA and IgM have many different catalytic activities. Catalytic antibodies have been found both in a number of autoimmune and inflammatory diseases and in healthy people. For instance, the first example of abzymes in healthy people was human milk IgA, which catalyzes protein phosphorylation. Subsequently, lipid kinase and polysaccharide kinase activities of these antibodies were discovered. Plenty of catalytic antibodies hydrolyzing RNA, DNA, nucleotides, lipids, oligopeptides, proteins and oligosaccharides have been identified in patients with various autoimmune and infectious diseases. Abzymes with oxidoreductase activities began to be researched recently and so far the ability of IgG to dismurg hydrogen superoxide and peroxide is studied. It is assumed that catalytic antibodies with oxidoreductase features can compensate the deficiency of antioxidant systems in diseases and play a significant role in protecting humans from oxidative stress. Generalov with co-authors in 1998 showed that IgG preparations from the blood serum of healthy donors and patients with various forms of hepatitis had peroxidase activity. In the works of A.S. Tolmacheva already in 2015 year it was shown that IgG which are taken from serum of healthy. Wistar rats have peroxidase activity in the presence of hydrogen pyroxide and oxidoreductase activity in the absence of hydrogen pyroxide. More peroxidase activity of IgG has not been researched. Therefore, the purpose of this work was to study the presence and properties of NADPH-dependent peroxidase activity of human IgG in comparison with similar catalase activity.
The blood serum of healthy individuals was used as the material for the study. For blood sampling Vacuette tubes with a clot activator were used. To separate the serum, the tube was centrifuged at 2000 g for 20 minutes in a centrifuge with cooling.
Research methods: Purification of IgG was carried out by affinity chromatography on columns with ProteinG-Sepharose on an AKTA purifier (GE) chromatograph. Antibody dialysis was performed against 20 mM Na-phosphate buffer, pH 7.0. The IgG concentration was determined by a Varioskan LUX multimode reader (Thermo Scientifie, USA) at 280 nm. The homogeneity of isolated IgG preparations was tested by Lemilly electrophoresis in gradient 4-18% PAAG. Gels were visualized with the help of iBright Imaging Systems FL1500 (Thermo Scientine, USA, a device is located in sharing centre "medical genomics", TNRMC). Gel-filtration in pH-shock conditions was carried out on a Superdex-200 HR 10/30 column using 50 mM Gly-HCl buffer, pH 2.6 with pre-incubation of IgG with 1 M acidic buffer. NADPH-dependent peroxidase activity of IgG was determined on a SPECORD M-40 spectrophotometer (Carl Zeiss) at 340 nm by the oxidation of NADPH in the conjugate glutathione reductase reaction of the reduction of tertiary butyl hydroperoxide and expressed in units of activity U. (U=mkM NADPH/min/mg IgG). The catalase activity of IgG was also determined on a SPECORD M-40 spectrophotometer (Carl Zeiss) by the decrease in the concentration of hydrogen hydroperoxide after adding the sample. Catalase activity of IgG was determined in units of activity U, (U=mkM H2O2 /min/mg IgG). The inhibitory analyse was made with the usage of sodium azide (NaN3) and ethylenediaminetetraacetate (EDTA) which are non-specific metal-dependent inhibitors. IgG were incubated with NaN3 at final concentrations from 1 mM to 0.005 mM and their NADPH-dependent peroxidase activity was determined. Similarly, we worked with EDTA at concentrations from 1.25 mM to 50 mM. To calculate the concentration of half-maximal inhibition IC50, a graph was built using the Very Simple IC50 Tool Kit online program with the GNUPLOT package. Statistical data processing was carried out in the Statistica 12.0 program.
As a result of the study, it can be concluded that immunoglobulins G of healthy donors are able to catalyze catalase and peroxidase reactions and this activity is an own property of the studied antibodies. To check whether the studied activity belongs to abzymes, three strict criteria were used: isolation of IgG on a specific affinity sorbent, electrophoretic homogeneity of antibodies, and retention of the studied AT activity after gel-filtration in an acidic environment. It was found that sodium azide and 3-amino-1,2,4-triazole inhibited the catalase activity of serum IgG, and at the same time 3-amino-1-2-triazole is more effective inhibitor (IC 50 :16,06 mkM against 140 mkM for sodium azide), because it requires lower concentrations to achieve maximum effect. Also, sodium azide and EDTA inhibited NADPH-dependent IgG peroxidase activity, confirming the metal-dependence of this activity

in immunoglobulins, but had an effect at higher, millimolar concentrations. An inverse correlation between these activities was also shown in the work. The correlation analyse of catalase and NADPH-dependent IgG peroxidase activities revealed a strong negative correlation (r = -0.85; p=0.001).
Thus, the obtained results suggest that the greatest contribution into the utilization of hydroperoxides is made by antibodies with NADPH-dependent peroxidase activity, and not with catalase.
The study was carried out on the topic of research work: Biopsychosocial mechanisms of pathogenesis and clinical polymorphism, adaptive potential and predictors of the therapy efficiency in patients with mental and behavioral disorders in the Siberian region, registration number 122020200054-8.

### S1.34. Dps can interact with phospholipid vesicles and integrate into outer membranes of E. coli cells

Antipov S.S.[1,2]*, Markelova N.Yu.[2], Shavkunov K.S.[2], Shilova E.V.[1], Prejbrazhenskaya E.V.[2], Skorobogatov M.S.[1], Ozoline O.N.[2]
[1]*Voronezh State University;*
[2]*Institute of Cell Biophysics of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences";*
* ss.antipov@gmail.com

Attention to polyfunctional proteins is characteristic of modern molecular biophysics and structural biology. Most of these proteins are multidomain or multisubunit. Different functions of these proteins can be provided by the work of different modules, evolutionarily combined into one protein globule. However, recently proteins have been identified that are capable of performing various functions without having special structural modules for this. Several in silico methods have been proposed for their identification of such proteins, called "moonlight proteins", as well as proteins with experimentally confirmed multifunctionality, which are collected in the MoonProt database, which includes Dps.
It was clear that, being one of the main proteins of the bacterial nucleoid, predominantly produced during the stationary phase of bacterial growth, Dps also acts as ferritin, oxidizing toxic iron ions and accumulating Fe(III) in an internal cavity formed by 12 identical subunits. The oxidation of Fe(II) is catalyzed by hydrogen peroxide in the ferroxidase centers located at the junctions of subunits. Dps-mediated condensation of a bacterial nucleoid in the stationary phase of growth mainly occurs due to the presence of positively charged amino acid residues in its unstructured N-terminal modules. By electrostatically attaching to the negatively charged DNA backbone, they can facilitate the interaction of the protein with any loci of the genome. However, in vitro Chip-seq and SELEX experiments revealed a nonrandom distribution of Dps binding sites throughout the bacterial genome.
Another interesting feature of Dps is its spatial distribution in bacterial cells. Discovered as a nucleoid protein of starving cells, it was found, as expected, in association with DNA. However, the impact of environmental bacteriophages on E. coli MG1655 led to the selection of phagotolerant bacteria with increased biofilm formation, which produced fibril-like structures and accumulated Dps in the outer membrane protein fraction. The membrane location of this protein is poorly understood, but by studying the outer membrane proteins involved in streptomycin resistance, it has been shown that along with the activated proteins (TolC, OmpT and LamB) of E. coli, three proteins are also present (FadL, OmpW and Dps) in a strain resistant to antibiotics. Probably, the presence of Dps on the membrane cannot be explained only by its leakage from dead cells together, for example, with DNA. Therefore, the purpose of this study was to evaluate the possibility of membrane localization of the multifunctional E. coli Dps oligomer.
To assess a principal ability of Dps to interact with lipids, the artificial liposomes widely used as drug delivery systems were obtainedfrom the soybean lecithin. The peak maximum in their size distribution corresponded to 103 nm, which gave 7.6 – 30.4 fold excess of the Dps particles taken in 0.55 mM concentration, when 20-5µl of liposome suspension was added to the protein solution. Although such a large excess hampered detection of liposome-mediated depletion of free Dps in band-shift experiments, we observed a decrease to 41.6 and 53.7% of unbound Dps in the presence of 20 or 15 microliters of freshly prepared liposomes. Indicating the ability of Dps to bind membrane phospholipids, probably in a manner similar to the sugar-phosphate backbone of DNA, this made further studies appropriate.
In the first step, we tested whether E. coli K12 MG1655 cells can exhibit Dps on their surface. Bacteria were grown in LB medium until stationary phase (16 and 72 hours), when the cellular concentration of Dps is maximal. They were prepared for confocal microscopy and the protein was visualized using green fluorescence dye Alexa Fluor 488 as described in Materials and Methods. As a result, we found about 20% of cells with green fluorescence with no apparent dependence of their number on the time of steady growth. Staining the bacteria with red fluorescent protein (RFP) derived from pET28b-mCherry confirmed the presence of Dps on the cell surface and revealed its ability to form distinctive granular structures. Nothing similar was observed for the dps-null mutant, assuming that these structures are formed at least with the participation of Dps and indicating the absence of significant cross-reactivity of antibodies with other E. coli surface proteins.
To assess the dependence of this phenomenon on cellular Dps concentration, we used E. coli XL-1 Blue cells. After 16 hours of cultivation, approximately the same percentage of cells exhibited Dps on their surface as estimated for E. coli k12 MG1655 cells. Harvesting bacteria at different optical densities (OD600 = 0.4, 0.6, 0.8 and 1.0) we observed an expected increase in the percentage of cells with green fluorescence. By transforming E. coli XL-1 Blue cells with pGEMΔXba_dps, carrying the dps gene under the control of the T7 RNA polymerase promoter, and inducing its expression with IPTG we obtained a sharply increased amount of green cells. However, most of them formed large aggregates where the percentage of cells with membrane bond Dps was impossible to estimate correctly. Therefore, we assessed it for separately located cells, and it turned out to be higher than for cells grown to the same OD without IPTG. Thus, it became clear that Dps migration to the cell surface depends on its cellular concentration. Since aggregates formed by E. coli K12 MG1655 cells grown to late stationary phase (72 h) were not usually significantly enriched in green cells, it is possible that IPTG-induced overproduction of this protein facilitates aggregation.
The study was supported by the RSF, project N 18-14-00348

### S1.35. Dynamic structural features of aqua complex formation for ice-binding protein from the fish Myoxocephalus octodecemspinosus based on the HDX-MS technique

Baranova S.V.[1]*, Zhdanova P.[1], Oleinik G.A.[1], Chernonosov A.A.[1], Koval V.V.[1,2]
[1]*Institute of Chemical Biology & Fundamental Medicine, Novosibirsk, Russia;*
[2]*Novosibirsk State University, Novosibirsk, Russia;*
* swb@niboch.nsc.ru

Ice-binding proteins (IBPs) are a special class of proteins that help organisms survive in cold ecosystems. This class of proteins controls ice growth by binding to its surface and aids living organisms avoid freezing damage. Ice-binding proteins are unique molecules that can bind ice crystals and can be active at the interface of two phases: solid and liquid.
In this work, we investigated the structural features of one of the IBPs class representatives: type-4 ice-structuring protein from

Myoxocephalus octodecemspinosus (Longhorn sculpin). The aim of this work was to determine the structure of the protein from the longhorn sculpin fish by HDX-MS technique and to carry out molecular dynamic modeling of the structure of the protein complex with ice. In this work, we made a peptide map of the protein and performed HDX-MS experiments to determine its three-dimensional structure. The structure of IBP in complex with ice was obtained by computer simulation, and the amino acid residues which form hydrogen bonds with the ice surface were determined. For the prepared structure of the complex, molecular dynamics simulations of 100 ns duration were performed in Amber20 force fields.

A combination of computer simulation techniques, HDX-MS with the assistance of other biophysical methods provided a complex notion about ice-binding proteins. The combination of these methods is used in the study of many proteins and macromolecular complexes and has a number of advantages over other biophysical approaches. One such advantage is the ability to study protein in solution.

The unique properties of ice-binding proteins found applications in the food industry, agriculture, and cryopreservation for preserving biological samples at low temperatures. Nevertheless, identification of the structural features of ice-binding proteins can help in the optimal choice of synthesis strategies to improve properties and yields. The successful embodiment of the natural cryoprotective capacity of ice-binding proteins has great promise but is still in its infancy.

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### S1.36. Effect of amphotericin B on the cytoarchitectonics of donor erythrocytes

Litvinov N.V.[1]*, Sokolova L.O.[1], Kalaeva E.A.[1], Sveklo L.S.[2], Artyukhov V.G.[1]
[1]*Voronezh State University;*
[2]*Voronezh Regional Blood Transfusion Station;*
* litvinov.nikolai97@inbox.ru

The polyene antibiotic Amphotericin B used for the treatment of invasive fungal diseases [2] can be used as a fluorescent probe due to its ability to bind to cholesterol, including in membranes, and form fluorogenic complexes with it [1]. The mechanisms of interaction of this antibiotic with biological membranes are complex and are subject to further studies.

In view of the above, the aim of this work was to study the effect of amphotericin B on the cytoarchitectonics of human blood erythrocytes. Erythrocyte cells from donor blood were obtained according to the standard technique [3]. The obtained erythrocyte suspension was brought to a concentration of 106 kl/mL. Then, 1 ml of amphotericin B was added to 4 ml of erythrocyte suspension at the concentrations of 2.5 - 10-5 and 5.4 - 10-5 M.

The surface architectonics of donor erythrocytes was studied using a scanning electron microscope JEOL JSM 6380-LV (Japan) at an accelerating voltage of 20 kV at the Center for Collective Use of Scientific Equipment of Voronezh State University (URL: http://ckp.vsu.ru). For a detailed analysis of the character of changes in the surface architectonics of erythrocytes the following parameters were calculated (in %): number of discocytes (D); number of reversibly deformed erythrocytes (OD); number of irreversibly deformed erythrocytes (ID); transformation index (IT), which is the ratio of the number of pathological and normal forms of erythrocytes; reversible transformation index (RTI) - the ratio of reversibly deformed and normal forms of erythrocytes; irreversible transformation index (INOT) - the ratio of irreversibly deformed and normal forms of erythrocytes [4].

The control suspension sample contained 93.9 % of discocytes, 4.2 % of OD cells, and 2.0 % of ND erythrocytes. The indexes of transformation had the following values: IT = 0.066±0.004 %; IOT = 0.045±0.003 %; INOT = 0.021±0.002 %. These parameters of cytoarchitectonics correspond to the morphological profile of healthy human erythrocyte cells and agree with the results of studies conducted earlier at the Department of Biophysics and Biotechnology of the VSU [4, 5]. After incubation of erythrocyte cells with amphotericin B at concentrations of 2.5 - 10-5 and 5.4 - 10-5 M for 15 min, the level of discocytes decreased to 3.6 and 6.2 % respectively, while the increase of OD to 11.4 and 8.6 % was observed; ND to 20.4 and 26.8 %; IT to 11.0 and 7.4 %; IOT to 3.7 and 1.4 % and INOT to 7.3 and 6.0 % compared to intact samples. Also, a significant increase in the number of spherocytes, echinocytes, erythrocytes in the form of "mulberry" and cells with one and with multiple growths was observed in the samples tested after incubation with the antibiotic.

Thus, exposure of erythrocyte suspensions to amphotericin B contributed to an increase in the number of reversibly and irreversibly deformed cells in the samples. Among the forms of erythrocytes detected, there was an increase in the number of cells with one and with multiple growths, as well as spherocytes, echinocytes and erythrocytes in the form of "mulberry", which indicates a violation of elasticity of the erythrocyte membrane due to cholesterol extraction from it.

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### S1.37. Effect of human serum albumin on the binding of vascular endothelial growth factor (VEGF-A165) to the monoclonal antibody bevacizumab

Shevelyova M.P.[1]*, Nemashkalova E.L.[1], Deryusheva E.I.[1]
[1]*Institute for Biological Instrumentation of RAS;*
* marina.shevelyova@gmail.com

Current therapy and diagnostic used in different diseases including cancer is based on specific full-length antibodies or their fragments treatment. Nowadays about a third of therapeutic proteins are antibodies. Laboratory methods in vitro used for high-affinity and high-specificity antibodies development such as surface plasmon resonance, immunoassay analysis, bio-layer interferometry and others are usually carried out with standard buffers. However, antibody exhibits its stability and affinity under in vivo conditions of physiological human fluids containing many other molecules. In human plasma and intercellular fluid that are normal functioning environment of antibodies such major components as serum albumin (HSA) and low-molecular-weight components and ions are needed to be considered. Knowing of the mechanism of the components influence is of interest to improve the antibodies efficiency.

Vascular endothelial growth factors (VEGFs) family is the most important regulator of a wide range of physiological and pathological

processes such as vasculogenesis, wound healing, bone formation, growth, and development. VEGF receptors are presented on the surface of endothelial and other cell types, and it causes multiple effects of VEGF. Such pathological processes as cancer (mammary cancer, leukemia, glioblastoma, colorectal cancer), macular degeneration, preeclampsia, diabetic retinopathy proceeds with VEGF as a key mediator. Thereby VEGF became one on the target during the mentioned diseases treatment, and anti-VEGF antibodies-based drugs are widely used today. Avastin (commercial name of bevasizumab) is a recombinant hyperhimeric humanized monoclonal antibody containing fully human framework regions with complementarity-determining regions of a mouse hyperchimeric antibody binding to VEGF.
In this work, human serum albumin influence on the most common and important growth factor VEGF-A165 binding to monoclonal antibody bevacizumab was studied with bio-layer interferometry using OCTET QKE SYSTEM. VEGF-A165 (0,5 μM) was chemically immobilized on biosensor layer. Bevacizumab concentrations were 15 and 20 nM. Determined equilibrium dissociation constant of VEGF-A165-bevacizumab (0,15±0,43) nM corresponds to the literature data. Addition of 600 μM HSA leads to an increase of the equilibrium constant in 350 times, wherein kinetic association constant decreased by an order of magnitude and dissociation constant increased in 20 times. For in silico analysis of antigen-bevacizumab interactions, as well as to establish a possible mechanism for the effect of serum albumin on the antigen-antibody complex, full-length antibody and VEGF-A165 models were modeled. Modeling was performed based on the available crystal structure of the bevacizumab fragment (mutant form), available in the PDB bank (PDB code 6BFT) and predicted by the AlphaFold (https://alphafold.ebi.ac.uk/) and I-Tasser (https:// zhanggroup.org/I-TASSER/) heavy and light chains of bevacizumab. The tertiary structures of the RBD (receptor-binding domain) and HBD (heparin-binding domain) domains were taken as the basis for VEGF modeling: 2VPF and 1VGH, respectively. The homodimeric form of the VEGF-A165 protein was predicted by the AlphaFold server in multimer mode. Alignment, refinement and visualization of three-dimensional structures were carried out using PyMOL v.2.5, the Pairwise Structure Alignment RCSB PDB online service and the Stride server. The content of secondary structure elements in the VEGF-A165 model, as well as the linear sizes of the molecule, corresponds to the experimental data obtained by circular dichroism and dynamic light scattering. The ClusPro server was used to predict the VEGF-A165–bevacizumab, HSA-bevacizumab, VEGF-A165-HSA complexes, as well as their ternary complex. Partial overlapping of the binding regions of VEGF-A165-HSA and VEGF-A165–bevacizumab was revealed; this fact may explain the change in the equilibrium dissociation constant.
Thus, for the first time, experimental data were obtained on the influence degree of human serum albumin on the interaction of VEGF-A165 with bevacizumab. The data may be used during therapy with the antibody, and development of another high-specific and functionally stable antibodies in different conditions.
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### S1.38. Effect of the electrostatic environment on the spectral properties of carotenoids

Yaroshevich I.A.[1]*, Mamchur A.A.[1]
[1]*MSU named after M.V.Lomonosov;*
* iyapromo@gmail.com

Carotenoids are one of the most common classes of biological pigments. The spectral properties of these molecules are determined by their conjugated π-system, the properties of which depend on the local environment. Carotenoproteins are stoichiometric complexes of protein and carotenoids, allow modifying the structured environment of bound pigments, which will lead to a change in their spectral properties. In this work, we have created a three-dimensional map of the effect of electric charge on the shift in the absorption spectrum of cantoxanthin. The main research method is computational quantum chemistry, in particular time-resolved density-matrix functional theory (TDDFT). This approach has already been used in the rational design of pigments based on lumiflavin [1], but has now been applied to much larger objects - C40-carotenoids. The projections of the obtained maps can be used to predict the spectral changes of new mutant forms of carotenoproteins, which are necessary within their rational design.
The study was supported by a grant from the Russian Science Foundation No. 22-74-00012.
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### S1.39. Experimental detection of conformational transitions between DNA forms: problems and prospects

Zubova E.A.[1], Kovaleva N.A.[1]*, Strelnikov I.A.[1]
[1]*N.N. Semenov Federal Research Center for Chemical Physics Russian Academy Of Sciences;*
* natykov@gmail.com

Depending on the environment, the DNA double helix can change its geometry. Besides the usual B-form, the A, C and Z conformations are more known, while the D, Hoogsteen and X forms are rarely mentioned. However, the non-canonical DNA forms play an essential biological role. The DNA molecule takes (locally or as a whole) one of these forms in vivo in critically important complexes with proteins (for example, in transcription complexes or nucleosomes), and in vitro under conditions of low dielectric constant, presence of salt, low water activity, or small volume.
In DNA crystals, the helix geometry (as well as the crystallographic cell) can be determined by X-ray methods. Comparison of the Raman and IR spectra from fibers of different DNA forms allowed to single out bands - markers corresponding to DNA vibration modes that change frequency after conformational transitions. These markers make it possible to separately estimate the helix geometry and the share of deoxyriboses and phosphates in noncanonical conformations. The spectral markers can be used to determine the DNA conformation in solutions, in nonoriented gels, and in cells, in DNA-protein complexes. In oriented gels, the DNA forms can be distinguished by the linear dichroism method, and in nonoriented gels or in solutions – by the circular dichroism method. Phosphorus-31 NMR studies allow to determine the fraction of phosphates in the non-canonical BII conformation depending on the type of the neighboring nucleobases. We review the existing experimental methods for distinguishing the DNA double helix conformations and discuss the difficulties and prospects for a reliable determination of the helix geometry, deoxyribose and phosphate conformations.
This work was supported by the Program of Fundamental Research of the Russian Academy of Sciences (project FFZE-2022-0009, registration number 122040500069-7). The calculations were carried out in the Joint Supercomputer Center of the Russian Academy of Sciences.

### S1.40. Fabrication and investigation of nanostructures based on DNA

Kasyanenko N.A.[1]*
[1]*Saint-Petersburg State University;*
* n.kasyanenko@spbu.ru

The usage of biopolymers, especially DNA molecules, to create nanostructures is currently used in various technological developments. Due

to its high specificity, DNA origami technology makes it possible to program and obtain various 3-D structures by self-assembly, which can be used as a material for fabrication nanoscale structures for various purposes, which are used in nanomedicine, nanophotonics, nanooptics, etc. An alternative approach uses induced conformational transitions based on the polyelectrolyte properties of DNA and its unique characteristics - high rigidity, the ability to fairly easily transform a unique double-stranded structure into flexible single-stranded polymers, and its amphiphilicity (a combination of hydrophobic bases and a hydrophilic sugar-phosphate backbone). The use of high molecular weight DNA capable of compacting as a result of complexation with oppositely charged agents (polyamines, polycations, multiply charged metal ions) in solution makes it possible to induce the formation of ordered nanostructures. This approach uses non-specific interactions of DNA with ligands. Manipulations with the quality of the solvent, variation in the pH value and ionic strength of the solution, the use of surfactants can make it possible to regulate the process of formation of nanostructures by determining their properties and physicochemical characteristics in advance. If necessary, such structures can be "decorated" with nanoparticles and biologically active agents. The specific binding of various ligands to the macromolecule provides targeted modification of the properties of the formed nanostructures. Optimization of this approach can ensure its usage in creating multicomponent systems. As is known, noble metal nanoparticles have unique plasmonic properties that are used in various technologies: in biomedical applications in the creation of biosensor devices, biocatalysts, carriers of biologically active substances, optical signal amplifiers, etc.

Depending on the nature and method of synthesis, nanoparticles can have different properties. One of the promising methods for the synthesis of nanoparticles is based on the use of a DNA molecule as a template. Nanomaterials obtained in this way attract attention with a wide range of applications. "Smart" multifunctional nanomaterials formed by self-assembly of complexes in solution attract attention not only for a wide range of possible applied developments, but also for the need to solve fundamental problems related to conformational analysis, polyelectrolyte properties, and possible phase separation in such systems.

One of the goals of this work was to select the conditions for the synthesis of DNA-coupled particles of gold, silver, and palladium. The possible effect of noble metal nanoparticles integrated with DNA on the conformational and optical properties of the macromolecule, on the luminescence of DNA-bound ligands, and on the compaction of the macromolecule induced by various agents was considered.

A new method for creating palladium nanoparticles (PdNPs) without a reducing agent using a synthetic copolymer has been proposed. The size and properties of the obtained nanoparticles are compared with gold (AuNPs) synthesized in a similar way, and the possibility of forming bimetallic Au/PdNPs nanoparticles is shown. A method for DNA metallization with gold and palladium nanoparticles in the presence and absence of a surfactant using sodium borohydride as a reducing agent is proposed. Complexes of DNA-nanoparticles-anticancer drugs based on metal coordination compounds are considered.

The systems were characterized by dynamic light scattering, atomic force microscopy, spectrophotometry, hydrodynamic methods for assessing the size and rigidity of macromolecules.

### S1.41. Fourier method of IR spectroscopy in the study of the anomeric purity of α- and β-forms of crystalline D(+)-glucopyranoses

Nechiporenko A.P.[1], Plotnikova L.V.[2]*, Vezo O.S.[2], Sitnikova V.E.[1]
[1]*National research University of information technology, mechanics and optic. Kronverksky Ave., 49, Saint Petersburg, 197101, Russian Federation;*
[2]*St. Petersburg State University, Universitetskaya Embankment, 7-9, St. Petersburg, 199034, Russian Federation;*
* ljusja@mail.ru

The Fourier method of IR spectroscopy was used to study a series of samples of α- and β-forms of crystalline D(+)-glucopyranose in order to assess the possibility of detecting an admixture of a concomitant anomer. The α- and β-forms of the monosaccharide, which differ markedly in optical properties, showed that, unlike α-D(+)-glucopyranose, whose spectrum is characterized by an intense maximum of 1009 cm^-1, in the spectrum of β-D(+)glucopyranoses in this area there is a complex band with a maximum of 993 cm^-1 and a differentiated doublet of 1020/1012 cm-^1. An important sign that the structure of glucopyranose belongs to the β-form is the presence of a band of 838 cm^-1, and for α-D(+)-glucopyranose – the presence of a band of 851 cm^-1. In addition, wide structured bands in the region of 1500-1400 cm^-1 are of interest, which clearly convey changes in the optical characteristics of OH groups as a result of the inversion of the pyranose cycle - a gentle slope of the right structured branch of the band with a maximum of 1460 cm^–1 in the β-form and a steep rise of the left structured branch of the band with a maximum of 1424 cm^-1. All the noted signs manifest themselves in the spectrum of the corresponding form of glucopyranose in the presence of an admixture of the second anomer, the severity of which increases with an increase in the content of the latter. The obtained data showed the effectiveness of the Fourier method of IR spectroscopy in the analysis of the anomeric purity of crystalline D(+)-glucopyranose, widely used in medical and pharmacological practice, cosmetology, and the food industry.

### S1.42. From DNA-protein interactions to understanding genome functioning in eukaryotes

Shaytan A.K.[1]*
[1]*Lomonosov Moscow State University, Faculty of Biology;*
* alex@intbio.org

More than 20 years have passed since the decoding of the human genome, but the mechanisms of the genome functioning as a whole are still far from clear. Initial optimism associated with the relative simplicity of the genetic code and the possibility of analyzing the genome as a text consisting of a linear sequence of discrete symbols collided with the realization that regulatory functions in the genome are carried out through a complex network of three-dimensional physical dynamic interactions between DNA, RNA and protein molecules. The atomistic structure of the basic complex of the eukaryotic genome - the nucleosome - was deciphered more than 25 years ago. Recently, there has been significant progress in understanding the dynamics of nucleosomes, the structure of various supranucleosome complexes, and the influence of nucleosome dynamics and modifications on the structure of chromatin. Ultimately, we begin to understand how various regulatory and functional programs are encoded in the genome by fine-tuning the dynamics of interactions between molecules in chromatin. The talk will review current ideas about the structure of chromatin, focus on discussing the growing amount of information about the interactions of nucleosomes with various proteins, and demonstrate the results showing the importance of nucleosome dynamics in the regulation of DNA accessibility for reading by transcriptional machinery.

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### S1.43. Histone tails and single strand DNA breaks stabilize intranucleosomal DNA loops during transcription through a nucleosome

Gerasimova N.S.[1,2], Pestov N.A.[3], Studitsky V.M.[1,4]*
[1]*Biological Faculty, Lomonosov Moscow State University, Russia;*
[2]*Institute of Gene Biology, Russian Academy of Sciences, Russia;*
[3]*Department of Pharmacology, Rutgers Robert Wood Johnson Medical School, NJ, USA;*
[4]*Fox Chase Cancer Center, Philadelphia, USA;*
* Vasily.Studitsky@fccc.edu

The majority of RNA molecules in eukaryotic cells are produced by DNA-dependent RNA polymerase 2 (RNAP 2). Nuclear DNA in eukaryotes is organized into a chromatin – a complex of nucleic acids and proteins with a nucleosome as a basic unit. Nucleosome consists of a 147-bp DNA fragment tightly packed on an octamer of core histone proteins [Luger et al., 1997] presenting a barrier for transcribing RNAPs [Bondarenko et al., 2006]. Moderate transcription through chromatin by RNAP 2 is accompanied by survival of the core histone proteins on the DNA due to specific RNAP 2 type mechanism of elongation conserved from yeasts to human [Kulaeva et al., 2009].

Transcription through nucleosomes by RNAP 2-type mechanism is accompanied by formation of small DNA loops on the histone octamer containing the enzyme (intranucleosomal loops, or i-loops) [Kulaeva et al., 2009; Pestov et al., 2015; Gerasimova et al., 2016]. Formation of these structures are presumably involved in survival of core histones on the DNA [Kulaeva et al., 2009; Gerasimova et al., 2016]. These i-loops form much more efficiently in the presence of single-strand DNA breaks in a non-template DNA strand (NT-SSBs) inducing arrest of transcribing RNAP [Pestov et al., 2015], and thus allowing detection of the damages by the enzyme. Structural studies of transcription intermediate containing i-loop reveals, that after RNAP passes the damage, the enzyme can backtrack, and DNA behind it recoils on the surface of the histone octamer, forming an i-loop that locks RNAP in the arrested state [Gerasimova et al., 2022].

Here we examined the role of N-terminal tails of core histone proteins and extranucleosomal NT-SSBs on the i-loop formation and arrest of RNAP during transcription of promoter-proximal region of nucleosomal DNA [Gerasimova et al., 2023]. It was found, that NT-SSBs in linker DNA induce arrest of RNAP at the positions +1 to +15 bp in the nucleosome from the promoter-proximal boundary, suggesting formation of the i-loops in these positions. The arrest of the enzyme is more efficient in the presence of the histone tails. Consistently, DNA footprinting assay reveals formation of an i-loop after stalling RNAP at the position +2 and backtracking to position +1. The data suggest that histone tails and NT-SSBs present in linker DNA strongly facilitate formation of the i-loops during transcription through promoter-proximal region of nucleosomal DNA.

Our newly obtained data suggests an important role of N-terminal tails of core histones in formation of i-loop structures. Since the i-loops are formed much more efficiently in the presence of SSBs positioned behind the transcribing enzyme, the loops likely play a role in transcription-coupled repair of DNA damages hidden in chromatin structure.

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### S1.44. Influence of pectin on the process of electropolymerization of methylene blue and toluidine blue

Ryabov E.I.[1]*, Cherenkov I.A.[1], Novakovskaya M.V.[1]
[1]*Udmurt State University;*
* r-expert@list.ru

Electroactive dyes of the phenazine series, methylene blue and toluidine blue, are widely used in the development of model and analytical biosystems. Dyes are used both in monomeric form and in the form of electroactive polymer films.

In order to assess the electrochemical activity and biocompatibility of polymer films of both dyes - methylene blue (MB) and toluidine blue (TB), obtained in the presence of natural pectins, and evaluating the possibility of further studies in relation to living cells on the obtained electroactive substrates, in particular the behavior of peritoneal lavage (PCL) cells in response to activation by the introduction of lipopolysaccharide (LPS)these electroactive materials were obtained and characterized.

In the course of the work, a unified polymerization scheme was adopted to both dyes. Equivalent amounts of natural pectin solutions with a concentration of 3% (wt.) were added to solutions of MB and TB (0.5 mM) and electropolymerization (EP) was carried out, Tris-Hcl solution (pH 8.31, 0,15M) was used as supporting electrolyte. The operating range of EP was +1200…-450 mV (for the MB reaction system) and +1200...-700 mV (for the TB reaction system). The residual activity of the polymers was evaluated in the potential range of +600...-600 mV (for the MB) and +700...-700 mV (for the TB).

Characterizing cyclic voltammograms (CVA) of the processes of EP MB and TB, as well as the CVA of the residual activity ones, let us suggest the formation of electroactive polymers on the surface of the working electrodes. There are three characteristic regions on the CVA during the EP-MB.

At high positive potentials, irreversible oxidation of the monomers occurs. In this region, in the presence of pectin, the values of the oxidation currents turned out to be somewhat higher (2.10 μA) than those observed in EP without polysaccharide (1.86 μA). As the cycles progress, the currents in this region decrease.

The second characteristic part of the CVA EP MB located in the potential range -200 ... -450 mV, it characterized the transformation of monomeric forms of MB dye. The effect of pectin is to limit the electrochemical transformations of dye - the oxidation and reduction currents are significantly lower (reach -1.35 μA and 0.44 μA) if pectin is present in the medium.

Noticeable changes were noted in the region of CVA corresponding to the growth of MB-polymeric form. These are the peaks of electrooxidation and electroreduction shifted towards positive potentials relative to the electrochemical transformations of the monomer and increasing as the cycles pass. In a pectin-containing electrolyte, the potential values corresponding to the electrooxidation of the polymer (E ~ -100 mV) are shifted relative to those obtained during polymerization without polysaccharide (E ~ -150 mV).

The current values turned out to be close (-0.40 μA and -0.60 μA, respectively). The potentials corresponding to the process of polymer electroreduction are almost equal (E ~ 150 mV), while the values of reduction currents at the end of polymerization were higher in the presence of pectin.

Three characteristic areas can be distinguished on the CVA during the EP TB. At high positive potentials, irreversible oxidation of the monomeric form of TB occurs. In this region, in the presence of pectin, the values of the oxidation currents turned out to be significantly lower (0.8 μA) than those observed with EP TB without polysaccharide (3.0 μA). As the cycles pass in the reaction system with TS, the currents in this region remain unchanged.
The second characteristic part of the CVA EP TS addicted to the potential range of 550 ... -250 mV characterizes the transformation of monomeric forms. The effect of pectin is to limit the electrochemical transformations of the dye, accompanied not only by a decrease in the values of recorded currents (from a total change of more than 3.5 μA to 0.2 μA), but also by a potential shift (from 300 ... -250 mV to -250 ... -400 mV) if pectin is present in the medium.
Noticeable changes are noted in the CVA reflecting the processes of TB polymerization. In the presence of pectin, there is a sharp decrease in the value of the recorded current in the system (at the same time, significant differences in the current strength are observed in the first and 20 cycles of polymerization -0.2 μA and -0.4 μA, respectively, for polymerization without polysaccharide, these values are -1.4 μA and - 1.8 uA).
A detailed analysis of the CVA polymerization of TB in the presence of pectin and without it reveals additional characteristic features in the region of polymer growth (potential range 0 ... 950mV mV in the presence of pectin, there is a slight increase in the value of the recorded current strength as the polymerization cycles pass, the increase is 0.2 μA). For the TB polymerization process in the absence of a polysaccharide, this potential range is significantly lower and is in the range of 500…1000 mV.
Thus, one can note the unequal electrochemical behavior of the described reaction systems at equal concentrations of electroactive dyes. When studying the response of polymeric forms of TB and MB, obtained in the presence of pectin, in response to the introduction of peritoneal lavage cells, changes in the characteristic curves of CVA were established. This confirms the possibility of further study of the properties of the obtained polymeric forms of electroactive dyes.

### S1.45. Influence of the Structural Organization of Nucleic Acids on the Interaction with Hypochlorite

Osinnikova D.[1]*, Moroshkina E.[1], Pavlova K.[1], Polyanichko A.[1]
[1]*Saint-Petersburg State University;*
* d.osinnikova@spbu.ru

The interaction of reactive chlorine-containing compounds with various bioorganic molecules, such as amines, amino acids, proteins, lipids, carbohydrates, and nucleic acids, is the focus of constant attention not only of chemists, but also of molecular biologists and physicians. Of particular practical importance is the study of the chemical mechanisms of the formation of various chlorine derivatives under physiological conditions. One of these compounds are hypochlorites - salts of hypochlorous acid. Being a strong oxidizing agent, hypochlorous acid acts as a natural antiseptic. With the development of inflammatory reactions, the production of hypochlorite in the body can lead to tissue damage and provoke the development of malignant neoplasms. The action of hypochlorite on various biological molecules in a living cell has been actively studied for a number of years. At the same time, the question of the influence of the structural organization of nucleic acids on their interaction with hypochlorite remains much less studied. To date, there are only a few works in which an indirect analysis of the effect of hypochlorite on the spatial structure of DNA was carried out.
In this work, using UV and IR spectroscopy, we analyzed the influence of the structure of nucleic acids on the course of the reaction with hypochlorite using the example of the three most common and biologically significant types of nucleic acids: double-stranded DNA in the B-form, single-stranded RNA, and nucleotide phosphates. It was shown that the structural organization of nucleic acids significantly affects their interaction with hypochlorite. The presence of complementary base pairs stabilized by hydrogen bonds seems to be the limiting factor in the onset of the reaction of hypochlorite with endocyclic nitrogen atoms. At the same time, the polymeric structure of nucleic acids significantly accelerates and increases the efficiency of the subsequent stages of the reaction associated with the chlorination of exocyclic nitrogen atoms and the destruction of the ring structure of nitrogenous bases.

### S1.46. Interaction of Sodium Citrate Coated Iron Oxide Nanoparticles with Chicken Egg Protein Lysozyme

Sarimov R.M.[1]*, Nagaev E.I.[1], Matveyeva T.A.[1], Binhi V.N.[1], Burmistrov D.E.[1], Serov D.A.[1], Astashev M.E.[1], Simakin A.V.[1], Gudkov S.V.[1]
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences; Vavilov St., 38, Moscow, 119991, Russian Federation;*
* rusa@kapella.gpi.ru

Superparamagnetic iron oxide particles coated with various shells are of interest as a contrast material in magnetic resonance imaging. In this work, nanoparticles of iron oxide Fe3O4 (FeO and Fe2O3) coated with sodium citrate (TSC-IONP) were obtained. Nanoparticles were obtained by the chemical precipitation of oxide with ammonium hydrate from an aqueous solution of a mixture of ferric and ferrous chloride salts according to the procedure described in [1]. Nanoparticles form self-organizing stable clusters ~10 and 50–80 nm in size, consisting of NPs 3 nm in size. Stability was controlled using the method of multi-angle dynamic light scattering and zeta potential, which was -32±2 mV.
Clusters from TSC-IONP can be destroyed when lysozyme (chicken egg protein lysozyme, HEWL) is added to a solution, and aggregates reaching micron sizes are formed from nanoparticles and protein quite quickly within a few minutes. These aggregates are stable for tens of minutes. The protein in the aggregates does not lose its enzymatic activity and can be released back using mechanical shaking.
Such aggregation was observed by several methods: multiangle dynamic light scattering, optical absorption, fluorescence spectroscopy, TEM, and optical microscopy. Rana spectroscopy and FTIR were also used, but the latter methods revealed almost no differences.
It is important to note that the concentrations of NPs at which protein aggregation occurred were also toxic to cells. There was a sharp decrease in the survival of mouse fibroblasts (Fe concentration ~ 75–100 μM), while the ratio of apoptotic to all dead cells increased. Also, at low concentrations of NPs, an increase in cell size was observed, as well as a decrease in the mitochondrial membrane potential. The investigated iron oxide nanoparticles due to cytotoxicity can be used as a material for contrasting only at low concentrations <50 μM. However, the effects shown during interaction with HEWL molecules make it possible to use nanoparticles in other biomedical problems. For example, they can be used to deliver protein drugs or concentrated in tumor tissues using magnetic fields.
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### S1.47. Interaction of serotransferrin and C1-esterase inhibitor with amyloid-β peptide

Nemashkalova E.[1]*, Vologzhannikova A.[1], Kazakov A.[1], Deryusheva E.[1], Shevelyova M.[1], Levashov P.[2], Shukurov R.[3], Litus E.[1]
[1]*Institute for Biological Instrumentation, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences;*
[2]*Lomonosov Moscow State University, Department of Chemistry;*
[3]*JSC "GENERIUM";*
* elnemashkalova@gmail.com

One of the main factors provoking the development of Alzheimer's disease (AD) is the accumulation of amyloid-beta peptide (Aβ) in the brain. A number of plasma and cerebrospinal fluid proteins have been shown to be powerful inhibitors of Aβ fibrillation, which can be used in AD therapy. Serotransferrin (Tf) and C1-esterase inhibitor (C1I) belong to a group of plasma proteins associated with the pathogenesis of AD. Tf is the main carrier of iron ions in blood plasma. According to some reports, its content in the brain and serum of patients with AD is reduced. C1I plays an important role in the functioning of blood proteolytic systems and in the regulation of homeostasis under critical conditions. It has been shown that in the brain of patients with AD there is a lack of activation of C1I expression, which in turn leads to an increase in the activity of the components of the complement system and contributes to the progression of AD. At the same time, the direct interaction of these proteins with Aβ has been little studied. In our work, we studied the interaction of Tf and C1H with the monomeric form of Aβ, as well as the effect of these proteins on the kinetics of the process of Aβ fibrilation. The interaction of proteins with the monomeric form of Aβ40/42 was quantitatively studied using the method of surface plasmon resonance spectroscopy. A direct interaction of C1I with the monomeric form of Aβ was found, which was not previously described in the literature. Also, the kinetic and equilibrium parameters of the interaction of Tf with the monomeric form of Aβ40/Aβ42 were studied for the first time. The values of the lowest equilibrium dissociation constant obtained after data approximation by the heterogeneous ligand model were 1.5*10^(-9) and 7.8*10^(-9) M for the complex with Aβ40 and Aβ42, respectively. Using a fluorescent test with thioflavin T, the kinetics of Aβ fibril formation in the presence of selected proteins was studied. It was shown that Tf and C1I at a concentration of 8 μM effectively inhibit the growth of Aβ40 fibrils. Thus, it can be assumed that the effect of transferrin and C1-esterase inhibitor on the development of AD, among other things, is mediated by direct interaction with Aβ.
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### S1.48. Investigation of Nd:YAG laser radiation interaction with protein solutions

Nagaev E.I.[1]*, Sarimov R.M.[1], Matveeva T.A.[1], Simakin A.V.[1], Baimler I.V.[1]
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences;*
* nagaev_e@kapella.gpi.ru

The effect of laser radiation on the properties of protein solutions is an urgent task since in recent decades lasers have found wide applications in medicine, particularly in surgery. The use of lasers as laser scalpels reduces the consequences associated with the use of conventional steel scalpels. When using lasers in tissues, ablation, coagulation, a connection of tissues, and destruction of tissues through the formation of shock waves can occur. The study of the effect of optical breakdown on the properties of proteins will help develop new therapeutic techniques that can improve the characteristics of existing medical equipment.
This paper investigates the effect of optical breakdown on the properties of protein solutions. Bovine serum albumin (BSA) and Henn egg white lysozyme (HEWL) were used as model proteins. The phenomenon of optical breakdown in protein solutions has not been practically studied, while there are many examples of studies with metals and their nanoparticles [1]. The breakdowns formed due to short laser pulses contribute to the formation of submicron and nanoparticles and their further fragmentation from the initial micron-sized particles [2]. Aqueous solutions of proteins were irradiated at the facility described in detail in [3]. A nanosecond Nd:YAG laser with second harmonic generation (λ=532 nm) was used. The solutions were irradiated for 30 minutes. After irradiation experiments, the solutions were examined by optical methods (absorption spectroscopy, refractometry, fluorescence spectroscopy, refractometry, and Raman spectroscopy). The results showed acoustic waves and plasma formed during experiments with proteins.
After irradiation, the absorption of protein solutions decreased in the spectral range corresponding to amino acid residues. In experiments with dynamic light scattering (DLS), it was shown that the peak, corresponding to protein molecules, decreases, and the peaks corresponding to large aggregates (>100 nm) grow. Raman spectroscopy has shown that there is a decrease in intensity at a wavelength of 1570 cm-1, which may indicate a possible degradation of the α-helix. There were no significant changes in the refractive indices and the shape of the fluorescent maps. However, after irradiation of the lysozyme solution, a significant decrease in the peak associated with the fluorescence of amino acids was observed and an additional peak of fluorescence was observed at the excitation wavelengths of 350 nm and 434 nm. Previously, researchers associated this peak with the formation of amyloid fibrils [4]. However, further studies using thioflavin-T and circular dichroism spectroscopy did not show the presence of amyloid fibrils. Thus, it can be assumed that partial denaturation and aggregation took place in both studied samples.
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### S1.49. Investigation of the Large-Scale Chromatin Organization in HeLa Nuclei by Small-Angle Scattering (SAS) Methods

Iashina E.G.[1,2]*, Varfolomeeva E.Yu.[1], Pantina R.A.[1], Bairamukov V.Yu.[1], Kovalev R.A.[1], Fedorova N.D.[1], Gorshkova J.Ye.[3], Grigoriev S.V.[1]
[1]*Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute»;*
[2]*Saint-Petersburg State University;*
[3]*Joint Institute for Nuclear Research;*
* yashina_91@inbox.ru

Interest in the question of how a double-stranded DNA strand, gigantic by the standards of biochemistry, is packed in a cell nucleus several microns in size has not weakened for several decades, despite the enormous progress in biology, genetics, and especially in technologies for studying biological cells. This work is part of a cycle of works devoted

to the search for universal principles of large-scale organization of chromatin, as well as the study of its physical properties and their changes under various influences [1–6].
The organization of chromatin in HeLa nuclei [2, 5] was studied using the small-angle neutron scattering (SANS) and ultra SANS methods using the KWS-2 and KWS-3 facilities (MLZ, Munich, Germany). The intensity of neutron scattering in the range of momentum transfer [4 10−2 - 10−1] nm−1 is described by a power function with the exponent $\nu$=2.5, which in the framework of small-angle scattering on fractal objects corresponds to the volume fractal model with fractal dimension DF=2.5. The intensity of neutron scattering in the range [10−3 - 4·10−2] nm−1 is described by a power function with exponent $\nu$=3, which corresponds to the logarithmic fractal model. The transition point between two fractal levels Qc=4·10−2 nm−1 reveals the characteristic maximum size of the volume fractal of 150 nm. That. on scales from 20 to 150 nm, chromatin is a homogeneous self-similar classical fractal structure (volumetric fractal), described by a power measure in which the fractal dimension is DF = 2.5, while on scales from 150 to 6000 nm (the size of the entire nucleus), the structure Chromatin is a hierarchical structure, which is described by a logarithmic measure (logarithmic fractal), which is formed according to the principle of volume conservation when the scale changes [6].
The study of changes in the structure of chromatin in transcription-repressed HeLa nuclei using small-angle X-ray scattering showed a correlation between the volume-fractal structure and the transcriptional activity of chromatin. It was shown that in the sample of HeLa nuclei cultured under nutrient deficiency and in the sample of HeLa nuclei cultured with the transcription inhibitor axtinomyceous D, the structure of the volume fractal (DF=2.5) was not detected, in contrast to the nuclei of the actively dividing HeLa cell.
Thus, using SANS and SAXS, one can identify the presence of a bulk fractal structure in the nucleus, as the appearance of regions of active chromatin responsible for the transcriptional activity of the nucleus. At the same time, inactive chromatin is a dense, homogeneous medium that fills the entire space of the nucleus and serves as a contrast for MUR, against which the bifractal structure of active chromatin is observed.
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### S1.50. Investigation of the effect of phosphatidic acid on the temperature of the conformational transition of complexes [cytochrome C + phospholipids]

Zhuravleva E.R.[1]*, Stepanov G.O.[1], Osipov A.N.[1]
[1]*Pirogov Russian National Research Medical University;*
* evel99@mail.ru

The study of molecular and cellular mechanisms of apoptotic processes is one of the most urgent problems of modern medical science. Thus, one of the ways to initiate apoptosis is the mitochondrial mechanism, which is characterized by an increase in the peroxidase activity of cytochrome C. However, this event is preceded by the interaction of cytochrome C with anionic phospholipids of mitochondrial membranes. This interaction leads to a conformational rearrangement of the active center of cytochrome C.
The purpose of this work was to study the dependence of changes in the structure of cytochrome C complexes with phosphatidic acid when heated, as well as to determine the temperatures at which this conformational transition occurs in the presence of phosphatidic acid. The study was carried out using spectrofluorimetry at excitation and emission wavelengths of 280 and 335 nm, respectively. The study was performed in the temperature range of 25-90°C with a step of 5°C. The samples contained cytochrome C complexes with phosphatidic acid or phosphatidylcholine.
Previously, using chemiluminescence, it was shown that the change in the peroxidase activity of cytochrome C occurs most pronounced with the addition of phosphatidic acid. In the presence of phosphatidic acid, the shape of the kinetic curve changed, showing a sharp increase in intensity. And when studying the temperature dependence of fluorescence for similar cytochrome C complexes with phospholipids, it was shown that the temperature of the conformational transition for the complexes [cytochrome C+ phosphatidylcholine] and [cytochrome C+ phosphatidic acid] was 66°C and 75°C, respectively.
The results obtained confirm the previously studied functional changes of cytochrome C and the ability of phosphatidic acid to influence them. It can be assumed that these differences are related to the fact that conformational transitions for [cytochrome C + phosphatidylcholine] and [cytochrome C + phosphatidic acid] complexes occur at different temperatures.

### S1.51. Lipid peroxidation induced by cytochrome c in the presence of phosphatidic acid. Prospective role in the development of apo- and ferroptotic processes

Volkov V.V.[1]*, Stepanov G.O.[1], Osipov A.N.[1]
[1]*Pirogov Russian National Research Medical University;*
* volkov.vv.work@mail.ru

There are many diseases, the pathogenetic link of which is a violation of the mechanisms of regulated cell death. Currently, a number of mechanisms such as apoptosis, ferroptosis, pyroptosis and others have been discovered and are being actively investigated. In recent years, a clear connection of various mechanisms of cell death with the oxidation of specific phospholipids has been shown: phosphatidylserine in phagocytosis, cardiolipin in apoptosis, phosphatidylethanolamine with arachidonic acid in ferroptosis. This feature of phospholipids to play the most key participation in cellular processes made it possible to distinguish a new direction in science - regulatory lipidomics[1].
As noted, in the mechanisms of development of apoptosis, the role of cardiolipin is well studied, leading first to structural changes in cytochrome C (CytC), and then to a change in its peroxidase activity, which leads to lipid peroxidation of biological membranes (LPO). The peroxidase activity of CytC increases dramatically (tenfold) in the presence of unsaturated mitochondrial cardiolipin, which leads first to membrane oxidation and then to pore formation. However, mitochondrial membrane peroxidation processes, are induced not only in the presence of cardiolipin, but even more pronounced in the presence of phosphatidic acid and its role has not been previously studied.
In this work, using chemilumineescence, it is shown that the intensity of CytC-induced LPO with an increase in membrane composition from 10 to 50% of the content of tetraoleoylcardiolipin (TOCL) or dioleoyl phosphatidic acid (DOPA) increases up to 24 times in comparison with control samples containing only dioleoyl phosphatidylcholine (DOPC). It should be noted that when this model is titrated with increasing concentrations of CytC, the dependence of chemiluminescence intensity, which characterizes LPO, is nonlinear. It becomes maximal at a ratio of anionic phospholipids to CytC equal to 200-250 to 1 for phosphatidic acid and 100-150 to 1 for cardiolipin.

At the same time, changes in the concentration of phospholipid hydroperoxides (LOOH) were also detected using the EPR method in the presence of the POBN spin trap. An increase in the amount of hydroperoxides was observed only in the system with liposomes containing TOCL or DOPA, while liposomes containing only DOPC showed no change in the LOOH concentration.
Thus, it is shown that:
1. The ability of CytC to initiate LPO in the presence of TOCL- or DOPA-containing liposomes increases up to 24-fold (relative to DOPC).
2. As the amount of CytC increases, its ability to induce LPO changes nonlinearly (first increases and then sharply decreases), which may be related to the specifics of structural changes of CytC, which are known to depend on the phospholipid/CytC ratio.
3. EPR confirmed the formation of peroxidation products - phospholipid hydroperoxides in systems with TOCL or DOPA liposomes, liposomes containing only phosphatidylcholine showed no production of LOOH during CytC-dependent LPO.
4. This ability of CytC to enhance LPO may play a key role in the development of both apoptotic and ferroptotic processes. Moreover, while in the presence of TOCL we are most likely talking about the active development of the LPO process, DOPA rather affects the processes of initiation of peroxidation processes.
The study of the initiation process of apo- and ferroptotic processes, will help in the search for regulation mechanisms and hence the treatment of various pathologies such as oncology, cardiovascular and many other diseases.
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### S1.52. Mathematical modeling of quantum yields of chemiluminescence activated by coumarins c-314 and c-334 under the action of a complex of cytochrome C with cardiolipin

Levchenko I.L.[1]*, Vladimirov G.K.[2], Volodyaev I.V.[3]
[1]*Moscow State University, Faculty of Physics, Moscow, Russia, 119234, st. Kolmogorova, 1, building 2;*
[2]*First MGMU them. I.M. Sechenova, Institute of Regenerative Medicine, Moscow, Russia, 119048, st. Trubetskaya, d. 8;*
[3]*Moscow State University, Faculty of Biology, Moscow, Russia, 119991 Moscow, st. Leninskie Gory d.1, building 12;*
* irnlevchenko@yandex.ru

Comparing mathematical modeling of the quantum outputs of physical activators C–314 and C–334, which intercept the excitation of triplet-excited ketones and have values of quantum chemiluminescence yields 3-4 orders of magnitude higher than the excited ketones themselves, we obtain chemiluminescence activated by coumarins C–314 and C–334, which shows an intensity value of ~1500 times and ~1600 times higher than the spontaneous chemiluminescence of lipids, while it does not differ from it in the parameters of kinetic curves and has velocity constants of the same order. The accuracy of the comparison of mathematical modeling of quantum outputs is determined by the presence of cardiolipin for pH stabilization, the quenching of Fe2+ and the presence of physical activators C–314 and C–334. Among the factors that distort the value of mathematical modeling of quantum yields, insufficient addition of hydrogen peroxide, excessive amounts of nitrogen (II), methanol, protein denaturation, as well as a change in the conformation of CytC in the CytC-CL complex are highlighted.
In search of optimal excitation conditions, the systems of lipoperoxidase and quasi-lipoxygenase reactions activated by physical activators - coumarins C–314 and C–334 were analyzed.
In our work, based on the analysis of the parameters of cytochrome C with cardiolipin, physical activators C–314 and C–334, as well as horseradish and luminol peroxidase, comparisons of studies of the sensitizing ability of coumarins C–314 and C–334 as physical activators were carried out in order to compare the quantum yields of C–314* and C–334*.
The cytochrome C complex with cardiolipin differs from the native cytochrome C in the following properties:(1)has the fluorescence of tyrosine and tryptophan residues; (2) loses absorption in the Cope band(405-410 nm) reflecting the existence of the Fe(heme)•••S(Met80) bond;(3)has peroxidase activity and catalyzes the formation of lipid radicals in the membrane;(4)C–334, a physical activator of chemiluminescence, as well as C–314, is actively oxidized by the CytC-CL complex, while the rate of this oxidation is limited only by the concentration of cytochrome C itself, which is also destroyed as part of the CytC-CL complex under the action of hydrogen peroxide.

### S1.53. Mathematical modeling of the occurrence of open states in the DNA molecule depending on the concentration of deuterium in the surrounding liquid medium

Dorohova A.[1,2]*, Drobotenko M.I.[2], Svidlov A.A.[1,2], Dzhimak S.S.[2,1], Lyasota O.M.[1]
[1]*Federal Research Center the Southern Scientific Center of the Russian Academy of Sciences;*
[2]*Kuban State University;*
* 013194@mail.ru

It is known that the concentration of deuterium plays a significant role in the metabolic processes occurring in biosystems of different levels of organization, while the change in the D/H isotope gradient in the body is used to increase its adaptive capabilities. Deuterium atoms, for example, being included in the structure of hydrogen bonds of double helixes of DNA molecules due to rapid protium-deuterium isotope exchange, can influence the "storage-reading" time of genetic information (tH/tD = 0.43), including by modifying the state of the genetic material ("opening" and "closing" of individual pairs of nitrogenous bases in the DNA molecule). It is possible that replacing a protium atom with deuterium increases the energy required to break the bond and "open" the pairs of nitrogenous bases. The theoretical study of these processes should be carried out using mathematical modeling methods, when one of the key conditions for the adequacy of the mathematical model of DNA is to take into account "open" states (OS) [1–3].
In the present work, a mathematical model is constructed for the occurrence of OS in a DNA molecule depending on the deuterium concentration in the surrounding liquid medium at different values of the hydrogen bond breaking energy. To simulate the processes of unwinding the DNA double helix and OS formation, we used a mathematical model that describes the rotational movement of nitrogenous bases around the sugar-phosphate backbone of the DNA molecule. To build it, we used an analogy between a DNA molecule and a mechanical system consisting of two chains of interconnected pendulums. At the same time, nitrogenous bases correspond to the rotating pendulums, and sugar-phosphate chains of the DNA molecule correspond to the elastic thread to which these pendulums are attached. The hydrogen bond of a pair of complementary nitrogenous bases corresponds to the elastic bond of the corresponding pendulums [4]. This mathematical model is based on Newton's equations and represents the Cauchy problem for a system of 2n ordinary differential equations [5].
It has been established that the probability of occurrence of open states between nitrogenous bases in double-stranded DNA depends on the concentration of deuterium in the liquid medium surrounding the molecule and on the value of the hydrogen bond breaking energy (Ecr). At a hydrogen bond breaking energy of $0.335 \cdot 10{-}22$ J, there is an almost linear decrease in the probability of the appearance of open states between nitrogenous bases in double-stranded DNA (for the first 10 base pairs of the gene encoding interferon alpha 17) in

the range of deuterium concentrations from 156 to 40 ppm in liquid environment surrounding the molecule. In this case, the probability of breaking hydrogen bonds between nitrogenous bases in the case of the introduction of even one deuterium atom into a DNA molecule exceeds the probability of a similar break in the same molecule containing only protium atoms (P′/P0 > 1), which indicates a decrease in the stability of the DNA molecular structure. If the hydrogen bond breaking energy is equal to 0.345 · 10−22 J, then in the range of deuterium concentrations from 156 to 40 ppm in the liquid medium surrounding the DNA molecule, there is an almost linear increase in the probability of the occurrence of open states between its nitrogenous bases.

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### S1.54. Mathematical models describing cooperative interactions during oxygen binding by hemoglobin

Lavrinenko I.A.[1]*, Vashanov G.A.[1], Hernández Cáceres J.L.[2], Buchelnikov A.S.[3], Nechipurenko Y.D.[3,4]
[1]*Voronezh State University;*
[2]*Cuban Center for Neurosciences;*
[3]*Sevastopol State University;*
[4]*Institute of Molecular Biology, V.A. Engelhardt RAS;*
* lavrinenko_ia@bio.vsu.ru

Biological systems are characterized by self-regulation, and one of the manifestations of such regulation is cooperativity. Despite advances in the study of the structure of these macromolecules, the mechanisms of cooperative binding of ligands are not completely clear. The emergence of new experimental data makes it necessary to improve the existing classical models, develop new concepts, and also look for alternative interpretations.

Historically, hemoglobin has become a classic object in the study of cooperative systems in biology. G. Hüfner proposed the first equation for hemoglobin oxygenation, which, however, could not satisfactorily describe available experimental data. A. Hill presented his equation with a reasonable approximation to the experimental data and the assumption that the hemoglobin molecule is capable of aggregation. Further studies of cooperative effects began to rely on the mathematical apparatus of statistical physics already developed by that time. Based on the revealed fact of the existence of four ligand binding centers and the idea of sequential oxygenation, G. Adair proposed his own equation, which became the most accurate in describing the hemoglobin dissociation curve. L. Pauling rethought this equation, taking into account the possible options for the spatial position of the oxygen binding centers. The model in the form of a tetrahedron, which is close to the natural structure of the gem protein, has led to the most accurate solution of this problem. D. Koshland, G. Némethy and D. Filmer, based on data on the structural rearrangement of oligomers, developed their own phenomenological model and oxygenation equation based on the induced fit hypothesis (Pauling-KNF model). J. Monod, J. Wyman and J. Changeux, taking into account the data of X-ray diffraction analysis of hemoglobin, proposed a symmetrical model of oxygenation (MWC model) and the corresponding equation.

New experimental data with a higher spatial resolution of the hemoglobin molecule, data on the nanosecond kinetics of ligand binding, as well as a number of other discovered facts, required the development of new models of cooperative interaction: the “Cooperon” model by M. Brunori et al., models by A. Szabo and M. Karplus (SK model), based on the stereochemical mechanism of M. Perutz, which was later generalized and revised by A. Lee and M. Karplus (SKL model), the “Tertiary Two-State” model (TTS model) by E. Henry et al., as well as the “Morpheein” concept proposed by E. Jaffe as well as a number of ensemble models based on the ideas of E. Ising.

In our presentation, we try to briefly outline the main ideas, mechanisms and models of cooperative interactions proposed by different researchers, connecting them with some of our research in this area [1-8]. In this regard, we have considered the equations of oxygenation, which are based on exponential and power dependences, where it is shown that the latter better approximates the oxyhemoglobin dissociation curves. The use of the relative cooperativity coefficient introduced by us makes it possible to divide the Hill coefficient into two components: the number of oligomer subunits and, in fact, the relative cooperativity coefficient characterizing the strength of the interaction between these subunits. Mathematical models of oxygenation are also proposed, based on the Hill equation, where the cooperativity coefficient is modulated by the Gauss and Lorentz distributions as functions of the oxygen partial pressure. Models developed by us have been based on modern molecular biophysics conceptions whereas allowing a better fit to experimental data.

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### S1.55. Mechanism of uranyl ion molecular toxicity towards DNA-binding domain of PARP-1 protein revealed throughout in silico simulations

Bulavko E.S.[1,2]*, Ivankov D.N.[2]
[1]*A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of RAS;*
[2]*Skolkovo institute of science and technology;*
* egor.bulavko@skoltech.ru

Not only radioactive isotopes of uranium appeared to be harmful for living cells. Similar to other heavy metals, in high concentrations uranium is able to change or even destroy the tertiary structure of proteins and DNA. It was shown that uranyl-ion (the most stable uranium form in physiological conditions) targets metal-containing proteins - cytochromes, transferrins, DNA-binding domains, etc. At the level of the organism, chronic poisoning results in malfunction of kidneys, nervous system, hematopoiesis.
Zinc fingers are one of the most common DNA-binding domains in eukaryotic cells. The zinc ion is needed in them for DNA-binding interface spatial structure stabilization. Recently, it was shown that zinc fingers functionality is impaired upon their incubation with uranyl acetate, which turned out to change the domain's tertiary structure. The aim of this work was to study the possible molecular mechanism of the uranyl toxic effect, as well as accompanying conformational changes in structure of PARP-1 protein zinc finger.
To model chemical transformations involving uranyl ion, amino acid side chains in potential binding sites and catalytic water molecules we used QM/MM dynamics approach in combination with bias potentials (Umbrella sampling). We parametrized resulting stable complexes in terms of the classical force fields to calculate long molecular dynamics trajectories. The latter was necessary for conformational sampling and estimation of system's dynamical and energetical parameters (conformational entropy, solvent entropy, enthalpy).
According to our simulations results, the state in which uranium is located in the native binding site is destroyed due to spontaneous internal hydrolysis of the U–Cys162 bond. The enthalpy of hydrolysis is 3.1 kcal/mol, but due to structure loosening, the final value of the free energy of the reaction becomes 1.5 kcal/mol. The subsequent reorganization of binding site includes association of uranyl ion with (Glu190, Asp191) cluster and significant changes in the tertiary structure of domain. The final stage free enegry turned out to be -13 kcal/mol, which makes the whole process energetically favorable. Disorganization of the DNA-binding interface seemingly appears to result in DNA affinity loss.

### S1.56. Mechanisms of Ion Selectivity in Voltage-Gated Cation Channels

Chugunov A.[1]*, Scherbakov K.A.[2], Trofimov Yu.A.[1], Vassilevski A.A.[1]
[1]*Institute of bioorganic chemistry;*
[2]*Institute of Biomedical Chemistry;*
* batch2k@yandex.ru

Transmembrane ion gradients underlie life itself and are inherent for all three domains of life (eukaryotes, bacteria, and archaea) as the basis for bioenergetics, cell excitability, and signal transduction. Moreover, different ions, e.g., sodium (Na+), potassium (K+) and calcium (Ca2+) cations, play individual roles with their concentrations varying dramatically in different cell compartments. Ion gradients are created, maintained and dissipated by ion pumps (ATPases) and ion channels, often selective to a certain ion type. Thus, the problem of ion selectivity is one of the most fundamental in molecular biology.
A giant leap to deciphering the mechanism of ion selectivity was made by the determination of the 3D structure of the potassium channel KcsA some 25 years ago [1], which became prototypical for all potassium and most other voltage-gated cation channels. That structure revealed a selectivity filter (SF) consisting of several layers of pore-lining carbonyl groups, perfectly desolvating K+ ions surrounded by eight water molecules in a square antiprism stereometry. Other ions (Na+ and Ca2+) possess different hydration shells and therefore cannot be desolvated by the potassium channel without energetic penalty, as it occurs for K+, which is, in fact, the mechanism of selectivity.
Although dozens of various potassium and sodium channel structures [2] have been studied since those times, the mechanism of Na+ selectivity still slips away from researchers. In this work, we try to figure it out by starting from purely geometric principles of ionic solvation shells and moving on to molecular dynamics (MD) simulations of ions passing through the channel pores.
First, we analyzed most of the available potassium channel structures (circa 200) using a Python-based geometric approach. Among all channel atoms, we selected the combinations that match the square antiprism, which describes the K+ hydration shell. To avoid combinatorial explosion, only atoms that are capable of ion coordination in a close vicinity are considered. Having performed a thorough scan, we demonstrate that conducting SF structures strictly follow the K+ hydration shell template. In contrast, non-conductive pores always exhibit a distorted SF geometry.
Second, in MD simulations of the symmetric tetrameric bacterial sodium channels, we found "shared" water molecules in the Na+ hydration shell (a bipyramid with six vertices). In this case the solvating water molecules are not substituted by the protein atoms (as in potassium channels), but are coordinated by them, supporting the hydration geometry. Interestingly, there is no such effect for K+ ions inside the bacterial sodium channels, suggesting that the "shared" hydration may play an important role for the Na+ selectivity, based upon fully solvated ion passage through the pore (compared to fully desolvated K+ in KcsA).
In this ongoing research we seek to explain Na+ selectivity in the eukaryotic voltage-gated sodium channels. This is the most complex case due to the asymmetric SF structure in these channels representing fused pseudo-heterotetramers, and absence of clear geometric principles underlying the selectivity.
The work is supported by the Russian Science Foundation (grant no. 22-14-00395).
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### S1.57. Model of large scale chromatin organisation in biological cell nuclei based on Small Angle Scattering (SAS) data

Iashina E.G.[1,2]*, Varfolomeeva E.Yu.[1], Pantina R.A.[1], Bairamukov V. Yu.[1], Kovalev R.A.[1], Fedorova N.D.[1], Grigoriev S.V.[1]
[1]*Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute»;*
[2]*Saint Petersburg State University;*
* yashina_91@inbox.ru

One of the most exciting questions in cellular biology is how can meters of DNA be packed inside the 5 to 10 μm nucleus. The concept of the fractal organization of chromatin is the most productive hypothesis that, however, is not well established yet. The Small-Angle Neutron Scattering (SANS) experiments have shown bi-fractal organization of the chromatin in nuclei of three different types of cells: rat lymphocytes, chicken erythrocytes and HeLa cells. The large scale structure from hundreds of nanometers to some micrometers is well described

by the logarithmic fractal, while a smaller scale structure from tens to hundreds of nanometers appears to be a volume fractal with dimension slightly less than 2.5. The volume fractal units are self-similar and built of the DNA and architectural proteins. The maximal size fractal unit corresponds to a crossover point in SANS curves between two fractal levels. These fractal units are densely packed and occupy rather homogeneously almost all of the nucleus space. The logarithmic fractal structure stands out from the background of chromatin and is attributed to a chromatin-free space shaped as a system of the diffusion channels those sizes are hierarchically changed upon scaling obeying the volume preserving principle. We believe that these channels provide fast diffusion of functional proteins. To demonstrate compliance of our model to real large scale chromatin structure we have simulated SANS experiment building the logarithmic fractal a structure obeying volume reserving principle. We show the equivalence in the power law of scattering intensity for the proposed model and the real experiments.

### S1.58. Modification of the AS1411 aptamer to modulate its affinity for the nucleolin protein depending on the pH of the medium

Gabrusenok P.V.[1]*, Sokolov P.A.[1]
[1]*St. Petersburg State University;*
* p.v.gabrusenok@gmail.com

Targeted delivery of drugs to tissues and certain cells is still an unresolved problem. DNA aptamers seem to be a promising means of targeted delivery of anticancer drugs to body tissues with desired properties [1]. The use of aptamers in this case can reduce immune response and various side effects. This is made possible by adjusting of aptamer activity to specific tissue (for example, cancer tissues have low pH value). The possibility of changing the affinity of the ATP aptamer for the target depending on the pH value has already been shown earlier [2]. This feature was achieved by adding to the aptamer sequence a certain regulatory sequence that interferes with the aptamer structure in certain pH ranges. In this work, we have experimentally shown the possibility of adapting this mechanism of pH dependence to an aptamer with a different structure.

In our work, we have demonstrated the efficiency of the method of modifying the conserved AS1411 aptamer to the nucleolin protein [3], which modulates its affinity for the target depending on the pH value of the medium. Nucleolin was chosen as a target for anticancer therapy [4, 5] because in healthy cells it is located mainly in the cell nucleus, and in cells that have undergone cancerous transformation it is also observed on the cell wall and in the cytoplasm [6], which makes it a reliable marker of tumor tissue. Since the method of titration with a target of a modified ATP-aptamer is not applicable to the nucleolin aptamer due to the low stability of the nucleolin itself and the complexity of its production and isolation, an alternative approach was developed to test the performance of the pH-dependent system. The proposed approach makes it possible to exclude the use of target molecules (proteins) from in vitro experiments when working with conservative aptamers (such as AS1411), the transition of which into a functional conformation is caused not by the presence of a target, but by environmental parameters.

In order to implement the pH-dependent modification, the AS1411 aptamer sequence was supplemented with a 3'-terminal region (interfere-tail) complementary to the 5'-end of the aptamer (quad-tail) and containing one A·G mismatch. These sequences were joined by a polythymine (polyT) linker. A decrease in pH leads to the protonation of adenine in the A·G pair, which increases the stability of the quad-tail:interfere-tail duplex [7]. The initial AS1411 aptamer forms a quadruplex structure with a melting point of about 55°C at the ionic strengths and pH used in the work. Therefore, we believe that in the absence of hybridization of the interfere-tail and quad-tail regions, the AS1411 aptamer sequence goes to a functional conformation, similarly to how it occurs with the initial AS1411 aptamer at temperatures close to room temperature. For this reason, specified modification allows to change the fraction of functional aptamers in solutions depending on pH. The stability of the quad-tail:interfere-tail duplex was assessed by the fluorescence of the Cy3 dye located at the 5'-end of the modified aptamer, which depended on the distance to the BHQ2 quencher at the 3'-end, making it possible to indirectly judge the size of the "enabled" aptamer fraction. An original method of competitive binding of signal oligonucleotides to an interfering interfere-tail region was used to test the performance of the proposed aptamer design. It consisted in titration of the modified aptamer solution with a solution of signal oligonucleotides at a given pH. The luminescence of the cyanine label stayed at low level when titrated with signal oligos in solutions with low pH. However, in solutions with pH > 6.5, a significant increase in the luminescence intensity was observed during titration, indicating the transition of the aptamer to a functional state.

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### S1.59. Molecular Complex of Rutin with Hederasaponin C

Yakovishin L.A.[1]*, Ratnikov V.D.[1]
[1]*Sevastopol State University;*
* chemsevntu@rambler.ru

Triterpene glycosides are able to form various molecular complexes with biologically active substances [1]. Complexes of licorice and ivy glycosides are most studied. Recently, complex of triterpene saponin glycyram with Rutin (**Rut**) was obtained [2]. **Rut** is one of the best known phenolic plant glycosides [3]. Glycyram is a monoammonium salt of glycyrrhizic acid (main saponin of licorice). Complexes of ivy glycosides with **Rut** have not been described. Therefore, a new molecular complex of **Rut** with the main ivy triterpene glycoside hederasaponin C (**HedC**) was obtained.

Complex formation was studied by UV-Vis and IR-Fourier spectroscopy. A comparison of the IR spectra showed that there is a shift in absorption bands of stretching vibrations of O–H bonds: 3415→3343 and 3343→3259 $cm^{-1}$ (for **Rut**) and 3329→3259 $cm^{-1}$ (for **HedC**). In addition, shifts of absorption bands of stretching vibrations of C–O bonds in C–O–C and C–OH groups were noted: 1405→1359, 1059→1053, 1013→1027, 999→1027 $cm^{-1}$ (for **Rut**) and 1027→1024 $cm^{-1}$ (for **HedC**). In the IR spectra shifts of absorption bands of stretching vibrations of C=O bonds were found: 1656→1651 $cm^{-1}$ (for **Rut**) and 1722→1730 $cm^{-1}$ (for **HedC**). The shift of the absorption bands

indicates the presence of hydrogen bonds between components of the complex.
Stability constant of the complex ($K = (1.2 \pm 0.5) \cdot 10^6$ $M^{-1}$) was calculated according to A.K. Babko method based on isomolar curve.
A preliminary study of antioxidant activity of **Rut** complex with HedC was carried out. Analysis of antioxidant capacity of the complex (in terms of trolox) showed an increase in ACW of 16.89 % (compared to **Rut** standard).
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### S1.60. Molecular Machines as a Tool for Coupled Symmetry Breaking and Energy Form Transformation in a Working Cycle

Tverdislov V.A.[1]*
[1]*Physical Dept of Lomonosov Moscow State University ;*
* tverdislov@mail.ru

The report is devoted to a discussion of a number of new general provisions developed in relation to biological molecular machines and considered in a non-standard aspect of the ideas of symmetry, specifically - chirality. In developing the non-trivial fundamental ideas of Emmy Noether and Freeman Dyson on the physical conditionality of connections between conservation laws and different types of symmetries, as well as on the connectedness of the evolution of systems through a series of symmetry breaking, we put forward the idea that in their conjugation, molecular machines of living nature are a key find Nature in the formation and functioning of Life on Earth.
The message is focused on protein-enzymes, receptors, pumps that convert energy, matter and information. The functioning of specialized information molecular machines based on nucleic acids and their complexes with proteins, as well as protein-lipid supramolecular membrane machines, we believe, is based on the same physical principles.
A machine can be called a device (construction) that is capable of converting the form of energy in a cyclic mode, performing “useful work”, due to the presence in it of “dedicated” mechanical (translational, oscillatory, rotational), including quantum mechanical, degrees of freedom, kinetically separating work and dissipation. The molecular machine is a tool for transforming the form of energy associated with symmetry breaking in a non-equilibrium nonlinear system (constructions with nonlinear/valve elements) to perform "useful work". The "useful work" of machines is the essence of their biological functions. Evolutionarily established molecular machines are hierarchically organized dynamic chiral devices (constructions).
Previously, we formulated and substantiated the concept, according to which the physical basis of molecular biology and molecular machines is a periodic system of sign-alternating chiral structures connected by symmetrical relationships in intra- and supramolecular hierarchies of structures of the most important classes of biomacromolecules. A general pattern was revealed: starting from the level of asymmetric carbon in deoxyribose and amino acids, a tendency of alternation of the sign of chirality of intramolecular structural levels D-L-D-L for DNA and L-D-L-D for proteins was traced. Manifestations of chirality are not only homochiral amino acids and sugars, but also helicity and superhelixity of secondary and higher order structures, as well as chiral features of different types of B-structures. The core of molecular biology composed by these hierarchies integrally constitutes an achiral invariant, and hese structures themselves directly implement the selected mechanical degrees of freedom of molecular machines.
The main idea of the position on molecular machines developed by the authors is the idea of conjugate symmetry breaking and change of the form of energy when the machine moves through the working cycle in the course of performing “useful work”. Such conjugation is the physical basis for performing electro-mechano-biochemical "useful work" focused on morphogenesis and physiological functions.
The phenomenon of chirality allows the formation of discrete chirally sign-alternating hierarchies of structures in macromolecular machines in the process of folding, and also ensures the unidirectional movement of machines along the thermodynamic cycle due to the nonlinear valve properties of secondary and hierarchically higher helical and other regular intra- and supramolecular structures. Structural elements of molecular machines are at the same time a "working substance", converters of energy forms, as well as non-linear elements that control the sequence and conjugation of elementary acts in a cycle.
It is essential that the chiral structures of water, which are in contact with the intramolecular and surface structures of large molecules, are direct participants in the generalized symmetry rearrangements in the operating cycle of the machine. A theoretical analysis of the mechanical valve properties of helical and superhelical structures of protein macromolecules has been carried out.
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### S1.61. Molecular determinants of zinc-dependent interactions of beta-amyloid as an innovative platform for the creation of a therapeutic technology for neurodegenerative diseases

Kozin S.A.[1]*
[1]*Engelhardt Institute of molecular biology, Russian Academy of Sciences;*
* kozinsa@gmail.com

Amyloid-beta was discovered in the mid-1980s as the main peptide component of fibrillar aggregates (amyloid plaques) that are located on the surface of neurons in the brain of patients diagnosed with Alzheimer’s disease, as well as patients with other neurodegenerative diseases. Monomeric molecules of amyloid-beta are present in the blood and cerebrospinal fluid in low nanomolar concentrations both in normal and pathological conditions. Aggregation of amyloid-beta with subsequent formation of amyloid plaques is a necessary condition for initiating the pathological cascade of neurodegeneration and neuroinflammation in Alzheimer’s disease. The physiological role of amyloid-beta may be to regulate synaptic function, protect against infections, restore damaged areas of the blood-brain barrier, and compensate for the consequences of brain injury. The current directions of research in the field of creating effective technologies for the treatment of neurodegenerative diseases are: (1) determining the reasons why endogenous amyloid-beta begins to undergo pathological aggregation; (2) elucidation of the role of amyloid-beta in the initiation of neuroinflammatory and neurodegenerative processes. Comparative analysis of the structural and functional properties of various natural amyloid-beta isoforms in humans and a number of mammals made it possible to establish the role of the 1-DAEFRHDSGYEVHHQK-16 amino acid region as the metal-binding domain of amyloid-beta. It has been established that amyloid-beta through its fragment 11-EVHH-14 forms zinc-dependent oligomers and aggregates, and also participates in the formation of non-covalent zinc-bound complexes with specific

molecular partners (proteins, nucleic acids, hormones, signaling molecules, etc.). A number of experimental observations indicate that such complexes, in contrast to free amyloid-beta, are active players in both normal and pathogenic physiological processes. Accordingly, modulation of the formation of zinc-induced amyloid-beta complexes by rationally designed agents that specifically bind to the 1-16 domain and/or the 11-14 region is a promising tool for controlling processes involving amyloid-beta molecules in health and disease.

**S1.62. Molecular dynamics of cobra cytotoxins in a highly mobile mimetic membrane is an effective tool for studying their structure-functional properties**

Dubovskii P.V.[1]*, Konshina A.G.[1], Efremov R.G.[1]
[1]*Institute of Bioorganic Chemistry named after Shemyakin M.M. and Ovchinnikov Yu. A.;*
* pvdubov@ya.ru

Cytotoxins (CT) from cobra venom are membrane-active 59-61 amino acid residue-long polypeptides. They feature antimicrobial and anticancer activities. This is due to capability of CT to destabilize the plasma membrane of bacterial and tumor cells and/or their intracellular organelles. CT belong to the family of three-finger proteins. Their structural feature is the presence of a hydrophobic core formed by 4 conservative disulfide bonds, from which three beta-structural hairpins, or fingers, protrude. CT have been proven to interact with lipid membranes via the termini of these fingers [1]. From a fundamental viewpoint, the relationship between the amino acid composition of the finger termini and capability of CT to incorporate into membranes of various lipid compositions is of interest. The most detailed information on a protein in a water-lipid environment can be obtained through molecular dynamics (MD) simulations in the full-atomic approximation. However, to date, there is only single example of such a successful study: the incorporation of cytotoxin 2 (CT2) from venom of the Central Asian N. oxiana cobra into the bilayer of palmitoyloleoylphosphatidylcholine (POPC) [1]. It has been demonstrated that during 1 mks-long MD, the CT2 molecule embeds sequentially the termini of the first, second, and then third fingers into the bilayer. When considering a CT with less hydrophobic finger termini, for example, cytotoxin 1 (CT1) from N. oxiana venom, this process becomes much more longer. The implementation of a “Highly Mimetic Membrane Model” (HMMM) [2] allows to not only preserve the molecular details of the protein-membrane interactions, but also to accelerate the process of the toxin incorporation into the lipid bilayer.

In HMMM model, the full-sized lipid molecules are replaced with shortened analogs, and a layer of an organic solvent, in particular, dichloroethane, is added in the center of the membrane. In such a membrane, lipid molecules are characterized by lateral diffusion coefficients that are two orders of magnitude higher than for conventional membranes. At the same time, the atomic density profile of the HMMM system is almost identical to the profile of the full-size model. Thus, HMMM model becomes especially attractive for studying the incorporation into the membrane of peripheral membrane proteins and peptides, to which CT belong.

In the present work, we studied the incorporation and dynamics of CT1 in POPC bilayer. At the first stage, HMMM approximation was used. The structure of CT1 in a dodecylphosphocholine micelle was used as a starting model for CT1 [3]. The toxin molecule was placed outside the bilayer consisting of 200 lipid molecules (100 per each monolayer). The variable parameter of HMMM model is the so-called lipid scaling factor (Rsa), or the ratio of the area per lipid molecule in an HMMM bilayer to the corresponding area in the full atom bilayer. A pair of bilayers was considered with Rsa=1.0 and 1.1. In both cases, the number of carbon atoms in the acyl group of the lipid molecules was 6. At Rsa=1.1, CT1 molecule completed its cycle of the incorporation of the three-finger loops into the HMMM bilayer within 200 ns (CHARMM36m force field was used). At Rsa=1.0 – for ~500 ns. To study in detail the effect of the inserted CT1 molecule on the POPC lipid bilayer (and vice versa), at the next stage, the HMMM membrane was converted into the full-atom bilayer. After the system has been equilibrated, MD simulations were performed during 200 ns. In the equilibrium part of the full-atom trajectory, the values of the area per lipid molecule in the upper and lower monolayers, as well as the area occupied by the toxin molecule, and the deuterium order parameters of the acyl chains of POPC molecules were determined. Both for Rsa=1.0 and 1.1, the corresponding sets of the parameters turned out to be practically coincident. This means that the value of Rsa=1.1 is optimal for studying the incorporation of the toxin molecule into the lipid bilayer since it provides good agreement with the results of all-atom MD and, at the same time, can significantly reduce the overall time of the simulations. The proposed two-step approach for the study of CT incorporation into lipid membranes can be expanded to other CT and peripheral membrane proteins.

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**S1.63. Molecular mechanisms of antibiotic resistance associated with the hydrolysis of β-lactam antibiotics**

Krivitskaya A.V.[1]*, Khrenova M.G.[2,1]

[1]*Federal research center "Fundamentals of biotechnology" of the Rassian Academy of Sciences;*
[2]*Lomonosov Moscow State University;*
* al_krivickaya@mail.ru

The dominant mechanisms of resistance for β-lactam antibiotics are modification of penicillin-binding proteins and inactivation of antibiotics by bacterial enzymes called β-lactamases. Molecular modeling has been used to study these processes, including examples such as the inhibition of penicillin-binding protein 2 by ceftriaxone and the inactivation of imipenem by metallo-β-lactamases L1 and NDM-1. Substrate activation effects and molecular mechanisms of reactions were described using combined quantum mechanics/molecular mechanics methods, while classical molecular dynamics methods were used to analyze conformational rearrangements in the investigated structures. Penicillin-binding proteins 2 from Neisseria Gonorrhoeae are critically important enzymes in the formation of bacterial cell walls. Inhibition of PBPs is used in the treatment of various diseases, including gonorrhea. Ceftriaxone is the only drug currently used to treat gonorrhea, as it irreversibly inhibits the action of the enzyme by incorporating into the active site of PBP2 instead of the natural substrate. The cases of PBPs resistance to this antibiotic are known: wild-type strain FA19 showing no resistance to penicillin, and mutant strains 35/02 and H041 are resistant. Experimental data of secondary acylation constants (k2/Ks) demonstrate that the inhibition efficiency decreases by 150 and 2300 times for 35/02 and H041, respectively. The acylation constant and binding constant are known for FA19, but it is not possible to determine them experimentally for mutant forms. Due to slow deacylation the

reaction is considered irreversible. Thus, the growth of resistance is associated with a decrease in either the affinity of PBP2 for ceftriaxone or the rate of acylation.
Molecular modeling of the mechanism of PBP2 inhibition in these strains by ceftriaxone revealed that the G545S mutation in the active sites of mutant strains affects the substrate position. This change affects the formation of the oxyanion hole and the height of the activation energy barrier. Electron density based analysis of the set of enzyme-substrate complexes allowed identification of a set of reactive and non-reactive structures. It was shown that the proportion of reactive structures decreases with increasing resistance. The molecular mechanism of the reaction was established by the scanning of Gibbs free energy surface. The reaction mechanism in the mutant PBP2 and the wild-type strain is different: the C-N bond cleavage and the detachment of the antibiotic fragment occur sequentially in the wild-type strain protein and simultaneously in mutant proteins. The new position of the substrate in the catalytic pocket also leads to changes in the affinity to the antibiotic. Analyzing conformational changes in the β3-β4 loop, it was shown that with increasing resistance, the affinity of PBP2 to ceftriaxone decreases.
The main mechanism for inactivating β-lactam antibiotics is the expression of bacterial β-lactamase enzymes. It is known that the reaction is initiated by a nucleophilic attack of a catalytic hydroxide anion. Then the β-lactam ring is broken and a negatively charged reaction intermediate is formed. In this intermediate, the charge is delocalized between three atoms of the pyrroline ring of imipenem - NC2C3-. The intermediate is then protonated, but it is unknown which atom, N or C3, is protonated. Experimental data demonstrate that the final product of the reaction for both enzymes is the carbon-protonated R-isomer of imipenem ((R)-imine). However, it is unclear whether (R)-imine is formed in the active site of the enzymes or through tautomerization from the N-product of imipenem (enamine) in solution.
The study investigated the structural features of the enzyme-substrate complexes L1 and NDM-1. The different amino acid composition of loop 10 is a significant structural feature. Loop 10 covers the active site in both enzymes. In L1, there is a hydrophobic, rigid amino acid residue Pro226 above the active site, making the position of the substrate in the active site more structurally rigid and immobile. In NDM-1, flexible Gly219 is located above the substrate, which makes the NDM 1 active site more mobile. These structural features account for the difference in the activation of imipenem and the reaction mechanism.
The analysis of molecular dynamics trajectories of enzyme-substrate complexes showed that the formation of a more rigid active site in L1 leads to more efficient activation of the substrate by the enzyme. As a result, this leads to a lower energy barrier and greater stabilization of the intermediate of the first stage of the reaction.
The molecular mechanism of reaction was established by scanning the potential energy surface. Enamine is formed as the main product in the active site of NDM-1 and as the only product for L1. Molecule of water enters the active site of NDM-1 due to the greater mobility of loop 10. This determines the existence of an alternative reaction pathway leading to the formation of (S)-imine. This process occurs with higher energy barriers.
The behavior of the products of the enzymatic reaction in solution has been studied. Classical molecular dynamics simulations of the enamine and the negatively charged reaction intermediate were used. Analysis of the obtained conformations based on key geometric criteria showed that tautomerization of the enamine to (R)-imine is more probable. The obtained results are consistent with known experimental data.

### S1.64. Molecular mechanisms of initiation of ferroptotic processes under the action of cytochrome c complexes with phophatide acid

Suchkov M.Y.[1]*, Stepanov G.O.[1], Osipov A.N.[1]
[1]*RNIMU;*
[2]*RNIMU ;*
* max.suchkov3001@yandex.ru

The biophysical mechanisms of apoptosis, which are characterized by an increase in the peroxidase activity of cytochrome C after its interaction with mitochondrial phospholipids, are now well understood. Today, the attention of scientists around the world is drawn to the study of the molecular and cellular mechanisms of ferroptosis associated with the action of free iron. So the question of the source of free iron, which is a catalyst for the peroxidation of biological membranes that occurs during ferroptosis, is very difficult [2].
The aim of this work was to investigate between the ability of cytochrome c to lose an iron ion at increasing concentrations of hydrogen peroxide, as well as the change in its peroxidase activity during this process. The dependences obtained were compared both for samples containing only cytochrome c and for cytochrome c forming complexes with various anionic phospholipids (phosphatidylcholine, cardiolipin, and phosphatidic acid).
The assessment of the content of iron ions in cytochrome C was performed using spectrophotometry (by the intensity of the Soret band), these spectra were compared with the kinetic curve of luminol-dependent chemiluminescence, reflecting the peroxidase activity of cytochrome C
It is well known that when a high concentration of hydrogen peroxide interacts with cytochrome C, the Soret band at 410 nm decreases, which explains the release of heme iron, which can affect the development of ferroptotic processes. It was shown that the decrease in the Soret band of cytochrome c in the presence of phosphatidic acid begins much faster (at hydrogen peroxide concentrations of 300 μM and cytochrome c 5 μM) than in control samples, where the change in absorption began at 500 μM peroxide concentrations. It was also seen that samples containing phosphatidic acid initially (before the Soret band began to fall) already exhibited peroxidase activity, which was about 10 times higher than that of complexes of cytochrome c with phosphatidylcholine.
As a result of the experiments, it was shown that a decrease in the intensity of the Soret band is accompanied by an increase in the intensity of chemiluminescence, which in turn indicates an increase in the peroxidase activity of cytochrome C, which accompanies, incl. release of iron from heme.
Thus, it has been shown that cytochrome C, when interacting with hydrogen peroxide, contributes to an increase in the concentration of iron, which in turn can induce the process of ferroptosis.
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### S1.65. Molecular modelling for studying molecular mechanisms of antibacterial and antiviral activity of cationic photosensitizers

Kholina E.G.[1]*, Fedorov V.A.[1], Khruschev S.S.[1], Kovalenko I.B.[1,2], Strakhovskaya M.G.[1,2], Rubin A.B.[1]
[1]*Lomonosov Moscow State University, Moscow, Russia;*
[2]*Federal Scientific and Clinical Center of Specialized Types of Medical Care and Medical Technologies, Federal Medical and Biological Agency of Russia, Moscow, Russia;*
* tenarra@mail.ru

Photodynamic inactivation, which uses photoactivated biocides – photosensitizers, is an approach to combat antimicrobial-resistant pathogens. In addition to their bactericidal action, cationic photosensitizers are highly effective against enveloped viruses. Octakis(cholinyl) zinc phthalocyanine (PC) is one of the most effective photosensitizers

against a broad spectrum of pathogens including enveloped viruses. The aim of this work was to study intermolecular interactions during the binding of PCs to the envelope components and its transport through the membrane structures of microorganisms.

To study the molecular nature underlying the antibacterial activity of PC, coarse-grained molecular dynamics was applied. Using the umbrella sampling technique, we described the process of PC translocation through bacterial membranes and demonstrated high affinity of PC to bacterial membranes. We found out that this process is energetically favorable and leads to overall disturbance of the model bilayer and formation of the aqueous pore. The results of our simulations confirmed the hypothesis of PC "self-promoted uptake" inside the outer bacterial lipopolysaccharide membrane and explained the molecular nature of PC antibacterial activity [1].

To study the antiviral activity of cationic photosensitizers and identify their binding sites on the viral envelope, we calculated distribution of electrostatic potential on the surface of S proteins of three coronaviruses [2,3] and the whole SARS-CoV-2 [4] and performed Brownian dynamics calculations. We obtained several thousand of electrostatically favorable encounter complexes of PC molecule with each of the coronavirus S protein and reveal the major binding site for all S proteins, located at the junction of the "stem" and the "head" at a distance of about 10 nm from the viral membrane. Since the diffusion distance of singlet oxygen generated by PS is 10–55 nm, it can cause oxidative damage to both the S proteins themselves and the lipid bilayer of virions, and thereby virus inactivation.

The next stage of the study was to study the interaction of PC with the whole model of the SARS-CoV-2 envelope. Using a coarse-grained model of the entire viral envelope developed by D. Korkin and S.-J. Marrink's scientific groups [5], we created an electrostatic map of the external surface of SARS-CoV-2 and found a highly heterogeneous distribution of the electrostatic potential field of the viral envelope [5]. Numerous negative patches originate mainly from negatively charged lipid molecules POPI (-1), POPS (-1), CDL2 (-2) and negatively charged amino acids of S proteins. To investigate which components of SARS-CoV-2 viral membrane attract PC molecule, we performed 40 thousand independent Brownian dynamics simulations of PC molecule relative to immobile viral envelope. About 43% of the PC molecules were found in encounter complexes with proteins of viral envelope. Among them, about 80% formed electrostatic contacts with S proteins. In the remaining 57% of the complexes of PC molecules, close contacts with membrane proteins are not required, and they were bound to negatively charged lipids only. Thus, all negatively charged components attract photosensitizer molecules and are potential targets for singlet oxygen generated by PS molecules. The theoretical results obtained by computer modelling are consistent with the previously observed spike loss and membrane destruction, as a result of the photodynamic inactivation of the coronavirus with the same PS. Thus, the study of the detailed electrostatic map of the whole virion using a computer model provides unique opportunities to reveal the binding sites of charged molecules on the surface of the virus.

Application of computer modeling methods made it possible to describe specific pathways for the transfer of cationic photosensitizers through the bacterial cell wall and to identify the binding areas of photosensitizers on the viral envelope. Knowledge of the molecular details underlying the antiviral and antibacterial activity of biocides contributes to their rational use for medical purposes and necessary for the design of new effective antimicrobial compounds.

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### S1.66. Molecular-dynamical characteristics of interactions between glycoprotein Ib and von Willebrand factor

Fedotova I.V.[1]*, Belyaev A.V.[1]
[1]*Lomonosov Moscow State University, Faculty of Physics, Department of Biophysics, Moscow, Russia;*
* fedotova.iv18@physics.msu.ru

Platelet hemostasis is is the first stage in bleeding cessation mechanism in cases of vascular damage. Blood platelets are the main link in this process. These cells that can prevent hemorrhage by adhesion to subendothelial matrix proteins and subsequent aggregation. The attracting specific non-covalent interaction between the von Willebrand factor protein (vWF) and platelet membrane receptor glycoprotein Ib (GPIb) has a decisive importance for the initial stages of platelet aggregation in vessels with high near-wall shear stress – arterioles, venules and arteries.

Von Willebrand factor circulates in the bloodstream as long linear multimers that assume a compact conformation in the absence of external mechanical forces. An increase in shear stresses in the bloodstream entails a change of the vWF conformation to a linear one. In this form vWF becomes capable of reversibly binding to collagen and platelet receptor glycoprotein Ib (GPIb) using the A1 domain. Thus, the von Willebrand factor can provide a deceleration of a platelet up to its complete stop in the area of vessel damage [1].

It is known from the literature that mutations in the A1 domain of von Willebrand factor affect its interaction with glycoprotein Ib and can lead to von Willebrand disease, a hereditary disorder of platelet aggregation [2].

Nowadays an understanding of the mechanisms of platelet adhesion and aggregation has been obtained, as well as an understanding of the structural features of protein mutations leading to von Willebrand disease, however, the molecular mechanisms of the interaction of the wild-type von Willebrand factor A1 domain and its mutant structures with GPIb remain insufficiently studied. The aim of this work is to clarify the theoretical concepts of the molecular mechanisms of platelet adhesion under conditions of arterial blood flow.

In this work, the interaction of GPIb and the wild-type vWF A1 domain, as well as its mutations leading to von Willebrand disease types 2M and 2B, is studied using molecular dynamics methods. Using the GROMACS software package and the Lomonosov-2 supercomputer, a computer model was constructed that implements the stretching of the GPIb–A1vWF protein complex by shear forces under physiological conditions. The dissociation energy of the GPIb–A1vWF bond was calculated. The method of weighted histogram analysis was used to construct the average strength potential for attractive interactions of wild-type GPIb and vWF proteins. An explanation of the mechanism

of enhancement of protein adhesion at high shear stresses is proposed. The strength characteristics were obtained and the mechanochemical features of the bond dissociation processes were analyzed for various vWF mutant structures corresponding to von Willebrand disease types 2M and 2B.
In the future, it is planned to use the approach proposed in this work for the numerical analysis of potentially dangerous mutations of proteins that ensure platelet aggregation. The study of the mechanisms governing the interaction of von Willebrand factor with platelets is of fundamental and medical importance and can help predict optimal strategies for the treatment of hemostasis disorders in patients with von Willebrand disease.
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### S1.67. Multiplicative noise effect on output signal of DNA-biosensor

Arakelyan V.B.[1], Aloyan L.[1], Parsadanyan M.A.[1], Vardevanyan P.O.[1]*
[1]*Yerevan State University;*
* p.vardevanyan@ysu.am

DNA-biosensor is more important among other biosensors. Actuality of these biosensors is connected to the fact that they are applied for solution of wide spectrum of topics of fundamental and practical problems in the spheres of biomedicine, pharmacology, molecular biology and genetic diagnosis. The chief advantages of DNA-biosensors are high sensibility and selectivity, as well as high rate of analysis, which is incompatibly higher, than that of traditional analytical methods.
Despite the significant successes at the usage of DNA-biosensors in scientific and practical studies, there is a number of problems connected to improvement of DNA-biosensors that are not solved to date. These are mainly the problems, connected to decreasing of DNA-biosensor output signal noise. This fact dictates the necessity to more detained study the process of output signal formation, aimed at the revelation of the possible types and noise sources , as well as characteristic peculiarities of these noises. In vast majority of cases DNA-biosensor "works" in medium, the properties of which are non-strictly constant, but randomly fluctuating, and these random fluctuations inevitably lead to the fluctuations of DNA-biosensor output signal. This type of noise is called an external noise [1].
It was shown that DNA-biosensor output signal in complicate way depends on the external noise action. Depending on the fact which parameter of adsorption system fluctuates under the effect of the external noise, the output signal may be exposed to the action of both Langevin's and multiplicative noises. If under the effect of the external noise the number of adsorption centers fluctuate, which can take place as a result of affinity change of adsorption center under the influence of randomly changing factors of medium, the noise is Langevin's one. If under the effect of the external noise the number of ligands in the solution fluctuates, the noise turns to be multiplicative one [2]. Action of Langevin's and multiplicative noises on the output signal of DNA-biosensor differs qualitatively and quantitatively from each other. For the case of small fillings the stochastic differential equation (SDE) is received of multiplicative type, describing the change of number of adsorbed ligands on DNA-duplexes in time. Further, for SDE the respective equation of Fokker-Plank is written, then using this equation we obtain the equation for moments. As far as the fluctuating parameter is approximated by Gauss white noise, in this case SDE is interpreted in sense of Startanovich. Calculating the first and second moment the dispersion of adsorbed ligands on DNA-duplex is determined. Then, understanding that DNA-biosensor output signal through the unit of biosensor surface, conditioned by ligand adsorption on DNA-duplex, is proportional to the number of adsorbed ligands on DNA-duplex, the dependence of non-stationary dispersion on time is determined.
Analysis of the stationary dispersion of DNA-biosensor output signal, at small values of the external noise intensity, shows that fluctuations of the output signal linearly depend on the external noise intensity. It was also shown that along with ligand concentration increase in the solution the fluctuations of DNA-biosensor output signal decrease.
The obtained data permit determining the possible types and noise source from analysis of noise peculiarities of DNA-biosensor output signal.
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### S1.68. Nanobiointeractions of serum albumin and shungite carbon

Rozhkov S.P.[1], Goryunov A.S.[1]*, Rozhkova N.N.[2]
[1]*Institute of Biology, Karelian Research Centre of RAS;*
[2]*Institute of Geology, Karelian Research Centre of RAS;*
* goryunov@krc.karelia.ru

The purposes of bionanodesign and biomedicine as well as assessing environmental nanorisks requires further understanding of the molecular mechanisms of biological activity of nanostructures and nanomaterials. Proteins are the most important object and component in such studies. Serum albumins (SA) for many reasons are among the most convenient models to this end as they are often used as sensors for conformational and phase changes during the interaction of biomolecules with nanoparticles (NPs) of various nature and the formation of protein corona, as surface coatings and biosurfactants, for the functionalization of bionanoconjugates, induction and inhibition in fibril formation. SA owes its wide variety of applications to its commercial and preparative availability as well as to a fairly complete picture of its properties including protein conformational state, intermolecular interaction, and phase behavior in dispersions with different microenvironments. This makes SA an ideal object for studying the effect of proteins on the colloidal stability of nanoparticles in dispersion, their phase, redox, and drug delivery properties.
The combination of the above properties of SA allowed us to advance our study of the molecular mechanisms of the biological activity of nanomaterials of natural origin - abiogenic shungite carbon nanoparticles [1-3]. Shungite carbon NPs (ShC NPs), obtained by green chemistry from shungite rock and stabilized in aqueous dispersion, received experimental and theoretical evidence of their graphene nature.
The study of the biological activity of ShC NPs at the molecular level showed that ShC affects the binding of ligands (fatty acids) to serum albumin, its conformation and aggregation through the transfer of a part of the ligand from protein to nanoparticles. When interacting with ShC, the ratio of SA fractions in the dispersion changes toward the fraction with the low ligand content. At the same time, the stability of SA aggregates containing a physiological amount of the ligand is increased compared to the protein without the ligand. An increase in the uniformity of the size distribution of molecular aggregates, microenvironment, and ligand binding sites is also observed.
Shungite nanocarbon has been shown to affect the degree of oxidation of blood proteins both by direct increase of the degree of oxidation (heme iron of hemoglobin) and by reducing the degree of free radical

oxidation (cys-34 of serum albumin). The interaction of nanoparticles with a protein and formation of their complexes and protein corona may result in a significant change in the conformational state of the protein. This can also leads to a decrease in their affinity to physiological ligands (fatty acids for albumin, oxygen for hemoglobin). The ShC NPs in an aqueous dispersion can function as an acceptor of ligands and electrons. This characterizes its role in the regulation of the processes of oxidation and transfer of ligands in systems involving proteins аы well as its hemocompatibility in general.

One of the mechanisms of ShC NPs bioactivity is conditioned by the effect of NPs on phase transformations in protein systems connected with the formation of protein complexes and associates as pre-nuclear amorphous clusters of an intermediate (hidden) phase. Our data of Raman spectroscopy in the range 3200-3600 cm-1 show that hydrogen bond network reacts in a similar manner - initial hardening and further loosening – on the variations in the concentration of components in dispersions of carbon nanoparticles and protein macromolecules despite the obvious differences in these systems. The primary associates that arise in this case are typically close in size and do not change with varying the concentration of components. This suggests that primary defects in the hydrogen bond network are filled first, but then larger defects are formed that can contain larger associates, and the system of hydrogen bonds is loosened. Colloidal stability to phase separation in protein dispersion is maintained by the formation of protein associates (clusters) of two main types and sizes as latent phase clusters. In presence of ShC NPs, colloidal stability of the dispersion is provided almost exclusively by NPs-protein association with the formation of protein corona. Our approach can describe the colloidal properties and stable behavior of particle dispersions of both biogenic and abiogenic nature.

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### S1.69. Natural IgG autoantibodies hydrolyze recombinant extracellular fragments of the NR1 and NR2 subunits of the NMDA receptor

Smirnova L.[1]*, Ermakov E.[2], Boksha I.[3,4], Kamaeva D.[1], Ivanova S.[1]
[1]*Tomsk National Research Medical Center RAS;*
[2]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences;*
[3]*Federal State Budgetary Scientific Institution Mental Health Research Centre;*
[4]*The National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation;*
* lpsmirnova2016@gmail.com

The production of antibodies to glutamate receptors (NMDAR) has been found in patients with many neurological and psychiatric disorders. Antibodies against various glutamate receptors, including AMPA-GluR3, NMDA-NR1, NMDA-NR2A/B, mGluR1 or mGluR5, are present in subpopulations of patients with epilepsy, encephalitis, cerebellar ataxia, systemic lupus erythematosus (SLE) and neuropsychiatric SLE, Sjögren's syndrome, schizophrenia, mania or stroke. These autoimmune glutamate receptor antibodies can bind neurons in several areas of the brain, activate glutamate receptors, decrease glutamate receptor expression, impair glutamate-induced signaling and function, activate blood-brain barrier endothelial cells, kill neurons, damage the brain, cause behavioral, psychiatric, or cognitive disorders. But the pathogenetic mechanisms of these phenomena remain unexplored. Studies of the proteolysis of neurospecific proteins by serum antibodies (abzymes) are of high relevance because they could reveal the molecular features of impaired neuroimmune interactions underlying the above diseases. In this work, the catalytic activity of IgG against the recombinant subunits NR1 and NR2 of the NMDA receptor was studied in an in vitro experiment. The recombinant subunit model included peptide fragments from the most functionally active regions of the receptor subunits. For this, two chimeric proteins DBD-NMDAR1 and DBD-NMDAR2 containing fragments of the NR1 and NR2 subunits were constructed based on the pL761 plasmid.

Plasmid pL761 was constructed based on the pR1504 vector containing the nucleotide sequence of the DBD1 dextrans-binding domain from Leuconostoc mesenteroides, a GS-spacer, and a selected nucleotide sequence encoding the NMDAR1 or NMDAR2 glutamate receptor subunit.

Serum IgG from healthy individuals were purified by affinity chromatography on a Protein-G Sepharose column. The belonging of the studied activity directly to IgG was confirmed by electrophoretic homogeneity; highly efficient gel filtration at pH 2.6; determination of "in situ" activity.

The reaction mixture containing 0.5 mg/ml DBD-NMDAR1 or DBD-NMDAR2 proteins and 0.1 mg/ml IgG from healthy individuals was incubated for 9 hours at 37°C. The hydrolysis products of NR1 and NR2 subunits by IgG were evaluated by electrophoresis and chromato-mass-spectrometric analysis on an OrbiTrap Elite mass spectrometer (Thermo Scientific, Germany) connected to an Easy-nLc 1000 nanoflow chromatograph (Thermo Scientific, USA). The results were processed using the Thermo Xcalibur Qual Browser and PEAKS Studio-7.5 software.

Incubation of the recombinant DBD-NMDAR1 and DBD-NMDAR2 proteins with IgG preparations from healthy donors led to a decrease in the intensity of the recombinant protein band on the electropherogram, and hydrolysis of the DBD-NMDAR2 protein was more efficient.

The mass spectrometric analysis data after appropriate processing showed the following results. In the case of hydrolysis of the DBD-NMDAR1 protein, the number of identified peptides (after hydrolysis) from the NR1 domain (mean: 23 peptides) was significantly higher ($p=0.005$) than from the DBD carrier domain (10 peptides). In the case of DBD-NMDAR2, the number of identified peptides from the NR2 domain (60 peptides) was also significantly higher ($p=0.017$) than from the DBD carrier domain (45 peptides).

Thus, it was shown for the first time that serum IgG of healthy individuals hydrolyze the NR1 and NR2 subunits of the NMDA receptor in an in vitro experiment, and the hydrolysis of the receptor subunits significantly exceeds the hydrolysis of other parts of the chimera. In this case, the DBD-NMDAR2 protein undergoes more active hydrolysis by antibodies.

Given the high pathogenetic significance of NMDA receptor disruption in mental and neurological disorders, new knowledge about proteolytic abzymes can help identify new molecular mechanisms of disease development.

The work was carried out within the framework of the research topic: Biopsychosocial mechanisms of pathogenesis and clinical polymorphism, adaptive potential and predictors of the effectiveness of therapy in patients with mental and behavioral disorders in the Siberian region, registration number 122020200054-8.

### S1.70. New biocatalysts based on cysteine proteases in a complex with chitosan micro- and nanoparticles

Holyavka M.[1]*, Goncharova S.[1], Redko Yu.[1], Lavlinskaya M.[1], Sorokin A.[1], Pankova S.[1], Koroleva V.[1], Paimetieva D.[1], Artyukhov V.[1]
[1]*Voronezh State University;*
* kholyavka@bio.vsu.ru

Micro- and nanoscale materials are widely used in various fields of human activity including biomedicine. They are widely applied to create innovative forms of medicines capable of targeted delivery and controlled release of biologically active substances. Due to the large surface area, micro- and nanoparticles are characterized by high drug substance loading, which makes it possible to rationally use the drug and reduce its toxic effect. For such pharmaceutical forms, the problem of excretion of the drug from the body is acute, associated with the steric impossibility of the passage of particles of many polymers through the renal tubules. One of the possible solutions is the creation of micro- and nanoparticles based on biodegradable carriers that can be metabolized in the human body and excreted from it without any difficulties. Chitosan is one of the most promising polymers of this type.

Chitosan and its derivatives have unique properties as carriers for various enzymes used in the pharmaceutical industry. Low cost, availability, antimicrobial activity, biodegradability, and adhesive properties make chitosan a promising carrier for drug delivery. Chitosan is biocompatible and practically does not cause adverse reactions when in contact with human cells.

Ficin, papain, and bromelain (Sigma, USA) were chosen as objects of study; micro- and nanoparticles were obtained from medium-molecular-weight (200 kDa) and high-molecular-weight (350 kDa) chitosan (Bioprogress, Russia).

Methods for obtaining micro- and nanoparticles of chitosan, as well as their complexes with proteases, are described in detail in [1-3]. The sizes of micro- and nanoparticles were measured on a Nano Zetasizer ZS device (Malvern Instruments, USA). Backscattered light from a 4 mW He/Ne laser (632.8 nm) was collected at an angle of 173°.

The stability of enzyme complexes with micro- and nanoparticles was evaluated after incubation in 0.05 M Tris-HCl buffer with pH 7.5 at 37°C for 7 days by measuring the proteolytic activity of samples after 1, 4, 8, 24, 48, 72, 96, 120, 144 and 168 h according to the amount of the colored reaction product as a result of the cleavage of the azocasein substrate [4].

It has been established that the associates of ficin, papain, and bromelain with particles of medium and high molecular weight chitosan obtained wuth ascorbic acid differ significantly in size from associates with blank particles. During the interaction of chitosan particles with the enzyme, the sizes of associates significantly exceed the sizes of free particles.

The activity of ficin complexes with nanoparticles of medium and high molecular weight chitosan turned out to be higher compared to complexes of microparticles of medium and high molecular weight chitosan by 8 and 5% when particles were formed without ascorbic acid and by 9 and 13%, respectively, when particles were formed with ascorbic acid.

When creating complexes of papain with microparticles of medium and high molecular weight chitosan without ascorbic acid, the enzymatic activity decreased by no more than 8% compared to free papain; when complexed in the presence of ascorbic acid, the activity of papain increased by 18 and 10%, respectively. In the formation of associates of papain with nanoparticles formed with ascorbic acid, its catalytic ability increased by 3% for medium molecular weight chitosan and by 16% for high molecular weight chitosan.

The activity of bromelain complexes with nanoparticles of medium- and high-molecular-weight chitosan turned out to be higher compared to complexes of microparticles of medium- and high-molecular-weight chitosan by 5 and 7% when obtaining particles without ascorbic acid and, respectively, by 9 and 8% when obtaining particles with ascorbic acid.

The complexes formation of cysteine proteases with micro- and nanoparticles of medium and high molecular weight chitosan, obtained both without and with the addition of ascorbic acid, led to the stabilization of the proteolytic activity of the samples.

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### S1.71. New theoretical and experimental data on the mechanisms of catastrophes and rescues of tubulin microtubules

Alexandrova V.V.[1,2], Anisimov M.N.[1,2], Gudimchuk N.B.[1,2]*
[1]*Lomonosov Moscow State University, Physics department, Moscow, Russia;*
[2]*Center for Theoretical Problems of Physico-Chemical Pharmacology, RAS, Moscow, Russia ;*
* nikita_gb@mail.ru

Microtubules are essential polymers of the tubulin that exhibit intermittent phases of elongation and shortening in all eukaryotic cells. Stochastic transitions of microtubules between assembly and disassembly are called catastrophes and rescues. They are tightly regulated throughout the life cycle. For example, during cell division the frequency of microtubule catastrophes increases significantly, while the frequency of rescues, on the contrary, is decreased to adjust the behavior of microtubules to the current tasked faced by the cell. Microtubule dynamics help reorganize the cytoskeleton, change the cell shape, generate mechanical forces for membrane remodeling, repositioning of organelles, and segregating duplicated chromosomes during mitosis. Biochemical data and electron cryotomography data accumulated over the recent years, suggest that the previously widely accepted model of microtubule assembly should be revised. In contrast to the earlier classical model, it has now been established that microtubules assemble by addition of curved tubulin dimers in complex with guanosine triphosphate (GTP) to the tips of curved tubulin protofilaments, both in cells and in vitro. Based on these new structural data and using a Brownian dynamics approach, we have recently built a detailed microtubule assembly model, which suggested that bent tubulin protofilaments straighten by thermal fluctuations, allowing formation of lateral bonds [1]. The constructed model describes the assembly and disassembly of microtubules at various concentrations of tubulin and the generation of mechanical forces on a time scale of several seconds. In the present work, we have extended computer simulations to the time scale of hours, sufficient to describe such rare events as microtubule catastrophes and rescues. Our new Monte Carlo model considers both the nucleotide and conformational states of tubulin. Model analysis offers insights into the mechanisms of microtubule catastrophes and "aging" - a gradual decrease in the stability of microtubules over time. Interestingly, a fully constrained model predicts an dramatic reduction in the rate of spontaneous microtubule rescues. They occur in the simulations only if the microtubule wall contains patches of embedded GTP-tubulin. To test this theoretical prediction, we have performed in vitro

experiments by observing microtubule assembly on the micropedestals, fabricated on the surface of the coverslip. This novel assay reveals microtubule dynamics away from contact with any surfaces, making it possible to avoid non-specific effects that appear when microtubules come into contact with the coverslip. We have observed a significant decrease in the frequency of incorporation of GTP-tubulins into the sites of microtubule lattice defects and a decrease in the frequency of rescues compared to the results of the conventional assay. These results are in agreement with our Monte Carlo model's predictions. Overall, our study provides a unified framework for describing the processes of microtubule assembly and their nonequilibrium dynamics.

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### S1.72. On the codon usage bias

Komarov V.M.[1]*, Samchenko A.A.[1], Kondratiev M.S.[1]
[1]*Institute of Cell Biophysics of RAS;*
[2]*Institute of Cell Biophysics of RAS;*
* komarov_vm@mail.ru

In modern genomics, the question of the codon usage bias in the functioning of a living organism remains very important and intriguing.

Traditionally, here the general position is used about the determining role of the GC composition in the formation of the main structural properties of genomic DNA. According to this concept, assessments of the contribution of guanine and cytosine to the third position of the codon (GC3) in the composition of protein-coding genes are often used as a possible measure of codon bias. However, in real circumstances, based, for example, on the specific composition of the human genome, such a "GC3 preference" turns out to be no more than 25%! Therefore, at the moment, many are still inclined to believe that under the existing conditions of the degeneracy of the genetic code, the observed inequality of synonymous codons in both pro and eukaryotes is a consequence of a non-trivial balance of two factors - the influence of natural selection and mutational predisposition.

Earlier in our work, we have already pointed out the important contribution of the "hidden" ambiguity of the initial form of complementary H-pairing of nitrous bases in initiating the observed features of the structural and functional organization of nucleic acid molecules. This ambiguity is caused by the bi-stable nature of the pyramidal structure of the exocyclic amino groups of adenine, guanine, and cytosine involved in the hydrogen bonding. The reduced, 2-fold polymorphism of the structure of AT-pairs compared to the 4-fold polymorphism of GC-pairs determines the observed dominance of A/T tracks over G/C tracks in the DNA double helix of any organisms. Thus, the natural selection of pairs is realized in ensuring the reliability of the processes of preservation and transmission of genetic information in the cell.

There is reason to expect that the increased ambiguity of GC base pairing form of compared to AT-pairing should somehow be reflected in the case under discussion, when forming the preferred composition of nucleotide triplets in the "codon-anticodon" structure of the nucleic acid binding center to maintain high accuracy and stability of the course of genetic processes.

Using the GenBank data and the www.kazusa.or.jp/codon resource, we performed a spectral analysis of the frequencies of occurrence of all 64 types of codons in the genes of a wide representation of pro- and eukaryotes, covering a scale range of sizes of the studied genomes (from 1.6 Mb to 140 000 Mb) with different GC-content. The genomes of amoeba (Amoeba proteus), tardigrade (Targidrada), horseshoe crab (Limulus polyphemus), and mollusk (Nautilus pompilius) were studied as an example of relic eukaryotes. Other eukaryotes as well as prokaryotes were represented by the genomes of a human (Homo sapiens), a chimpanzee (Pan triglodites), a mouse (Mus musculis), a marble lungfish (Protopterus aethiopicus), a frog (Xenopus tropicalis), a fly (Drosophila melanogaster), a flower (Arabidopsis thaliana ), slime mold (Dictyostelium discoideum), parasite (Leishmania major), yeast (Saccharomyces cerevisiae), malaria parasite (Plasmodium farciparum), bacteria (Escherichia coli), and very small bacteria (Candidatus Pelagibacter). The spectral analysis itself was carried out on the basis of a developed own computer program with a description of the algorithm in [A.A.Samchenko et al, Biophysics,2016, 61 (6),813-824].

The results obtained generally confirmed the assumption made. It has been shown that each organism has two sets of codons bias. The first group is the most numerous. It concentrates approximately 64 to 95 percent of these codons with either an A or T(U) base in the second position. Thus, the priority of the contribution of the "most reliable" bases with initially low structural polymorphism of complementary H-pairing is implemented. This achieves a consistently clear spatial fixation of the central link of the "lock-key" recognition system (codon-anticodon) in the functional complexes of DNA-mRNA, mRNA-tRNA, tRNA-rRNA.

In the second, very small group, the remaining codon usage bias with a central G or C nitrous base gathered.

In general, the resulting split of codon bias into two such groups is quite significant, can reach a ratio of 95:5 (%), and depends on the composition of a particular genome.

### S1.73. Pacemaker currents are involved in the regulation of 3-week-old rat's atrial myocardial contractility

Kuptsova A.M.[1]*, Khisamieva L.I.[1], Faskhutdinov L.I.[1], Shakirov R.R.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University;*
* anuta0285@mail.ru

Hyperpolarization-activated cyclic nucleotide-regulated channels (HCN4) are key membrane proteins involved in the initiation and regulation of heartbeat. The pacemaker cells in the sinoatrial node generate the electrical impulse that underlies the contraction of atrial and ventricular cardiomyocytes. HCN4 channels contribute to the control of resting membrane potential and rhythmic activity of excitable cells expressing these channels. In addition to abundant expression in the pacing and conduction system, the membrane of adult rat working myocardium cells is characterized by low levels of HCN channels. However, in the early embryonic stages, HCN4 channels is abundantly transcribed throughout the heart and makes an important role in ventricular myocyte automatism triggered by If. By birth, HCN4 channels transcription is suppressed in working-type cardiomyocytes and remains at low levels in the adult body, which prevents pathological remodeling of the heart.

The aim is to study the effect of pacing currents blockade, in the regulation of atrial myocardial contractility in 3-week-old rats.

An object of the study were chosen 3 weeks old rats, which are at the initial stage of heart sympathetic innervation.

Myocardial contractile activity was studied in an in vivo experiment on right atrial myocardial strips on a Power Lab setup (AD Instruments, Australia).

Registration of spontaneously generated action potentials was performed on a microelectrode unit. The blocker of currents activated by

hyperpolarization, ZD7288, at a concentration of 10-6 M, was used as pharmacological agents.
The initial contraction force of the isolated right atrial myocardium was 0.26±0.13 g. After adding the blocker ZD7288 (10-6 M) to the working solution, there was a gradual decrease of contraction force during the 21st minute of the experiment. During the 1st minute of If blockade, the contraction force of isolated atrial myocardial strips decreased to 0.25±0.13 g ($p<0.01$). By the 14th minute of the experiment, contraction force decreased to 0.23±0.13 g ($p<0.05$). By the final minute of the experiment, contraction force decreased to 0.22±0.12 g ($p<0.01$). The decrease of contraction force of isolated atrial myocardial strips was 15% of the initial value.
When ZD7288 at a concentration of 10-6 M was injected into the perfused solution, the action potential duration at the 50% level increased from 11.86±1.21 ms to 19.14±1.77 ms ($p\leq0.01$). At the 7th minute of the study, the greatest increase in action potential duration at the 90% level was recorded from 22.43±3.6 ms to 30.71±2.69 ms ($p\leq0.01$), the parameter of total action potential cycle length from 160.28±7.85 to 171±9.14 ms ($p\leq0.01$). The baseline value of the action potential generation frequency parameter was 375.04±16.84 units/min. At the 7th and 15th minutes of the experiment, the values of this parameter decreased to 351.71±18.12 units/min ($p\leq0.01$) and 354.58±17.23 units/min ($p\leq0.01$), respectively.
Thus, the results showed that If blockade has a significant effect on the working atrial myocardium of 3-week-old rats.
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### S1.74. Phase separation of SARS-CoV-2 N-protein and viral RNA: possible mechanism and regulation by nucleoside derivatives

Svetlova J.[1], Tsvetkov V.[1], Knizhnik E.[1], Vedekhina T.[1], Varizhuk A.[1]*
[1]*Lopukhin Federal research and clinical center of physical-chemical medicine, FMBA, Moscow, Russia;*
* annavarizhuk@gmail.com

The nucleocapsid protein (N) of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) tends to form biomacromolecular condensates with RNA in aqueous media via the liquid-liquid phase separation (LLPS) mechanism. N-RNA LLPS in the cytoplasm of the host cell supposedly enables viral immune evasion by blocking an intracellular signaling pathway and may contribute to viral replication. Within the framework of the "scaffold-client" model, which is typically used to describe multicomponent condensates, N protein can be regarded as the main driver ("scaffold") of LLPS, and its "clients" include the components of the replication/transcription machinery. Their concentration and redistribution on SARS-CoV-2 genomic RNA are needed for the onset of replication-transcription and the switch from classical to discontinuous transcription, respectively, resulting in the production of genomic and subgenomic RNA.
Previously, small-molecule LLPS modulators that reduce condensate density, have been shown to enhance the activity of viral polymerase inhibitors by facilitating their access to the target. Such modulators and inhibitors of N-RNA LLPS may find application in the development of combination strategies for antiviral therapy, and further investigation of N-RNA phase transitions may contribute to the rational design of new antivirals. Condensate-stabilizing compounds, in turn, are theoretically capable of disrupting the viral life cycle by the unpacking of the nucleocapsid right after infection. Thus, they may also prove to be therapeutically relevant. Despite the growing interest to this matter, studies of N-RNA LLPS modulators are at an early stage. One limitation is the lack of a simple and adequate in vitro model of the condensates.
The aim of this work was to obtain a model of N-RNA condensates under physiological conditions and to evaluate the effects of known nucleoside/nucleotide-based antivirals on these condensates. The key difference between the proposed model and those described previously is the choice of RNA and N-RNA ratio. We used fragments of the SARS-CoV-2 genome that contain primary and secondary structure elements recognized by N protein, while in previous studies random-sequence oligoribonucleotides were typically used. We also used an N:RNA ratio comparable to that expected in SARS-CoV-2 virions. Nucleoside/nucleotide analogs were considered as possible modulators of N-RNA LLPS based on the reports of condensate sensitivity to ATP, which is arguable the key endogenous modulator of biopolymer phase transitions.
The condensates were obtained using a fluorescently labeled recombinant N protein and an RNA sequence from the 5'-untranslated region of the viral genome, which forms a branched hairpin and contains sequences recognized by N protein. Phase separation was confirmed using fluorescence microscopy and turbidimetry. The model of the condensates was validated by comparing observed and predicted effects of pH, temperature, solution ionic strength, and known modulators. To assess the effects of nucleoside/nucleotide modulators, changes in N partitioning coefficient and the total area of the condensates per surface unit were calculated based on fluorescence microscopy analyses of N-RNA mixtures in the presence/absence of the modulators. Finally, the effects of nucleoside/nucleotide analogs on the condensates were correlated with antiviral activity in cells.
Analysis of condensate sensitivity to external factors supported the recently proposed hypothesis explaining the relationship between specific electrostatic N-RNA interactions and nonspecific hydrophobic interactions within unstructured N regions. According to that hypothesis, the mechanism of the RNA-dependent phase transition includes the following steps: 1) recognition of sequence motifs characteristic of the 5'-untranslated region of the viral genome by the RNA-binding domain of N increases promotes contacts between the backbone of the adjacent RNA region and the positively charged N fragment near the dimerization domain; 2) RNA binding alters the conformation of the dimerization domain, causing its partial denaturation; 3) partial protein denaturation and exposure of hydrophobic regions initiate the phase transition.
Analysis of the effects of nucleoside/nucleotide analogs revealed weak correlation between LLPS modulation and antiviral activity in the series of 5'-norcarbocyclic nucleoside derivatives and fleximer-containing nucleoside analogs [1]. Non-nucleoside modulators, such as perylene-based antivirals, showed no such correlation. In the nucleoside analog series, top effects were obtained for 5'-norcarbocyclic derivatives, which caused up to 15-fold increase in the efficiency of N separation. These compounds might hold promise for the development of therapeutic agents. However, to take into account possible side effects, their effects on host cell condensates (membraneless organelles) should be tested.
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### S1.75. Photodimerization of thymine chromophores in poly-T aqueous solutions at room temperature

Malkin V.M.[1]*, Rapoport V.L.[1]
[1]*Saint-Petersburg University;*
* vlmalkin@yandex.ru

Our interest in the study of photophysical and photochemical processes in aqueous solutions of poly-T was associated both with the fact that similar processes occur in living organisms and with an attempt to approach the implementation of photochemical recording of information on thymine or its derivatives [1, 2].

Using the methods of luminescent and absorption spectroscopy, we established the heterogeneity of aqueous solutions of polytimidylic acid (poly-T, 5•10-5 mol/liter, pH≈7.0) at room temperature: their luminescence excitation spectra differed from the absorption spectra, and the luminescence spectra depended on the lengths excitation waves [3]. It should be noted that even in [4], absorption spectra and photodimerization effects differing in maximum in aqueous solutions of poly-T were presented. We suggested that thymine chromophores in aqueous solutions of poly-T can be both in relative isolation and in stacking aggregates favorable for photodimerization [5, 6].

Observation of a reversible change in the absorption spectra of solutions under the action of ultraviolet irradiation (297 nm) showed not only a decrease in absorption, but also a change in the shape of the absorption spectrum. The study of the differences between the absorption spectra of solutions that received different doses of irradiation made it possible to detect two types of photochemically active stacking aggregates of thymine chromophores, the first of which corresponds to the absorption bands at 250 and 280 nm (exciton splitting 4300 cm-1), and the second - 260 and 290 nm (exciton splitting 4000 cm-1). The photochemically inert fraction was also observed to have an absorption spectrum with a maximum at 270 nm, without exciton splitting. The last fraction consists of chromophores, the mutual position of which prevents the cyclobutane photodimerization from entering into the reaction. It is likely that the chromophores included in it are either remote from other chromophores or have an orientation that is unfavorable for entering into photochemical reactions [3, 5, 6].

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### S1.76. Photoelectronic properties of peptide nanotubes based on various amino acids

Bystrov V.S.[1]*, Paramonova E.V.[1], Yurkova D.O.[2], Ledeneva O.R.[2], Belova E.V.[2]
[1]*Institute of Mathematical Problems of Biology RAS - the Branch of Keldysh Institute of Applied Mathematics of Russian Academy of Sciences ;*
[2]*Faculty of Physics, Lomonosov Moscow State University, Moscow, Russia;*
* vsbys@mail.ru

The study of structural self-organization and physicochemical properties of peptide nanotubes based on various amino acids and their dipeptides is of considerable interest for fundamental molecular biology and for practical applications. Peptide nanotubes based on diphenylalanine (FF PNT) are an example of already quite well studied and widely used nanostructures in various fields [1–5], including biomedicine. However, relatively recently it was found that they also exhibit pronounced photoelectronic properties [6,7], which differ for cases of structures of different chirality of the initial amino acids L-FF/D-FF [1,2,8].

In this work, we report similar properties of nanotubes based on other amino acids and dipeptides: dileucine (Leu-Leu/LL PNT) and diisoleucine (Ile-Ile/II PNT). Initially constructed model structures of nanotubes for FF PNT based on assembled stacks of parallel rings (ring structures) [1,2,6,8] were different and had other characteristics (including large values of the band gap Eg = E_LUMO - E_HOMO ), compared with their experimentally self-assembled structures, which have the form of coils of spirals according to X-ray data [1,3,8].

Nanotubes based on leucine and their dipeptide, dileucine LL: L-LL PNT, also exhibit a similar character [2]. In this case, too, calculations for helical structures built on the basis of X-ray data also give values of Eg that are much smaller than for model structures from layers of ring structures. Currently, except for FF PNT, all experimentally known structures of dipeptides are based on amino acids of left chirality L [4,5,8] (Table 1). They have sets of rather intricately organized molecular crystals, from which it is not always possible to extract a clear structure of a helical nanotube [5]. In this case, similarly to other tubular structures of dipeptides, it is possible to build a model nanotube from dipeptide molecules in an annular layer, which has all the properties (high polarization, piezoelectric effect [1,2,8] and photoelectronic properties [6,7] with energy levels giving large values of Eg) characteristic of ring PNT structures. And to carry out artificial "spiralization" of this structure and build spiral structures with a step equal to the period of this molecular-crystalline structure along the axis of the nanotube. This is how all helical FF PNTs with 6 FF dipeptides per layer are arranged, for PNT based on leucine, 4 LL dipeptides each. An isoleucine-based L-II PNT of 4 dipeptides per layer is also considered here as an example.

Calculations performed on the basis of semi-empirical quantum-chemical PM3 methods in the restricted Hartree-Fock (RHF) approximation showed that in this case, too, the band gap Eg of such an artificially created helical PNT is also noticeably smaller than Eg for the L-II model ring nanotube (Table . 1). Dileucine-based ring and helical PNTs of right chirality D for D-LL were constructed similarly.

Thus, it can be concluded that helical structures based on dipeptides of various amino acids will have Eg values less than their similar ring structures. It can be assumed that this effect is of great evolutionary importance for biomolecular structures, since they all have helical amino acid/dipeptide sequences. In the processes of their self-assembly, it is energetically more profitable to switch from ring structures to helical ones: this is a kind of topological transition, to a more stable organization of biomacromolecules [8]. In addition, it is important to note that the Eg values turn out to be in the range of spectral characteristics close to the solar ultraviolet. It probably has a biological significance as well. This range is in the region of solar-blind ultraviolet, associated with the absorption of sunlight by the ozone layers of the atmosphere. In the absence of such absorption, in the case of "ozone holes", the radiation passes through the atmosphere and can be detected using photodetectors based on such PNTs [8]. It is important that the width Eg changes under the influence of the electric field, which makes it possible to adjust the recorded range. Polymer ferroelectrics and layers of graphene/dichalcogenodes are suitable here [7, 9]. Such works are now actively developed in the world.

Table 1. Eg values (eV) calculated by the PM3 RHF method for PNT based on various
amino acids in a-helix conformation and L&D chirality, and corresponding dipeptides.

| PNT type | PNT model: Helix-2-coils model, Helix-2-coils-model, 2-rings-model | experimental x-ray data | artificial coils |
|---|---|---|---|
| L-FF | 8.5865 | 3.4410 | |
| D-FF | 8.7027 | 3.5750 | |
| L-LL | 8.3411 | 3.2296 | |
| D-LL | 9.0976 | 6.5440 | |
| L-II | 8.5940 | 5.3967 | |

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### S1.77. Physical basis of the relationship between the structural organization and molecular mobility of polymeric systems for medical purposes and their chemical structure

Smyslov R.Yu.[1], Gorshkova Yu.E.[2,3]*, Nekrasova T.N.[1]
[1]*Institute of Macromolecular Compounds, RAS;*
[2]*Joint Institute for Nuclear Research;*
[3]*Institute of Physics, Kazan Federal University;*
* Yulia.Gorshkova@jinr.ru

Poly-2-alkyl-2-oxazolines (PAOZ) are a promising class of polymers for medical applications. This is largely due to their biocompatibility, nontoxicity, and resistance to the action of enzymes. At present, they are used to make drug carriers, anti-burn coatings with a controlled drug release rate, thermolabile gels for bleeding arrest, materials with antithrombogenic properties, etc. Interpolymer complexes expand the possibilities of their application.

Interpolymer complexes (IPC) formed in solution during the interaction between two macromolecules of different chemical structure due to the formation of hydrogen bonds or electrostatic interactions are of interest for the creation of new highly functional polymeric materials as molecular nanocontainers for drug transportation, supersorbents, membrane materials, living tissue substitutes. IPCs have different properties than the individual macromolecules that make them.

To study how polymethacrylic acid (PMAA) forms IPC with linear poly-2-alkyl-2-oxazolines, the polarized luminescence method was used. The nanosecond relaxation times τ_IMM characterizing the intramolecular mobility (IMM) of macromolecule chain sites. τ_IMM were determined from luminescence polarization (P) measurements of the solution using the formula: $\tau_IMM = (1/P'o + 1/3) \times 3\tau_fl/1/P - 1/P'o)$, where τ_fl — luminescence duration of the luminescent tag, 1/P'o — parameter accounting for the contribution of high-frequency movements of the luminescent label.

The supramolecular structure of the obtained IPCs in the interaction of PMAA with linear and branched polymers was studied by the method of small-angle X-ray scattering. The influence of the polymer chain topology on the complexation was studied by the example of PMAA interaction with poly-2-ethyl-2-oxazoline chains grafted along the upper or lower rim of calyx-8-arene. The aim of the study is to establish the factors determining the structure and functioning of new polymer systems of complex architecture and composition in solutions.

Luminescently labeled PMAA* was obtained by free-radical copolymerization of methacrylic acid and 9-anthrylmetacrylamide in solution. The luminescent labeling content was 0.15 mol %. Linear polymers were synthesized at Reading School of Pharmacy, University of Reading, UK (Prof. V. V. Khutoryanskiy). Star-shaped copolymers were obtained in the Institute of Macromolecular Compounds, Russian Academy of Sciences (Prof. A.V. Tenkovtsev). The degree of polymerization of n examined samples PMAA* was 3100 = 264 kD, poly-2-alkyl-2-oxazolines ~ 505 kD, in the star-shaped copolymers the degree of poly-2-ethyl-2-oxazolines rays polymerization was 30 kD.

The dependences of nanosecond relaxation times τ_IMM of PMAA* macromolecules in water when adding a solution of poly-2-alkyl-2-oxazoline at different ratios of interacting components poly-2-methyl-2-oxazoline, poly-2-ethyl-2-oxazoline with n = 500 were obtained.

The addition of 2-alkyloxazoline to the polyacid solution leads to a sharp increase in τ_IMM values and a corresponding decrease in the mobility of the polyacid chain sites. This indicates the interaction with macromolecules of poly-2-alkyloxazoline and the formation of IPC. The key factor in the formation of IPC between PMAA* and PAOZ is the H-bonds between the non-ionized proton-donating COOH group and the carbonyl oxygen of PAOZ. Since the change in τ_IMM with an increase in the [PAOZ]/[COOH] ratio is practically independent of the nature of the substituent in PAOZ, the role of hydrophobic interactions in the formation of the PAOZ/PMAA complex can be considered insignificant. When the ratio of the interacting components β = [PAOZ]/[COOH]= 0.7, opalescence appears in the solution, which indicates the aggregation of the formed IPCs. When a poly-acid interacts with PAOZ, not an extended cooperative system of hydrogen bonds between individual macromolecules is formed, as, for example, observed for poly-N-vinylamide complexes with carboxylic acids, but numerous cross-links (H-bonds) between proton-donor and proton-acceptor groups removed along the chain, which results in the formation of a net-like structure. The formation of such a structure seems to be caused by the geometrical factor — the distance between the interacting groups and the presence of massive hydrophobic substituents, and it prevents the formation of an extended system of H-bonds.

The influence of the degree of ionization of COOH groups on the formation of IPC was detected by the change in the inverse value of luminescence polarization 1/P, proportional to IMM, from the degree of ionization of PMAA. PMAA is a weak polyelectrolyte; in aqueous solution, the degree of ionization of COOH groups of the macromolecule, α, does not exceed 0.03%. In the non-ionized and weakly ionized state in PMAA is in a compact conformation stabilized in addition to H-bonds by hydrophobic interactions of α-methyl groups. As a result, the IMM of PMAA chains is significantly retarded compared to, for example, the IMM of polyacrylic acid under the same conditions. When PMAA is ionized, the mobility of the chain sections increases with increasing α due to the destruction of the compact structure by electrostatic repulsion. In this case, the ionization of COO groups is more difficult in IPC, due to the formation of more stable H-bonds with PAOZ macromolecules. However, if we compare the PAOZ/PMAA/their IPCs with the "classical" poly-N-vinylpyrrolidone/PMAA IPCs, their strength is noticeably weaker, and they are stable in a relatively narrow pH interval, indicating a significant difference in the structural organization of the IPCs.

The luminescence of aqueous solutions of curcumin in the presence of calix-8-arene with rays (poly-2-ethyl-2-oxazoline) and aqueous solutions of curcumin in the presence of its complex with polymethacrylic acid demonstrate the promising use of IPC as nanocontainers for the delivery of difficultly soluble substances.

### S1.78. Physical principles of lampbrush chromosomes chromatin organization

Lagunov T.A.[1,2]*, Nurislamov A.R.[1,2], Gridina M.M.[1], Kulikova T.V.[3], Krasikova A.V.[3], Fishman V.S.[1,2]
[1]*ICG SB RAS;*
[2]*Novosibirsk state university;*
[3]*Saint Petersburg State University;*
* t.lagunov@g.nsu.ru

The emergence of new methods for studying chromatin (cytological – based on the FISH method; molecular – such as 3C, Hi-C) demonstrated the patterns of spatial DNA organization in the cell nucleus. The results of these methods usage suggest that the three-dimensional

organization of chromatin plays a significant role in the regulation of genes, as well as the spatial organization disorder of chromatin is connected with certain diseases. Physical modeling of chromatin as a polymer demonstrated that the basis of genome stacking is based on physical patterns and physical processes (entropic elasticity, mechanical repulsion of DNA, the mechanism of "stretching the loop" by cohesins and condensins, and others). Lampbrush-type chromosome is very interesting object for research and modeling due to the fact that each such chromosome individually can be described quite fully using direct imaging methods (light microscopy and FISH), and then compared with the indirect method of obtaining spatial organization (Hi-C). Since lampbrush chromosomes are formed only at a certain stage of development of avian and amphibian oocytes, we optimized the Hi-C method to obtain the spatial organization of chromatin in lamp brush type chromosomes to get high-quality data for single cells. Additionally, our colleagues conducted an RNA-seq experiment on single oocytes to compare expression patterns with chromatin contact domains and lateral loops. Based on the results of Hi-C and RNA-seq experiments, it can be assumed that active transcription of the genomic locus contributes to the extraction of DNA sites, and also suggest two main physical mechanisms for the formation of chromatin domains (analogues of topologically-associated domains) that are not related to the mechanism of "loop stretching": by colliding cohesins with RNA polymerase and by superspiralization of DNA chains.

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### S1.79. Physical principles of organization of 3D structure and dynamics of biopolymers

Shaitan K.V.[1]*
[1]*M.V.Lomonosov Moscow State University, Moscow, Russia;*
* shaytan49@yandex.ru

The physical features of the chemical structure of linear biopolymers, which turned out to be important from the point of view of the formation of unique 3D structures, are considered. The kinematic bonds between the nodes of the polymer chain and the dynamic effects of viscosity, which tend to form various helical structures, are discussed. It is shown that the dynamics of folding of a long polymer (biopolymer) chain in a viscous medium (liquid) obeys two extreme principles:
- the maximum rate of loss of potential energy and
- minimum energy dissipation rate

The rules for the movement of a representative point along the multidimensional potential energy surface are also obtained:
- average rates of change of potential energy are uniformly distributed over the nodes of the chain and
- the average rates of energy dissipation are also uniformly distributed over the nodes of the chain.

These statistical rules for the movement of a representative point along a multidimensional potential energy surface [1] dictate the choice of the smoothest trajectories of movement, which contributes to overcoming the Levinthal paradox and achieving a global minimum of energy in a reasonable time.

A new approach to the study of multidimensional energy landscapes of polymers and biopolymers has been proposed, based on the principles of symmetry with respect to permutations of identical monomer units in the polymer chain and the topology of the configuration space of macromolecules [2]. The analysis of the topography of the energy landscapes of macromolecules is carried out using the Morse theory and expansion of the potential energy surface into a multidimensional Fourier series. In the Gaussian approximation, the effect of weak symmetry breaking with respect to permutation of monomer units (for example, due to some modification of the side groups) is studied, which leads to the topography of energy landscapes with many nested energy funnels. In this case, there is the deepest central funnel and shallower satellite funnels separated from each other by energy barriers. The discussed energy funnel topographies correspond to a number of effects observed in the kinetics of protein folding. In particular, a volcano-like profile of the free energy surface arises, the sensitivity of protein refolding to the method of denaturation (unfolding of the spatial structure), the dependence of the kinetics and the result of folding on the region of the initial chain conformations. The latter may be essential for understanding the mechanisms of co-translational folding.

When calculating the topography of the free energy surface, the characteristic temperature parameter T' arises, which is defined as the energy gain when the chain is folded by one conformational degree of freedom. It is shown that at a temperature T>0.26T' the spatial structure of the globule is destroyed. The parameter T' and the denaturation temperature in the system under consideration follow from the basic topological and geometric principles for the formation of energy landscapes in the configuration space of torsion angles and symmetry considerations regarding the permutation of identical monomer units. Note that at biopolymer denaturation temperatures of about 60°C, this ratio leads to an estimate of the energy of non-valent bonds of monomers of about 2.5 kcal/mol per conformational degree of freedom, which is very similar to hydrogen bonds in polypeptides in an aqueous medium.

The effects of viscosity and symmetry with respect to the permutation of monomeric units can, under certain conditions, act together. The resulting effects may be of interest from the point of view of the physicochemical evolution of macromolecules towards the formation of a pool of linear polymers with unique spatial structures (we believe that they can lead to the "arrow of molecular prebiological evolution"). The text of the report is illustrated by the results of molecular dynamics calculations and videos of the folding processes of various types of linear polymers in viscous and nonviscous media.

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### S1.80. Physicochemical mechanisms of self-assembly of enveloped viruses

Denieva Z.G.[2], Popova M.M.[2], Shtykova E.V.[1], Batishchev O.V.[2]*
[1]*FSRC "Crystallography and Photonics", Russian Academy of Sciences;*
[2]*Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences;*
* olegbati@mail.ru

Enveloped viruses are a broad class of pathogens that are the causative agents of the most dangerous viral diseases, such as SARS-CoV-2, influenza virus, human immunodeficiency virus, and many others. A common feature of all these viruses is the presence of a lipoprotein envelope, in which the main structural proteins of the virion are located. These proteins ensure the implementation of most of the processes of viral infection and its multiplication, such as reception on the cell surface, fusion with its plasma membrane or membranes of cell organelles, release of the viral genome, and, ultimately, assembly and release of newly synthesized virions from the infected cell. At the same time, capsid or matrix proteins of enveloped viruses play an important role in almost all the described stages. These proteins, represented by the largest number of copies inside the viral particle, are the most conserved among viral proteins, but, nevertheless, their functional role at various stages of the life cycle of enveloped viruses is still not fully understood. For this reason, these proteins are not yet targets for any antiviral drugs. In our work, we used various biophysical and structural

methods to demonstrate that the capsid or matrix proteins of many enveloped viruses use common physicochemical mechanisms for the self-assembly of the viral scaffold and the organization of the release of progeny virions from the infected cell. These mechanisms include various variants of the membrane activity of structural proteins of viruses, including surface activity, electrostatic interactions, influence on the structure of lipid and lipid-protein domains, and incorporation into the lipid membrane. Thus, despite the differences in the structure of these proteins, their functional role can be reduced to a limited set of physicochemical mechanisms common to all enveloped viruses. Based on the obtained models of such physicochemical processes, we propose new ways to search for possible antiviral drugs aimed at self-organization of viral proteins and lipid-protein interactions.

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### S1.81. Possible polymorphism of non-canonical structures formed by DNA contacts containing G-repeats and C-repeats: modeling strategy, classification, and most probable forms

Tsvetkov V.B.[1,2]*
[1]*I.M. Sechenov First Moscow State Medical University;*
[2]*Y.M. Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine;*
* v.b.tsvetkov@gmail.com

In this study, I used molecular modeling methods to investigate all possible structures of the contacts between (G3T)nG3/(C3A)nC3-containing duplexes, taking into account all possible quadruplex (G4) and i-motif (IM) forms, as well as variability of their orientation relative to each other, both with strand exchanges or mutual girths and without them. Sequences with different numbers of G/C repeats were considered. To estimate changes in the structures during modeling for testing their stability, I proposed and used a system of G4/IM-specific geometric parameters that describe the localization of nucleotides in non-canonical forms relative to each other. By scoring the main contributions to the free energy of the modeled structures within the framework of the Generalized Born approach, the most probable of them were determined. My research allowed for proposing and modeling a significantly larger number of options for contacts through non-canonical forms compared to those proposed previously based on a schematic approach. The geometry of these forms turned out to be much more complicated and diverse than the previously described cases of non-canonical structures. Modeling allowed me to establish many new ways of folding of chains containing G and/or C repeats into non-canonical structures that have never been considered before. Previously published hypotheses about the structure of G/C-rich DNA-DNA contacts formed during enhancer-promoter interactions, chromatin loop formation, translocation, and some other stages of chromatin reorganization contained only simplified models of contacts by means of strand entanglement or stacking of outer tetrads in G4s. In most cases, only G4s were considered, although both G4s and IMs exist in the nuclei of living cells. In addition, the schemes relied on the available classification of G4 structures described for short single-stranded oligonucleotides. The previously used schematic approach did not allow the authors to reflect the whole diversity of duplex contacts. In particular, in this study, the possibility of chain exchange through the formation of G4/IM was considered for the first time, and the cross-shaped junctions s of the complexes proposed by me are much more complicated than those proposed by Holliday and contain G4s/IMs at the boundary of the central “hole”. Also, for the first time, I predicted and studied the possibility of stacks formed from right- and left-handed quadruplexes, and layers formed from quadruplex grids. The structures have considered me make it possible to suggest what kind of non-canonical structures are formed during the exchange of chains of duplexes containing G-repeats and C-repeats in the process of homologous recombination. To implement this study, I developed a strategy for creating G4 / IM models of any complexity, which can be used for in silico studies of non-canonical DNA and RNA structures. This strategy includes the following key steps. First, 3D models of all possible G4s and IMs for a given sequence are obtained and combined in various orientations relative to each other, taking into account the possibility exchanging and girthing of strands. Then, the duplex flanks are added. The resulting models are checked for the presence of internal steric hindrances, and all geometrically impossible ones are discarded. The remaining structures are then subjected to MD simulation to test their stability based on free energy contributions to determine the most probably variant. The structures I have described can serve as a starting point for modeling similar and possibly more complex DNA and RNA systems. The proposed strategy for the structural in silico analysis of G/C-rich DNA can be a useful alternative to structural studies based on X-ray diffraction, cryomicroscopy or NMR, which are rarely applicable to conformationally polymorphic nucleic acids. The results of this study have been published in bioRxiv: https://doi.org/10.1101/2022.10.10.511558

### S1.82. Protein engineering based on hyperstable Sm-like proteins. First results and prospects

Mikhaylina A.O.[1], Lekontseva N.V.[1], Khairetdinova A.R.[1], Marchenkov V.V.[1], Nikonov O.S.[1], Balobanov V.A.[1]*
[1]*IPR RAS;*
* balobanov@phys.protres.ru

Engineering of new protein materials is currently one of the topical areas of bioengineering. The combination of natural domains in a hybrid protein can give it properties that are not inherent in each of the domains individually. This gives a wide scope for creativity in creating proteins with new properties. We applied this approach to oligomeric proteins. Sm-like proteins became the basis for hybrid oligomeric proteins in our work. These proteins are convenient in that they have an extremely high stability. In addition, among them there are oligomers with a different number of subunits, which makes it possible to choose for the necessary tasks. The addition of target domains to the oligomeric base brings them closer and determines their mutual orientation, enhancing their interaction. In order to determine the possible advantages and limitations of such a system, we have designed, obtained and studied several hybrid proteins.

The first fusion protein we present in our work is the combination of the Hfq protein from E. coli and the amyloidogenic A-beta peptide 1-40. When obtaining and studying this protein, we used a significant difference in the stability of the structures of the base and attached peptides. This approach allowed us to purify the protein under denaturing conditions, and then, stepwise renaturing it, to study the folding of each of the parts of this construct. The Sm-protein base fold into the expected oligomer, on which the A-beta peptide then forms a Thioflavin T binding structure. At the same time, due to a significant local increase in the concentration of amyloidogenic peptides, their interaction occurs mainly within the oligomer without the formation of fibrillar structures.

The second fusion protein presented in this work is the combination of a heptameric Sm-like protein from Sulfolobus acidocaldarius (SacSm) and the apical domain of the GroEL chaperone protein (ADGroEL). In our design, the hyperstable SacSm holds the seven ADGroELs together forcing them to oligomerize. For this protein, stepwise assembly and self-organization was also shown. First of all, the SacSm base is assembled, and then ADGroEL is folded on it. We shown that the fusion protein has a thermal stability higher than the individual ADGroEL and even higher than the full-length GroEL. The resulting fusion protein is capable of binding non-native proteins bound by the full length GroEL chaperone. It also reduces the aggregation of a number of proteins when they are heated, which confirms its chaperone activity.

Our result shows the productivity of our tool for the stabilization of oligomeric proteins. This tool can be used for a number of molecular biological tasks requiring stabilization of circular oligomeric proteins.
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### S1.83. Protein translational diffusion as a marker of protein-protein interactions

Kusova A.[1]*, Sitnitsky A.E.[1], Zuev Yu.F.[1]
[1]*Kazan Institute of Biochemistry and Biophysics;*
* alexakusova@mail.ru

Translational diffusion is the main way of molecular transport in organisms that defines numerous vital activities of the living systems. In simple cases, the diffusional process is well described by the classical Stokes–Einstein model. Translational diffusion of macromolecules significantly deviates from the classical representation in real living systems. Living systems contain various types of macromolecules such as DNA, RNA, proteins and there are many non-specific "soft" intermolecular interactions. Soft interactions include hydrogen bonds and charge–charge, solute–protein, van der Waals and hydrophobic interactions. Of these, only the strong electrostatic repulsion of like charged molecules prevents their approach. Other soft interactions are attractive and destabilizing because they favor expanded conformations that allow access to the attractive surfaces. Effect of the intracellular environment modulating protein-protein interactions (PPI) is important because the totality of weak interactions in cells forms the crowded cellular interior.
One of the commonly accepted approaches to estimate PPI in aqueous solutions is the analysis of their translational diffusion. Phenomenological approach was applied to analyze PPI effects via concentration dependencies of self- and collective translational diffusion coefficient for several spheroidal proteins derived from the pulsed field gradient NMR (PFG NMR) and dynamic light scattering (DLS), respectively [1,2]. The technique of pulsed field gradient nuclear magnetic resonance (PFG NMR) operates on time scales exceeding those of the intermolecular collisions. The long-time self-diffusion coefficient Ds is observed as the averaged result of protein diffusivity over a long observation time. In dilute solutions, the molecules move independently of each other, whereas in the semi-dilute solutions, the intermolecular interactions result in appearance of new class of motion-collective modes described by collective diffusion coefficient Dc (DLS) [3,4]. Dc depends on the microscopic fluctuations in the local concentration of particles and the corresponding local heterogeneities in the refractive index of the medium [5]. The self- and collective diffusion coefficients are always identical with diffusion coefficient in dilute solution when interactions between diffusing species are absent. At intermediate concentrations, Dc is different from D0 and Ds and strongly depends on intermolecular interactions.
The concentration dependence of molecular diffusion coefficient contains information on the contributions of various types of PPI. The combination of protein concentration dependencies of self- and collective diffusion coefficients with phenomenological approach based on the formalism of non-equilibrium thermodynamics allows one to obtain sets of friction and virial coefficients. The second and higher virial coefficients were obtained for evaluation of paired and multi-particle intermolecular interactions in solution for chymotrypsin (ChTr), human serum albumin (HSA), α-casein (α-CN), and β-lactoglobulin (β-Lg). The McMillan–Mayer theory can be used for quantitative estimation of the non-specific PPI [6]. Balance of attraction-repulsion potentials between the two protein molecules in solution was considered within the DLVO theory. The positive value of the second virial coefficient of spheroidal ChTr, HSA, α-CN, and β-Lg at low ionic strengths (0.003 M–0.01 M) indicates the dominance of the intermolecular repulsion. Increase of ionic strength (0.1–1.0 M) leads to screening of the protein charges and, as a result, electrostatic potential decreasing was observed. The increase of the van der Waals attraction of ChTr and α-CN can be explained by propensity to manifest weak unstable attractive interactions. The decrease of van der Waals interaction of β-Lg molecules is associated with well-known association process [7].
Self- and collective diffusion are sensitive differ to a short-lived HSA molecular forms. Short-time collective diffusion shows one type of diffusing species of pure HSA solution and two molecular forms of HSA with the presence of metal ions in the either concentration range. While the long-time diffusion detected an averaged self-diffusion coefficient among fast exchanging HSA oligomer forms.
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### S1.84. Proteins: from design to construction

Anashkina A.A.[1]*, Nekrasov A.N.[2]
[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia;*
[2]*Shemyakin-Ovchinnikov Institute of bioorganic chemistry RAS Moscow, Russia ;*
* anastasia.a.anashkina@mail.ru

The study of the entropy characteristics of fragments of protein sequences showed that for 5 successively located residues, a reduced level of informational entropy is observed and, therefore, blocks of just this size must be considered as elementary units of the sequence. This approximation made it possible to develop a method that reveals the hierarchical structure in protein sequences - the information structure analysis method (ANIS method) (Nekrasov A.N. et al., 2021).
An analysis of the conformational stability of pentapeptides by the molecular dynamics method showed that all pentapeptides can be conditionally divided into three types. Pentapeptides, which are in the predominant topology for more than 80% of the modeling time, we called structure-forming. Structures that have two predominant conformations, in each of which the pentapeptide was located for more than 40% of the modeling time, we called trigger. The remaining peptides do not have a predominant topology. We believe that such regions in the structure of proteins adapt to the environment and form a dense packing of protein domains. We called such peptides structure-stabilizing.

The use of the ANIS method for the analysis of the primary structures of proteins makes it possible to identify hierarchically organized elements of the information structure (ELIS) in them. ELIS are characterized by their position in the primary structure of proteins and rank - level in the hierarchical structure. The application of the ANIS method to a set of proteins with a known spatial structure made it possible to obtain fragments of PDB files corresponding to ELIS of all possible ranks and classify them according to topological stability. The identification of the patterns described above, which describe the organization of natural polypeptide chains, makes it possible to move from the modification of native proteins to their design.

### S1.85. Puckering states of iononic rings of carotenoids

Surkov M.M.[1]*, Mamchur A.A.[1], Yaroshevich I.A.[1]
[1]*Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University;*
* macsurmak.m02@mail.ru

The properties of the protein-pigment complex are determined by the pigment included in its composition and the way it is coordinated in the active center. In case of carotenoproteins, the configuration of the pigment binding site depends on the conformational dynamics of the carotenoid in their composition. One of the ways of changing the conformation for a carotenoid is the transition of its iononic ring between different variants of a non–planar structure – puckering states. In our study, the conformational dynamics of five different carotenoids was investigated within the framework of molecular modeling methods.
The iononic rings of astaxanthin (AST), beta-carotene (BCT), canthaxanthin (CAN), lutein (LUT) and zeaxanthin (ZEA) were selected as the objects of the study. The energies of puckering states were calculated within the molecular dynamics method using the GROMACS package. The duration of the simulation was 1 microsecond for each carotenoid. As a result, the energy profiles of puckering states (puckering maps) were characterized for the studied series of carotenoids. It has been shown that energy barrier for transition between two local minima for iononoic rings containing two sp2 hybrid carbon atoms (BCT, LUT, ZEA) is twice lower than for iononic rings of AST and CAN containing three sp2 hybrid carbon atoms. For AST, the removal of energy degeneracy for two puckering states due to the formation of an intramolecular hydrogen bond is shown. For each puckering state, within the framework of computational quantum chemistry methods using the ORCA package, profiles of the potential energy of rotation around C6 – C7 and C6' – C7' bonds were constructed. A significant modification of the corresponding profiles depending on the puckering state of the lateral cyclic group is shown.
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### S1.86. Quantum chemical modeling of intermolecular interaction of hyaluronic acid with amino acids from the composition of targeted delivery nanocontainers and target substances

Bryxin K.A.[1]*, Plastun I.L.[1], Naumov A.A.[1]
[1]*Saratov State Technical University;*
* kir.bryksin@yandex.ru

Recently, theranostics, a direction associated with the targeted delivery of a therapeutic and diagnostic agent to affected cells, has become increasingly widespread in medicine. One of the target substances is not only cells, but also mucin – protein tissue from the mucosa. One of the means of drug delivery are polymer capsules and nanogels based on protein structures. To study the complex formation during the delivery of a medicinal substance based on protein capsules, the analysis of the degree of hydrogen binding of hyaluronic acid as a delivered drug with amino acids from the composition of protein capsules and from the composition of amino acids included in the target substance was carried out. These amino acids can be included both in the composition of protein capsules and in the composition of the target substance. Lysine-proline and lysine-serine complexes were considered as the object of the study. It is well known that nitrogen-containing amino acids, in particular, lysine, serine and proline, play an important role in the formation of intermolecular interaction of protein structures with each other and with various substances, therefore, the assessment of the degree of complexation and the strength of hydrogen binding can be applied both to the assessment of the degree of interaction of the molecular layers of the capsule and to the assessment of the degree of stability of the attachment of the capsule. to the target.
Modeling of intermolecular interaction was carried out by methods of density functional theory with B3LYP functional and 6-31G(d) basis using Gaussian software package, preliminary optimization of molecules was carried out in Avogadro and GaussView software complexes. At the first stage, the calculation and analysis of the lysine-proline molecular complex was carried out. The calculated IR spectrum of the complex has a peak at a frequency of 3501 cm-1, which corresponds to the oscillation of the –OH group. This peak was chosen for the study of complex formation during the addition of a hyaluronic acid molecule. At the next stage, a hyaluronic acid molecule was attached to the molecular complex, and the resulting structure and IR spectrum of the resulting molecular complex were calculated and analyzed. In the calculated IR spectrum of the complex, the peak corresponding to the oscillation of the –OH group shifted to a frequency of 3396 cm-1, and the peak also became more pronounced, which indicates an increase in hydrogen bonds. The lysine-serine molecular complex was also investigated. The calculation and analysis of the structure and IR spectrum of the complex was carried out. The calculated IR spectrum revealed a peak at a frequency of 3586 cm-1, corresponding to the oscillation of the –OH group. This peak was selected for the analysis of the molecular complex after the addition of the hyaluronic acid molecule.
At the next stage, a hyaluronic acid molecule was attached to the lysine-serine complex, after which the resulting molecular complex was calculated and analyzed. On the calculated IR spectrum of the lysine-serine-hyaluronic acid molecular complex, the peak corresponding to the –OH group oscillation shifted to a frequency of 3325 cm-1, and also became more pronounced, which indicates an increase in hydrogen binding in the resulting complex.
Based on the analysis of the molecular complexes of amino acids that make up the protein delivery container and the target substance, as well as molecular complexes obtained by attaching a hyaluronic acid molecule to amino acid pairs, it can be concluded that in the case of the formation of a complex with a hyaluronic acid molecule, the strength of hydrogen binding increases, which may be useful in further research in the field of theranostics. In addition, when analyzing complexes consisting of one amino acid and a hyaluronic acid molecule, the hydrogen bonds formed in molecular complexes were weaker than in triple complexes.

### S1.87. Rat cardiomyocytes electrical activity during α2-adrenoreceptors stimulation after If currents blockade

Kuptsova A.M.[1]*, Faskhutdinov L.I.[1], Vakhitov L.I.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University;*
* anuta0285@mail.ru

The α2-adrenoreceptors (α2-AR) in mammalian heart perform the functions of regulatory effects modulation. Activation of α2-AR inhibits the synthesis of cyclic adenosine monophosphate (cAMP) by adenylate cyclase. If currents of the myocardium cells are modulated by the level of cAMP, sympathetic and parasympathetic departments of the autonomic nervous system. Modulation of the If currents activity via cAMP is essential in the increase or decrease of heart rate by the departments of the autonomic nervous system. It has been shown that activation of adrenergic receptors activates If and thereby increases the chronotropic function of the heart through β-adrenoreceptor dependent increase in the level of cAMP. Since HCN channels and α2-AR are present in cardiomyocytes, it is possible that If currents are an effector of adrenergic regulation of the heart through this type of receptor as well. The aim of this study is to investigate the effect of α2-adrenoreceptor stimulation after If preliminary blockade on the electrical activity of rat cardiomyocytes.

The object of the study was 6 weeks old white rats, at the stage of the beginning of pubertal period of development. The preparation of the right atrium was prepared, keeping the sinoatrial node, then placed in the tray with the endocardial layer up and fixed. Thyrode's physiological solution was passed through the tub. Action potential registration was performed using the standard method of intracellular action potential registration. Microelectrodes filled with 3M KCl solution and 30 MOm resistance were fixed in the holder and immersed in the drug. The pharmacological drug used was ZD7288, a blocker of currents activated by hyperpolarization, in concentrations of 10-9 M and clonidine hydrochloride, an alpha2-adrenoreceptor agonist, in concentrations of 10-9 M. Such parameters of action potential as duration of action potential at 20%, 50%, and 90% repolarization, amplitude of action potential, and cycle length were studied.

Perfusion of clonidine hydrochloride at a concentration of 10-9 M against ZD7288 (10-9 M) increased action potential duration at the 20% level in 6-week-old rats from 6.34±2.86 to 8.2±3.14 ms ($p \leq 0.05$). The baseline value of action potential duration at the 50% level was 14±4.25 ms. At 7 minutes after administration of the If blocker, there was an increase in this parameter to 19.85±3.57 ms ($p \leq 0.05$). After administration of clonidine hydrochloride after If blockade, there was a significant increase in action potential duration of 50% to 22.13±4.6 ms ($p \leq 0.05$). Clonidine hydrochloride after ZD7288 promoted a 90% increase in action potential duration from 30.45±6.54 to 32.78±4.16 ms ($p \leq 0.05$) by the 7th minute of experiment, to 32.54±4.66 ms ($p \leq 0.05$) by the 15th minute of observation. Total cycle length increased from 115.1±8.56 to 128.51±8.19 ms ($p \leq 0.01$), and the frequency of action potential generation decreased from 326.8±43.22 to 319.16±54.33 units/min ($p \leq 0.05$) by the 7th minute of experiment.

Thus, stimulation of alpha2-adrenoreceptors after preliminary blockade of hyperpolarization activated currents caused an increase in atrial myocardial action potential duration due to prolongation of the repolarization phase of atrial myocytes of 6-week-old rats.

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### S1.88. Rearrangement of the conformational structure of biomacromolecules on the surface of an oblate metallic nanospheroid in an alternating electric field

Kruchinin N.Yu.[1]*, Kucherenko M.G.[1]
[1]*Orenburg State University;*
* kruchinin_56@mail.ru

At present, the use of spheroidal gold nanoparticles with biomacrochains adsorbed on their surface to create nanoprobes with adjustable plasmonic characteristics in biophysical and biomedical research, as well as in sensors based on the giant Raman scattering effect, is of great interest. In this case, the conformational structure of adsorbed macromolecules can be rearranged under the influence of either a static electric field [1–2] or electromagnetic radiation [3–4]. If an oblate metal spheroid is placed in an external uniform electric field that is directed along its axis of rotation, then charges will be induced on its surface, the distribution of which will differ significantly from the case of a polarized spherical metal nanoparticle: on the surface of an oblate spheroid when displaced from its center along the axis of rotation the surface charge density changes sharply, reaching values close to the maximum at a small distance from the neutral equator.

Using molecular dynamics simulation, conformational changes in polyampholytic polypeptides with different distances between oppositely charged units in the macrochain adsorbed on the surface of an oblate gold nanospheroid with a periodic change in time of its polarity along the axis of rotation were studied. At a low temperature and the lowest considered peak value of the dipole moment of an oblate nanospheroid in the absence and presence of sodium and chlorine ions, the conformational structure of the polypeptide changed from the initial enveloping nanoparticle to a conformation in the form of a narrow ring around the nanospheroid near the equator. At higher values of the peak value of the dipole moment of the nanospheroid, the macromolecular ring around the nanoparticle narrowed and swelled, and with a further increase in the amplitude of the polarizing alternating electric field, the desorption of the polypeptide occurred. The resulting conformational structures of polyampholytic polypeptides obtained from modeling with ions turned out to be similar to the conformations of the same polypeptides obtained from modeling without ions. At the same time, changes were observed on the distribution curves of the linear and radial atomic densities of polypeptides, associated with the partial neutralization of the charged subpolar regions of the nanospheroid by ions.

When modeling at high temperature, periodic changes in the conformational structure of adsorbed polyampholytic polypeptides on the surface of an oblate gold nanospheroid were observed at the frequency of an external polarizing alternating field. At times when the dipole moment of the nanospheroid was equal to zero, the conformational structure of the polypeptide was close to the starting conformation, completely enveloping the nanospheroid. And at times when the values of the dipole moment of the nanospheroid were maximum in absolute value, most of the charged units of the polypeptide were adsorbed on oppositely charged subpolar regions of the polarized nanospheroid. At the same time, most of the adsorbed charged amino acid residues were located on the edge of a vast subpolar region near the equator. The ejection of macrochain loops along the direction of the dipole moment vector of an oblate nanospheroid was also observed.

Within the framework of the quasi-stationary field approximation, an analytical model was constructed for the formation of the conformational structure of polyampholyte chain links interacting with the surface of an oblate nanospheroid polarized in an external alternating electric field. At sufficiently high frequencies of changes in the external field (but not violating the conditions of its quasi-stationarity), it is necessary to take into account the temporal dispersion of the permittivity of the metal of the nanoparticle. In the region of low frequencies, much lower than the plasma frequency of the metal, the formula obtained under conditions of a constant external field becomes valid for the field potential of a polarized nanospheroid. In the final expression of the model for the distributed density of polyampholyte chain links, the values responsible for the entropy effects of conformation formation and the effects of remote interaction of dipoles of polyampholyte links with an extended external field and the polarization field of the spheroid are singled out as separate factors. The calculation of the entropy factor is made in an approximate way by approximating the surface of the spheroid by fragments of the sphere. In this case, the correct account was taken of the curvature of the adsorbing surface over most of the compressed spheroid.

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**S1.89. Relationship between the equilibrium parameters of lysozyme adsorption on the surface of living cells of bacteria of various bacterial families and the kinetics of enzymatic lysis (destruction) of bacterial cells on the example of Escherichia coli, Bacillus subtilis and Lactobacillus plantarum**

Levashov P.A.[1]*, Rastriga N.V.[1], Smirnov S.A.[1]
[1]*Lomonosov Moscow State University, Department of Chemistry;*
* levashov@yahoo.com

For complex polymeric substrates, the importance of the type of enzyme adsorption has long been known. It can be either productive, i.e., preceding the catalytic stage of the process, or unproductive, in which it blocks the action of the enzyme. During the past two decades, new aspects have been discovered in association with a special form of bacterial resistance with respect to the action of antibacterial factors of the immune system. Increasingly, new molecular structures of the surface of bacterial cells are being revealed to be capable of purposely binding the antibacterial enzyme lysozyme, thereby blocking the mechanisms of bacterial disintegration during phagocytosis by an immune cell, which in turn blocks the process of neutralizing the bacterium and the subsequent presentation of bacterial antigens, the latter of which is necessary for the formation of specific antibodies.
In this study we examined the parameters of adsorption of the enzyme lysozyme directly on living whole bacterial cells under conditions of enzymatic lysis (destruction) of bacteria. Chicken-egg lysozyme, which is currently used in medical preparations and has a structure similar to that of human lysozyme, was used as a model enzyme. Gram-negative bacteria Escherichia coli of the Enterobacteriaceae family, Gram-positive spore-forming bacteria Bacillus subtilis of the Bacillaceae family, and Gram-positive Lactobacillus plantarum of the Lactobacillaceae family were studied as bacteria.
It was shown that the adsorption of lysozyme is reversible and that equilibrium is established in less than three to five minutes at a temperature of 37°C. Once equilibrium is established, the adsorption isotherms in all cases are satisfactorily approximated by the Langmuir equation. An analysis of the experimental data did not reveal the simultaneous presence of different lysozyme binding sites on bacterial cells, thus indicating that the equilibrium adsorption parameters differ significantly. With a different proportion of lysed cells, following incubations of a mixture of bacteria with lysozyme for 5 minutes and 20 minutes, respectively, the adsorption characteristics did not change in excess of the error margin, indicating that the nature of lysozyme adsorption on whole cells and on cell fragments is the same. The adsorption capacity is the maximum amount of lysozyme that can bind to bacterial cells under specified conditions (Amax). Amax, however, decreases as the pH value rises and falls from the activity optimum. Amax ranges from 2.7·10^7 to 1.5·10^8 lysozyme molecules per cell, depending on the type of bacteria. In the case of bacterial cells E. coli and B. subtilis, a twofold decrease in the rate of enzymatic cell lysis (VL) was found along with a decrease in the ionic strength of the solution in the range of 80 mM to 5 mM. The study of kinetic parameters showed that a decrease in the rate of cell lysis occurs due to an increase in the Michaelis constant (Km) but that the maximum rate of cell lysis (Vmax) remains unchanged. It was found that, with a decrease in ionic strength, the desorption constant of lysozyme (Kd) decreases while Amax remains unchanged. Kd decreases up to 10 times for E. coli (upto 1.2·10^-7 M), and Kd decreases up to three times for B. subtilis (upto 2.4·10^-7 M). An increase in the contribution of unproductive adsorption of lysozyme on bacteria E. coli and B. subtilis can be assumed but with decreasing ionic strength. In the case of L. plantarum cells, when the ionic strength of the solution changes in the range of 80 mM to 5 mM, there is no significant change in the adsorption parameters of lysozyme and VL. In the case of E. coli, an increase in VL by a factor of 1.5 to 2.0 is observed in the presence of free amino acids glycine, histidine and charged amino acids (Gly, His, Asp, Glu, Arg, Lys) at a concentration of 1–5 mM. In the presence of these amino acids, Amax does not change, but Kd decreases. Consequently, the additives that activate enzymatic lysis probably increase the contribution of productive sorption.
Bacterial infections of the Enterobacteriaceae and Bacillaceae families (e.g., typhoid fever, salmonellosis, bacillary dysentery, anthrax) are especially dangerous because these bacteria can bind the phagocyte lysozyme and use phagocytes as containers for spreading the bacteria throughout the body. Therefore, new information on effectors that prevent unproductive adsorption of lysozyme is particularly important in the development of new antibacterial drugs.
The work was performed at the Department of Chemical Enzymology of the Faculty of Chemistry of Lomonosov Moscow State University within the framework of the state task “Molecular design, structural and functional analysis and regulation of enzyme systems, cellular structures, bionanomaterials: fundamental foundations and applications in technology, medicine, environmental protection” Number CITIS: 121041500039-8.

**S1.90. Silicon analogues of biomolecules in the focus of drug design methods**

Kondratyev M.S.[1]*, Badalov A.A.[1]

[1]*Institute of Cell Biophysics RAS, FRC PNTsBI RAS, Pushchino, Russia;*
* ma-ko@bk.ru

Silicon-based compounds are often referred to in concepts describing non-carbon, alternative life forms. The electronic configurations of the valence shells of silicon and carbon are similar, silicon is tetravalent and is theoretically capable of forming complex, branched molecules. For these reasons, the study of silicon analogs of amino acids, sugars, components of nucleic acids, and a number of other biomolecules is of interest. To do this, we used the theoretical methods of computer modeling, which are usually used in exploratory studies in the search for new medical substances (drug design).
The primary optimization of the structures was carried out in HyperChem through molecular mechanics (AMBER). At the second stage, the structures of the studied molecules were calculated using the quantum mechanical (QM) package MOPAC2016 with PM7 parameterization. Also, geometric optimization and search for the energy minimum for the silicon and carbon peptides in alpha-helix and beta-sheet conformations, consisting of 12 Ala residues, were also carried out. When calculated in PM7, both for the alpha helix and for the beta sheet, the silicon peptide demonstrated a more pronounced thermodynamic stability than the carbon one, while the global extremum of the heat of formation of such a silicon molecule corresponds to the alpha helix conformation.
The results obtained make it possible to develop hypotheses about the fundamental possibility of the existence of systems in which silicon atoms form covalent bonds, forming a wide variety of conformationally labile structures. Such ensembles of atoms can be considered as analogues of terrestrial biomolecules, and it can be assumed that they have some similar functional activities.

### S1.91. Some curious features of kinetics of water freezing new view on the mechanism of biological activity of the antifreeze protein

Finkelstein A.V.[1,2,3]*, Melnik B.M.[1,2], Garbuzynskiy S.G.[1]
[1]*Institute of Protein Research of the Russian Academy of Sciences;*
[2]*Biotechnology Department of the Lomonosov Moscow State University;*
[3]*Biology Department of the Lomonosov Moscow State University,;*
* afinkel@vega.protres.ru

An examination of the freezing kinetics shows that (i) at small negative temperatures, the ice formation in bulk water takes enormous time and, therefore, can occur neither in lakes nor bodies; (ii) ice nucleation at small negative temperatures requires some ice-bonding surfaces, but (iii) even then, ice formation can typically occur at temperatures a few degrees below 0°C. However, some surface shapes can initiate the ice formation at virtually zero temperatures. Antifreeze proteins are responsible for the adaptation of organisms to low temperatures, but the mechanism of their action was still unclear. By examining the effect of the ice-binding protein cfAFP on the freezing point of water and the melting point of ice, we showed that the task of the antifreeze protein is not, as it was commonly believed, to bind to ice crystals that have been already formed and stop their growth, but to bind to the "ice-nucleating" surfaces of the cell where ice nuclei can form, screen then, and thereby completely prevent the ice formation.

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### S1.92. Spatial Structure of Soymorphin-6 Molecule

Agayeva L.N.[1]*, Abdinova A.A.[2], Akhmedova S.R.[3], Akhmedov N.F.[1], Akhmedov N.A.[1]
[1]*Baku State University, Institut for Physical Problems;*
[2]*2Azerbaijan State Pedagogical University;*
[3]*3Azerbaijan Technical University;*
* leylanamiq@mail.ru

Opioid peptides are of animal and plant origin. A number of exogenous peptides, obtained from food, have opiate-like properties. Such peptides were named exorphins. The discovery of the opioid activity of the peptide components of food has led to the assumption that certain types of food can act on the central nervous system like opiate drugs. Exorphins have been isolated from various plant species. Soymorphins-5, -6 and -7 derivatives of soybean β-coglycinin have been discovered relatively recently. Soymorphins are formed in noticeable amounts during the digestion of soy: when the β-subunit of soybean β-coglycinin is cleaved by pancreatic elastase in vitro, soymorphin-5 makes up 9.1% of all products.

We have studied the structural and functional organization of opioid peptides, and at present the spatial structure of exorphins is being studied. This work is a continuation of our previous studies.

The soymorphine-6 molecule was calculated using the method of theoretical conformational analysis. The potential function of the system is chosen as the sum of non-valence, electrostatic and torsion interactions and the energy of hydrogen bonds.

The spatial structure of the soymorphine-6 molecule (Tyr1-Pro2-Phe3-Val4-Val5-Asn6-NH2) was calculated based on the low-energy conformations of soymorphine-5 and N-acetyl-L-asparagine methylamide. The results of the calculation of the soymorphine-6 molecule showed that energy differentiation occurs between the forms of the main chain and conformations. Conformations of eight shapes fall into the energy interval 0-30 kJ/mol. In low-energy conformations, the energy of non-valence interactions varies in the range (-127.3) - (-90.3) kJ/mol, electrostatic interactions 6.3-10.5 kJ/mol, torsion interactions 10.1-16.8 kJ/mol. The global conformation of the soymorphin-6 molecule is B3RR1R2R2R11 of the effff shape. In this conformation, only the first Tyr1 residue is in the B form of the main chain, the remaining residues are in the R form of the main chain and form a helical structure. The side chains of amino acid residues are directed in such a way that effective interactions occur between the atoms of the main chain and the atoms of the main and side chains. Hydrogen bonds are formed between the C=O Pro2 and N – H Val5 atoms; between C=O Phe3 and N – H atoms of the C-terminal group of NH2. In this conformation, the contribution of non-valent interactions is greatest, and due to non-valent interactions, the conformation is global. It is unfavorable in terms of electrostatic interactions; the contribution of electrostatic repulsions is the largest. The B3RB1R2R2B31 conformation of the efeff shape with a relative energy of 0.8 kJ/mol differs from the global conformation in the shape of the Pro2 backbone and in the arrangement of the Tyr1 and Phe3 side chains in space. The conformation is favorable for electrostatic interactions, electrostatic repulsions are the smallest. The B3RR1R2B2R21 conformation of the efffe shape has a relative energy of 11.3 kJ/mol. Here, the first residue of Tyr1 and Val5 is in the B form of the main chain, the remaining amino acid residues are in the R form of the main chain and form a semi-folded structure. In the B3RR3B2B2B31 conformation of the effee shape, the N-terminal dipeptide fragment Tyr1-Pro2 and the C-terminal tripeptide fragment Val4-Val5-Asn6 are in the unfolded form of the main chain, Pro2-Phe3 forms a bend, and effective interactions are created between these regions.

In the B2BB2B2B2B31 conformation of the eeeee shape, all amino acid residues are in an unfolded form, the side chains of all amino acid residues, except for Asn6, are oriented in the same direction. The conformation is favorable in terms of electrostatic interactions and has a relative energy of 19.3 kJ/mol. The B3RB3B2B2B31 conformation of the efeee shape differs from the previous one in the shape of the Pro2 main chain and in the position of the Tyr1 and Phe3 side chains; therefore, its relative energy is only 1.2 kJ/mol higher than that of the fully unfolded form.

In the B1BB2R2R2R33 conformation of the eeeff shape with a relative energy of 27.3 kJ/mol, the first three amino acid residues of Tyr1-Pro2-Phe3 are in a completely unfolded form, and the next three amino acid residues are in a completely folded form of the main chain. The B1BR2B2B2B31 conformation of the eefee shape has a relative energy of 28.6 kJ/mol and differs from the fully unfolded conformation in the shape of the Phe3 backbone. The N-terminal dipeptide fragment and the C-terminal tripeptide fragment are completely unfolded and are separated from each other by the third amino acid residue phenylalanine.

Thus, the spatial structure of the soymorphine-6 molecule can be represented by eight structural types, and it can be assumed that the molecule performs its physiological functions in these structures. Based on these structures, it is possible to propose its synthetic analogues and calculate the spatial structure of the soymorphine-7 molecule. The theoretical conformational analysis of the soymorphine-6 hexapeptide molecule led to such a structural organization of the molecule that does not exclude the implementation of a number of functions by the molecule that require strictly specific interactions with various receptors.

### S1.93. Stabilisation of the full-length S-protein of SARS-Cov-2 in SMA polymer for electron microscopy studies

Mamaeva N.[1], Glukhov G.[1], Novoseletsky V.[1], Derkacheva N.[2], Sokolova O.S.[1]*
[1]*Moscow Lomonosov University, Faculty of Biology;*
[2]*A.I. Evdokimov Moscow State Medical and Dental University, Moscow, Russia;*
* sokolova184@gmail.com

The study of integral membrane proteins is one of the main objectives of modern research in structural biology. In native conditions, membrane proteins are embedded in the lipid bilayer, which is involved in maintaining the stability of their structure and is necessary for their proper functioning. However, for detailed structural and functional studies, membrane proteins need to be isolated from the lipid medium while maintaining their stability and activity. Therefore, purification of membrane proteins is more challenging than purification of soluble proteins. The full-length S-protein of the wild-type coronavirus SARS-CoV-2 (strain Wuhan-Hu-1), which is the causative agent of COVID-19, was chosen as the object of study in this work. Because of its functions, the S-protein is one of the most important targets for COVID-19 vaccine and therapeutic research. This work aims to study the conformational variability of the coronovirus S-protein and the possibility of obtaining the structure of a full-length wild-type S-protein as part of lipodiscs.
The full-length S-protein of coronavirus SARS-CoV-2 is a very flexible molecule due to the need to bind ACE2 cell receptors. Molecular modelling of the full-length S-protein showed that the major conformational changes of the S-protein occur at two points of the spike-protein "steam", which form three axes of rotation. The use of styrene maleic anhydride (SMA) as a solubilizing agent is a promising method for purifying membrane proteins to obtain three-dimensional structures using electron microscopy techniques. In this work, we for the first time used SMA to purify the S-protein of coronovirus and obtained a reconstruction with a resolution of 2.4 nm .
This research has been supported by the Interdisciplinary Scientific and Educational School of Moscow Lomonosov University «Molecular Technologies of the Living Systems and Synthetic Biology»

### S1.94. Stability and kinetic characteristics of bacterial luciferases at various temperatures

Deeva A.A.[1]*, Sukovatyi L.A.[1], Lisitsa A.E.[1], Melnik T.N.[2], Nemtseva E.V.[1,3]
[1]*Siberian Federal University;*
[2]*Institute of Protein Research RAS;*
[3]*Institute of Biophysics SB RAS;*
* adeeva@sfu-kras.ru

Luciferases are responsible for light emitting reactions in luminous organisms, among which bacteria are the most widespread in nature. They can be isolated both from the tropical waters of the global ocean, and in northern latitudes, in fresh water and on land. However, bacterial luciferases (BLs) belong to thermolabile enzymes, which undergo inactivation at ~37°C [1]. This makes it difficult to implement them as reporters or labels for in vivo molecular analysis, as well as biosensor elements in field studies.The stability of BL to thermal inactivation in vitro could vary depending on the bacterial species from which it was isolated. A recent phylogenetic analysis of BL amino acid sequences showed that they fall into two highly homologous groups [2]. Each group comprises BLs with the similar type of decay kinetics: “fast” or “slow”. Several "slow" luciferases are characterised by relatively high thermal stability, while among the "fast" luciferases, psychrophilic enzymes can be found more often. Thus, the study of the influence of different temperatures on the activity and structure of BLs with different types of kinetics is a topical issue considering their application in analytical methods.
This research aimed to reveal the effect of temperature on the reaction kinetics and structure of two types of bacterial luciferases: "slow" Vibrio harveyi and "fast" Photobacterium leiognathi. In addition, the role of natural extremolite (sucrose) in maintaining the activity of these enzymes under unfavorable temperature conditions was studied.
The following characteristics of two types of BLs were experimentally studied at different temperatures: (1) activity, (2) the rate of thermal inactivation, and (3) heat-induced denaturation. The reaction kinetics of V. harveyi and P. leiognathi luciferases (OOO Biolumdiagnostics) at a temperature of 5–45 °C was measured by the stopped flow method on an SX-20 analyzer (Applied Photophysics). The reaction components were incubated at a given temperature for 5 min before measuring the kinetics. The thermal inactivation rate of the luciferases was estimated by measuring the remaining activity of the enzymes (at 20 °C) after their incubation for various times under the required temperature in the range 40–55 °C. Solid-state thermostat Gnom (DNA technology) was used for enzyme incubation. Calorimetric measurements were made using a precision scanning microcalorimeter SCAL-1 (Scal Co. Ltd.) at a scanning rate of 1 K/min and under 2.5 atm pressure. In addition, three 100-ns runs of molecular dynamics (MD) simulations were performed for both enzymes at 5, 10, 27, 45, and 60 °C using the GROMACS 2020.4 software package.
The study of reaction kinetics at 5–45 °C revealed the different sensitivity of two luciferases to the temperature of solution. In particular, P. leiognathi luciferase responds by the pronounced activity change for each temperature shift of 5 ◦C, while V. harveyi luciferase provides about the same peak intensity within a wide range of 20–35 °C. The results of MD showed that a higher activity of P. leiognathi luciferase at low temperatures can be due to the peculiarities of the mobile loop dynamics, which forms the active center. The structure of V. harveyi luciferase was more stable at 60°C, as evidenced by the lower standard deviation of the RMSD parameter during the simulation. The observed thermal lability of P. leiognathi luciferase and its higher activity under optimal conditions (at 20–25°C) as compared to V. harveyi luciferase are consistent with the idea of the structural and functional features of cold-adapted proteins.
In addition to activity, the structural stability of proteins, which plays an important role in maintaining their function under temperature changes, was studied. It was found that under the same conditions, the rate of thermal inactivation of V. harveyi luciferase is always lower than that of P. leiognathi enzyme. Sucrose slows down the thermal inactivation of the enzymes by a factor of two to four times, but without a significant reduction in the activation energy of the process, which in buffer and sucrose solution was 237 ± 30 and 224 ± 7 kJ/mol for V. harveyi luciferase, and 255 ± 27 and 243 ± 47 kJ/mol for P. leiognathi enzyme, respectively. The revealed effect of sucrose may be associated with a decrease in mobile loop oscillations at high temperatures, which was observed during MD. The study of denaturation of BLs using differential scanning calorimetry showed that the melting temperature of V. harveyi luciferase is higher than that of P. leiognathi enzyme (47.0 and 45.4 °C, respectively). In the presence of sucrose, the melting point of both enzymes increases to 50.1 and 52.0 °C for P. leiognathi and V. harveyi, respectively. Thus, both approaches showed that V. harveyi luciferase is more thermostable than P. leiognathi, and sucrose is able to protect proteins from thermal inactivation and denaturation.
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### S1.95. Structural study of SARS-COV-2 envelope protein E by AFM method

Gifer P.K.[1,2]*, Batishchev O.V.[2]
[1]*Moscow Institute of Physics and Technology;*
[2]*IPCE RAS;*
* gifer.pk@phystech.edu

The COVID-19 pandemic is one of the most significant epidemics in recent history. Understanding the physicochemical mechanisms of interaction between the SARS-CoV-2 coronavirus and the host cell plays an important role in finding strategies to combat the virus. Coronaviruses are enveloped viruses whose lipid bilayer typically includes three proteins: spike protein (S), membrane protein (M), and envelope protein (E). These proteins are being studied for their important role in receptor binding and virion budding. While some of the coronavirus proteins are well characterized, others remain poorly understood.
Coronaviruses, including SARS-CoV-2, express an envelope protein (E) involved in many aspects of the virus life cycle. The E protein of coronaviruses is a small 76 amino acid integral membrane protein with one putative transmembrane α-helical hydrophobic domain 20-30 amino acids long, surrounded by a short N-terminus (<10 amino acids) and a longer C-terminal tail.
The E protein promotes virus packaging and propagation and the removal of this protein reduces or even eliminates virulence. Another proposed role of the E protein is the stimulation of apoptosis. It has also been found that this protein oligomerizes to form a pentameric structure that displays ion channel activity. A full understanding of the role of the E protein in the penetration of the virus into the cell, replication and formation of progeny virions requires knowledge of the structure and physicochemical properties of this protein. Atomic force microscopy (AFM) makes it possible to visualize the dynamics of adsorption, diffusion and interactions of various biomolecules in their natural liquid medium at the molecular level in real time. In this study the structure of the E envelope protein of SARS-CoV-2 was studied using high-resolution AFM under physiological conditions. The purpose of this work was to elucidate the mechanism of self-organization of the E protein of the coronavirus and the conditions for the formation of pores in the lipid envelope of the virus. The results of the studies showed that the appearance of pores occurs even from protein monomers. It has also been demonstrated that pentamers are formed at protein concentrations of 100 nM and higher.
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### S1.96. Structural Analysis of Fibril-Forming Peptides SEM1(86-107) and SEM1(68-107) Involved in Increased Hiv Infectious Activity

Osetrina D.A.[1]*, Yulmetov A.R.[1], Bikmullin A.G.[1], Klochkov V.V.[1], Blokhin D.S.[1]
[1]*Kazan Federal University;*
* d.sanchugova@yandex.ru

The human immunodeficiency virus (HIV) is one of the most dangerous diseases that affects the cells of the human immune system. Over time, HIV causes acquired immunodeficiency syndrome (AIDS), which can lead to susceptibility to various infections and tumors and eventually death. Since the main route of transmission of the virus is unprotected intercourse, seminal fluid is considered as the main factor in increasing HIV activity.
The main component of the seminal fluid is the semen coagulum, which is mainly composed of spermatozoa and various proteins, including the protein semenogelin 1 (SEM1). It is secreted in the seminal vesicles, and during ejaculation, SEM1 is cleaved into small peptide fragments by internal proteases. Some of these semenogelin1 peptide fragments (SEM1(86-107), SEM1(68-107), SEM1(49-107) and SEM1(45-107)) have been shown to form amyloid fibrils that increase HIV infectivity. To understand the process of fibril formation and the structure of fibrils, information on the spatial structure of the peptides forming them is required.
In this work, peptide fragments of semenogelin1 SEM1(86-107) and SEM1(68-107) were studied by high-resolution nuclear magnetic resonance (NMR) spectroscopy, circular dichroism (CD) spectroscopy, and SEM1(68-107) was studied by the method molecular dynamics (MD). Analyzing 1D, 2D, 3D NMR spectra, we obtained the values of chemical shifts of the 1H, 13C, 15N nuclei, as well as geometric restrictions, which were used as input data for calculating the spatial structures of the peptides SEM1(86-107) and SEM1(68-107) using molecular modeling by the method of nonlinear annealing, as well as for SEM1(68-107) all-atom MD modeling was performed. According to the results of the study, the established spatial structures of the peptides were included in international databases.
The information obtained will help identify the regions of the SEM1(86-107) and SEM1(68-107) peptides that are directly involved in the formation of amyloid fibrils.
The work was supported by the Russian Science Foundation (project №. 20-73-10034).

### S1.97. Structural Organization of the Rubiskolin-6 Molecule

Akhmedov N.A.[1]*, Agayeva L.N.[1], Abbasli R.M.[1], Ismailova L.I.[1]
[1]*Baku State University, Institute for Physical Problems;*
* Namiq.49@bk.ru
Structural Organization of the Rubiskolin-6 Molecule
Akhmedov N.A., Agayeva L.N., Abbasli R.M, Ismailova L.I.
Baku State University, Institute for Physical Problems
Z. Khalilov Str.23, Baku, Azerbaijan
e-mail: Namiq.49@bk.ru

Opioid peptides are of animal and plant origin. A number of exogenous peptides obtained from food have opiate-like properties. Such peptides have been named exorphins. Exorphins have been isolated from various plant species. Rubiscolins were first isolated from spinach leaves. However, the degree of homology of the large RUBISCO subunit in different species of higher plants is extremely high (more than 90%), and the content of RUBISCO in green leaves is up to 50% of the total protein. Consequently, rubiscolins in significant amounts can enter the body when eating not only spinach, but also lettuce, sorrel, parsley, etc. The green leaves of these plants are an important component of a balanced diet, so studies of the structural organization of rubiscolins are of practical value. We have studied the structural and functional organization of opioid peptides, and at present the spatial structure of exorphins is being studied. This work is a continuation of our previous studies.
The calculation of the molecule was performed using the method of theoretical conformational analysis. The potential function of the system is chosen as the sum of non-valent, electrostatic and torsion interactions and the energy of hydrogen bonds. Non-valent interactions were evaluated using the Lennard-Jones potential. Electrostatic interactions were calculated in the monopole approximation according to the Coulomb law using partial charges on atoms. The conformational capabilities of the rubiscolin molecule were studied under conditions of an aqueous environment; therefore, the value of the dielectric permittivity was taken equal to 10. The energy of hydrogen bonds was estimated using the Morse potential.
The three-dimensional structure of the rubiscoline-6 (Tyr1-Pro2-Leu3-Asp4-Leu5-Phe6-NH2) molecule was studied fragmentarily. First, on the basis of low-energy conformations of the corresponding amino acid residues, the spatial structure of the pentapeptide molecule of

rubiscoline-5 (Tyr1-Pro2-Leu3-Asp4-Leu5-NH2) was studied and its stable conformations were determined. At the second stage, based on the low-energy conformations of the rubiscoline-5 molecule and the Phe6 amino acid residue, the spatial structure of rubiscoline-6 was calculated. The results of the calculation showed that there is an energy differentiation between conformations, forms of the main chains and shapes. Conformations of eight shapes fall into a wide energy range of 0-60.0 kJ/mol. The energy of non-valent interactions in low-energy conformations varies in the energy range (-139.9) – (-103.7) kJ/mol, electrostatic interactions (-13.9)–9.7 kJ/mol, torsion interactions 13.9-23.1 kJ/mol. The global conformation of the rubiscolin-6 molecule is the B3RR12R1R21R3 conformation of the effff shape. The conformation is favorable both for non-valent and electrostatic interactions. Here, only the first amino acid residue of Tyr1 is in the form of the main chain, the remaining amino acid residues of the Pro2-Leu3-Asp4-Leu5-Phe6 region are coiled. Therefore, effective interactions arise here between the atoms of the main chain and between the atoms of the side chains. Hydrogen bonds arise between the N-H atom of the main chain of Tyr1 and the C=O atom of the side chain of Asp4: between the C=O atom of the main chain of Tyr1 and the N-H atom of the main chain of Leu5; between the C=O atom of the Pro2 main chain and the N-H atom of the Phe6 main chain, which also stabilize the global structure. In this conformation, the side chains of all amino acid residues are directed from the molecule to the solvent and can easily interact with other molecules and receptors.

In the B3RR21B3B32B3 conformation, a structure is formed in which Tyr1 and Leu3 effectively interact with atoms of other amino acid residues. Amino acid residues Pro2, Asp4, and Leu5 effectively interact with neighboring amino acid residues.

The B2RB21B3B32B3 conformation of the efeee shape has a relative energy of 26.0 kJ/mol. This conformation is mainly stabilized by di- and tripeptide interactions. The relative energy of the other low-energy conformations of the rubiscolin-6 molecule presented in Table 1 varies in the energy range (38.6-52.5) kJ/mol. In all conformations, the contribution of non-valent interactions is less than in the previous conformations. They have electrostatic repulsions. The contribution of electrostatic interactions is positive for them.

Thus, the spatial structure of the rubiscolin-6 molecule can be represented by four structural types, and it can be assumed that the molecule performs its physiological functions precisely in these structures. Based on the obtained three-dimensional structures, it can be assumed that this molecule has its synthetic analogues. The theoretical conformational analysis of the hexapeptide molecule of rubiscolin-6 led to such a structural organization of the molecule that does not exclude the implementation of a number of functions by the molecule that require strictly specific interactions with various receptors.

### S1.98. Structural and functional organization study of hydrobionts dermal covers fibrillar proteins

Tarasova D.V.[1]*, Lihodzievskaya M.V.[1], Borodina M.M.[1], Antipova L.V.[2], Artiukhov V.G.[1], Antipov S.S.[1]
[1]*Voronezh State University;*
[2]*Voronezh State University of Engineering Technologies;*
* di48880@gmail.com

Collagen is the main protein of connective tissue, consisting of three subunits in the form of an alpha helix. The three-dimensional structure of collagens is studied using both bioinformatic approaches and diffraction methods. More than 20 types of collagens are known, while in mammals most of the proteins of this family are type I collagen. However, the question of the structural and functional organization of hydrobiont collagens remains open. Thus, only a limited number of structures of animal collagen molecules has been published in the PDB database, and data on the structure of collagen molecules in aquatic organisms are completely absent. Therefore, the study of the structural and functional organization of hydrobiont collagen is of even more acute scientific and applied interest.

The starting point of this work was the assessment of the amino acid composition of the dermal integument of the African catfish (Clarias gariepinus). The highest content was noted for nonpolar aliphatic amino acids, namely, for glycine (33.27%), proline (18.49%), and alanine (10.37%), and the lowest for tryptophan (0.2%) and tyrosine (0.34%), while the presence of cysteine was not was recorded. In general, the content of aromatic amino acids in the dermal integument of the African catfish was insignificant, which is typical for tissues consisting mainly of collagen proteins.

At the next stage, a solution of African catfish dermal integument proteins was obtained, having a pH of 5.4, containing a mass fraction of protein - 1.10%, a mass fraction of fat - 0%, and a mass fraction of carbohydrates - 0.7%. During the preparation of such a solution, the content of individual amino acids was redistributed, in particular, a decrease in the content of arginine from 4.08% to 0.05% and proline from 18.4% to 0.09% was registered, and the presence of tryptophan was not detected. It is interesting that the content of proline in the dermal integument was one of the most significant. The main difference in the amino acid composition of hydrobiont and cattle collagens is arginine and proline, the content of which differs by several orders of magnitude.

If in the resulting emulsion most of the proteins belong to collagens, then the differences in the content of some amino acids in the samples obtained from hydrobionts and from mammals are quite obvious due to differences in the habitat. Therefore, an assessment was made of the profile of the proteins present in the samples. These data indicate that proteins corresponding to molecular masses from 120 to 212 kDa and higher are present in the solution of proteins in the dermal integument of the southern catfish. This indicates that even under conditions of denaturing electrophoresis, a significant part of the drug is a high molecular weight protein. The presence of bands on the electrophoregram, corresponding in their mobility to a molecular weight of less than 200 kDa, raises doubts about their belonging to collagens.

Next, the charges of backs present in the samples were evaluated using electrophoretic fractionation under native conditions from the cathode to the anode and from the anode to the cathode. The data obtained indicate that all proteins of the emulsion have a negative charge, since the registered protein bands migrated only to the positive pole. The weak anion exchange resin Sephadex DEAEA-25 was used for chromatography. Two maxima were recorded, corresponding to 36 and 67 ml of elution. The data obtained indicate that the proteins present in the emulsion have different affinities for the resin, with the intensity of the first maximum approximately 4.5 times higher. This allows us to draw an analogy with mammalian collagens, in which a significant part of collagens is collagen type I. The results of electrophoretic fractionation of samples obtained by chromatography indicate that the highest protein content falls on fractions 4, 5, and 6, the molecular weight of which is over 120 kDa, which also supports the assumption that these proteins may belong to the type I collagen group. To identify proteins of the collagen family, these fractions were treated with collagenase from Clostridium histolytica for 1, 6, and 24 h at 37°C. The results obtained are consistent with the assumption that these proteins belong to the collagen family. The most interesting fact is that after 1 hour of incubation in the presence of collagenase, proteins with a molecular weight in the range from 150 to 200 kDa, the collagen nature of which was questioned, undergo enzymatic hydrolysis. At the same time, the general tendency to cleavage of all proteins present in all fractions is preserved. Thus, it can be concluded that all proteins present, with a high degree of probability, belong to collagens.

The overall assessment of the structural and functional properties of proteins gives grounds to consider the resulting emulsion as a source of functional biopolymers, in particular, collagen proteins. During the research, the following were determined: fractional composition,

charge state, molecular weight, ability to hydrolyze under the action of C. histolytica collagenase. Evaluation of the amino acid composition makes it possible to reveal the analogy and features of collagen proteins of animal and fish origin. It has been established that the studied emulsions contain negatively charged proteins with a molecular weight of over 100 kDa, the fractions of which contain predominantly collagen. Data have been obtained that support the assumption that a significant part of hydrobiont collagens belongs to type I collagen, which makes it possible to consider them in the future as a basis for various substances and serve as a starting point for understanding the physicochemical essence of functioning and evaluating the possibility of using them in technologies medicine and cosmetology.
The study was supported by the Ministry of Science and Higher Education of Russia under agreement N 075-15-2021-1351

**S1.99. Structural basis of the signal transduction via transmembrane domain of bitopic receptors studied by high-resolution NMR**

Bocharov E.V.[1,2]*
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS;*
[2]*Moscow Institute of Physics and Technology ;*
* edvbon@mail.ru

Signal transduction by bitopc receptors of type I, such as receptor tyrosine kinases (RTK) and cytokine receptors (utilizing Janus kinase), has been in the spotlight of scientific interest owing to the central role of these single-spanning membrane receptors in the regulation of development, cell motility, proliferation, differentiation, and apoptosis. The representatives from the epidermal growth factor receptor (ErbB) and insulin receptor (InsR) families, as well as growth hormone receptor (GHR) serve as excellent models of bitopic receptor to illustrate how ligand-induced conformational rearrangements and specific dimerization of extracellular domains lead to the allosteric activation of the cytoplasmic domains, resulting in signal propagation across the membrane. Dysregulated signaling from these receptors has been shown to play significant roles in promotion of number of human diseases, and inhibitors of these receptors have been among the most successful examples of targeted therapies to date. Many essential aspects of ErbB, InsR and GHR signal transduction at the molecular level have been elucidated lately. Nevertheless, there are several issues yet to be resolved, including the particular role of single-span helical transmembrane domain (TMD) and flexible juxtamembrane regions in the receptor activity switching in terms of an apparent loose coupling between structural rearrangements of the extracellular and intracellular regions. In this work the alternative dimeric conformations of the ErbB, InsR and GHR TMDs were experimentally determined in different membrane-mimicking environments using high-resolution NMR spectroscopy combined with MD-relaxation in explicit lipid bilayer. Based on the available mutagenesis data, observed conformations correspond to the dormant and active states of the receptors. Fine adaptation of intermolecular polar and hydrophobic contacts that was found to accompany the different ErbB TMD dimerization modes (observed in detergent micelles or in lipid bicelles) suggests that certain membrane properties can govern the TMD helix-helix packing and, thus, their alteration can trigger the receptor state. The conformational variability of TMDs among insulin family receptors and their interactions with surrounding lipids were found to differ significantly between these three representatives, suggesting a unique molecular mechanism of the receptor activation distinct in some details from other RTKs. Whereas two distinct dimeric modes of GHR TMD (coexisting in micellar environment) revealed the functional role of juxtamembrane region rearrangements in alternation between protein-protein and protein-lipid interactions that can be initiated by ligand binding. Observed the TMD helix-helix packing diversity appears in favor of the lipid-mediated activation mechanism, which implies that the sequence of structural rearrangements of ErbB, InsR and GHR domains is associated with perturbations of the lipid bilayer in the course of ligand-induced receptor activation, considering the receptor together with its lipid environment as a self-consistent signal transduction system. The proposed mechanism explains a number of paradoxes observed during the activation of wild-type receptors, as well as receptors with pathogenic mutations.
The work is supported by the Russian Science Foundation (project no. 23-44-10021).

**S1.100. Structural changes in chromatin are reflected in the surface features of mechanically deformed nuclei observed by atomic force microscopy**

Bairamukov V.Yu.[1], Kovalev R.A.[1], Fedorova N.D.[1], Pantina R.A.[1], Iashina E.G.[1], Grigoriev S.V.[1], Varfolomeeva E.Yu.[1]*
[1]*Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre «Kurchatov Institute»;*
* e_varf@mail.ru

Chromatin inside the nuclei is highly organized. Thus, heterochromatin is often separated from transcriptionally active euchromatin, which leads to the formation of compartments. Euchromatin itself is organized into transcriptionally inactive domains interspersed with foci of transcriptional activity [1]. At the same time, it is believed that the actively transcribed DNA is less densely packed.
Currently, approaches to direct measurements of transcriptionally active chromatin stiffness are very limited. The use of additional methods of direct observation, even those related to the need for specific processing of nuclei, could expand our understanding of the principles of the organization of cell nuclei. Isolated cell nuclei fixed in suspension and placed on a substrate are often characterized by a relatively smooth shape without distinctive morphological features. Obviously, in this form it is impossible to use AFM to study the internal structure of the nuclei. But the natural elasticity of the nuclei allowed us to implement a new approach to the study of the organization of the nuclear structure through AFM. Mechanical action (centrifugal acceleration) transforms the features of the internuclear organization into a change in the relief of the nuclei surface.
The nuclei of normal fibroblast cells were completely flattened by mechanical action, whereas the nuclei of HeLa cancer cells were extremely stable. In the deformed HeLa nuclei, AFM revealed a highly branched landscape assembled from ~400 nm globules with a closed package, and their structure changed in response to external influences. Isolated nuclei of normal and cancer cells differed strikingly in the resistance of DNA to mechanical action. Paradoxically, the more transcriptionally active and less optically dense chromatin of cancer cell nuclei demonstrated higher physical rigidity. The high concentration of the transcription inhibitor actinomycin D led to a complete flattening of the HeLa nuclei, which may be due to the relaxation of supercoiled DNA prone to deformation [2].The action of topoisomerase inhibitors I and II led to the removal of supercoiling and significant flattening of the nucleus, whereas the action of the DNA intercalator, on the contrary, led to an increase in supercoiling and, as a consequence, chromatin stability to mechanical action [3].
We have shown that the surface relief is a consequence of the structural features of the nuclei. The nature of chromatin's high resistance to deformation is due to DNA supercoiling. And the resistance to deformation of nuclear chromatin correlates with fundamental biological processes in the cell nucleus, such as transcription.
1. Transcription organizes euchromatin via microphase separation Lennart Hilbert, Yuko Sato, Ksenia Kuznetsova, Tommaso Bianucci, Hiroshi Kimura,Frank Jülicher, Alf Honigmann, Vasily Zaburdaev & Nadine L. Vastenhouw NATURE COMMUNICATIONS | (2021) 12:1360 | https://doi.org/10.1038/s41467-021-21589-3|

2. AFM imaging of the transcriptionally active chromatin in mammalian
cells' nuclei V.Yu. Bairamukov, M.V. Filatov, R.A. Kovalev, N.D. Fedorova, R.A. Pantina,A.V. Ankudinov, E.G. Iashina, S.V. Grigoriev, E.Yu. Varfolomeeva, BBA - General Subjects 1866 (2022) 130234
3. Structural Peculiarities of Mechanically Deformed HeLa Nuclei Observed by Atomic-Force Microscopy V. Yu. Bairamukov, M. V. Filatov, R. A. Kovalev, R. A. Pantina, S. V. Grigoriev, E. Yu. Varfolomeeva Structural Peculiarities of Mechanically Deformed HeLa Nuclei Observed by Atomic-Force Microscopy. J. Surf. Investig. 16, 854–859 (2022). https://doi.org/10.1134/S1027451022050263

### S1.101. Structural organization of chromatin proteins HMGB1, H1 and their complexes

Chikhirzhina E.[2], Polyanichko A.[1,2]*
[1]*Saint-Petersburg State University;*
[2]*Institute of Cytology RAS;*
* a.polyanichko@spbu.ru

DNA in the nucleus of a eukaryotic cell is a part of DNA-protein complex, which provides not only functioning of DNA, but also, if necessary, a high degree of compaction. DNA-binding proteins in the cell nucleus can be roughly divided into two large groups: histone and non-histone chromatin proteins. The former include core histones (H2A, H2B, H3, and H4), which form nucleosomal core particle around which the DNA double helix is twisted, and the linker histone H1, which interacts with DNA at internucleosomal (linker) sites. Among the remaining (i.e., non-histone) chromatin proteins, the most numerous are representatives of an extensive group of proteins with high electrophoretic mobility (High Mobility Group, or HMG), some of which, the so-called HMGB proteins, also function in the internucleosomal regions of chromatin. These proteins are actively involved not only in the regulation of chromatin structure, but are also directly involved in many cellular processes such as transcription, repair, recombination, etc.
The functions of linker proteins are closely related to their conformational state. Currently, the structure of proteins that play a key role in the formation of higher levels of chromatin structural organization is being actively studied. In this work, a comparative analysis of the secondary structure of the linker histone H1 and the non-histone protein HMGB1 was carried out. Using UV circular dichroism and FTIR spectroscopy, it was shown that the positively charged histone H1 binds to the C-terminal fragment of HMGB1, stabilizing the resulting complex and inducing the formation of additional α-helical regions in both proteins. It has been shown that the interaction of HMGB1 and H1 proteins very quickly leads to the formation of fairly large scattering complexes in solution, which makes it difficult to analyze CD spectra. However, even such relatively large protein complexes do not scatter light in the infrared region of the spectrum, which makes it possible to analyze the secondary structure of the proteins in the complex from their IR absorption spectra.
Based on the analysis of the IR spectroscopy data, we assumed that the initial formation of the complex occurs due to the electrostatic interaction between the negatively charged C-terminal region of HMGB1 and the positively charged groups of H1 histone. The subsequent change in the secondary structure of the proteins in the complex leads to the formation of additional α-helical regions in both proteins.
The work was performed using the equipment of the resource centers of the Science Park of St. Petersburg State University ("Centre for Optical and Laser Materials Research", "Center for the Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics", "Cryogenic Department").

### S1.102. Structural studies of human semen amyloid peptides

Osetrina D.A.[1], Abramova M.O.[1], Kusova A.M.[1], Yulmetov A.R.[1], Bikmullin A.G.[1], Klochkova E.A.[1], Klochkov V.V.[1], Blokhin D.[1]*
[1]*Kazan Federal University;*
* dblohin@kpfu.ru

Amyloid fibrils are ordered aggregates of proteins and their fragments. Amyloids are involved in more than 20 human diseases, including Alzheimer's, Parkinson's, and HIV infection [1]. It has been found that semen amyloid fibrils increase the probability of HIV infection by 105-106 times, by reducing the electrostatic repulsion between the HIV virion and the target cell [2]. Semen amyloid fibrils consist of peptide fragments of three semen proteins: prostatic acid phosphatase (PAP), semenogelin 1 (SEM1), and semenogelin 2 (SEM2) [3]. Nine peptide fragments were identified: PAP(248-286), PAP(85-120), SEM1(45-107), SEM2(45-107), SEM1(49-107), SEM2(49-107), SEM1(68 –107), SEM2(68–107), and SEM1(86–107). Structural studies of amyloid fibrils by experimental methods are difficult. Therefore, the main method for their study is molecular simulations of amyloids. To run the calculations, the structure of the peptides is required. In this regard, for the study of amyloids, an actual task is to establish the spatial structure of amyloid peptides.
Our work is devoted to structural studies of human semen amyloid peptides. To study peptides in solution, the methods of nuclear magnetic resonance spectroscopy, circular dichroism spectroscopy, and molecular dynamic simulations were used. We are defined the spatial structures of peptides: PAP(85-120), SEM1(86-107), SEM1(68-85), SEM1(68-107), and the structure of the SEM1(86-107) peptide in solution with dodecylphosphocholine micelles. Micelles acted as a model of membrane charged surface. The obtained results are planned to be further applied to the simulations of semen amyloids.
The work was supported by the Russian Science Foundation (project no. 20-73-10034).
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### S1.103. Structure and properties of polysaccharide hydrogels for delivery of therapeutic enzymes, vitamins and diagnostic markers

Bogdanova L.R.[2], Zueva O.S.[1]*, Salnikov V.V.[2], Faizullin D.A.[2], Zelenikhin P.V.[3], Ilinskaya O.N.[3], Zuev Yu.F.[2]
[1]*Kazan State Power Engineering University;*
[2]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS;*
[3]*Kazan Federal University;*
* ostefzueva@mail.ru

Hydrogels based on proteins and polysaccharides are promising biotechnological materials for delivery of drugs and diagnostic substances [1]. This is due to natural origin, the similarity of structure and basic

physical and chemical properties with intercellular matrix of living systems, which ensures their biocompatibility. The most technologically advanced are physical and ionotropic hydrogels, in which the three-dimensional polymer network is maintained by mechanical interweaving of polymer molecules and/or stabilized by intermolecular interactions such as ionic bridges, hydrogen bonds and hydrophobic interactions.

Hydrogels based on polysaccharides exhibit original physicochemical and biotechnological properties in drug delivery, tissue engineering, regenerative medicine, molecular diagnostics and therapy [2,3]. Polysaccharides from natural sources have low toxicity, biocompatibility and high stability under physiological conditions. The present work demonstrates our results on the use of polysaccharide hydrogels for delivery of therapeutic enzymes, vitamins, and diagnostic markers.

Two polysaccharides were used in our work. Sodium alginate is a linear polymer from brown algae. Gelation of alginate is stimulated by divalent cations at a constant temperature. The second polysaccharide, κ-carrageenan, is a linear anionic polymer extracted from red seaweed. These polysaccharides are actively used in tissue engineering and drug delivery. In this work, a comprehensive study of the influence of technological features of hydrogel preparation and their encapsulating properties was carried out.

One of the main technologies used in drug delivery is microencapsulation. The presence of regions with different polarity in the structure of polysaccharide hydrogels makes it possible to encapsulate both hydrophilic and hydrophobic target bioactive compounds.

The results of our comprehensive study on the structure and properties of polysaccharide-based systems for delivery of drug and diagnostic formulations are presented for chemical compaunds of various functional origin, namely, enzymes (binase and lipase), vitamin B9 (folic acid), and brilliant green as a model of medical contrast agent [4]. The report presents a brief review of data on the structure of polysaccharide hydrogels stabilized by physical and ionic bonds, discusses the methods of their preparation and conditions of use.

It has been established that the use of κ-carrageenan and alginate, along with properly selected conditions, creates prospects for obtaining effective materials for biomedicine. The advantage of using alginate as an object for encapsulation of biologically active substances lies in the instantaneous formation of ionotropic gels dispersed in a medium of a partially structured solution with a lower concentration of crosslinks, which affects the release of carried substances from hydrogels. In addition, as a rule, all materials stabilized with ionic crosslinkers exhibit pH-sensitive swelling and release of drug compounds due to diffusion through their porous structure, which allows the use of designed systems to prolong the action of drug compounds.

It has been shown that potential for successful biomedical applications of hydrogels may be hindered by the toxicity of crosslinking cations and the presence of carbon nanotubes (CNTs), used to modify delivery systems and impart new properties to them.

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## S1.104. Structure of an ice-binding protein from the fish longhorn sculpin Myoxocephalus octodecemspinosus based on circular dichroism spectra

Oleinik G.A.[2]*, Baranova S.V.[2], Zhdanova P.V.[2], Chernonosov A.A.[2], Koval V.V.[1,2]

[1]*Novosibirsk State University, Novosibirsk, Russia;*

[2]*Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of RAS, Novosibirsk, Russia;*

* zakabluk@niboch.nsc.ru

Organisms whose habitat is in a zone of low temperatures survive due to the properties of ice-binding proteins (IBPs). These proteins have the ability to bind to ice and influence ice crystal growth in several ways: inhibit ice crystal formation, lower freezing temperatures, prevent ice recrystallization, and promote nucleation [1]. Thus, ice-binding proteins allow organisms to exist harmoniously at low temperatures, preventing cell damage and, as a result, their death.

These proteins could be used in various fields of human activity. They could help to solve both everyday consumer tasks and more complex medical ones. For example, they could be used to preserve the quality of products during freezing. In medicine, IBP could be used in cryosurgery, as well as cryoprotectants for organ transportation. In literature, works describes practically positive effect of ice-binding proteins on long-term hypothermic storage of bovine embryos, for example [2].

Thus, the benefits of these proteins are obvious, therefore, for optimal use, it is necessary to understand the ice-binding mechanisms, to define their structure. For most ice-binding proteins, only theoretical models based on homology are known. Defining the structure is difficult in practice due to inability of proteins to make a detectible crystalline structure. In current work, the object of study is the class IV ice-binding protein, found in fish of the species Myoxocephalus octodecemspinosus (Longhorn sculpin) (P809061).

This work aimed to determine the secondary structure of the P809061 protein using the circular dichroism method. An electrophoretically homogeneous protein preparation obtained by IBP class IV was obtained and characterized by a mass spectrometric method. Three independent experiments were carried out, and the spectra of circular dichroism were obtained. The data of this work give an idea of the secondary structure of the class IV protein from fish of the species Myoxocephalus octodecemspinosus.

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## S1.105. Structure of human serum albumin upon interaction with divalent metal ions

Paston S.[1]*, Gorokh A.D.[1], Fedotova E.V.[1]

[1]*St. Petersburg State University;*

* svpaston@list.ru

The interaction of proteins with metal ions plays an important role in their functioning in the organism. The active centers of many proteins contain a metal ion. In addition, metal ions can disrupt the structure of proteins, provoking their aggregation, which entails pathological processes up to amyloidosis. A number of neurodegenerative pathologies

(for example, Alzheimer's, Parkinson's, prion diseases) are conformational diseases, their cause is a violation of the processing of a number of neuronal proteins, leading to a alteration of their conformation, irreversible complexation and accumulation in the cell in the form of toxic insoluble amyloid fibrils and larger aggregates (plaques) [1]. Pathogenic complexes self-organize due to hydrophobic interactions, forming a characteristic cross-β-structure consisting of β-layers directed perpendicular to the fibril axis [2]. There is evidence that transition metal ions, which have a high affinity for peptides and proteins, can induce the formation of intermolecular beta layers when bound to them [1]. In this regard, the study of the protein structure during complex formation with metal ions can serve as an impetus for the development of methods for the prevention and treatment of conformational diseases.

Serum albumin (SA) is the most abundant water-soluble protein in mammals, accounting for about 60% of plasma proteins. It is the main transporter of many low molecular weight ligands and the regulator of blood osmotic pressure. It is widely used as a model globular protein due to its availability. In this work, we studied the influence of a number of divalent metal ions (Mg2+, Mn2+, Cu2+, Co2+) on the structure of human SA. The study of the secondary structure was carried out by Fourier IR spectroscopy on an ATR attachment with the analysis of the Amide I band. The state of the tertiary structure of the protein globule was judged from the spectra of UV absorption, SA intrinsic fluorescence, and also the zeta potential of protein molecules in solution was determined.

It was found that the interaction of HSA with Mg2+ ions occurs under the action of electrostatic forces and reaches saturation at the ratio [Mg2+]:[HSA] = 1000, while the protein structure is not disturbed. The interaction of HSA with manganese and cobalt ions does not cause disturbances in the tertiary structure of the protein, however, Mn2+ is able to form coordination bonds and, probably, provoke the formation of beta layers.

The most significant changes in the protein structure are observed in solutions containing copper ions. At a low content of Cu2+, the secondary structure of HSA does not differ from the native one. At the ratio [Cu2+]:[HSA]=1:1, partially reversible protein aggregation in solution is observed, while the secondary structure of HSA is not disturbed both for the protein found in the precipitate and for the protein in solution. A further increase in the concentration of copper [Cu2+]:[HSA]=2:1 leads to a sharp violation of the secondary structure of the protein: the content of alpha-helices decreases, and the protein in solution shows a greater content of alpha-helices than the protein in the sediment.

The potential of the diffuse layer of HSA molecules is very sensitive to the ionic environment of the protein. In an aqueous solution at neutral pH, the zeta potential of HSA is approximately –40mV [3]. When salt is added to the solution, HSA interacts predominantly with cations, as a result of which the negative zeta potential of the protein decreases in absolute value. When interacting with divalent magnesium ions, the albumin charge approaches zero at the concentration ratio [Mg2+]:[HSA]=1000, and the protein structure is not disturbed. The study of systems containing manganese ions was carried out in this work up to a concentration ratio of [Mn2+]:[HSA] = 50, and at these ratios, the change in the charge density on the HSA molecule in the presence of manganese ions occurs similarly to that observed for magnesium, but less pronounced. The interaction of HSA with Cu2+ leads to a change in the sign of the protein charge. A zeta potential equal to zero is achieved at a ratio of 1:1. This ratio corresponds to an increase in absorption and scattering and a decrease in the HSA fluorescence intensity. A further increase in the concentration of copper ions leads to the appearance of a positive charge on the HSA surface, and a decrease in scattering is observed in the absorption spectra, i.e., the protein solubility increases again. The recharging of albumin molecules indicates the formation of a strong complex with copper cations. Thus, copper ions form a strong coordination bond with serum albumin.

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### S1.106. Structure, dynamics and membranotropic properties of antimicrobial peptides. Spectroscopy and computer modeling

Zuev Yu.F.[1]*, Khairutdinov B.I.[1], Kurbanov R.Kh.[1], Skvortsova P.V.[1], Ermakova E.A.[1]
[1]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS;*
* yufzuev@mail.ru

Antimicrobial peptides (AMPs) are important components of immune systems of mammals, cold-blooded reptiles, insects and plants.

In this work, a complex of spectroscopic methods (NMR, IR, CD, UV) and computer simulation (molecular docking and coarse-grained molecular dynamics (CGMD)) were used to study structure, conformational dynamics and membranotropic properties of a number of cationic, cysteine-containing AMPs. The report provides information on the structure of several AMPs, obtained by a complex of spectroscopic methods and mechanisms of their action on model membrane structures, estimated using computational methods. In some cases, we tried to supplement the overall picture with data on functional activity of AMPs. Taken together, our data give an idea of the structure, dynamics, and mechanisms of action of AMPs on membrane structure [1–5]. The mechanism of FMP action is a complex multistage process, one of the main stages being the interaction of AMPs with cell membranes. Using the molecular dynamics method, it has been shown that AMPs form pores in the studied model membranes or are localized on membrane surface forming stable complexes with membranes of various compositios according to the "carpet" model. The AMP binding on membrane surface leads to a change in distribution of lipids in membrane and to the change in electrostatic potential of membrane. Deformation of membrane electrostatic potential can cause the formation of defects in membrane structure, facilitate incorporation of AMPs into its structure, and cause cell death.

The construction of model peptides based on natural AMPs is a promising direction both in study of mechanisms of AMP action and in development of new drugs. For example, as a model analogue of AMP, a cationic β-peptide with the sequence RMCKTPCGKFYCYKPCP was constructed based on amino acid sequence of defence protein, pine defensin PsDef1, and its interaction with multicomponent membranes was studied. The coarse-grained molecular dynamics (CGMD) simulations have shown that, in contrast to defensin itself, the model peptide can be incorporated into membrane and form stable pores in it. The observed pores do not have a central water channel, and water molecules penetrate membrane through narrow gaps between peptides or between peptides and charged head groups of anionic lipids. It has been shown that peptide oligomerization is a necessary step for formation and stabilization of pores.

As another example, the cationic peptide megin, obtained from the skin secretion of Australian amphibians, was used. The three-dimensional structure of two forms of this peptide was determined using NMR spectroscopy in solution and refined by optical spectroscopy. The thermodynamic characteristics of peptide transformation from a linear to a cyclic form have been obtained. The antibacterial and antimycotic

properties of peptide and its inhibitory activity against serine proteases were analyzed.

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### S1.107. Study of Methyl Violet Binding to Human Serum Albumin

Shahinyan M.A.[1], Mikaelyan M.S.[1], Antonyan A.P.[1], Poghosyan G.H.[1]*
[1]*Yerevan State University;*
* g.poghosyan@ysu.am

Interaction of human serum albumin (HSA) with low-molecular compounds (ligands) is of great interest, since HSA is the main transport protein in human organism, which transmits various exogenous and endogenous compounds, including drug preparations. Methyl violet (MV), also known as crystal violet, has a molecular mass of 408 Da and is a triphenilmethane dye, owning positive charge, which used as a biological stain, fungicide in agriculture and drug for external application at skin diseases. Besides, MV possesses good sterilization, low toxicity and property of hormesis. It means that MV is able to initiate different processes in cells, if it enters bloodstream and is transmitted to cells. It causes its external application. On the other hand, it is interesting to reveal how MV will interact with HSA, if, for instance, it enters into blood. From this point of view, it is very important to reveal how the properties and conformation of HSA will change at model studies of MV binding to HSA.

In this work the fluorescence study of HSA interaction with MV has been carried out. The fluorescence study was carried out on spectrofluorimeter Cary Eclipse (Australia). The excitation of HSA solutions was made at wavelength 280 nm. In the table 1 the values of fluorescence intensities of HSA in the absence and presence of MV were presented, while at the titration of HSA solutions by MV solution the concentration ratio ligand/albumin changed from 1/2 to 1/10.

Data, presented in table 1, indicate that MV binding to HSA results in increasing of fluorescence intensity of HSA at the wavelength 353 nm, though, the wavelength shift does not occur. The last fact indicates that the only fluorescing tryptophan is not shifted, moreover it is in polar surrounding [1,2].

Table 1.Values of fluorescence intensities of HSA at the interaction with MV

Fluorescence intensity (a.u.) at wavelength$\lambda$=353 nm
HSA 516
HSA-MV (MV/HSA=1/10) 531
HSA-MV (MV/HSA=1/2) 554

Meanwhile, the fluorescence intensity increases, which can be connected to charge change in the vicinity of tryptophan, which in turn occurs due to the binding of MV to HSA. Apparently, in the vicinity of tryptophan the cationic form of MV neutralizes the protein charge, which positively affects the enhancement of fluorescence intensity of HSA.

Thus, the data obtained by the fluorescence method indicate that HSA conformation does not change at the binding to MV, though, the protein fluorescence increases due to the change of adjacent surrounding of tryptophan, which takes place as a result of MV binding to HSA.

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### S1.108. Study of conformational mobility of tubulin protein in the presence of taxol by molecular modeling methods

Fedorov V.A.[1,2]*, Kholina E.G.[1], Gudimchuk N.B.[1,2], Kovalenko I.B.[1]
[1]*Lomonosov Moscow State University ;*
[2]*Center for Theoretical Problems of Physical and Chemical Pharmacology RAS;*
* xbgth@yandex.ru

Microtubules are essential components of the cytoskeleton and play a major role in cell division. Microtubules are composed of αβ-tubulin, which assembles longitudinally into protofilaments. About 13 protofilaments bind laterally, thereby forming a microtubule wall. Essential to microtubule function is the property of dynamic instability, the ability to spontaneously switch between microtubule growth and shortening associated with GTP binding and hydrolysis. Microtubule dynamics is regulated in vivo by a number of microtubule-associated proteins. In medicine, antimitotic chemotherapeutic agents such as taxol, which bind to and stabilize microtubules, suppress dynamic instability and prevent cell division, are widely and very successfully used. Taxol and other stabilizing compounds also interfere with interphase microtubules, thereby affecting many important cellular processes. Since its discovery, taxol has been the subject of many studies aimed at elucidating its mechanism of action. The molecular mechanisms of action of such drugs can be studied in silico using the molecular dynamics method.

To reveal differences in the mechanical properties of tubulin protofilaments bound to GTP, GDP, and GDP+taxol, we performed a series of computational experiments using the full atom molecular dynamics method in an explicitly specified solvent. An important improvement compared to previous calculations of the dynamics of GTP- and GDP tubulin protofilaments was the use of new data from works (Manka S.W., Moores C.A. Nat Struct Mol Biol. 2018; Zhang R et al. Proc Natl Acad Sci U S A. 2018), which for the first time determined the structures of tubulins associated with GDP and a non-hydrolyzable analogue of GTP in the composition of 13-protofilament microtubules. Using our previously developed method for analyzing the bending angles of tubulin protofilaments (Fedorov et al., Plos Comp. Biol. 2019), based on the calculation of modified Euler angles, we evaluated the conformational mobility of GTP-, GDP-tubulins and GDP-tubulins with taxol. Taxol addition has been shown to result in changes at both intra- and inter-dimeric interfaces. The change in both the magnitude of the bending stiffness and the bending direction was evaluated in the presence of taxol.

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### S1.109. Study of the effect of curaxin CBL0137 on the structure of nucleosomes using spFRET-TIRF microscopy

Geraskina O.V.[1], Maluchenko N.V.[1]*, Gerasimova N.S.[1], Lyubitelev A.V.[1], Feofanov A.V.[1], Studitsky V.M.[2]
[1]*Biological faculty of Lomonosov Moscow state university;*
[2]*Cancer Epigenetics Program, Fox Chase Cancer Research Center, Philadelphia;*
* mal_nat@mail.ru

Curaxin CBL0137 belongs to the group of DNA-binding anticancer drugs. By binding, curaxin interferes the interaction of a number of enzymes, such as topoisomerases and DNA methyltransferases, and histones with DNA, and also promotes the capture of the FACT multisubunit protein complex in chromatin [1]. In the cell, curaxin acts on multiple signaling pathways, activating p53 and suppressing the activity of NF-κB and HSF1 factors [2]. The molecular mechanisms of the action of CBL0137 on chromatin and, in particular, on its structural unit, the nucleosome, are the subject of active study. For in vitro studies, nucleosomes can be assembled using core histones and template DNA containing a high affinity nucleosome positioning sequence. Mononucleosomes make it possible to study certain aspects of complex processes occurring in chromatin with the participation of histone chaperones, chromatin remodelers, and transcription factors [3–5]. Previously, we found that curaxin CBL0137 causes concentration-dependent changes in the structure of nucleosomes. [6]. In this work, structural changes and conformational transitions occurring in nucleosomes under the action of curaxin were studied using single particle fluorescence microscopy based on total internal reflection effects and Förster resonance energy transfer (spFRET-TIRF microscopy).
Immobilized nucleosomes were used to carry out experiments by TIRF microscopy. Fluorescently labeled mononucleosomes were constructed with the DNA linker region biotinylated at the 5' end. Template DNA with the introduced Cy3/Cy5 FRET pair of labels and a TspRI restriction site in the terminal region was synthesized by PCR. The labels were positioned in such a way that, after the assembly of nucleosomes, they were located on adjacent DNA supercoils at a distance from each other less than the Förster radius. Nucleosome assembly was performed as described previously [7]. Nucleosomes were ligated to a biotinylated DNA fragment containing a TspRI restriction site. Biotinylated nucleosomes were immobilized on glass modified with biotinylated polyethylene glycol silane and streptavidin [8]. The concentration of nucleosomes was chosen so that after immobilization they were located at a distance of more than a few microns from each other. Measurements were performed using an experimental setup based on an inverted fluorescence microscope with a laser module and an adapter for TIRF microscopy, as described previously [8]. Based on a series of images obtained with a time resolution of 300–500 ms, we analyzed the dynamics of the FRET value for individual nucleosomes and the distribution of time-averaged values of this value in the population of particles. Under experimental conditions, ~30% of immobilized nucleosomes retained their native structure with a FRET level of 0.5–0.7. At a curaxin concentration of 5 μM in nucleosomes, significant stochastic changes in FRET efficiency were found, indicating a weakening of interactions between nucleosomal DNA and core histones and the formation of unstable conformational states in dynamic equilibrium. At a curaxin concentration of 10 μM in nucleosomes, the FRET efficiency decreased to zero, which indicates the separation of adjacent supercoils of nucleosomal DNA over a distance of more than 10 nm. In this case, no reverse conformational transitions leading to the convergence of supercoils were found in a time interval of several tens of seconds. After the removal of free curaxin from the solution, the initial structure is not restored in most nucleosomes for at least ten minutes, which indicates either a slow dissociation kinetics of curaxin from the complex with DNA or an irreversible dissociation of the nucleosomes themselves into core histones and a DNA template.
Financing. The study was supported by a grant from the Russian Science Foundation (project no. 21-74-20018). In the studies, the equipment of the TsKP IBCh 2020 was used.
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### S1.110. Study of the electrostatic nature of the protein-ligand interaction in the orange carotenoid protein

Mamchur A.A.[1]*, Yaroshevich I.A.[1]
[1]*Lomonosov Moscow State University, Biological faculty;*
* al.mam4ur@yandex.ru

Orange carotenoid protein (OCP) plays an important role in the photosynthetic apparatus of cyanobacteria. Cyanobacteria, the oldest organisms involved in oxygenic photosynthesis, face the need for non-photochemical quenching in intense light to prevent oxidative stress. Unlike green plants, the quencher function in cyanobacteria is performed by the orange carotenoid protein. OCP is a 35 kDa photoreceptor that is activated by blue light. It consists of two domains - a fully α-helical N-terminal domain and a mixed α-helical/β-sheet C-terminal domain, between which the photoactive canthaxanthin molecul is non-covalently fixed. Upon photoactivation, OCP transforms into a physiologically active red state through the formation of numerous intermediate compounds, and the quantum yield of such a transition is extremely low (about 0.2%). OCP is unique in that it is the only known photoactive protein in which carotenoids are used as photosensitive chromophores. As part of this study, we calculated the molecular dynamics of a dark OCP variant with a duration of 1 μs using the GROMACS software package version 2020.1 [1] and the OPLS-AA force field [2]. An integration step of 1 fs and periodic boundary conditions were chosen. The simulation was carried out at a temperature of 300K and a pressure of

1 atm, which were maintained using the V-rescale [3] and Parrinello-Rahman [4] algorithms, respectively. For Coulomb and van der Waals interactions, a cutoff radius of 12 Å was set. Electrostatic effects were controlled by the PME algorithm [5]. The protein was solved in water (model TIP4P), 7 sodium ions were added to neutralize the system. Before conducting molecular dynamics, the system was subjected to an energy minimization procedure and subsequent heating from 5 to 300K. Data analysis was carried out using the Python programming language version 3.9.12. To simplify the analysis, the atoms of the pi-conjugated chain of canthaxanthin were numbered from 0 to 25, starting from the oxygen of the keto group located closer to the N-terminus of the protein.
We calculated the electrostatic potential at each atom of canthaxanthin - the sum of the potentials created by the protein atoms as point charges. There was shown the reduction of a trajectory-averaged electrostatic potential along the pi-conjugated canthaxanthin chain with an amplitude of 25 mV. In this case, the phenomenon turned out to be stepwise: from 0 to 10 atom, a linear potential drop by 10 mV occurs, and from 11 to 22 atom, a linear potential drop by 15 mV occurs with a large slope of the straight line. The potential of 22-25 atoms does not change significantly and fluctuates around -40mV.
Interestingly, the amino acids ARG155, GLU244, PHE315 make the greatest contribution to the formation of the electrostatic potential created by the protein. The results obtained will form the basis for further studies involving the point mutations and the use of a quantum chemical approach to assess the effect of changes in the protein structure on the spectral properties of carotenoids.
The study was supported by a grant from the Russian Science Foundation No. 22-74-00012.
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### S1.111. Study of the influence of silver ions on the stability of duplexes with different lengths of the cytosine-cytosine region

Baryshev A.[1]*, Sokolov P.A.[1], Kasyanenko N.A.[1]
[1]*Saint-Petersburg State University;*
* andry-barash@mail.ru

Since the discovery of the DNA structure, scientists have not ceased to modify it in all sorts of ways, both to alter its properties and to obtain new ones. One of such modifications, for example, is the replacement of Watson-Crick base pairs with non-canonical pairs mediated by metal ions. In this work, cytosine-cytosine pairs mediated by silver ions were studied. For such pairs, high chemical and thermal stability has already been shown earlier. Generally, either individual pairs of mismatches, which can be used, for example, to detect free silver ions in solutions, or rather long sequences of cytosines, the formation of structures of which occurs only in the presence of silver ions are studied in the articles. Moreover, the studied base pairs can be used in DNA nanotechnologies or DNA origami due to the high specificity of silver binding to the cytosine-cytosine pair. Thus, this research topic is relevant, and the results obtained in the work may be applied in various fields of science.
In this study, oligonucleotides with different sequence lengths of cytosines (from 8 to 16), which are framed by complementary regions of length. The original aims of the study were to test the effect of different numbers of silver ions on duplex formation and stability, so this work will fill in the region between single pairs and significant sequences of cytosines. For these tasks, the methods of melting and gel electrophoresis were applied. The melting method revealed two melting temperatures, one of the order of 30 C. and the other over 40 C, with a different amount of silver ions per duplex (the amount of silver varied from 0 to n, where n is the number of cytosine-cytosine pairs in the duplex). An increase in the amount of silver led to a shift by 1-2 C of a lower melting temperature to the region of high temperatures, as well as the appearance of a higher temperature. The measurements were carried out in sodium borate buffer pH 7.0 with various ionic strengths. Then, silver ions associated with cytosines were reduced with sodium borohydride to check the formation of silver nanoclusters. In this part of the work, an important role was played by the oligonucleotide matrix, which may lead to the reduction of silver nanoclusters with different properties.

### S1.112. Study of the interaction mechanism of bromelain, papain and ficin with graft copolymers based on chitosan and carboxymethylcellulose

Sorokin A.V.[4,2,1]*, Holyavka M.G.[4,1], Lavlinskaya M.S.[4,2,1], Goncharova S.S.[4], Koroleva V.A.[4,3], Redko J.A.[4], Malykhina N.V.[4], Pankova S.M.[4,3], Belyaeva T.N.[4], Dubovitskaya A.N.[4], Paymetieva D.S.[4], Narazina D.A.[4], Artyukhov V.G.[4]
[1]*Sevastopol State University;*
[2]*Voronezh State University of Engineering Technology ;*
[3]*Voronezh State Medical University named after N.N.Burdenko;*
[4]*Voronezh State University;*
* andrew.v.sorokin@gmail.com

The study of the microenvironment influence on conformational changes and activity of enzymes is one of the fundamental problems of modern biophysics solving of the one opens up possibilities for the design of new selective enzyme inhibitors or activators and expands the scope of biocatalysts practical application. The aim of this work is to study the mechanism of cysteine proteases interaction – bromelain (EC 3.4.22.32), papain (EC 3.4.22.2) and ficin (EC 3.4.22.3) – with graft copolymers of sodium salt of carboxymethylcellulose (CMC) or chitosan (Cht) side grafted chains of poly(N-vinylimidazole) (PVI) and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA).
Conjugates of cysteine proteases with graft copolymers were obtained by complexation in borate buffer with pH 9. The interaction mechanism was studied by flexible molecular docking and FTIR. The protein content in the resulting conjugates was determined by the modified Lowry method, and the proteolytic activity was assessed by the rate of the hydrolysis reaction of the azocasein substrate [1-6].
As a result of the study, both the carbohydrate backbone of the polysaccharides and the side grafted chains are involved in interactions with cysteine protease molecules. An in silico study showed that conjugation is spontaneous and characterized by negative affinity values. Ligands are located in the catalytic pocket of protease globules. The amino acid residues of the enzymes forming the active center form hydrogen bonds with graft copolymers based on the sodium salt of carboxymethyl cellulose and chitosan with poly(N-vinylimidazole) for bromelain and papain, while for ficin, a similar interaction is observed only with the CMC-PDMAEMA graft copolymer. In addition, ligands enter into weak physical interactions, mainly hydrophobic ones, with catalytically significant amino acids. It should be noted that, on the part of graft copolymers, the polysaccharide backbone of macromolecules of graft copolymers takes part in the formation of new bonds, with the exception of ficin, where the azole cycles of grafted chains also form

H-bonds. It is noteworthy that the polar azole cycles, as well as the carbon skeletons of the side chains, enter into hydrophobic interactions in all cases considered.

The FTIR data confirm the results of molecular docking pointing to the same graft copolymers' functional groups and fragments of macromolecules as being involved in the interaction with enzymes. In addition, there is a shift in the position of the Amide I band in the FTIR spectra of conjugated enzymes compared to native ones. Considering this fact, we can conclude that enzyme globules undergo conformational changes taking on more elongated shapes. In the cases of conjugates with carriers based on chitosan, a decrease in the regions containing α-helices and an increase in the regions of β-barells are clearly revealed.

Based on the studies carried out, it can be concluded that such structural changes can have a significant effect on the catalytic ability of bromelain, papain, and ficin. Therefore, the next stage of the work was the evaluation of the conjugated enzymes proteolytic activity. In the case of conjugation of enzymes with graft copolymers based on the sodium salt of carboxymethyl cellulose, and for papain and ficin with a graft copolymer of chitosan with grafted chains of poly(N-vinylimidazole), there is an increase in both total and specific activity of conjugated enzymes. We would like to note that this effect (the phenomenon of hyperactivation) is due not to the high content of the enzyme in the preparations, but to conformational changes in globules and modulation of the structure of the active center of cysteine proteases.

Thus, the mechanism of interactions of bromelain, papain, and ficin with graft copolymers of the sodium salt of carboxymethyl cellulose or chitosan with grafted side chains of poly(N-vinylimidazole) and poly(N,N-dimethylaminoethyl methacrylate) has been studied. It has been established that the interaction with carriers based on carboxymethyl cellulose and a copolymer of chitosan with N-vinylimidazole leads to an increase in the proteolytic activity of enzymes.

The work was supported by the Russian Science Foundation, project No. 21-74-20053.

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## S1.113. Study of the mechanism of interaction between ficin and N-(2-hydroxy)propyl-3-trimethylammonium chitosan

Lavlinskaya M.S.[1,2,3]*, Holyavka M.G.[1,2], Sorokin A.V.[1,2,3], Goncharova S.S.[1], Koroleva V.A.[1,4], Redko Yu.A.[1], Malykhina N.V.[1], Pankova S.M.[1,4], Belyaeva T.N.[1], Dubovitskaya A.N.[1], Paymetyeva D.S.[1], Narazina D.A.[1], Artyukhov V.G.[1]
[1]*Voronezh State University;*
[2]*Sevastopol State University;*
[3]*Voronezh State University of Engineering Eechnologies;*
[4]*Voronezh State Medical University named after N.N. Burdenko;*
* maria.lavlinskaya@gmail.com

The study of the mechanism of biomacromolecules interaction with various elements of the microenvironment can significantly expand the understanding of the biopolymers functioning, as well as predict the ways of modulating their biological activity. Promising components for the creation of enzyme preparations for biomedicine and biotechnology are natural polysaccharides, materials that combine availability, low cost, non-toxicity, non-immunogenicity and biocompatibility. In this regard, the aim of this work is to study the mechanism of interaction between the cysteine protease ficin (EC 3.4.22.3) and N-(2-hydroxy) propyl-3-trimethylammonium chitosan (HPTAC), a chitosan derivative exhibiting polycationic properties in a wide range of pH values. .

Conjugates of ficin and HPTAC with molecular weights of 200, 350, and 600 kDa were obtained by complexation in borate buffer with pH 9. The interaction mechanism was studied by flexible molecular docking and FTIR. The protein content in the resulting conjugates was determined by the modified Lowry method, and the proteolytic activity was assessed by the rate of the hydrolysis reaction of the azocasein substrate [1-4].

Based on molecular docking, it was found that the interaction of ficin with HPTAC proceeds spontaneously, with the latter being located in the gap between two domains of the ficin globule containing the active center of the enzyme. Four hydrogen bonds are formed between the protein and the ligand, including one formed by a catalytically significant Gln19 residue. The Cys25 residue, which is directly part of the active site of the enzyme, enters into hydrophobic interactions with hydroxypropyl fragments. On the ligand side, hydrogen bonds are formed mainly due to pyranose cycles, with the exception of Lys145, which interacts with the OH-group of the side fragment.

The results of FTIR correlate with the data obtained by in silico studies and confirm the same functional groups and fragments of carrier macromolecules involved in the interaction with ficin. Based on the position of the Amide I band of ficin in the FTIR spectrum of the conjugates, one can make an assumption about changes in the secondary structure of the enzyme: the conformation changes from globular to more elongated and enriched in β-structures.

As a result of the study of the catalytic ability of the samples obtained by us, it was found that the total proteolytic activity (in U/ml of solution) for native ficin and the enzyme conjugated on a carrier with molecular weights of 200, 350 and 600 kDa is 96 ± 4 and 106 ± 4, 108±5, 99±3, respectively. These values are quite close and, in general, somewhat higher for conjugated ficin. The specific activity values (in units/mg of protein) of the conjugated preparations significantly exceed this value for free ficin and are 948±3, 1037±10, 1591±12, and 787±4, respectively, for the native enzyme and the enzyme conjugated on a carrier with molecular weights 200, 350 and 600 kDa. Thus, phenomenon of ficin hyperactivation is occurred, mainly due to conformational changes in the enzyme globule.

From the presented data, it follows that the highest activity values are observed for preparations obtained using HPTAC with a molecular weight of 350 kDa, and the lowest values are observed for a carrier with a molecular weight of 600 kDa. To interpret this phenomenon, a morphological study of the carrier surface was carried out. As a result, it was found that, in contrast to supports with a molecular weight of 200 and 600 kDa, N-(2-hydroxy)propyl-3-trimethylammonium chitosan with a molecular weight of 350 kDa has a porous structure with a cavity size of about 80 nm. Apparently, during conjugation, ficin, whose globule size is about 5 nm, is located in the pores of the carrier, and this contributes to an increase in the activity of the enzyme.

Thus, it has been shown that the combined effect of modulation of the active site of ficin and the surface morphology of N-(2-hydroxy)

propyl-3-trimethylammonium chitosan with a molecular weight of 350 kDa leads to an increase in the proteolytic activity of ficin.
The work was supported by the Russian Science Foundation, project No. 21-74-20053.
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### S1.114. Study of the structure and reactivity of carnosine dipeptide by the density functional theory method

Demukhamedova S.D.[1]*
[1]*Institute for Physical Problems, Baku State University;*
* svetlanabest@mail.ru

Carnosine (β-alanyl-L-histidine) is a very interesting natural dipeptide molecule consisting of β-alanine and L-histidine amino acid residues. Particularly high concentrations of carnosine have been found in skeletal and cardiac muscle, in brain, kidneys, skin and gastric mucosa. The interest to carnosine and its derivatives, such as anserine and homocarnosine is primarily related to the fact that these molecules play an important functional role, exerting a complex effect on cells and various systems of the human and animal organism. First of all, the antioxidant properties of carnosine should be mentioned [1]. In recent years the positive effect of carnosine-based drugs in treating cancer, immune system disorders, diabetes, kidney, liver, stomach, cataracts, as well as in treating Parkinson's, Alzheimer's diseases and various neurological disorders after an acute ischemic stroke and some heart problems were testified. Carnosine has been successfully used for slowing down the aging process and treating senile dementia. Such a wide range of applications of carnosine opens up prospects for the creation of new medications based on it. The non-toxicity of carnosine is of great importance; it does not accumulate in the organs and can be safely used for the treatment of many diseases. However, the mechanism of action of carnosine on the molecular level is still not fully understood, which proves the expediency of further investigations of the structure-function relationship of the carnosine molecule. It is known that the variety of biological functions of the molecules is determined by their spatial and electronic structures. To date, quantum-chemical calculations are considered to be the best theoretical method for determining the structure and properties of polyatomic molecules.
Over the years many of our investigations based on semiempirical methods of quantum chemistry have been devoted to the study of carnosine, its derivatives anserine and homocarnosine and their complexes with transition metals. In [2] the spatial and electronic structure of a neutral carnosine molecule in the N3H tautomeric form in aqueous and gaseous media was studied by the quantum-chemical method of the density functional theory DFT. In the present work, in order to study the structural, electronic, spectral characteristics and to determine the reactivity of carnosine dipeptide with the N1H tautomeric form of the imidazole ring, a similar study by the same method of quantum chemistry in gas and aqueous media was carried out.
All calculations were performed by the density functional theory method DFT with a hybrid three-parameter B3LYP functional. A reliable and economical basis set with polarization and diffusion functions 6-31+G (d, p) was used for calculations. All calculations were performed using the Gaussian 09 application software package [3]. GaussView 6.0.16 [4] was used to visualize and analyze the results of investigations. After the optimized structure was obtained, its IR and NMR spectra were calculated at the same level of theory. The absence of imaginary frequencies confirms that the optimized structure obtained in this calculation is a global minimum on the potential energy surface. The results of the optimization of the carnosine dipeptide in the N1H tautomeric form including bond lengths, valence and dihedral angles, values of electronic energy, energies of HOMO and LUMO orbitals, the energy gap between them, values of partial charges on atoms, values of dipole moments were analyzed in the presented work. The values of the ionization potential and the electron affinity were obtained. The global descriptors of the carnosine reactivity in the N1H tautomeric form, correlating with its specific physico-chemical properties such as electronegativity, chemical potential, chemical hardness and softness, electrophilicity, maximum charge transfer and polarizability, were calculated on the basis of the boundary orbitals energy values. The obtained reactivity indices for two forms of the carnosine imidazole ring were compared. HOMO and LUMO boundary orbitals were visualized to identify regions where electronic transitions occurred. Molecular electrostatic potential (MEP) surfaces clearly demonstrate potential binding sites. Natural bonding orbitals (NBO) analysis was performed to investigate intramolecular interactions that determine structural stability and to obtain information on electron density delocalization and charge transfer. Theoretical IR and NMR spectra of carnosine dipeptide in N1H tautomeric form were obtained for gas and aqueous media. The structural rearrangements and changes of various parameters depending on the dielectric permittivity of the medium were analyzed.
A comparative analysis of the results for two N1H and N3H tautomeric forms of carnosine dipeptide was performed. Calculations show that the proposed models of carnosine dipeptide in both tautomeric forms form stable structures.
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### S1.115. Study of the structure of SARS-CoV-2 and human rotavirus A virus-like particles by cryo-electron, transmission and analytical microscopy

Cherepushkin S.[2], Moiseenko A.[1], Trifonova T.[1], Savochkina T.[2], Filatov I.[2], Karlova M.[1], Grebennikova T.[2], Sokolova O.S.[1]*
[1]*Moscow Lomonosov University, Faculty of Biology;*
[2]*The National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya;*
* sokolova184@gmail.com

Virus-like particles (VLP) are a promising platform for vaccine development and production. To date, there are registered vaccines as well as a wide range of candidate vaccines based on VLP against various infectious agents. VLP vaccines are more immunogenic than subunit vaccines and do not contain virus nucleic acids, making them safer

than live and inactivated vaccines. VLPs mimic the virion structure and methods are needed to study the particle structure and confirm their authenticity as well as routine quality control of VLP vaccines. Various types of electron microscopy, primarily transmission electron microscopy, are the gold standard for these tasks. The aim of this work was to develop electron microscopy methods to study the structure of virus-like particles of SARS-CoV-2 and human rotavirus A.

SARS-CoV-2 and human rotavirus A virus-like particles were obtained using a baculovirus expression system in insect cells. A culture of Trichoplusia ni butterfly cells was infected with several recombinant baculoviruses, each expressing the target gene of the structural virus protein. These viral proteins have the ability to self-assemble to form VLPs. The VLP of human rotavirus A included 4 of the 6 structural proteins of the virus: inner layer proteins VP2 and VP6 and outer layer proteins VP4 and VP7. Particles consisting of 2 and 3 proteins were obtained: VP2/6, VP2/6-VP7 (genotypes G1, G2, G4, G9) and VP2/6-VP4 (genotypes P4, P8). SARS-CoV-2 virus-like particles consisting of E, M, N, and S proteins of different SARS-CoV-2 strains were also obtained. The VLPs were purified by ultracentrifugation in sucrose solution and examined by cryo-electron, transmission, and analytical microscopy.

Samples were examined by transmission electron microscopy (TEM) in negative contrast as well as by single particle analysis in cryo-electron microscopy. PEM images were obtained on a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. A Gatan Orius SC200D CCD camera was used to acquire images in negative contrast. A Gatan Elsa cryotransfer holder, a Direct Electron DE-20 direct electron detection detector, and SerialEM data acquisition software were used to obtain serial cryo-electron microscopy images on the same microscope.

The preparations were applied to standard copper microscopy grids with a carbon substrate prehydrophilized in a glow discharge. The sample on the grid was incubated for 60 seconds at room temperature, then a drop of 2% uranyl acetate solution was applied to the grid, incubated for 30 seconds, and the excess of the solution was removed from the grid with filter paper. According to the results of transmission electron microscopy, the VLP samples of rotavirus A are homogeneous due to a small amount of protein aggregates. The average VLP size (70-80 nm) corresponds to the size of a full-length virion, and the particles have a characteristic "wheel-like" shape. The VLP samples of SARS-CoV-2 are also homogeneous, the size (about 100 nm) corresponding to the size of the viral particle.

Staining with antibodies conjugated with colloidal gold was used to confirm the protein composition of VLP. The nets with the preparations were placed on a drop of 0.3% BSA-FSBT blocking solution. After blocking, the grid was placed on a drop of primary antibody solution (10 μg/ml in 0.3% BSA-FSBT solution) and incubated for 14 h at +4°C. After incubation, the grid was washed 3 times 5 min each on drops of 0.3% BSA-FSBT solution, then placed on a drop of a solution of secondary antibodies conjugated with gold nanoparticles (Sigma) at a dilution of 1:250, incubated for 14 h at +4°C. VLP of rotavirus A was stained with antibodies to the VP6 protein and VLP of SARS-CoV-2 with antibodies to the receptor-binding domain of protein S. The presence of these proteins in virus-like particles was shown.

A statistical analysis of virion projections using two-dimensional classification was performed using single particle analysis by cryo-electron microscopy data, and a set of 2500 virion projections was selected. From this set of projections, a VLP reconstruction of rotavirus A was assembled at 7 Å FSC resolution. The processing was done in the Curacris software. The reconstruction showed that the arrangement of the VP2 and VP6 proteins in the VLP was similar to that of the viral particle. Analytical microscopy demonstrated the absence of nucleic acid in the VLP of rotavirus A.

Thus, the structure and morphology of virus-like particles according to electron microscopy data correspond to those of the original virions.

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### S1.116. Study on the interaction of some DNA-specific ligands with human serum albumin

Vardevanyan P.O.[1]*, Parsadanyan M.A.[1], Antonyan A.P.[1], Shahinyan M.A.[1], Petrosyan N.H.[1]
[1]*Yerevan State University;*
* p.vardevanyan@ysu.am

Study of interaction of different ligands with transport proteins is one of actual topics, since it can lie at the basis of understanding of influence of biologically active compounds on organism. One of transport proteins is albumin, which functions deprotonating and transition of wide spectrum of low- and high-molecular endogenous and exogenous compounds. Taking into account the important role of albumin we have carried out the studies on interaction of DNA-specific ligands, particularly, intercalator methylene blue (MB) and DNA groove binder Hoechst 33258 (H33258) with this protein.

The obtained results indicate that intercalator MB and DNA groove binder H33258 bind to albumin. Meanwhile, MB, which interacts with DNA by several modes, among which the intercalation refers to specific type, binding to albumin does not show any specificity to this protein. Moreover, the preferable binding sites for MB on albumin are relative hydrophilic regions (site I), since the non-specific electrostatic interaction plays the main role at the binding of this ligand [1]. For H33258 some preference to hydrophobic regions of this protein is revealed. It is indicated by the results of fluorescence studies, since the fluorescence intensity of H33258 at the binding to protein decreases at low concentrations of albumin and increases at relavively high concentrations of albumin. It also indicates that at complexformation the hydrophobic interactions play the important role, and result in enhancement of fluorescence intensity of H33258-albumin complexes at higher concentrations of the protein.

It is obvious that the fluorescence changes of the complexes are caused by micro-environment changes, bound ligand molecules to ligand and indicate the existence of two types of centers in protein – hydrophilic and hydrophobic. Though, the hydrophobic interaction of of H33258 with albumin is the consequence of hydrophilic binding, as a result of which H33258 invokes unwrapping of protein dimensional structure. In the result of this, most apparently, the protein conformation changes, due to which hydrophobic aminoacid residues become available for bisbenzimidazole (hydrophobic) groups of this ligand. Consequently, at the formation of hydrophobic contacts between them, the ligand molecules will be screened from quenchers (water molecules, solved oxygen) [2].

The results reveal that both ligands initiate multidirectional conformational reconstructions in protein molecule, which is reflected on stability of macromolecule structure: in the case of H33258 a loosening of tertiary structure is observed, in the case of MB – vice versa – more folding (increase of compactness degree) of protein dimensional structure [3].

Based on the obtained results it is concluded that H33258 forms hydrogen bonds with albumin, as well as interacts via van-der-Waals forces, and MB – via electrostatic forces. It was also found out that the binding of both H33258 and MB to albumin is caused by enthalpy-entropy compensate mechanism, which is indicated by negative value of Gibbs free energy change.

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### S1.117. Terahertz dynamics of hydrate shells of biomolecules

Penkov N.V.[1]*
[1] *Institute of Cell Biophysics of RAS;*
* nvpenkov@rambler.ru

Hydration is a fundamental process in biology necessary to bring biomolecules to their native state. At the same time, the reverse side of hydration, namely the formation of a hydrate shell, is much less studied. Hydrate shells of biomolecules have been studied for a long time and by various methods, but a full understanding of their structure and functions is still very far away. A powerful impulse in the development of this area of research was the appearance of the method of terahertz (THz) spectroscopy in the early 2000s. This method turned out to be much more sensitive to the structural-dynamic characteristics of water than other methods. This is due to the specifics of the THz range (0.3-3 THz). It corresponds to the characteristic frequencies, times and energies of the intermolecular structure and dynamics of water. It is shown that hydrate shells are not limited to one or two layers of strongly bound water, but also include more distant regions of the water phase (up to several nm from the surface of the biomolecule) with altered molecular dynamics, which in a number of works has been called dynamic hydrate shells. However, despite the 20-year history of their study, universal research approaches have not yet been proposed and generalizing characteristics of dynamic hydrate shells of biomolecules have not been described. The main problem is that the spectra of aqueous solutions in the THz range are not characteristic, which makes it difficult to determine specific spectral parameters characterizing the structure and dynamics of hydrate shells.
In our work, an approach has been developed for the study of hydrate shells of biomolecules based on the THz time-domain spectroscopy (THz-TDS). This type of THz spectroscopy makes it possible to measure the spectra of complex dielectric permittivity (DP), which are much more informative than absorption spectra. The essence of the approach is as follows.
1) The DP spectra of the solution of biomolecules ε_s^*, solvent ε_w^(0*) (without biomolecules) and the dry biomolecules ε_i^* are recorded.
2) Using the effective medium models, the dielectric contribution of biomolecules ε_i^* is subtracted from the DP solution ε_s^* and the spectrum of the water phase of the solution ε_w^*=f(ε_s^*,ε_i^*) is calculated.
3) There are differences between ε_w^(0*) and ε_w^* due to the presence of hydrate shells in the water phase of the biomolecule solution. Based on these differences, information about the hydrate shells of biomolecules is extracted.
Particular attention was paid to the selection of effective medium models suitable for each type of biomolecules. In particular, based on the formalism of electrodynamics of continuous media, we have theoretically developed and experimentally confirmed the effective medium model applicable to solutions of extended biopolymers (DNA, polysaccharides, etc.).
The spectra of ε_w^(0*) and ε_w^* were not compared directly, but were decomposed into components describing all the main types of intermolecular dynamics of water in the representation of the theory of dielectric spectroscopy:

$$\varepsilon^* = (\Delta\varepsilon_1)/(1-i\omega\tau_1) + (\Delta\varepsilon_2)/(1-i\omega\tau_2) + A/(\omega_0^2-\omega^2-i\omega\gamma) + \varepsilon_\infty + i\,\sigma_0/(\varepsilon_0\,\omega), \quad (1)$$

$\tau_1$ and $\Delta\varepsilon_1$ – time and amplitude of orientation relaxation of bound water molecules (Debye relaxation); $\tau_2$ and $\Delta\varepsilon_2$ – time and amplitude of orientation relaxation of free water molecules; A, $\omega_0$, $\gamma$ – amplitude, resonant frequency and damping parameter of intermolecular stretch vibrations of water molecules bound by hydrogen bonds; $\varepsilon_\infty$ – high frequency DP, $\sigma_0$ – dc-conductivity, $\varepsilon_0$ – dielectric constant, $\omega$ – cyclic frequency, i – imaginary unit.
Each parameter of the equation (1) has a certain physical meaning, and its change during the transition from the solvent to the water phase of the solution uniquely characterizes the structural and dynamic characteristics of hydrate shells. Thus, a decrease in $\Delta\varepsilon_1$ means an increase in the binding of water in hydrate shells, the parameter $\Delta\varepsilon_2$ is proportional to the number of free water molecules, $\tau_2$ is the time of their orientation relaxation, the change in A correlates with the change in the number of hydrogen bonds, and the parameters $\omega_0$ and $\gamma$ determine the average energy and the width of the energy distribution of hydrogen bonds.
The described approach was used to study the hydration of all major types of biomolecules, which demonstrated its informativeness and versatility. It is shown that when the conformation of the protein changes, the relaxation parameters of water molecules in hydrate shells change. In phospholipid liposomes, phase transitions are accompanied by a change in hydrogen binding in hydrate shells, the thickness of which exceeds 5 nm. The Mg•ATP complex forms a special hydrate shell with the formation of additional hydrogen bonds, which is not observed for either ATP or Ca•ATP. This may make biological sense, since Mg•ATP is involved in most biologically significant reactions. The hydrated shell of DNA exhibits three regions different from undisturbed water: more strongly bound molecules, a region with an increased number of free molecules, and a region with an increased number of hydrogen bonds. K+ ions with intracellular concentration significantly weaken all the effects of DNA hydration, which may also make biological sense. DNA exhibits cooperative hydration effects compared to individual nucleotides. A number of features of sugar hydration have been established. The dependence of hydration on the orientation of OH-groups and the type of glycosidic bonds is shown. In contrast to DNA, polysaccharides exhibit anticooperative hydration effects.
Taking into account the fact that the thickness of dynamic hydrate shells is comparable to the average distance between biomolecules inside a living cell, their role in the biomolecules interaction is of particular interest. It seems that research in this direction at the junction of physical chemistry and molecular biophysics has great prospects.

### S1.118. Terahertz spectra of DNA molecules

Galanov E.K.[1]*
[1]*St.Petersburg State Transport University;*
* galanov-evgenijj@rambler.ru

An analysis was made of the infrared absorption and Raman spectra of DNA molecules in the region λ=2–1000 μm (h•ν=5000–10 cm-1 ). At terahertz range h•ν=80—10cm-1 bands of deformation oscillations sugar ring rotational vibrations of the ion - RO-4 - around the C3 –C5 axis sugar-phosphate chain, torsional vibrations of bases, twist vibrations complementary base pair and others. It is shown that the discrete nature of the conformational states (B, B′ ,B″ ,…A, A′ , A″ ,…) of molecules DNA is determined by the discreteness of metastable electronic states, obeying Fermi statistics. Filling quantum vibrational levels of oscillators of DNA molecules is determined by Bose statistics. It is shown that upon absorption of electromagnetic terahertz radiation the structural parameters of conformations B, B′, B″,…, A, A′,A″ ,… and in the case of absorption of high-density radiation, transitions between electronic states are likely. It

has been shown that domains from DNA unit cells containing 50-200 base pairs can be considered as elements of long-term memory time.

### S1.119. The Relationship between Entropy, Symmetry and Information of the Supramolecular Structure of DNA Molecules

Gorovoy Y.M.[1]*
[1]*Yaroslavl State Technical University;*
* gorovoyj@mail.ru

The purpose of this work is to establish the relationship between entropy, symmetry and information of the supramolecular structure of DNA molecules. The structure and functions of DNA molecules are determined by their interaction with their aquatic environment. The correct model of the supramolecular structure of DNA, as a complex system consisting of two interacting subsystems. This model is based on the statistical physics of complex systems [1]. Liouville's theorem for complex systems is proved. The result is: Ic parameter is constant in time if energy of interaction of subsystems is a constant. The Ic parameter is equal to the difference between the entropy of a complex system and the total entropy of the interacting subsystems that make up this complex system. The meaning of the Ic parameter is a decrease in the entropy of a complex system during the transition from a stable state to a metastable one. Such a transition is not feasible as a result of heat exchange. It is possible only as a result of working on a complex system Experimental confirmation of this theory is found in the work of E.L. Andronikashvili and G.M. Mrevlishvili [2]. Experimental data were obtained on the heat capacity of DNA molecules with their aqueous environment and the heat capacity of destroyed DNA molecules with an equivalent amount of water in a wide temperature range. The entropy of DNA molecules is less than the entropy of destroyed DNA molecules by 3.5%. There is not only a quantitative difference: the water around whole DNA molecules did not turn into ice.
The physical meaning of the Ic parameter is clarified by the work of L.A. Blumenfeld [3], who proved the inapplicability of the combinatorial approach to describing the quantity of information contained in biological systems, since biological systems contain "constructions", that is, structures separating and deforming the Gibbs phase space. "From the standpoint of statistical physics, the presence of structures means the presence of boundaries between regions of phase space, the intersection of which is prohibited for figurative points of the statistical system" [3]. In the interpretation of statistical physics, the Ic parameter characterizes the depth of deformation of the Gibbs phase space, which occurred as a result of the interaction of subsystems of a complex system. Deformation means a change in the spatial structure of a complex system and the appearance of collective vibrational or rotational degrees of freedom [1]. The Noether's theorem for complex systems is proved. The result of Noether's theorem: the parameter Ic is invariant under any transformations that keep the interaction energy of subsystems unchanged. Deformation of the structure of a complex (supramolecular) system is accompanied by a change in the symmetry of this system. The Ic parameter changes when the chirality of the supramolecular structure of DNA molecules changes. The article [2] presents data on the heat capacity of DNA molecules with altered symmetry: "unwound" molecules. The entropy of such molecules is 2.9% higher than the entropy of DNA molecules that have retained their symmetry.
In the reaction of molecular recognition, in which the supramolecular structure of the DNA molecule is a receptor, information is received. The Ic parameter has an informational interpretation. Ic is the amount of mutual information: information that interacting supramolecular systems have about each other: ligand and receptor. The Ic parameter is well known in quantum physics as the quantity of mutual information. Ic is equal to the von Neumann entropy difference between the entropy of a complex quantum system consisting of interacting (entangled) quantum subsystems and the total entropy of non-interacting (non-entangled) subsystems. Ic characterizes the degree of entanglement of states of a complex quantum system. The Ic parameter is important for quantum computing. Only entangled quantum systems can produce efficient quantum computing.
The processes of changing the entropy, structure, symmetry and reception of information of the supramolecular structure of DNA are interdependent. The transition to a metastable state (entropy reduction) means a deformation of the structure and a change in the symmetry of the supramolecular structure of DNA molecules. An increase in the complementarity of the receptor to the ligand, due to the deformation of the receptor structure, leads to an increase in the quantity of mutual information. All these processes are characterized by a single parameter Ic. The relationship between entropy, symmetry and the ability to receive information of the supramolecular structure of DNA molecules has been found.

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### S1.120. The Role of Hydrogen Bond Networks in Type-2 Photosynthetic Reaction Centers

Vasilieva L.G.[1]*
[1]*Institute of basic biological problems of RAS, Pushchino, Russia;*
* vsyulya@mail.ru

In recent years, data on the spatial structures of membrane proteins with high resolution have significantly increased. This makes it possible to study the relationships between the structure and function of transmembrane complexes. In particular, clusters of hydrogen bonds in the structures of integral proteins near the membrane surface, which are formed due to the interactions of ionizable amino acid residues and water molecules, attract considerable attention. Currently, the role of hydrogen bond networks in the structures of a number of membrane transporters and receptors (in particular, in the photoreceptor bactriorhodopsin), on the acceptor side of photosynthetic reaction centers (reaction centers of purple bacteria, photosystem-2 of plants), as well as on the donor side of the photosystem-2, has been most studied. The role of hydrogen bonds in providing structural plasticity and stability of membrane complexes is shown. Less studied is the effect of hydrogen bond clusters on the redox potential of cofactors, as well as on the interaction of membrane complexes with mobile electron carriers. A review of published works and our own results on this topic is planned.

### S1.121. The ab initio DNA synthesis by Bst DNA polymerase in the presence of nicking endonucleases and the peculiarities of products structure as a platform for new DNA-based biomaterials

Zyrina N.V.[1,2]*, Antipova V.N.[2], Reveguk Z.V.[3]
[1]*Institute for Biological Instrumentation, RAS, Pushchino, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
[3]*Centre for Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics of St. Petersburg State University, St. Petersburg, Russia;*
* zyrina.nv@gmail.com

The essential part of modern DNA nanotechnology is the development of approaches to the formation of systems with a high yield at relatively

low cost. Ab initio DNA synthesis is a noncanonical synthesis of dsDNA by thermophilic DNA polymerases of prokaryotes only from free dNTPs. We found that very intensive ab initio synthesis by thermophilic DNA polymerase Bst takes place in the presence of nicking endonucleases (NEases) Nt.BspD6I, Nt.AlwI, Nb.BbvCI and Nb.BsmI. The resulting DNA contained mainly the repeats of short non-palindromic (Nt.BspD6I) or palindromic (Nt.AlwI, Nb.BbvCI, Nb.BsmI) sequences with several recognition sites of the NEases and a small random AT-rich sequence between them. More than 10 μg of DNA was synthesized by 1 a.u. of Bst DNA polymerase in 1.5 h. In the presence of a large amount of NEase were synthesized products of 1-2 kbp. The decrease in the amount of NEase caused the increase in the size of products up to 100 kbp. Restriction endonucleases whose recognition sites coincided with that of the NEases completely hydrolyzed low-molecular-weight 1-2 kbp products of the ab initio synthesis. The DNA products over 20 kbps were extremely difficult to further digest with the same restriction enzymes. The treatment of all the synthesized DNAs with the Mung bean nuclease, which is specific to ssDNA, loops, branches etc considerably reduced their size to short DNA fragments. Atomic force microscopy revealed that high-molecular-weight dsDNA products branched and formed net-like structures. The DNA contained single-stranded and triple-stranded segments. Low-molecular-weight DNA molecules did not form networks and consisted of single linear ds molecules with small number of branches. Thus, ab initio DNA synthesis allows specifying the sequence and length of DNA and generates the products with complex spatial structures. This fast high-yielded and inexpensive reaction may be very useful to develop techniques for the fabrication of DNA-based biomaterials.

### S1.122. The basis of metabolism is the transport of ortho-para spin isomer-H2O via aquaporin channels across membranes

Pershin S.M.[1]*
[1]*Prokhorov General Physics Institute of RAS;*
* pershin@kapella.gpi.ru

It is known that metabolism, as a process, reflects the transport of substances in the body, the main of which is water due to the large mass fraction: brain, heart, lungs ~ 80%, blood ~ 90%. It is physically clear that the most critical site of water transport in the body are membranes with aquaporin channels, which allow only H2O monomers to pass through at a rate of 3E9 molecules/s. We recall here that the H2O monomers differ in the orientation of the proton spin and are ortho- and para-isomers. At the same time, ortho-H2O have a magnetic moment (the spins are parallel) and always rotate. In contrast, para-H2O is not a magnet (the spins are antiparallel) and some of them do not rotate in accordance with the Boltzmann distribution over rotational states. The discovery of aquaporin channels (Peter Agre) was awarded the Nobel Prize 20 years ago [1]. Note that the aquaporin channel has a diameter of ~0.3 nm with a H2O monomer size of ~0.28 nm and a dipole key in the middle (Fig. 1). Despite these factors, human kidney membrane channels can pass up to 200 liters of water per day [1]. P. Agre et al. [2] notes that the mechanism of formation of a chain of H2O monomers inside the channel with the breaking of hydrogen bonds in the vicinity of the dipole key remains unclear (Fig. 1, bottom panel). The totality of data obtained over two decades in different scientific centers gives us a rationale for the presence of spin isomers and their conversion, as well as the place of their localization in water and aqueous solutions.
50 years ago, the pioneering work of Akhmanov S.A. et al. [3] opened a new era of nonlinear optical active Raman spectroscopy (CARS). Here, using laser beams in the visible range, nonlinear optics provided a unique opportunity to study [4, 5] the motion of molecules in water and aqueous solutions in the region of giant absorption at GHz–THz frequencies. Thus, free rotations of ortho-para spin isomers of H2O molecules in water [6] and selective binding of para-isomers of H2O during the formation of hydration shells in aqueous solutions of proteins were discovered by the CARS method [6]. Later [7], the spin isomers of H2O were also found in water. It remained unclear where the H2O monomers can be localized in water and hydration shells?
Early [8], we substantiated that the ice-like structures of ice Ih in water [9–11] and hydration shells [12–15] are capable of localizing H2O monomers, as in fullerene [16], in hexagonal cavities along the c axis, the transverse size of which is 0.57 nm is almost twice the diameter of the aquaporin channel [1, 2]. So it was established [13] that the elasticity and strength of the structure of hemoglobin hydration shells inside erythrocytes is very high in order to destroy them by shell pressure. Only the melt of the structure [13] due to para-ortho conversion of H2O [12] is accompanied by the loss of ~55% of water through aquaporin channels and provides deformation of erythrocytes for movement through capillaries. A similar melt (para-ortho conversion of H2O) of hydration shells (up to 90% water) of lysozyme protein takes place in a chicken egg in an incubator at a temperature of ~37.5 0C and the transport of H2O monomers through the aquaporin
channels of the yolk membrane for irrigation of the nucleus and its subsequent division [15], which is located at the top of the yolk under the membrane. We believe that the key factor in this representation is thermal fluctuations, which create mixed quantum states of closely spaced rotational levels and provide para-ortho spin conversion. Then, the rotating ortho-para H2O spin isomers pass through the dipole key of the aquaporin channel of the membrane [1, 2] at a rate of 3E9 molecules/s without stopping and maintain the body's metabolism at the required level.

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### S1.123. The role of biopolymers phase transitions in the formation and functional activity of nuclear stress-granules (A-bodies)

Gavrilova A.A.[1]*, Fonin A.V.[1], Fefilova A.S.[1]
[1]*The Institute of Cytology, Russian Academy of Sciences;*
* asultanbekova@incras.ru

The study of the molecular basis of processes occurring in a living cell under normal and unfavorable (stress) conditions is one of the fundamental tasks of molecular and cellular biology. Over the past decades, a large amount of data has accumulated that indicates a significant role of non-membrane organelles in the spatial organization of the intracellular space and the regulation of signaling pathways in response to stress.
As a rule, non-membrane organelles are liquid droplets formed as a result of nonspecific multivalent interactions between disordered regions of RNA-binding proteins and RNA molecules. In some cases, liquid-drop condensates of biopolymers can transform into gel-like aggregates and even more ordered structures, both pathological and functionally significant. A-bodies are an example of non-dynamic functionally significant structures formed as a result of phase transitions of biopolymers.
A-bodies arise in the nucleolus in response to stress caused by changes in temperature, pH, osmotic pressure, etc. They contain in the amyloid state hundreds of proteins responsible for proliferative activity, transcription, and other cellular functions. The formation of A-bodies begins with the activation of transcription from intergenic spacers of ribosomal DNA (ribosomal intergenic spacers, rIGS) of a long noncoding RNA (lncRNA) called rIGSRNA (ribosomal intergenic spacer RNA). rIGSRNA acts as a structural element and nucleator of A-bodies. Further maturation of A-bodies occurs due to the recruitment of rIGSRNA partner proteins. The rIGSRNA transcripts are negatively charged sequences with a low degree of complexity and consist of (CU)n/(AG)n tandem repeats. An increase in the concentration of these molecules in the vicinity of genomic rIGS loci leads to the formation of bimolecular condensates, to which, due to electrostatic interactions, internally disordered and amyloidogenic proteins containing disordered positively charged regions are recruited. The presence of hydrophobic ACM (amyloid-converting motif) motifs in these proteins, provided that they are highly concentrated in A-bodies, creates conditions for the transformation of condensates into a gel-like state and, further, into aggregates of amyloid fibrils. This stage completes the maturation of A-bodies. The end of stress exposure causes the disassembly of A-bodies with the help of the chaperone system of the cell and the dissolution of amyloid fibrils. Thus, A-bodies provide storage of proteins under stress conditions in the amyloid form without the need for their degradation and de novo synthesis.
It is known that the formation of pathological amyloid fibrils accompanies a number of severe diseases, including neurodegenerative diseases. In this regard, it seems essential to study the structure, mechanisms of disassembly of functional fibrils, as well as the reasons for the transition of these fibrils to an insoluble state.
This work is aimed at studying the mechanisms of formation and the functional role of A-bodies. We have developed approaches for the visualization of A-bodies. The signal for the formation of A-bodies is the transcription of lncRNA rIGSRNA, which binds to the ubiquitin-ligase VHL (von Hippel-Lindau protein). To visualize A-bodies in the studied cells, plasmids encoding fusion proteins of the fluorescent EGFP protein with VHL isoforms (VHL30 and VHL19) were created. Next, the obtained constructs were transfected into MCF-7 human adenocarcinoma cells and incubated in the temperature range from 40 to 43 degrees Celsius to induce cellular heat shock. Osmotic stress was created by introducing NaCl into the cells, and the induction of a stress response in the studied cells due to a change in pH was carried out by acidifying the cell culture media and incubating the cells in the presence of 1% oxygen.
It has been shown that, regardless of the type of stress, there is an increase in the nuclear localization of VHL isoforms in MCF-7 cells exposed to stress, as well as the formation of compartments stained with dyes specifically interacting with amyloid fibrils. This indicates the formation of A-bodies. The dynamism of A-bodies formed as a result of stress exposure was studied using the method of EGFP fluorescence recovery after its photobleaching (FRAP). It has been established that A-bodies formed as a result of the incubation of cells for 1 hour at 43 degrees Celsius lose their dynamism.
The formation of A-bodies in stressed cells was also examined using electron microscopy. For these purposes, ultrathin sections of the cell culture were used, placed on nickel grids and additionally treated with a contrast solution. It has been shown that heat stress causes the formation of amorphous and fibrillar electron-dense structures in the nucleoli of the studied cells.
FISH was performed to visualize the target rIGSRNA transcript in fixed cells. The formation of rIGS16RNA clusters in the nucleoli of MCF-7 cells exposed to heat stress was established.
The ability of the VHL protein to form fibrillar structures in vitro was investigated. For these purposes, VHL preparations were incubated in solutions with neutral pH in the presence of high concentrations of crowding agents and in solutions with acidic pH. It has been established that in solutions with neutral pH, VHL molecules form amorphous aggregates, while in solutions with acidic pH, they form amyloid fibrils.
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### S1.124. The role of correlated movements of individual protein fragments in their function: photophysics of color fluorescent proteins

Savitsky A.P.[1]*, Khrenova M.G.[1]
[1]*FRC Biotechno;ogy of the RAS;*
* apsavitsky@inbi.ras.ru

Fluorescent proteins have a fairly rigid beta-barrel structure; however, local conformational mobility significantly affects their properties. For example, the correlation of movements of individual protein fragments leads to the possibility of cis-trans isomerization of the chromophore group, and the presence of different conformers of the side chains of individual residues determines the rate of these transformations. The study of the distributions of geometric parameters within the chromophore related to the alternation of double and single bonds in the system makes it possible to judge the shape of the absorption band and the position of its maximum, and by analyzing the dynamic behavior of the C-O bond of the phenyl fragment of the chromophore, one can judge the pKa of this group. Thus, the analysis of the dynamic characteristics of proteins obtained from calculations by molecular modeling methods makes it possible to determine the most diverse properties of fluorescent proteins.

### S1.125. The structure and functions of nuclear proteins HmgB1 and HmgB2

Chikhirzhina E.V.[1]*, Starkova T.Y.[1], Tomilin A.N.[1], Tsimokha A.S.[1], Polyanichko A.M.[1]
[1]*Institute of Cytology of the Russian Academy of Sciences;*
* e.chikhirzhina@incras.ru

Chromatin is a highly dynamic system that includes many factors. The length of DNA in a chromosome significantly exceeds the size of the nucleus of a eukaryotic cell, and the issue of DNA packaging is very

important in biology, not only from a structural point of view, but also because it is closely related to the correct functioning of the entire genetic apparatus of the cell. A high degree of DNA compaction in the nucleus is achieved due to its interaction with various histone and non-histone proteins. Histones (H2A, H2B, H3 and H4) form a protein particle (nucleosome) around which DNA is wound. These particles are connected by a linker region of DNA, with which the fifth histone H1 interacts. Also proteins of a large family of non-histone proteins, proteins with high electrophoretic mobility (High Mobility Group), are associated with linker DNA. From our point of view the HmgB1 and HmgB2 proteins are the most interesting among these proteins. HmgB1 and HmgB2 are actively involved not only in the regulation of the chromatin structure, but are also directly involved in many cellular processes, such as transcription, repair, and recombination. In addition, domains homologous to the domain of the HmgB1 protein were found in many transcription factors as DNA-binding domains. Proteins HmgB1 and HmgB2 interact with DNA by a non-specific way. However, just like histone H1, they are able to recognize and bind to DNA regions with various structural disorders. It is also interesting that, under certain conditions (due to post-translational modifications or changes in redox status), HmgB1 leaves the nucleus, moves to the cytoplasm, and then enters into the extracellular space. All these processes are closely related to the development of various human diseases, ranging from cardiac pathologies to disorders in the development of the embryo, and hence with cell damage and with its death. When HmgB1 exits into cytoplasm, its amount in the nucleus decreases, while the HmgB2 protein continues to function normally. In connection with the above, it is important to understand the subtle structural differences between the HmgB1 and HmgB2 proteins, which undoubtedly affect the mechanisms of their interaction with DNA and other partners.

The HmgB1 and HmgB2 proteins are very similar in structure and amino acid sequence. Both consist of a short N-terminal region, two DNA-binding domains A and B connected by a linker, and a random C-terminal sequence of glutamine and aspartic amino acid residues. The most common method of the research of the protein secondary structure is the method of circular dichroism in the UV range. This method makes it possible to track changes in the structure of both the proteins themselves and their complexes with DNA, including the assessment of the degree of α-helicity of proteins.

In this work the secondary structure of proteins HmgB1 and HmgB2 of the calf thymus and the peculiarities of their interaction with DNA, we studied by the methods of circular dichroism (CD) and UV spectroscopy. In addition, a comparative analysis of post-translational modifications (PTMs) of HmgB1 and HmgB2 proteins was carried out using mass spectrometry.

We have shown that, despite the high conservation of their amino acid sequences of the HmgB1 and HmgB2, these proteins differ significantly from each other, which undoubtedly affects on the mechanism of interaction of these proteins with their main target in the cell nucleus, DNA. In this work it was shown that the nature of the location of PTMs in the studied proteins is different. While HmgB1 PTMs are predominantly located in the A DNA-binding domain and in the linker region between the A and B domains, PTMs of HmgB2 are concentrated in its B domain and also in the linker region. It was shown that, despite the high degree of homology between HmgB1 and HmgB2, the secondary structure of these proteins is different. An analysis of the CD spectra of proteins showed that, under physiological conditions, the HmgB1 protein is characterized by a more ordered secondary structure than HmgB2. At the same time, HmgB2 exhibits great conformational flexibility under changing external conditions. We believe that this flexibility contributes to the structural adaptation of the HmgB2 protein to a much greater extent than for the HmgB1 protein. This circumstance undoubtedly influences their interaction with other proteins, DNA and the structure of DNA-protein complexes. The latter can determine the difference in the functions performed by the HmgB1 and HmgB2 proteins in the cell nucleus.

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### S1.126. Towards investigation of Influenza A matrix protein M1 role in infection process: molecular modeling study

Bulavko E.S.[1,3]*, Kalutsky M.A.[2], Batishchev O.V.[1]
[1]*A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of RAS;*
[2]*Max Planck Institute for Multidisciplinary Sciences;*
[3]*Skolkovo institute of science and technology;*
* egor.bulavko@skoltech.ru

Influenza A virus is an enveloped RNA virus, which could cause seasonal epidemics and global pandemics. Cell infection is mediated by endocytosis and subsequent fusion of host and viral membranes induced by pH decrease. The matrix protein M1 forms a continuous helical scaffold beneath the lipid envelope of the virus, binding to it with N-terminal domain, while C-terminal domain forms complex with viral RNA. M1 performs several important functions at various stages of the life cycle, but its role in the process of viral and endosomal membranes fusion remains a matter of controversy. In vitro experiments have recently shown that M1 is actively involved in viral membrane rearrangements and subsequent release of the genome. However, the details of the processes of protein scaffold reorganization and membrane deformation induction remain unclear. The aim of this work was to study the conformational dynamics of M1 N-domains di- and oligomers complexed the membrane upon pH changed from 7.7 to 4.0.

To model systems described above, we used the approaches of coarse-grained and all-atom molecular dynamics simulations. Environmental acidification was imitated by protonation of titratable amino acids (primarily His-110). We also estimated the free energy stored in the scaffold structure upon pH changed, for which we applied the thermodynamic integration method.

We showed that stability of the protein scaffold is modulated by interaction with membrane, since in its absence the lifetime of M1 dimers is no more than 500 ns. Long-time simulations of oligomers folded into an infinite periodic structure that imitates a scaffold ribbon have shown that, as pH decreases, M1 helices are partially immersed in the membrane. Additionally, mutual orientation of the monomers changes and now resembles that in the crystal structure of M1 dimer at pH 4.0. Using the dimer as an object, we also showed that pH decrease leads to the accumulation of an excess free energy around 9.7 kT (per mole of protein) in the system. The membrane bending modulus is around 20 kT, which means that the cumulative potential of the scaffold is sufficient to induce rearrangement of the viral envelope.

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### S1.127. Vibrational structure in thymine aqueous solutions luminescence spectrum

Vladimir M.[1]*, Tulina T.A.[1]
[1]*Saint-Petersburg University;*
* vlmalkin@yandex.ru

The structure of the energy levels of polyatomic molecules is complicated by the splitting of electronic levels into vibrational and rotational sublevels [1, 2]. Theoretically, it should correspond to the line spectra of absorption, emission, and excitation of luminescence. However, in solutions at room temperature, the spectra of molecules are broad bands [3-16]. As the temperature decreases (77 K), the luminescence spectra of molecules have a vibrational structure (the Shpol'skii effect) [2, 7, 8], but this method is practically impossible for experiments with biologically significant molecules in their most natural medium, aqueous

solutions, at room temperature. The structure of the vibrational levels of molecules in an aqueous medium is also difficult to obtain using IR spectroscopy, since water does not transmit light in the IR range [9].
We assumed that the seemingly random "noise" shoulders and small "peaks" of the luminescence spectra are not randomly distributed.
After analyzing the luminescence spectra of thymine in aqueous solutions, we constructed histograms of the distribution of such peaks and shoulders over wavelengths. It turned out that they are indeed distributed not randomly, but are most often observed at 295nm (33898 cm-1), 303nm (33003 cm-1), 312nm (32051 cm-1). , 319 nm (31348 cm-1), 327 nm (30581 cm-1), 336 nm (29762 cm-1), 344 nm (29068 cm-1), 353 nm (28329 cm-1), 363 nm (27548 cm-1), 372 nm (26882 cm-1).
We believe that statistical processing makes it possible to observe manifestations of the vibrational structure of the ground energy level of thymine in an aqueous solution in the luminescence emission spectra, and that each band on the histogram of the "noise" distribution corresponds to a transition from the zero or first vibrational level of the excited state to one of the vibrational levels of the ground state . Based on the data obtained, it was possible to obtain the vibrational frequencies of the IR spectrum of thymine at 895 cm-1 (close to 889 cm-1, obtained experimentally in a non-aqueous solution [10] and theoretically 907 cm-1). [11]), 952 cm-1 (cf. 959 cm-1[10] and 965 cm-1 [11]), 1655 cm-1 (cf. ]. ]), 767 cm-1 (cf. 763 cm-1[10] and 767 cm-1 [11]), 739 cm-1 (cf. ]), 781 cm-1, 666 cm-1 (cf. 662 cm-1[10] and 667 cm-1 [11]), 704 cm-1, etc.
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## S2. Biophysics of the cell. Membrane and transport processes

### S2.128. A nonlocal electrostatic approach to the cation selectivity of monovalent cations in the aqueous cavity of the K+ channel in a biomembrane and to the charge selectivity in tight junctions between epithelial cells

Rubashkin A.A.[1]*, Iserovich P.[2], Ostroumova O.S.[1]
[1]*Institute of Cytology RAS, St. Petersburg, Russia;*
[2]*SUNY Downstate Medical Center, Brooklyn, NY, USA;*
* andrey.rubashkin@gmail.com

The importance of the analysis of electrostatic interactions in the water cavity of the ion channel in the biomembrane for explaining the stabilization of the cation in the cavity was pointed out in [1,2]. In [1], the change in the chemical potential Δμ of the cation during its transition to the cavity of the KcsA K+ channel was not considered, since it was assumed that the static permittivity ε in the cavity does not differ from ε of water in an external solution. In [2], the passage of a cation into a cylindrical channel was considered; it was shown that ε of water in the channel is much less than its value in the external solution. However, the authors failed to explain the stabilization of the cation in the channel, since the classical Born formula was used to estimate the Δμ of the cation, the calculation by which, as is known, gives overestimated values for the ion solvation energy W in solution [3]. Probably for this reason, in [1], the value of ε in the cavity was chosen to be 80, i.e., its value in a free solution. Therefore, in [4], we analyzed the possibility of applying the classical theory of solvation based on the Born formula to explain both the stabilization of the cation in the water cavity of the channel and the appearance of cation selectivity in the cavity. This analysis showed the impossibility of explaining the stabilization of the cation in the cavity on the basis of classical electrostatics. Earlier, in [5], we considered the transition of a cation from solution to a selective channel filter; to calculate W, we used the nonlocal electrostatic (NE) theory, the foundations of which are described in monograph [3].
In the report presented here, we apply the ideas of our works [4, 5] to model the process of transition of monovalent cations into the water cavity of the channel, and use the NE theory to calculate the W of the cation both in free solution and in the water cavity of the channel.
The appearance of K+/Na+ selectivity in the water cavity (Cav) of the ion channel differs significantly from the mechanism of charge selectivity formation in tight junctions (TJ) between epithelial cells. The main effect here is the difference in ΔW for K+ and Na+ cations. The electrostatic interaction of the ion with the charges of proteins surrounding the cavity is important for the stabilization of the cation in the cavity, but not for the occurrence of cation selectivity, which is calculated by the formula: SK/Na(Cav)=exp[(−ΔμK)/kT]/exp[(−ΔμNa)/kT]. The selectivity calculation carried out using this formula predicts the existence of K+/Na+ selectivity in a nanometer-sized water cavity in the range 1.4<SK/Na<8. In this case, the value of ε in the channel cavity varied from 2 to 10. We were able to explain the stabilization of the cation in the cavity, which is impossible to do with such a large difference between ε in the cavity and its value in a free solution using the classical theory of solvation.
Let us now consider the general and the difference in the processes of occurrence of cation selectivity in channels and charge selectivity in tight junctions (TJ) between epithelial cells. We developed the theory of charge selectivity in TJ in [6–8], using the NE theory to calculate the solvation energies of ions. It was shown that Na+/Cl- selectivity arose due to a combination of two effects. The first of these effects is the electrostatic interaction of mobile ions with fixed charges of claudine macromolecules in TJ. The second effect is an increase in the correlation length of water in TJ and, as a consequence, large negative values of the Na+ and Cl- resolvation energies ΔW, leading to their low concentrations inside TJ. The high Na+/Cl- selectivity in TJ is due to low ionic concentrations in combination with the negative charges of claudine in TJ. The formula for calculating the charge selectivity of SNa/Cl in TJ includes changes in the chemical potentials of ions (Δμ=−ΔW) and the electrostatic potential in TJ (φTJ):
SNa/Cl(TJ)={exp[(−ΔμNa)/kT]/exp[(−ΔμCl)/kT]}exp[−2eφTJ/kT].
Note that the last factor was not a formula for cationic selectivity. Both the cation selectivity in the channel cavity and the charge selectivity in tight junctions cannot be explained using the classical theory of solvation.
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### S2.129. AFM studies of the electrophysical properties of the membrane of human buccal epithelium cells

Torkhov N.A.[1], Mosunov A.A.[1]*
[1]*Sevastopol State University;*
* aamosunov@sevsu.ru

Unilateral inhomogeneous electrical conductivity of the membrane of human buccal epithelium cells and its correlation with the topology of micromechanical properties were found.
Intensive development of bioelectric integrated human systems based on the propagation of electrical signals in his tissues requires the study of the electrical properties of the body at the cellular level [1]. In this regard, living human buccal epithelial cells (hereinafter referred to as cells) obtained by liquid cytology were used as the object of research. This method included mechanical sampling (scraping) from the inner surface of the cheek of the oral mucosa, washing the scraping in a phosphate buffer (3.03 mM phosphate buffer with the addition of 2.89 mM calcium chloride with a volume of 5 ml, pH 7.0), placing this mixture in a test tube and separating its contents in a centrifuge for 5 minutes with acceleration 1700g (5000 min-1), sampling from a centrifuge tube aliquots of a buffer solution containing a suspension of living buccal epithelial cells. The epitaxial structure of silicon of the p-type hole conductivity p- p+- Si{111} with the size of irregularities <20 pm was used as a substrate material.
After placing the aliquot on the epitaxial silicon surface Si{111}, it was dried in air at normal atmospheric pressure and temperature T<=40 C for <10 minutes. The living cells in the aliquot were naturally deposited on the epitaxial surface of silicon and preserved on it in this form for 3-4 hours after evaporation of the main amount of moisture. The presence of an adsorption layer of buffer solution on the surface of the cells maintained their viability during a relatively long stay in the air under normal conditions (NU).
Studies of the geometry of the surface relief of cell membranes and their electrical conductivity – the electron-hole spreading current Ipr (hereinafter referred to as the spreading current Ipr) were carried out in air at NU using the NTEGRA–AURA AFM with a scanning slide table in contact scanning mode and a resolution of 300x300 points providing constant mechanical and electrical contact of the tip of the cantilever needle with a constant by the force of pressing against the surface of the "Molecular structure of matter" conducted on the basis of the Center for Collective Use Sevastopol State University. Conductive HA-FM/W2C cantilevers with a radius of rounding of the needle tip r~35 pm were used as a measuring probe. The cantilever was grounded.
The location of the cell nucleus and surrounding organelles is clearly visible on the surface of the membranes. At the same time, the shell surface itself is not smooth, but has a sufficiently developed relief with a sufficiently developed morphological structure and mechanical organization [2, 3].
Studies have revealed one-sided inhomogeneous electron-hole conductivity of the membrane of human buccal epithelium cells. To determine the type of conductivity (electronic or hole), further research is necessary.
Analyzing the histograms of the distribution N = N (Iprr), it can be determined that the membrane is formed by regions with different electrical conductivity, which are formed by a set of smaller areas with similar values of spreading currents Iprf =Iprf(x,y). Studies have shown that the topology of these sites coincides quite well with the topology of local terrain irregularities and the distribution of adhesive forces over the membrane surface Fadh=Fadh(x,y), as well as the work performed by them Aadh=Aadh(x,y). A more detailed analysis showed that ion nanochannels penetrating the membrane have several times higher electron-hole conductivity compared to their surrounding surface. At the same time, the heterogeneity of membrane conductivity partially correlates with the nature of the location of some cell organoids. This indicates the existence of an electrical connection of such organoids with the cell membrane.
Unilateral electrical conductivity of the membrane was revealed. So for Ur<0, the average values of currents <Iprr>=20.8 pA significantly exceed the values of <Iprf>=0.01 pA for Uf>0.
Studies have shown that, depending on the state of the cell, the conductivity of different areas of the cell membrane can vary within a fairly wide range, which can be used to assess the state of the cell.
The research was conducted in accordance with the principles of the Helsinki Declaration. Permission to conduct studies with buccal epithelium sampling was obtained by the Ethics Committee of Sevastopol State University (Study No. 3, July 15, 2021).
Buccal epithelium was collected in accordance with the rules for conducting research on human material in the Russian Federation. All subjects have given written informed consent.
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### S2.130. Analysis of the participation of protons, calcium ions and hydrogen peroxide in changes in the content of phytohormones during the propagation of electrical signals

Kuznetsova D.V.[1]*, Ladeynova M.M.[1], Pecherina A.A.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* kuznetsova.dar0@gmail.com

For an effective response to changing environmental factors, plants have a complex system of generation and propagation of stress signals.

It is known that under the action of a damaging stimulus, such an electrical signal as a variable potential (VP) propagates and there is a change in the content of stress phytohormones. According to the literature, VP generation is associated with a significant change in the concentrations of various ions, which may be a possible inducer of changes in hormone concentrations. The probable involvement of hydrogen peroxide (H2O2) in the propagation of VP provides a basis for the assumption that the change in the concentration of H2O2 also plays a role in the change in the content of phytohormones. Thus, the aim of the work was to analyze the participation of protons, calcium ions, and hydrogen peroxide in the change in the content of phytohormones during the propagation of electrical signals.

For the experiments, 15-19-day old wheat plants (Triticum aestivum L.) were used. Heating of the leaf tip was used as a local stimulus to generate VP. A multichannel macroelectrode setup was used to record VP. The study of the dynamics of the concentrations of protons, calcium ions, and hydrogen peroxide during the propagation of VP was carried out on a surface optical imaging setup using BCECF, AM, Fluo-4, AM, and Ampliflu Red fluorescent probes, respectively. The vacuum infiltration method was used to load probes into plants. Determination of the content of phytohormones was carried out in a leaf fragment remote from the damage zone using the method of high-performance liquid chromatography-mass spectrometry. During sample preparation, a leaf fragment was frozen in liquid nitrogen and was homogenized in an extracting solution (80% methanol + 1% formic acid containing internal standards of the substances under study). The extraction was carried out in two stages for 15 minutes, then centrifuged at 20040 g, the supernatant liquid was evaporated 2 times using a concentrator. The inhibitory analysis was performed using the method of vacuum infiltration of wheat plants with a solution of 5 mM Na3VO4 (proton pump inhibitor) or 5 mM LaCl3 (calcium channel blocker); for the control group, vacuum infiltration was performed with a solution without the addition of inhibitors.

Under the action of heating, the generation and propagation of VP, as well as a change in the content of phytohormones in wheat plants, is observed. It was shown that the maximum amplitude of changes in the concentration of stress phytohormones, namely, jasmonic acid (JA), is observed 15 min after the stimulus, while abscisic acid (ABA) and salicylic acid (SA) are characterized by concentration maxima after 60 and 40 min, respectively. During VP generation, temporary acidification of the cytoplasm is observed. Inhibitory analysis showed a decrease in the amplitude of the pH wave and the amplitude of the VP under the action of Na3VO4. Inhibition of the proton pump also affects the concentrations of the studied phytohormones: under the action of Na3VO4, the content of ABA and jasmonates increases both during stimulation and without a stimulus. The intracellular concentration of Ca2+ temporarily increases during VP generation. Inhibitor analysis also showed that inhibition of calcium channels leads to a decrease in the VP amplitude, and changes in the shifts in phytohormone concentrations induced by the action of a damaging stimulus on the plant are also observed. The action of LaCl3 leads to blocking of the increase in jasmonates at the time point of 15 min and SA at the time point of 60 min after the action of the damaging stimulus.

During VP generation, a temporary increase in the H2O2 concentration is also observed. With the artificial addition of H2O2, changes in the content of phytohormones occur: the concentration of SA increases statistically significantly under the action of 5 mM H2O2, with an increase in the concentration of H2O2 to 20 mM, the effect is even more pronounced compared to the control group. A change in the content of the JA derivative, jasmonyl-isoleucine, occurs under the action of 10 mM H2O2. The dynamics of the phytohormone concentration under the action of a local stimulus is comparable to the dynamics of the hormone content upon the artificial addition of 10 mM H2O2: it was shown that the concentrations of ABA and SA increase at a time point of 45 min after the addition of H2O2, while jasmonates are characterized by a significant increase in concentration at a point of 15 min.

Thus, the inhibitory analysis showed the participation of protons and calcium ions in the change in VP parameters, as well as in the shift in concentrations of the studied stress phytohormones under the action of a local stimulus, caused by VP; which indicates a possible connection between electrical signals, changes in the concentration of hydrogen peroxide, and hormonal signals.

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## S2.131. Antiplatelet and antioxidant efficiency of lipoic acid nanoconstructions

Andreevich V.A.[1,2,3]*, Inshakova A.M.[1], Darnotuk E.S.[1], Shipelova A.V.[1], Baranova O.A.[2,3], Chekanov A.V.[2,3], Kazarinov K.D.[3], Shastina N.S.[1], Solovieva S.Yu.[2], Fedin A.I.[2]

[1]*MIREA-Russian Technological University (ITHT named after M.V. Lomonosov);*

[2]*Russian National Research Medical University named after N.I. Pirogov of the Ministry of Health of Russia;*

[3]*Kotel'nikov Institute of Radio Engineering and Electronics of the Russian Academy of Sciences;*

* vasiliy9999@yandex.ru

Abstract

Various nanoconstructions with lipoic acid (LA), with a particle size from 20 to 300 nm were obtained, characterized by its slow release from nanoparticles (NPs) and high dispersion stability during long-term storage at room temperature and at T +4 °C. It has been found that nanodispersions (ND) with LA decrease the activity of lactate dehydrogenase (LDH) in platelets (Pt) by 1.5-2 times, which indicates the absence of their cytotoxicity. It has been revealed that nanoparticles with LA suppress platelet aggregation by 45-85%, caused by arachidonic acid (AA) and reduce the concentrations of reactive oxygen species (ROS) and products of lipid peroxidation (POL).

Keywords: nanoconstructions, lipoic acid, platelets, arachidonic acid, lipid peroxidation, reactive oxygen species.

Cerebrovascular diseases are among the most common forms of CNS pathology with a high rate of deaths [1]. The main pathogenetic mechanisms of ischemic stroke include: the onset and progression of oxidative stress, disorders of vascular-platelet and coagulation hemostasis, damage to the BBB, etc. [2]. Therefore, in the complex therapy of this pathology, it is necessary to use drugs that exhibit antioxidant and antiplatelet effects. One of the most promising antioxidants is lipoic acid. However, LA is poorly soluble in water and, when it enters the body, it quickly binds to proteins, biodegrades, and is quickly excreted, which leads to a decrease in the therapeutic effect.

The purpose of this research work is to obtain various nanoconstructions with LA for its solubilization in aqueous solutions, prolonged release, as well as to study their effect on the functional activity of platelets.

In the research work, nanodispersions with LA based on phosphatidylcholine (PC), oligoglycerol (OG), and Pluronic F68 were obtained in a phosphate buffer solution (PBS, pH 7.4, 0.15 mM) with particle sizes from 20 to 350 nm. NPs with LA based on F68 and OG were heterogeneous and consisted of two fractions of NPs: 20–70 nm (25±5%) and 110–310 nm (75±5%). It has been shown that NPs with LA were dispersion stable during long-term storage (>20 months) at room temperature and at T +4°C nanoconstructions with lipoic acid were electroneutral.

Using the cryo-TEM method, it was found that nanodispersions with LA represent an heterogeneous system consisting of single-layer and multilayer nanostructures of mainly spherical particles with different size.

Then, the kinetics release of LA from nanostructures has been studied. It has been established that about 50±5% LA was released from

nanodispersions in 24 h. This process of LA release from NPs can provide a prolonged action of LA and long-term maintenance of its therapeutic concentration in the blood.
At the next stage of the research work, the effect of nanoconstructions with LA on LDH activity in platelets activated by arachidonic acid (AA) was evaluated. As a result of the studies, it was found that nanodispersions with lipoic acid (1-4 mM) decrease the activity of this enzyme by 1.5-2 times, which indicates the absence of their cytotoxicity.
Then, we studied the effect of nanoconstructions with LA on the aggregation of Pt in blood plasma isolated from the blood of healthy donors. The aggregation of Pt was induced by arachidonic acid (AA), since as a result of its action, various metabolites are formed, incl. POL and ROS products. It has been shown that ND LA (1-4 mM) reduces AA-induced Pt aggregation by 45-85%. Water-soluble forms and NPs without LA had virtually no effect on Pt aggregation. Apparently, LA in nanoconstructions is better able to penetrate into cells due to the interaction of lipids with the cell membrane or as a result of receptor-mediated endocytosis.
The antioxidant effect of LA NPs was assessed by the concentration of reactive oxygen species (ROS) and thiobarbiaturate active products (TBA-AP) in samples of plasma enriched with Pt incubated by AA. The addition of all types of NPs with LA to blood plasma samples led to the significant decrease in the amount of ROS (2-5 times) and TBA-AP (5-20 times). All types of ND with LA had the most effective antioxidant effect when using the maximum concentration of LA. Inhibition of the process of initiation of lipid peroxidation products and ROS by AA can be considered as the proposed mechanism of the antiplatelet effect of nanoconstructions with LA.
Thus, all types of nanodispersions with lipoic acid are promising candidates for further studies of molecular biological mechanisms in vivo.
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**S2.132. Astaxanthin is able to involve in processes of mitochondrial quality control**

Krestinina O.V.[1]*, Krestinin R.R.[1], Baburina Y.L.[1], Odinokova I.V.[1], Sotnikova L.D.[1]
[1]*Institute of Theoretical and Experimental Biophysics;*
* ovkres@mail.ru

One of the reasons due to which there is a violation of cerebral circulation is heart disease, as a result of which the blood flow in the arteries is disturbed and the blood supply to the brain decreases. Mitochondrial dysfunctions are involved in the etiology of various diseases such as neurodegenerative and cardiovascular diseases, diabetes, various forms of liver and musculoskeletal diseases, sepsis, and psychiatric disorders. In addition, mitochondria are important cell organelles because they are the main source of ATP, which is essential for cell survival and many vital cellular functions. Since mitochondria are involved in cell death, the quality of mitochondria must be well controlled. It is suggested that mitochondrial fission and fusion may serve as important quality control mechanisms for mitochondrial conservation.
Astaxanthin (AST) is a ketocaratinoid of the xantafil subclass, has a strong antioxidant capacity and can scavenge singlet oxygen and free radicals. It is known that AST significantly attenuates mitochondrial dysfunction associated with ischemic myocardial damage, can reduce oxidative stress and prevent the development of cardiovascular diseases.
In this study, we studied the effect of AST on the functional state of brain mitochondria in rats with heart failure and analyzed whether AST is involved in mitochondrial quality control. For experiments, rats were divided into four groups (four rats in each group). The group 1 of rats was the control, the rats of the group 2 were administered orally AST (150 mg/kg, in olive oil) for 2 weeks. Rats from group 3 were injected with isoproterenol (100 mg/kg, in 0.9% NaCl) twice 24 hours apart to induce heart failure. Isoproterenol (ISO) is a non-selective beta-adrenergic agonist. The injection of ISO is a model that mimics stress-induced damage in various cardiac pathologies. Animals of groups 1 and 3 received an equal amount of olive oil. Rats of the fourth group were administrated with AST (150 mg/kg) for two weeks. Two weeks later, the rats of the fourth group were injected with ISO twice with an interval of 24 hours. Changes in the content of proteins such as myoglobin, troponin I, and lactate dehydrogenase were studied in order to identify cardiac dysfunctions in the presence of ISO. The content of all studied proteins decreased in heart mitochondria isolated from group 3 of rats, while AST increased the level of these proteins (group 4). Histological analysis of samples of the left ventricle of the heart indicates that both in the group 3 and 4, there are signs of myocardial hypertrophy. However, in the samples of the group 4, the process was less pronounced and was limited to the subenocardial and median zone, compared with the samples from the group 3, where the damage was of a total nature. These results show that heart failure has been achieved. AST reversed the effect of ISO and reduced damage to the heart.
We have shown that under conditions of heart failure (group 3) the respiratory control index in rat brain mitochondria decreased compared to the control, while AST (group 4) increased this parameter to the control value and the functional state of mitochondria improved. It is known that the uncoupling of oxidative phosphorylation leads to a change in the permeability of the mitochondrial membrane. Therefore, in the next step of our study, we measured the swelling of mitochondria under our experimental conditions. The ISO injection resulted in an increased swelling rate. When AST was administered to rats followed by ISO injection, the swelling rate decreased.
To assess the effect of AST on quality control processes, we studied the content of marker proteins involved in mitochondrial fusion and fission. With the injection of ISO, the level of Drp1, which indicates the fission of mitochondria, decreased, while the level of OPA1, which is responsible for the fusion of mitochondria, increased. In brain mitochondria isolated from rats with heart failure, administration of AST resulted in an increase in Drp1 expression and a decrease in OPA1.
Mitophagy refers to the process by which cells selectively remove excess or damaged mitochondria through autophagy, which plays an important role in mitochondrial quality control and cell survival. If mitochondria are damaged, autophagic clearance is initiated. In the present study, we observed that the levels of autophagy markers LC3A/B-I, II increased in the brain mitochondrial fraction of rats with heart failure, while AST reduced the level of the markers.
Recently, in rat heart mitochondria, we have identified a protein with a molecular weight of 30 kDa as prohibitin (PHB). There are two isoforms of PHB, PHB1 and PHB2, which are two highly homologous subunits of the eukaryotic mitochondrial prohibitin complex. PHB1 has been shown to have cardioprotective and anti-inflammatory effects, which are partly related to the maintenance of oxidative phosphorylation and metabolic control. Ablation of PHB2 leads to cardiac mitochondrial dysfunction and plays an important role in the homeostasis of fatty acid metabolism in the heart. Therefore, it can be assumed that prohibitins are important proteins for the normal functioning of the heart. One of the functions of PHB in mitochondria is to control the quality of mitochondrial proteins. In the present study, we showed that

PHB content decreased in brain mitochondria isolated from group 3 rats (ISO injection), while AST increased PHB levels (group 4).
Based on the foregoing, it can be assumed that astaxanthin improves the functional state of brain mitochondria in rats with heart failure and may be involved in mitochondrial quality control.
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### S2.133. Atomic force microscopy of erythrocytes in experimental diabetes mellitus and its correction with 2,5-substituted 6H-1,3,4-thiadiazines

Emelianov V.V.[1]*, Leontiev D.V.[1], Ishchenko A.V.[1], Sidorova L.P.[1], Tseitler T.A.[1], Shadrin I.A.[1], Gette I.F.[2], Danilova I.G.[2,1]
[1]*Ural Federal University named after First President of Russia B.N. Yeltsin;*
[2]*Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences;*
* v.v.emelianov@urfu.ru

The study of the morphology of erythrocytes in diabetes mellitus (DM) in the experiment and clinic reveals a change in diameter and the appearance of abnormal cells in the bloodstream. The advantage of the atomic force microscopy (AFM) method is that it allows you to obtain information not only about the morphology of the cell, but also about the relief of the cell surface and its mechanical properties [1, 2]. The severity of morphological changes is determined by the accumulation of structural damage to the erythrocyte as a result of metabolic disorders (hyperosmolarity, activation of non-enzymatic glycation of membrane and cytoskeleton proteins, accumulation of lipid peroxidation products in the membrane) associated with hyperglycemia [1, 2, 3]. Our previous studies have shown the possibility of correcting metabolic disorders in experimental diabetes mellitus with synthetic organic compounds of a number of substituted 6H-1,3,4-thiadiazines combining antiglycative and antioxidant activity [4].When searching for new antidiabetic compounds, it is necessary to take into account not only their metabolic effects, but also the ability to correct the biophysical parameters of the erythrocyte, on which the state of microcirculation and the effectiveness of oxygenation of tissues affected by diabetes depend.
Objective: to evaluate morphological and biophysical parameters of peripheral blood erythrocytes in rats with experimental DM under conditions of its correction with substituted 6H-1,3,4-thiadiazines.
Diabetes mellitus in rats was modeled by intraperitoneal administration of alloxan at a total dose of 300 mg/kg of body weight divided into 3 fractions. The animals of the experimental groups were intramuscularly injected at a dose of 40 mg/kg with synthetic compounds L-17 and LT-27 from the class of substituted 6H-1,3,4-thiadiazines, differing in the nature of the substituent in the positions of the 2- and 5-heterocycle, diluted in water for injection. After 4 weeks, smears were prepared from the peripheral blood of animals on a substrate of freshly ground mica without the use of fixing reagents. AFM of dried preparations was carried out by a semi-contact method in an air environment on an "Integra Maximus" microscope (NT-MDT), with an NSG03 brand cantilever at an oscillation frequency of 90 kHz. The morphology of erythrocytes (diameter, height), the quantitative ratio of normal (discocytes) and abnormal (spherocytes, stomatocytes, echinocytes, etc.) cell forms, as well as the amount of cell surface adhesion were evaluated.
Erythrocytes of rats with alloxan DM were characterized, in comparison with intact animals, by large values of average diameter (9.75±0.20 and 8.8±0.32 microns, pSt <0.05) and height (448.6±19.57 and 377.5±26.33 nm, pSt<0.05), cell surface adhesion did not undergo statistically significant changes (104.5±6.73 and 90.6±19.01 nN). The introduction of compound L-17 led to a decrease in the average diameter of erythrocytes, statistically significantly lower than the values of control and intact animals (7.9±0.18 μm, pSt <0.05), the average height of cells did not change significantly (407.3±21.69 nm), and adhesion increased to 133.1±18.45 nN, pSt<0.05.
Against the background of the use of the compound LT-27, the average diameter of erythrocytes was also statistically significantly lower than the values of control and intact animals (7.2±0.27 microns, pSt<0.05), however, there was an increase in the average height of the erythrocyte to 472.4±35.27 nm, pSt<0.05, and a decrease in adhesion to 58.2±6.13, pSt<0.05, which indicates an increase in the stiffness of the membrane surface.The development of DM led to a decrease in the proportion of normal-form erythrocytes (discocytes) from 57% to 17%, px2<0.001, the predominant cell form was a spherocyte (42% vs. 20% in intact animals, px2<0.001). The introduction of compound L-17 led to an increase in poikilocytosis: discocytes were 13%, echinocytes 29%, spherocytes 23%, stomatocytes 20%, other forms 15%, px2<0.01. When correcting DM with LT-27 compound, 96% of erythrocytes were echinocytes, px2<0.001.
Thus, the study showed the ability of compounds of a number of 2,5-substituted 6H-1,3,4-thiadiazines to change the morphological and biophysical parameters of rat erythrocytes in experimental DM. The L-17 compound had the best corrective ability.
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### S2.134. Autowave nature of giant depolarization pulses preceding epileptic seizures

Zinchenko V.P.[1]*, Laryushkin D.P.[1], Teplov I.Yu.[1]
[1]*Institute of Cell Biophysics of the RAS, FITC PNCBI RAS, Pushchino;*
* vpz@mail.ru

Between epileptic seizures, after brain damage and stroke, high-amplitude (giant) synchronous oscillations/pulses of electrical activity recorded by EEG occur in the brain. Brain cells isolated from epileptic foci also generate periodic high-amplitude hypersynchronous discharges, which are based on a trigger mechanism of unknown nature. The latter phenomenon is considered as a cellular correlate of epileptiform activity of the neuronal network and is called paroxysmal depolarization shift (PDS). Since the discovery of PDS in the 1960s, the existence of an unusual mode of electrical activity of neurons in the network has been shown not only in epilepsy, but also in ischemia, and a number of other neurodegenerative diseases. At least three oscillatory systems are included in the formation of the PDS cluster: fluctuations of action potentials (AP), fluctuations of PDS and fluctuations of PDS clusters. All three systems have an autowave nature. The autowave

nature of PD has been studied in detail. The pacemaker cell generates periodic auto waves of PD, constantly depolarizing the membrane. The autowave attributes are: the constancy of the pulse amplitude (non-attenuation), the presence of a slow phase between the PD pulses and the mechanism of temporary inactivation (disconnecting of the environment) to ensure unidirectional of autowave motion, trigger positive feedback amplifying the generator signal, the presence of an excitation inhibitor, the presence of an energy source. With increasing depolarization, the frequency of PD can either monotonically increase, or a mode of PD "burst" activity is formed. Externally, the bursts are similar to PDS clusters, but differ in the amplitude of the control depolarization. In the PDS cluster, the maximum amplitude of depolarization may exceed the Na+ channel reactivation potential.

It is assumed that the initial formation of the PD bursts occurs due to the depolarization pulse generated by low-threshold Ca2+ channels. And the transformation into a PDS cluster occurs due to the loss of GABA-dependent inhibition and hypersynchronization of PD cluster and Ca2+ pulses. At the same time, periodic clusters of PDS and accompanying Ca2+ pulses have all the autowave attributes and properties. The PDS in the cluster also fluctuate. An unknown mechanism opens the PDS channel, which provides rapid depolarization to a level exceeding the Na+ channel reactivation potential. At a high rate of depolarization at the leading edge of the PDS, often only one PD manages to be generated. Thus, the PDS controls the frequency of PD generation in the cluster. PDS fluctuations also have the above-mentioned the autowave attributes. Unlike the first two oscillatory systems, neither the nature of the channels nor the mechanisms of their regulation are known for PDS.

Identification of the ion channels involved in the PDS clusters formation is an important task, the solution of which is necessary to identify new targets for the treatment of neurological diseases associated with hyperexcitation. The complexity of the task lies in the fact that various ion channels can simultaneously take part in the PDS cluster formation.

### S2.135. Bacteriolytic and anti-adhesive effect of Limosilactobacillus fermentum 3872 on methicillin-resistant strains of Staphylococcus aureus (MRSA)

Machulin A.M.[1]*, Abramov V.A.[2,3], Kosarev I.K.[2,3], Karlyshev A.K.[4]
[1]*Skryabin Institute of Biochemistry and Physiology of Microorganisms, Federal Research Center "Pushchino Scientific Center for Biological Research of Russian Academy of Science";*
[2]*Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor), Federal State Budgetary Institution "The Russian State Center for Animal Feed and Drug Standardization and Quality" (FGBU VGNKI);*
[3]*Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Health;*
[4]*Kingston University London;*
* and.machul@gmail.com

Methicillin-resistant strains of Staphylococcus aureus (MRSA) are multidrug-dose pathogens and one of the main drugs circulating inside hospitals and agricultural farms around the world. Some clones of S. aureus are able to acquire resistance to all classes of antimicrobial agents to which they are exposed. World Health Organization (WHO) experts have included MRSA in the list of pathogens requiring urgent development of new antibiotics to treat diseases caused by these pathogens. In humans and animals, S. aureus is part of the normal microbiota and present in the upper respiratory tract and on the skin and intestinal mucosa. About 20% of the world's population is persistent carriers of S. aureus. Antibiotic treatment disrupts the normal intestinal microbiota, which creates serious complications in the absorption of nutrients from the diet by the macroorganism. Probiotic bacteria are effective in the prevention and treatment of gastrointestinal infections in both humans and animals. So, the creation of new probiotics for the prevention and treatment of staphylococcal infections in humans and farm animals is relevant.

The aim of the work was to study the bacteriolytic and anti-adhesive effect of Limosilactobacillus fermentum 3872 (LF3872) on MRSA multiresistant strains isolated from humans and animals. Previously, we performed whole genome sequencing of LF3872. In the LF3872 genome, a gene encoding the bacteriocin protein (BLF3872), which has bacteriolytic properties, was found. The BLF3872 structure was modeled using the AlphaFold server. The resulting model revealed a two-domain structural organization of BLF3872, similar to the structure of the morphogenesis 1 protein from the phage Bacillus phi29. It was found that the N-terminal domain of BLF3872 is homologous to lysozyme, which hydrolyzes the β-1.4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine of the glycosidic chain of S. aureus peptidoglycan. The C-terminal domain of BLF3872 is homologous to metalloendopeptidase, which hydrolyzes amide bonds between the amino acid residues of short peptides connecting the glycosidic chains of the peptidoglycan of the S. aureus cell wall. To experimentally test the bacteriolytic and anti-adhesive effects of LF3872 on methicillin-resistant MRSA strains, two multi-resistant strains were used: a strain isolated from the human nasopharynx and from the oropharynx of pigs, which are resistant to methicillin, oxacillin, amoxicillin, ampicillin, cephalosporin, cefamycin, ciprofloxacin and nalidixic acid. According to electron microscopy, LF3872 cells produced by BLF3872 destroy the peptidoglycan of the S. aureus cell wall and cause the death of the pathogen. In addition, co-cultivation of LF3872 reduces the viability of multidrug-resistant MRSA strains by 6 logs. LF3872 was also found to inhibit the adhesion of multidrug-resistant MRSA strains to Caco-2 human enterocytes.

The obtained results are important for the creation of new effective drugs against multidrug-resistant MRSA strains circulating in humans and animals.

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### S2.136. Calcium-dependent activation of mitochondrial ROS production in primary brain cells

Stemashchuk O.A.[1]*, Vinokurov A.Y.[1]
[1]*Orel State University named after I.S. Turgenev;*
* o.stelmashchuk@oreluniver.ru

Mitochondria are organelles involved in various important processes in the cell. In addition to their primary function of generating ATP, mitochondria play a key role in the regulation of calcium signaling, generation of reactive oxygen species, and cell death. Although all these processes have been described in detail, their interaction during intracellular signal propagation remains unclear. At the same time, a number of diseases have been shown to be associated with mitochondrial Ca2+ overload, which, in combination with other triggers, leads to the opening of the mitochondrial pore (mPTP) and subsequently triggers programmed cell death. The interaction of ROS and mitochondrial calcium uptake was studied in cells of a primary co-culture of neurons and astrocytes using MitoTracker Red CM-H2XRos probes (indicator of ROS production in the mitochondrial matrix), Fluo-4AM (fluorescent indicator of calcium in the cytosol) and X-Rhod-1, AM (fluorescent indicator of calcium in mitochondria).

It was found that the addition of ATP or L-glutamate, which triggers calcium signaling in astrocytes and neurons and initiates calcium uptake by mitochondria, leads to stimulation of an increase in the level of mitochondrial ROS in neurons ($p = 0.035$) and astrocytes ($p < 0.01$). The Ca2+ ionophore ionomycin also activates the formation of ROS in mitochondria. The Ca2+-induced increase in ROS production depended on the presence of the FCCP uncoupler ($p < 0.05$ of the

baseline level in neurons) and the mitochondrial complex I inhibitor rotenone ($p < 0.05$ of the baseline level in astrocytes). When complex I was inhibited by rotenone, the observed ROS production increased significantly ($p < 0.001$) under conditions of increased intracellular Ca2+ induced by ionomycin. It is possible that the increase in ROS under the influence of ionomycin may be associated with the activity of complex II of the electron respiratory chain and the reverse transport of electrons from complex II to complex I.

Thus, the absorption of calcium by mitochondria of neurons and astrocytes in response to physiologically significant stimuli leads to the generation of mitochondrial ROS, which can play an important role in the development of physiology and diseases.

### S2.137. Calcium-induced lipid pore and its role in preventing mitochondrial calcium overload

Mironova G.D.[1]*, Belosludtseva N.V.[1]
[1]*Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences;*
* mironova40@mail.ru

Calcium is known to be a mediator of many processes occurring in the body. Under the action of hormones, electrical impulses or other factors, Ca2+ enters the cytoplasm and activates certain functions, after which the intracellular concentration of this ion should decrease to a control level. This process in the cell is controlled mainly by mitochondria, which have systems that bind Ca2+ with high affinity. However, with intensive cell functioning, as well as with pathologies, especially accompanied by prolonged hypoxia, when Ca2+ accumulates in the cytoplasm in increased amounts, mitochondria may overload with this ion. As a result, there is a danger of the appearance of nonspecific permeability in the inner membrane of mitochondria as a result of the formation of a mitochondrial permeability transition pore (MPT pore), leading to apoptosis or necrosis of cells. The mechanism of this permeability is associated with the discovery of a mega-channel sensitive to cyclosporine A (CsA), which has a protein nature. In our work, we have shown that in addition to the pathway associated with the opening of the protein pore, short-lived lipid pores can be opened in mitochondria, appearing in the membrane from the matrix side during the accumulation of palmitic acid complexes with Ca2+ ions. These pores are not regulated by CsA and, apparently, play a protective role, preventing mitochondria from accumulating large concentrations of the ion and the MPT pore opening.

We have previously shown that saturated long-chain free fatty acids bind Ca2+ with a high affinity, which is 1.5-2 orders of magnitude higher than the affinity for this ion of other fatty acids and lipids. These acids in the presence of Ca2+ are able to induce the ionic permeability of the bilayer lipid membrane, and the nonspecific permeability of both the liposomal membrane and the inner membrane of mitochondria isolated from different tissues. These studies have suggested a common universal mechanism of palmitate/Ca2+-induced permeabilization of bilayer lipid membranes, different from the mechanism of MPT pore formation.

Based on the experiments conducted on liposomes, it was assumed that the mechanism of formation of this pore in the lipid bilayer is associated with a chemotropic phase transition.

It is known that the entry of Ca2+ into mitochondria leads to the activation of phospholipase A2 and the appearance of free fatty acids, mainly palmitic and stearic. When they accumulate in the mitochondrial matrix, it is possible, according to our data, to form complexes of these acids with Ca2+ and to open short-lived pores according to the mechanism described above. Since these pores have a small diameter, they close quickly and, as we have shown, do not affect the work of the respiratory chain, while restoring ionic homeostasis. In the presence of Ca2+ (Sr2+)-dependent phospholipase A2 inhibitors, the accumulation of free fatty acids is not observed. At the same time, spontaneous cyclic changes in the concentration of K+, Ca2+, H+, membrane potential, swelling, oxygen consumption rate and the rate of H2O2 formation in mitochondria caused by Sr2+ in the presence of valinomycin are blocked. The suppression of the above-described oscillations by phospholipase A2 inhibitors is associated with the absence of free fatty acids in the matrix and the impossibility of opening a lipid pore under these conditions. As a result, both the output of K+ and Ca2+ ions from mitochondria and the entry of H+ into them along the concentration gradient are blocked, while the induction of pore formation balances the ionic gradients through the mitochondrial membrane. Consequently, the opening of the lipid pore provides K/H and Ca/H exchange in mitochondria. The participation of Ca2+-dependent phospholipase A2 in this process is confirmed by the fact that the inhibitor of Ca2+-independent phospholipase A2 did not affect the oscillations described above. This pathway probably plays a protective role against the toxicity of Ca2+ and ROS.

The opening of the lipid pore reduces the likelihood of the appearance of a protein pore in the mitochondria, which is known to be responsible for cell death. One of the features of the lipid pore is its ability to be induced by relatively small amounts of Ca2+, i.e., these pores appear in the mitochondrial membrane before the organelles are overloaded with Ca2+ to the extent that triggers the opening of MPT pore. This assumption was confirmed by comparative studies of the parameters of formation of lipid and MPT pores in the mitochondria of different tissues of rats with genetically modified resistance to hypoxia. Experiments have shown that the mitochondria of hypoxia-resistant animals are more susceptible to the opening of the lipid pore and more resistant to the opening of MPT pore, compared with the organelles of hypoxia-sensitive rats. The results obtained were further confirmed by studies on two rat lines (August and Wistar) with different levels of oxidative phosphorylation (OXYPHOS). At the same time, August rats had a higher respiration rate and OXYPHOS efficiency, as well as higher rates of potassium transport and mitochondrial swelling and lower rates of H2O2 production. They were also less sensitive to hypoxia and stress. At the same time, lipid pores formed more easily in the mitochondria of hypoxia-resistant rats. Thus, our results confirm the assumption that the more efficiently the lipid pore works, the more difficult it is to open the protein pore. The formation of lipid pores does not inhibit OXYPHOS in mitochondria, allowing organelles to maintain the supply of energy to cells during stress. Consequently, the lipid pore can provide protection against mitochondrial overload with Ca2+ ions. This assumption may also explain the effects of phospholipase A2 inhibitors on the opening of the MPT pore, documented by the Pfeiffer group in 2006.

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### S2.138. Changes in Mitochondrial Activity in the Presence of Promising Drugs

Zagryadskaya Y.A.[3], Lomakina G.Y.[1,2], Okhrimenko I.S.[3]*
[1]*Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia;*
[2]*Department of Fundamental Sciences, Bauman Moscow State Technical University, Moscow, Russia;*
[3]*The Research Center for Molecular Mechanisms of Aging and Age-Related Diseases of Moscow Institute of Physics and Technology, Dolgoprudny, Russia;*
* ivan.okhrimenko@phystech.edu

Some drugs affect the activity of human mitochondria. The Aβ(1-42) peptide involved in the pathogenesis of Alzheimer's disease also reduces the rate and amount of ATP produced by mitochondria. In our experiment the 1st experimental group of human neuroblastoma cell line SH-SY5Y was cultivated for 24 h in the presence of 200 nM Aβ

peptide monomerized according to the standard method (Jao, 1997; Džinić, 2018), the 2nd group of cells (control) did not contain any additives, the 3rd group contained 0.1% DMSO (this amount of DMSO was added to the 1st group with Aβ), and the 4th and 5th, in addition to Aβ, also contained D-peptides interacting with various forms of Aβ and its precursors (Bocharov et al., 2021; Van Groen et al., 2008), one these peptides has passed the second phase of clinical trials (Mathew, 2023). After cell cultivation, mitochondria were isolated according to the standard method (Martin, 1998; Daum, 1982). The substrates of complexes I, II and IV and the corresponding inhibitors of the OxPhos complexes were added to isolated mitochondria in separate experiments to focus on studying the change in the activity of each complex induced by Aβ and other substances. Immediately before the beginning of the luminescence measurements, ADP and luciferase with luciferin were added to mitochondria (Lomakina, 2022). For each experimental group, a luminescence graph (RLU) was plotted against time. The maximum values of the first derivatives of the left parts of the obtained bell-shaped curves (ATP production rate) were compared. Peak heights were also compared (they corresponded to the amount of ATP produced by mitochondria). The presence of DMSO led to a slight decrease in the amount and rate of ATP production by human mitochondria compared to the control group. Cultivation of cells with Aβ leads to a decrease in ATP synthesis by mitochondria and a halving of the synthesis rate, while the presence of D-peptides restores these indicators to control values. The work was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement 075-03-2023-106 dated January 13, 2023, subject number FSMG-2021-0002).

### S2.139. Changes in metabolic parameters in cells with multiple mtDNA mutations associated with diseases

Vinokurov A.Y.[1]*, Popov D.Y.[1], Pogonyalova M.Y.[1], Shitikova E.Y.[1], Kazakov M.S.[1], Kuznetcova E.A.[1]
[1]*Orel State University;*
* tolmach_88@mail.ru

Mitochondria play a key role in the development of most intracellular processes. The genetic regulation of its functioning is determined by nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), which contains the genes of 12S and 16S rRNA, tRNA, and individual polypeptides of the electron transport chain (ETC). mtDNA is characterized by a significantly higher level of mutation [1,2]. According to various estimates, the incidence of diseases associated with mtDNA mutations is about one case per 4000-5000 people [3]. Due to the presence of a large number of mtDNA molecules in the cell (it can reach several thousand), the symptoms of pathologies associated with mutations manifest themselves at the level of heteroplasmy of 60-90% [4, 5]. However, it should be noted that these data were obtained as a result of studies containing a limited (usually one or two) number of mtDNA mutations in the cell. While due to the high frequency of mtDNA damage, cases of a significantly higher level of mutational load are not uncommon. So, various interaction effects are possible. The molecular mechanisms of mtDNA mutations have not been sufficiently studied. The nature of changes in ATP synthesis, mitochondrial membrane potential (ΔΨm), the content and activity of ETC proteins, ROS production in various studies differs both qualitatively and quantitatively (even when considering the same mutations), which is probably due to both the values of heteroplasmy and the combination of mutations [6-9]. Thus, the development of research of the relationship between complex combinations of mtDNA mutations and various levels of cellular phenotypic changes is very relevant.

In our work we use lines of cybrids based on THP-1 cells and having 5-8 mtDNA mutations in the MT-RNR1, MT-TL1, MT-TL2, MT-CYTB, MT-ND1, MT-ND2, MT-ND5 and MT-ND6 genes with a heteroplasmy range 1%-68%. At this stage, the studies performed include the analysis of a number of parameters characterizing the bioenergetics of cells (the level and mechanism of formation of ΔΨm; mitochondrial content, the ratio of reduced and oxidized forms, as well as the rate of production of NADH and FADH2; the content and rate of ATP consumption; respiration of cells; formation of ROS; mitophagy level).

The results obtained allow us to make a conclusion about a significant effect of the studied mutations on cellular metabolism, even despite the significantly lower levels of heteroplasmy in comparison with those indicated for the presence of single mutations. In particular, mutations of tRNALeu genes turn out to be significant already at 20% content with the simultaneous presence in the cell of cytochrome b mutations (m.14846G>A) or subunits of the ETC complex I (m.5178C>A, m.14459G>A). All lines are characterized by a significant decrease in the level of ATP in the absence of a positive correlation of this parameter with the time of complete exhaustion of the macroerg when blocking the pathways of its biosynthesis. This indicates various causes of energy deficiency – from decreased ATP formation to hyperactivated actively consuming processes. The combinations of mutations presented in the cybrids are associated with a significant level of dissociation of oxidative phosphorylation, which may be a way to reduce the negative effects of ROS hyperproduction both in the MX matrix and the intermembrane space in the dysfunction of ETC complexes. Disorders of complex I associated with mutations of genes of individual proteins, as well as tRNA genes, are not always compensated by an increase in the expression or activity of succinate dehydrogenase encoded by nDNA, which indicates the limited use of complex II substrates as a tool for protecting cells in the presence of mtDNA mutations. A number of cybrids are characterized by an inverse mode of functioning of the complex V of ETC, which allows maintaining the level of ΔΨm due to consumption of ATP at the same time. Despite the revealed violations, some cybrids are characterized by defective mitophagy, leading to the accumulation of non-functional organelles. In some cases, combinations of mutations can lead to an improvement in the parameters characterizing the state of cells, which is observed, in particular, in the presence of mutations m.13513G>A and m.1555A>G in the MT-ND1 and MT-RNR1 genes, respectively.

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### S2.140. Changes in the electrical characteristics of identified neurons in the terrestrial snail as a result of the development of a conditioned situational reflex and reconsolidation of memory for this reflex

Bogodvid T.K.[1,2]*, Muranova L.N.[2], Andrianov V.V.[2], Deryabina I.B.[2], Chihab A.W.[2], Gainutdinov K.L.[2]
[1]*Volga Region State University of Physical Culture, Sport and Tourism, Kazan, Russia;*
[2]*Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan;*
* tat-gain@mail.ru

A plethora of experimental data indicate that cellular processes associated with learning are caused by long-term modifications in the efficiency of synaptic transmission and changes in the endogenous properties of the neuron and its membrane [1,2,3]. For a long time, the change in the efficiency of synaptic transmission was recognized as the main learning mechanism until later evidence of non-synaptic mechanisms appeared. Within the framework of such ideas, there is a sufficient number of studies of cellular learning mechanisms [4,5]. Therefore, in many works, studies have been carried out to establish a link between the outcomes of behavioral learning and the excitability of neurons and the electrical characteristics of the membrane [1,2,4,5]. Previously, we have shown membrane correlates (changes in the membrane and threshold potentials of premotor interneurons) for conditioned defensive reflexes of tapping on the shell and aversion to food [2,4,6]. Therefore, the question arose whether such changes are possible during the development of other types of conditioned reflexes. To do this, we studied the possible correlation of the development of a conditioned situational reflex [7], and the reconsolidation of its memory to the dynamics of changes in the electrical characteristics of the premotor interneurons of the defensive behavior LPa3 and RPa3 as well as the serotonin-containing neurons Pd2 & Pd4 of the pedal ganglion, which modulate this reflex in the terrestrial snail,. Therefore, we studied the changes in the membrane and threshold potentials of the premotor interneurons LPa3 and RPa3 of the terrestrial snail after the development of a conditioned defensive reflex to the situation and memory reconsolidation of this reflex.
The experiments were carried out using the mollusc Helix lucorum. In all animals, a situational conditioned reflex was developed according to the contextual paradigm "on the ball" where the animals were rigidly fixed to the shell. Before elaborating the conditioned reflex and after training, the amplitude of the defensive reaction was tested as an indicator of the formed long-term memory. Behavioral responses were tested in two environments (contexts): 1) on a ball (i.e., under standard learning conditions), 2) on a flat surface. In some snails, after the development of a conditioned reflex to the environment, long-term memory of the learning environment was reconsolidated [7]. For the study of reconsolidation, a "reminder" of the learning environment was introduced. The results showed that the membrane potential in neurons LPa3 and RPa3 decreases significantly (about 5 mV) after training. No significant further changes were found in the membrane potential after the reminder (initiation of reconsolidation relative to its post-training level). The threshold potential of these neurons decreased after training and remained unchanged after the reminder. At the same time, after the reminder, the membrane and threshold potentials significantly decrease relative to the initial level (before training). All in all, these neurons can participate in the process of reconsolidation of the situational reflex.
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### S2.141. Changes in the process of endocytosis of synaptic vesicles in various types of muscle mice after hindlimb unloading

Tyapkina O.V.[2], Rossomakhin R.A.[1]*, Yakovleva O.V.[1]
[1]*Kazan (Volga Region) Federal University;*
[2]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS;*
* rossomakhin@inbox.ru

Functional unloading of skeletal musculature (immobilization, bed rest, space flight) leads to atrophy, which mechanisms are well studied. Condition of motoneurons, innervating this muscles and launching muscle contraction process, is less studied. Action potential propagation along motoneuron axon provides transmission of excitation from the motor nerve ending to postsynaptic membrane of the muscle fiber by activation of acetylcholine receptors with mediator – acetylcholine. Previously shown, that levels of non-quantum secretion of acetylcholine change, and quantum induced and spontaneous secretion, in rats are changed after antiorthostatic suspension of the hind limbs (support unloading model according to the Morey-Holton method). The intensity of the quantum release of the mediator can be regulated both by exo- and endocytosis. In this work, we analyzed the processes of endocytosis of synaptic vesicles in the nerve endings of muscles of different functional types in mice after 30 days of support unloading (antiorthostatic rear limb suspension (AOS) according to the Morey-Holton method). The experiments were carried out on isolated neuromuscular preparations m. Diaphragma (mixed muscle), m. Soleus (slow muscle), m. EDL (m. extensor digitorum longus fast muscle) of laboratory white mice. The processes of endocytosis of synaptic vesicles were studied using a fluorescent marker FM 1-43 (3μM), which reversibly binds to the presynaptic membrane and during endocytosis of synaptic vesicles is inside the nerve terminal ("loading" of the terminal). An indicator of endocytosis and loading of the fluorescent dye into synaptic vesicles was the appearance of brightly glowing spots inside the nerve ending. The intensity and duration of stimulation of the nerve stump depended on the type of muscle: m. Diaphragma 50 imp/s in 1 minute, m. Soleus – 1 imp/s 5 sec and 10 imp/s 10 sec, m. EDL – 60 sec by hyperpotassic Krebs solution. In the control group of mice with high-frequency stimulation of the motor nerve of the phrenic muscle, the luminescence intensity was 87 r.u. ± 3 r.u. (n=14). In the group of animals after AOS, the terminal luminescence intensity was 75 ±

5 r.u. (n=5, p<0,05). Similar results were obtained on other muscles. The luminescence of the terminals of the soleus muscle was 68 ± 7 r.u. (n=5, p>0.05), which is significantly lower than the control values (74 ± 7 p.u., n=8). The luminosity of the EDL terminals was 71 ± 7 pu. n=5, p<0.05), which is also lower than the control values (109 ± 3 p.u., n=7). The data obtained may indicate both a slowdown in the processes of endocytosis of synaptic vesicles in the nerve endings of mice after support unloading, and morphological changes in the nerve terminal (decrease in the number of synaptic vesicles, disorganization of the cytoskeleton).
Thus, support unloading affects the processes of endocytosis not only in skeletal muscles that are in a state of functional unloading, but also in the respiratory muscle, which requires further study.
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### S2.142. Chronic impact of nano- and microfine oxide on the morphological physical and chemical parameters of erythrocytes

Zemlyanova M.A.[1], Ignatova A.M.[1]*, Stepankov M.S.[1], Koldibekova Y.V.[1], Zaytseva N.V.[1]
[1]*Federal Budget Scientific Institution "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies";*
* iampstu@yandex.ru

There is a high probability of regular exposure of the population to nano- and micro-sized CaO particles when consuming food, beverages and drinking water. Such chronic exposure with a sufficiently high probability leads to a change in the chemical and physical parameters of red blood cells, which affects the rheological properties of blood.
The purpose of the work. To determine how chronic exposure to nano- and micro-sized calcium oxide coming from the environment affects the morphological, physical and chemical parameters of red blood cells.
Materials and methods of research. The object of the study was the blood of sexually mature female white rats of the Wistar line. The experimental animals were divided into three groups of 12 individuals each. The animals of the experimental group were injected with a suspension of nanodispersed calcium oxide (10-70 nm). A suspension of microdispersed calcium oxide (100-250 nm) was administered to animals of the comparison group in a similar dose and method. The animals of the control group were injected with a suspension base (dis. water) without substances in an equivalent volume. The experimental scheme corresponded to the definition of cumulation by the Limm method. In the first 4 days, a dose equal to 1/10 LD50 (LD50 - 2000 mg / kg) was administered daily, then the dose was increased 1.5 times and the next 4 days were administered, with such a sequential dose increase every 4 days, the experiment was completed on day 20. Physical and chemical parameters of erythrocytes were determined by standardized methods of laboratory diagnostics, the following parameters were taken into account: the number of erythrocytes in a liter of blood, the concentration of hemoglobin, hematocrit, the average volume of erythrocytes, the average hemoglobin content in a single erythrocyte and the average concentration of hemoglobin in the erythrocyte mass.
Morphological analysis of erythrocytes was performed on images of hematological smears stained by the Romanovsky–Giemza method obtained using a polarization microscope (Carl Zeiss, Germany Nikon Eclipse LV100NPOL). The following parameters of the erythrocyte shape were taken into account: the reduced diameter of the ferrets, the sphericity coefficient, the aspect ratio and the convexity coefficient. Image processing was carried out using a calculation unit built into the ImageJ universal software. Statistical processing of the results was carried out by the method of single-factor analysis of variance.
Results and their discussion. The general trend is that the number of red blood cells under the influence of both micro- and nanodispersed calcium oxide increases by 6%, and the hemoglobin content in a single erythrocyte decreases by 4%, while the concentration of hemoglobin in the blood decreases by 8-9%.
The average volume of red blood cells decreased relative to the control indicator in the comparison group and the experiment by 3.8 and 4.7 fl, respectively. A similar phenomenon was found when assessing the average hemoglobin content in a single erythrocyte, the value of which statistically significantly decreases in the comparison group by 1.2 pg and in the experimental group by 1.7 pg relative to the control.
In the study of morphology, it was revealed that morphological changes in erythrocytes under the influence of micro-sized calcium oxide are manifested in a reduction in diameter by 0.5 microns and the aspect ratio by 0.03. When exposed to nanoscale particles, all morphological indicators differ statistically significantly: the diameter decreases relative to the control group by 1.2 times, and relative to the comparison group by 1.1 times, the sphericity coefficient increases relative to the control indicator by 0.1, the aspect ratio by 0.1, the convexity coefficient by 0.01.
Together, the analysis of changes in physical, chemical and morphological characteristics allowed us to establish that the effect of calcium oxide with repeated oral intake leads to a decrease in the size of red blood cells and a decrease in their saturation with hemoglobin. Morphological changes indicate that when exposed to nanoscale calcium oxide particles, erythrocytes acquire a more spherical shape, this indicates premature aging of erythrocytes, the spherical shape of old erythrocytes prevents their passage through the intraendothelial sinuses of the spleen.
Conclusion. With chronic exposure to nano- and micro-sized calcium oxide coming from the environment, the diameter decreases and the shape of red blood cells changes, while the hemoglobin content decreases both in individual red blood cells and in the red blood cell mass as a whole, hematocrit decreases. The revealed changes can lead to a violation of blood gas metabolism and a deviation of its rheological properties from normal parameters, in aggregate, the effect is characterized as premature aging of red blood cells.

### S2.143. Contractile activity of pulmonary artery in models of volume change smooth muscle cells

Gusakova V.S.[1]*, Prshemysky M.A.[1], Raskauskaite V.A.[1], Zaitseva T.N.[1], Smagliy L.V.[1], Gusakova S.V.[1]
[1]*Siberian State Medical University, Ministry of Health of Russia;*
* ryd4enkoviknoriya@mail.ru

An important aspect of the functioning of the cells and tissues of the body is the maintenance of normal cell volume. It is known that the development of hypoxic pulmonary hypertension leads to swelling of smooth muscle cells and remodeling of the smooth muscle layer of the pulmonary artery. What determines the need to identify the existing relationships between the processes of regulation of the contractile functions of vascular smooth muscle cells and changes in the volume of smooth muscle cells. The study was performed on pulmonary artery segments of Wistar rats using a non-selective blocker of chloride transport DIDS, a selective blocker of volume-dependent chloride channels DCPIB, and blocker of Na+,K+,2Cl- cotransport bumetanide. Contractions of smooth muscle cells in volume change models were achieved by placing the segments in a hyperosmotic solution containing 120 mM sucrose (hyperosmotically induced contraction), a hypoosmotic solution containing a reduced concentration of NaCl (hypoosmotically induced contraction) and changing hypoosmotic medium to normoosmotic medium (isoosmotically induced contraction). One of the mechanisms of volume-dependent regulation contractile activity of pulmonary artery smooth muscles is the activation

of Na+,K+,2Cl- cotransport and volume-dependent chloride channels. It was found that bumetanide causes a multidirectional effect on hyperosmotically induced contractions, depending on the concentration and time of pretreatment. Bumetanide reduces the amplitude of hypoosmotically induced contraction, while bumetanide increases the amplitude and eliminates the transient nature of contractile response during isosmotic cell striction. Blockers of chloride channels DIDS and DCPIB reduce the amplitude and duration of contractions in models of changes cell volume, the selective blocker of volume-dependent chloride channels DCPIB has a more pronounced effect.

### S2.144. Deuterated polyunsaturated fatty acids protect lipid membranes from photodynamic damage

Firsov A.M.[1]*, Kotova E.A.[1], Fomich M.A.[2], Bekish A.V.[2], Sharko O.L.[2], Shmanai V.V.[2], Antonenko Y.N.[1], Shchepinov M.S.[3]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russia;*
[2]*Institute of Physical Organic Chemistry, National Academy of Science, Minsk, Belarus;*
[3]*Retrotope, Inc., Los Altos, CA, USA;*
* firsov@belozersky.msu.ru

Lipid peroxidation (LPO) induced by reactive oxygen species plays a key role in the development of multitude pathologies, including neurodegenerative and cardiovascular diseases. LPO can cause damage to cell membranes both by disrupting the barrier function of the lipid bilayer and by modifying membrane proteins. To protect against LPO in vivo, polyunsaturated fatty acids (PUFAs) containing deuterium atoms instead of protons in bis-allylic positions (D-PUFAs) were proposed to be used [1]. Previously, we found that LPO initiated by ferrous ions together with ascorbate in liposomes was significantly suppressed in the presence of about 20% of lipids with D-PUFA in the membrane [2].

Here, we studied the protective effect of D-PUFA on photodynamically induced LPO in model lipid membranes using illumination in the presence of trisulfonated aluminum phthalocyanine (AlPcS3) as a photosensitizer. To assess the changes in the photodynamic effect on liposomes upon introduction of D-PUFA into membrane lipids, we measured: 1) the leakage of sulforodamine B from liposomes, detected by fluorescence correlation spectroscopy (FCS), as an indicator of membrane integrity loss, 2) the formation of diene conjugates during lipid peroxidation in liposomal membranes, measured by absorbance at 234 nm, and 3) the photoinactivation of ion channels formed in a planar bilayer lipid membrane (BLM) by the gramicidin A peptide. Illumination of liposomes formed from lipids without D-PUFA caused a sharp drop in the amplitude of the autocorrelation function of sulforodamine B fluorescence, showing the dye leakage, already after the first minute of light exposure, whereas when liposomes contained 50% of lipids with D-PUFA residues, illumination for one minute did not induce the leakage. Therefore, the presence of D-PUFA in the lipid structure prevents the impairment of membrane integrity upon the photodynamic treatment. Iinterestingly, the accumulation of diene conjugates in the course of photodynamically induced LPO was inhibited at a lower content of deuterated lipids in liposome membranes compared to the suppression of the dye leakage [3].

In experiments on gramicidin A (gA) channels in planar BLM without D-PUFA, after turning on the white light in the presence of the photosensitizer, an immediate drop in the gA-induced electrical current was observed, which was obviously associated with the oxidation of tryptophan residues in gA molecules. The presence of 20% of the D-PUFA-containing lipid in the membrane caused the delay in the process of gA photoinactivation, while in the presence of 50% of the lipid with deuterated linoleic acid, almost complete inhibition of gA photoinactivation was found.

This work was supported by the Russian Science Foundation (grant no. 21-14-00062).

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### S2.145. Development of more effective dosage forms of polyene antibiotics

Efimova S.S.[1]*, Ostroumova O.S.[1]
[1]*Institute of Cytology of Russian Academy of Sciences;*
* efimova@incras.ru

Polyene macrolide antibiotics, in particular, amphotericin B, are still the gold standard for the treatment of systemic mycoses. The main mechanism of action of polyene antibiotics includes formation of the transmembrane pores in the target cell membranes which leads to disturbing water-salt homeostasis and fungal cell damage. The latter is determined by low probability of developing the antibiotic resistance of pathogenic microorganisms. The high toxicity of polyene macrolide antibiotics limits their pharmacological application. Lipid-associated compositions of amphotericin B are successfully used to reduce the nephrotoxicity of the antibiotic at the maintaining its antifungal efficacy. In order to further increase the effectiveness and reduce the toxicity of the macrolide antibiotic, we have developed its innovative liposomal forms, including various phospholipids, sterols and plant flavonoids. It has been established that phloretin-modified amphotericin-containing liposomes are characterized by a greater ability to release a fluorescent marker from vesicles that mimic fungal cell membranes compared to liposomal complexes including biochanin A, genistein, and quercetin. Single polyene ion-permeable pores in lipid bilayers mimicking the composition of fungal cell membranes, have a symmetrical current-voltage characteristics, that indicate the functioning symmetrical amphotericin B channels, and, consequently, determine a decrease in the threshold concentration of the antibiotic compared to the concentrations required for the formation of asymmetric polyene channels. Based on the obtained data, the optimal composition of liposomal forms of amphotericin B was determined, which ensures their increased affinity to membranes of pathogenic microorganisms and reduced affinity to mammalian cell membranes. This work was supported by the Russian Science Foundation (No. 22-74-10023).

### S2.146. Effect of resveratrol on structure of mitochondrial membrane of pea germs in the presence of TNIC-thio

Nevrova O.[1]*, Gerasimov N.Yu.[1], Zhigacheva I.V.[1], Generozova I.P.[2], Goloshchapov A.N.[1]
[1]*N.M. Emmanuel Institute of Biochemical Physics of RAS;*
[2]*K.A. Timiryazeva Institute of Plant Physiology of RAS;*
* neova@mail.ru

Under certain conditions, antioxidants can act as prooxidants and can have a toxic effect on the body in large quantities. The negative effects of antioxidants on the cell can cause antioxidant stress. The

phenomenon of antioxidant stress is poorly understood, especially in relation to plants. The question of what conditions can lead to antioxidant stress remains open. It is assumed that the use of several antioxidants together will neutralize the reactivity of antioxidant radicals. In work the interaction of antioxidants - plant polyphenol resveratrol and nitric oxide donor, iron-sulfo-nitrosyl complex with thiosulfate Na2[Fe2(S2O3)2(NO)4]2● 4H2O (TNIC-thio), and their combined action on mitochondria of pea germ epicotyls was studied in vitro. Mitochondria were isolated from pea epicotyls by differential centrifugation in potassium phosphate buffer. The microviscosity of the lipid bilayer of the membranes was determined by the method of electron paramagnetic resonance (EPR) of spin probes. Stable nitroxyl radicals 2,2,6,6-tetramethyl-4-capryloyloxylpiperidine-1-oxyl and 5,6-benzo-2,2,6,6-tetramethyl-1,2,3,4-tetrahydro-y-carboline-3-oxyl were used as probes.

The antioxidant activity of resveratrol, 10^(–6) M, partially compensated the toxic effect of TNIC-thio at the high concentration, that is most likely associated with the exhibition of the prooxidant properties of high concentrations of the nitric oxide donor. The unidirectional changes in the microviscosity of the lipid and protein regions was observed when affect of resveratrol at concentrations of 10^(-6) M and 10^(-8) M on mitochondrial membranes treated with TNIC-thio at 10^(-8) M. Thus, the presence of resveratrol caused antioxidant stress and disruption of links in the system of regulation of membrane lipid peroxidation. The action of resveratrol at the dose of 2•10^(-5) M had the dual character and practically did not effect on the structural state of mitochondrial membranes.

### S2.147. Electric fields at the lipid membrane interfaces

Ermakov Yu.A.[1]*
[1]*A.N. Frumkin Institute of Physical Chemistry and Electrochemistry RAS, Moscow, Russia;*
* yury.a.ermakov@gmail.com

Liposomes, planar bilayer lipid membranes (BLM), and Langmuir monolayers have been used for many years to model the surface characteristics of biomembranes, most importantly in the part that determined by the presence of the lipid matrix surrounding membrane proteins. In our studies, we focused on the electrical phenomena associated with the adsorption of biologically significant ions, organic molecules, and polypeptides on the surface of membranes available for registration by various bioelectrochemical methods (1). Significant information about the structural changes in membranes caused by membrane-active compounds was obtained by registration a dipole component of the boundary potential (2). In particular, it was possible to experimentally prove that the phase state of the charged components of the lipid environment of proteins has a direct effect on the functioning of many processes in cell membranes mediated by specific transport and signaling systems in cell biomembranes (3;4). The key questions arising in the interpretation of experimental data relate to the elucidation of the role played by the state of hydration of phospholipid polar groups in the electric field distribution at the lipid membrane boundaries. Experimental facts indicate a significant contribution of the quantity, orientation of water molecules and their participation in the formation of hydrogen bonds with phosphate groups of phospholipids (5). The combination of electrochemical methods, X-ray reflectometry, and quantitative analysis of lipid systems simulated by methods of molecular dynamics make it possible to reveal many details of the physicochemical processes associated with the charging of lipid water interface (6). The report presents the main facts found in the study of the adsorption of multivalent inorganic ions and polylysine molecules of different sizes on the surface of lipid membranes.

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### S2.148. Electrical activity of atrial cardiomyocytes during stimulation of NPY receptors

Iskakov N.G.[1,2]*, Anikina T.A.[1], Zverev A.A.[2], Nasartdinova R.R.[2], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University;*
[2]*The Volga Region University of Sports and Tourism;*
* nikitaiskakov1992@mail.ru

Neuropeptide Y (NPY) is a highly conserved peptide that was first discovered in the pig brain. NPY is a peptide consisting of 36 amino acids that acts through a family of G-protein-coupled receptors and is associated with numerous physiological processes, including nutrition, circadian rhythms, stress, fear, anxiety, and blood pressure regulation. Currently, eight subtypes of receptors sensitive to NP Y (Y1-Y8) have been detected. The action of NPY is realized with the help of various types of receptors that have both pre- and postsynaptic location. All types of receptors, at least in mammals, use the same signaling pathways. First of all, it is a Gi/o signaling pathway that leads to inhibition of cAMP formation, mobilization of intracellular Ca2+ and modulation of the state of Ca2+ and K+ channels. NPY binds primarily to Y1, Y2, and Y5 receptors.

The study was conducted on 21-day-old white mongrel rats, which are characterized by a peak heart rate and a high level of sympathetic effects on the heart (n=18). Strips of the right atrium myocardium with preserved sinus node and spontaneous activity were prepared from the heart. The registration of the action potential (PD) was carried out in accordance with the classical technique of intracellular leads. Glass electrodes were made on the day of the experiment on a special horizontal puller P-1000 (Sutter Instruments, USA) from borosilicate glass capillaries BF 120-60-10 (Sutter Instruments, USA) with an outer diameter of 1.2mm, an inner diameter of 0.6 mm– The tip of the microelectrode is filled with a solution of 3M KCl. The initial resistance at the tip of the electrode was 30-80 MΩ. The recording part of the installation included an amplifier for intracellular leads with high input resistance (Neuroprobe 1600, A -M Systems, USA) and devices that visualize and record experimental data. The microelectrode holder is connected to the preamplifier by the amplifier head. The output signal of the amplifier was transmitted to an analog-to-digital converter E14 - 140 (L-card, Russia) connected to a personal computer, an oscilloscope, and then recorded on the computer using the Elph 3.0 program. Statistical processing of the results was carried out using the program "PowerGraph Profeessional 3.3" (Disoft). Changes in the parameters of the action potential in response to neuropeptide Y were calculated as a percentage of the initial one. The reliability of the differences was calculated by the Student's paired criterion ($p < 0.05$). Sigma chemical reagents were used. The effect of NPY in consistently increasing concentrations was studied on one drug. All solutions were prepared on the day of the experiment.

The aim of our study was to study the role of neuropeptide Y in the electrical activity of cardiomyocytes of 21-day-old animals.

Application of NPY at a concentration of 10-8M caused a decrease in the frequency of occurrence of the action potential by 13% ($p<0.05$).

The duration of the action potential at the level of 20%, 50% and 90% repolarization (DPD20, DPD50, DPD90) increased from 17.4 ± 1.3 to 19.3± 1.1 ms, from 44.4±3.1 to 49.9±2.2 ms, from 99.1±5.1 to 108.9±4.2, which is 10%; 11%; 9%, respectively ($p<0.05$; n=9). The rest of the studied parameters did not change significantly. An increase in concentration by one order of magnitude (NPY 10-7M) did not lead to significant changes in the studied parameters (n=9). NPY at a concentration of 10-6M caused a decrease in the frequency of occurrence of the action potential by 21% ($p<0.05$) The duration of the action potential at the level of 20% repolarization (DPD20) increased from 19.0±1.9 to 19.9±2.0 ms, which is 5%, respectively ($p<0.05$; n=9). The rest of the studied parameters did not change significantly.
Thus, the maximum effect was observed at a concentration of 10-8M. We attribute the observed changes to the fact that NPY acts on If currents through the alpha subunit of the G protein. As a result, there is a decrease in the frequency of occurrence of the action potential and a prolongation of the duration of PD.

### S2.149. Electrical activity of rat working myocardiocytes during alpha2-adrenoreceptor stimulation

Galieva A.M.[1]*, Zefirov A.L.[2], Ziyatdinova N.I.[1], Krylova A.V.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University, Kazan, Russia;*
[2]*Kazan state medical university, Kazan, Russia;*
* galieva_alina94@mail.ru

It is known that isolated rat myocardium contains beta1-adrenoreceptors, beta2-adrenoreceptors, beta3-adrenoreceptors, beta4-adrenoreceptors, as well as alpha1-adrenoreceptors and alpha2-adrenoreceptors. [1] Research indicate that α2-AR isoforms (α2A-, α2B- and α2C) are expressed in cardiac myocytes with the potential to safeguard cardiac muscle under adrenergic surge by governing intracellular Ca2+ handling and contractility. By adjusting the balance between protein kinase and phosphatase activities, sarcolemmal α2-ARs are capable of counterbalancing signaling cascades that provoke hypertrophic cardiac remodeling under chronic activation of adrenergic and angiotensinergic signaling. In this regard, the reprograming gene or cell based therapies aimed at cardiac specific restoration or enhancement of α2-AR signaling may represent future therapeutic directions for prevention or treatment of heart failure. [2]
The aim of our study was to identify the effect of alpha2- AR stimulation on the electrical activity of the heart of adult rats.
White outbred rats aged 3.5-4 months were used in the work. Experiments were performed using perfused preparation (solution Tyrode, 37ºC, pH=7.4) from the right atrium of the rat under conditions of rhythmic stimulation (5 Hz). The effect of clonidine hydrochloride (10-6 M) on the duration of action potential (AP) at the level of 50 and 90% repolarization (APD50%, APD90%) was assessed using standard microelectrode technique. During the experiments, all the requirements of ethical standards for working with laboratory animals were observed. Clonidine hydrochloride has been shown to cause a statistically significant decrease in APD50% and APD90%.
Modulation of adrenergic activity through pharmacological approaches plays an important role in a wide range of cardiac disorders. The electrophysiological method using a constant imposed rhythm allows us to conclude that α2-AR plays a significant role in the regulation of the electrical activity of working cardiomyocytes.
The study was supported by the Russian Science Foundation grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/.
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### S2.150. Electrophysiological studies of the effect of photocontrolled azobenzene and stilbene derivatives on rat neonatal cardiomyocyte cells

Frolova Sh.R.[1,2], Kovalenko S.G.[1,2], Agladze K.I.[1,2]*
[1]*State Budgetary Institution of Healthcare of the Moscow Region "Moscow Regional Research Clinical Institute named after V.I. M. F. Vladimirsky;*
[2]*Moscow Institute of Physics and Technology;*
* agladze@yahoo.com

It is known that azoTAB (azobenzene trimethylammonium bromide) and c-TAB (stilbene trimethylammonium bromide)[1], derivatives of azobenzene and stilbene, respectively, are capable of changing the excitability of neonatal cardiomyocyte cell culture in a photocontrolled manner. The energetically stable trans-form of azoTAB and c-TAB is capable of suppressing spontaneous activity and the propagation velocity of excitation waves. Restoration of the excitability of the culture of cardiomyocytes can be achieved by washing these substances from it. Isomerization of trans-azoTAB into cis-azoTAB, obtained as a result of irradiation with soft ultraviolet (λ~365 nm), the excitability of the culture of cardiomyocytes is restored. While the cis-form of c-TAB, obtained under the same conditions, fixes the blockade of cell culture excitability. A study was made of the effect of trans- and cis-forms of azoTAB and c-TAB on voltage-gated ion channels involved in the formation of the action potential. The aim of the work was to understand whether the change in the conductivity of cardiomyocytes under the action of photocontrolled substances (azoTAB and c-TAB) is mediated through the modulation of voltage-gated ion channels responsible for the formation of the action potential. The effect of trans- and cis-forms of azoTAB and c-TAB on voltage-dependent fast sodium (INav), L-type calcium (ICav), and potassium (IKv) currents was studied on isolated neonatal cardiomyocytes using the patch-clamp method in the whole-cell configuration. As a result, it was found that under the action of the above substances, the fast sodium and calcium current of the L-type is suppressed, but the slow potassium currents, on the contrary, increase. Moreover, complete suppression in the case of azoTAB occurs at a concentration of 100 μM [2], and in the case of c-TAB, at a lower concentration - 60 μM. According to the study of the toxicity of azoTAB and c-TAB to cardiomyocytes in our laboratory, it was found that the toxicity to c-TAB cells is less than azoTAB, and in c-TAB it begins at a concentration of more than 100 μM. suppression of voltage-dependent sodium and calcium currents, which are responsible for the formation of an action potential in cardiomyocytes. Since this process is reversible, and in the case of c-TAB it also varies depending on what effect we want to fix (wash and restore the excitability of the cardiomyocyte cell culture or irradiate with soft ultraviolet and fix the conduction block in cardiomyocytes), these substances are of interest, for example , for ablation.
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1) Patent: MIPT, RU Pat., RU 2515502 C1, 2012
2)Sheyda R. Frolova, OlgaGaiko, Valeriya A. Tsvelaya, Oleg Y. Pimenov, Konstantin I. Agladze Photocontrol of Voltage-gated ion channel activity by azobenzene trimethylammonium bromide in neonatal rat cardiomyocytes // PLoSONE 2016 V. 3

### S2.151. Erythrocyte age variations in atherosclerotic patients

Gisich A.V.[1,2]*, Yastrebova E.S.[1], Nekrasov V.M.[1], Maltsev V.P.[1,2]
[1]*Institute of Chemical Kinetics and Combustion RAS;*
[2]*Novosibirsk State University;*
* a.gisich@g.nsu.ru

Atherosclerosis is the leading lethal disease in the United States and most developed countries. Unstable atherosclerotic plaques can rupture and lead to acute disorders such as heart attacks and strokes. Forecasting these processes is an urgent world-class problem.
Erythrocyte dysfunctions are involved in the pathogenesis of atherosclerosis. Local hypoxia caused by a lack of oxygen delivered by erythrocytes affects the cell function in unstable plaques. Moreover, erythrocyte membrane rigidity is higher in atherosclerotic patients than in donors. In addition, old erythrocytes have a lower deformability than young ones due to increased hemoglobin concentration and membrane rigidity.
The aim of this study is to identify differences in the age indices of erythrocytes between donors and patients with atherosclerosis of the brachiocephalic arteries.
For measuring erythrocyte parameters whole blood was taken by venipuncture into a vacuum tube (9∶1 blood:anticoagulant). Then the cells were used in experiments on the Scanning Flow Cytometer (SFC) at room temperature (22°C) within 3 hours. The protocol was approved by the participating hospital's institutional review board. The current setup of the SFC can be found here (doi: 10.1002/cyto.a.24554)
We present the novel approach that allows retrieving the age of individual erythrocytes, constructing the age distribution and forming the age category by 8 indices. The approach is based on the analysis of erythrocyte morphology changes during vesiculation.
The analysis of erythrocytes of 40 donors and 60 patients with atherosclerosis of the brachiocephalic arteries was carried out. Increased resistance of erythrocytes to hemolysis was found in patients with unstable atheromas. Moreover, patients had a higher number of old erythrocytes than donors.
The constructed dependence of erythrocyte membrane rigidity on the erythrocyte age for donors and patients with atherosclerosis allows us to conclude about the presence of pathology. The patterns found can serve as a diagnostic factor and a reason for further research.

### S2.152. Evaluation of the effect of silicon dioxide nanoparticles on morphometric parameters of blood cell membranes by laser interference microscopy

Ignatova A.M.[1]*, Nikitiuk A.S.[1], Starostenko D.A.[2], Lvova M.N.[3], Burmistrova O.S.[1], Koshkina A.A.[1], Naimark O.B.[1]
[1]*Institute of Continuous Media Mechanics of the Ural Branch of Russian Academy of Science (ICMM UB RAS);*
[2]*Budker Institute of Nuclear Physics of Siberian Branch Russian Academy of Sciences (BINP SB RAS);*
[3]*Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences;*
* iampstu@yandex.ru

Blood cells are sensitive to environmental factors and under their influence can change their properties, including morphometric parameters. From the works presented in the monograph [1], it is known that altered and unchanged erythrocytes have unequal functionality. The authors associated the resistance of erythrocytes to hemolysis with their total number per unit of blood volume and size. Studies concerning the dynamics of cellular communities indicate that functional disparity occurs in relation to individual communities and within communities. Thus, the cells of one patient produce a less active enzyme than the cells of another patient, and with various anemia, the parameters of individual erythrocytes of one patient deviate from the norm in wide ranges, it is obvious that hypo-, hyperchromic, as well as altered erythrocytes with poikilocytosis, have different functionality.
Functional disparity of blood cells can be caused by external factors such as nanoscale particles [2]. Evaluation of the functional status of one cell relative to another is an important research task.
The aim of the study is to study the effect of nanoscale silicon dioxide particles on the morphometric parameters of blood cell membranes.
The research was carried out on an experimental sample of a laser interference microscope MIM-N (Russia) equipped with a laser with a wavelength of 650 nm. The microscope of this modification assumes work on the lumen and does not require slides with a mirror coating, as is usually required when working with microscopes of this type [3]. Laser interference microscopy makes it possible to obtain a "phase portrait" of individual blood cells in the native state without fixation and staining [4].
Blood from the ulnar vein of relatively healthy volunteers aged 30 to 36 years was used as the object of the study. Venous blood preparation was carried out as follows: sampling was carried out in a vacuum tube with heparin. Then the selected blood was divided into three portions of 1 ml, the first portion was considered as a control, the second portion was mixed with a sterile saline solution in proportions of 1:1, the third portion was mixed with a sterile saline solution containing 15 µl of a suspension of nanoscale particles of silicon dioxide (NCHDC), also in proportions of 1:1. The NCHDC suspension contained particles of 4-80 nm in size, the average particle size was 16.38 nm, the concentration was 1.21·10-6 g/ml. The second and third portions of blood were incubated in a thermostat at 37 ° C for 30 minutes. The control portion was not incubated, it was previously found that incubation of native blood with an anticoagulant under these conditions does not significantly differ from the selected control.
After incubation, preparations were made from the obtained portions of blood as follows: 10 ml of the portion was placed on the surface of the slide, then 5 ml of saline solution was introduced, after which a cover glass was applied to the surface of the slide and the interference phase portrait was removed. The drug from the control portion was made immediately after sampling. 15-20 erythrocytes and leukocytes were taken from each portion.
Image processing was carried out in the program MIM Visualiser and ImageJ-FiJi. The following parameters were measured: maximum (Hmax) and minimum (Hmin) phase height of the cell; maximum (Dmax) and minimum (Dmin) diameter of the cell; area (S) and perimeter (P) of the cell contour; sphericity coefficient (KS) and fractal dimension of the cell contour (D), and the analysis was carried out the contour of the cell for the detection of signs of multifractality by the 2D method. According to the recommendations in the article [5], the oxygenation index (IO) of the erythrocyte membrane was calculated as the ratio of the minimum phase height to the maximum. The comparison of group indicators was carried out using the Mann-Whitney method.
According to the results of the experiment, it was found that erythrocytes and leukocytes from the control group differ from similar cells in groups subjected to incubation. Cells incubated with a pure saline solution and a solution with the addition of nanoscale particles of silicon dioxide also differ from each other.
The effect of NCDC on the morphometric parameters of erythrocytes is expressed in the fact that their oxygenation index changes, as well as the fractal properties of the contour.
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### S2.153. Impact of γ - irradiation on biochemical content of nuclear fractions

Minasbekyan M.L.[1]*
[1]*Yerevan State University;*
* minlia@ysu.am

The effects of various doses of γ-radiation on the radio sensitivity of common wheat seeds have been investigated. Changes in the biochemical composition of the nuclear fractions of seedlings of irradiated wheat seeds under the influence of γ-radiation were determined. The research results indicate a direct dependence of changes in nuclear membranes and soluble nuclear fraction on the dose of ionizing radiation. There was a change in the content of DNA, RNA, and protein in the fraction of the nuclear membrane and the soluble nuclear fraction of the cell nuclei of the seedlings of irradiated seeds. We have found a drop in the value of the ζ-potential of the isolated nuclei from irradiated seeds seedlings in the gradient of the electrostatic field. Thus, under the action of ionizing radiation, a change in the content of nuclear fractions was obtained: a change in the total content of protein and nucleic acids. Exposure to radiation causes the destruction of the ionogenic groups of molecules, resulting in a change in the electromobility of the nuclei, which in turn entails a violation of the functional activity of the nucleus.

### S2.154. Influence of hypoxia on the contractile activity of smooth muscles of the pulmonary artery rats

Gusakova S.V.[1]*, Gusakova V.S.[1], Smagliy L.V.[1], Zaitseva T.N.[1], Gushchin E.I.[1], Golovanov E.A.[1], Totumacheva E.V.[1]
[1]*Siberian State Medical University;*
* gusacova@yandex.ru

We studied the effect of hypoxia on the contractile activity of smooth muscle segments of the rat pulmonary artery in models of changes in cell volume. The mechanical stress of the vascular segments was measured using a Myobath II four-channel mechanographic unit and LAB-TRAX-4/16 hardware and software (Germany). The change in cell volume was obtained by placing smooth muscle segments in solutions with different osmolarity. It has been established that the contractile activity of pulmonary artery smooth muscle cells induced by the action of a highpotassium solution or phenylephrine is inhibited during hypoxia, depending on the time of incubation in a hypoxic solution. The receptor-dependent contractile activity is more sensitive to hypoxic exposure, as evidenced by a stronger decrease in the amplitude of the phenylephrine-induced contraction with an increase in the time of exposure to hypoxia, in contrast to the contraction induced by highpotassium depolarization of the membrane. Under conditions of hypoxia, there is a decrease in the contractile activity of the smooth muscle segments of the pulmonary artery, induced by hypo- and isoosmotic solutions, while the hyperosmotically-induced contractile activity of the smooth muscle segments of the pulmonary artery does not change. This may indicate that the processes associated with the regulation of the processes of swelling of smooth muscle cells and the mechanisms of restoration of cell volume after swelling are more sensitive to hypoxic effects. Whereas the contractile activity induced by cell shrinkage in a hyperosmotic environment is not sensitive to a decrease in oxygen in solution.

### S2.155. Influence of structural features of decellularized organ matrices on proliferation and morphology of SKOV-3 human ovarian adenocarcinoma cells

Gefter S.D.[1]*, Pospelov A.D.[1], Trushina D.B.[2,3], Balalaeva I.V.[1]
[1]*Lobachevsky State University of Nizhni Novgorod;*
[2]*Sechenov First Moscow State Medical University ;*
[3]*Federal Research Center «Crystallography and Photonics» RAS;*
* sofia.gieftier.00@mail.ru

The ability of tumor cells to metastasize is one of the key criteria for the malignancy of neoplasia. An important role in the migration of tumor cells is played by the extracellular matrix, which is the main component of the tumor microenvironment. The diversity of the molecular composition of the matrix provides specific structural and biomechanical characteristics of various biological tissues. Active interaction of tumor cells with such a microenvironment can lead to appropriate adaptive morphological and phenotypic modifications. The model of cancer cell growth in the extracellular matrix allows simulating many characteristics of the tumor microenvironment. To obtain such a model, normal cells which are originally populating them are removed from biological tissues (decellularization method). Subsequently, the decellularized (DCL) matrices obtained by this method are subjected to colonization by tumor cells (recellularization).
The aim of the work was to determine the effect of the structural features of DCL matrices of mouse biological tissues (lung, liver, spleen, kidney, and ovary) on the morphology and proliferation rate of repopularized SKOV-3 human ovarian adenocarcinoma tumor cells.
To obtain DCL matrices, organ fragments were kept in detergent solutions based on phosphate buffer (PBS; pH = 7.4) in the following sequence: 0.5% Triton X-100, 0.5% sodium dodecyl sulfate (SDS), 1% sodium deoxycholate (SDC) and 0.075% SDS. Saturation of matrices with nutrients before repopulation was ensured by keeping the samples in DMEM medium with the addition of 30% calf serum.
The structural features of DCL matrices were studied using a Hitachi TM 4000 Plus scanning electron microscope.
Recellularization of SKOV-3 cells in DCL matrix was carried out by injection of cell suspension in the amount of $3\times10^5$ cells per matrix. Matrix cell occupancy and morphology were analyzed on the seventh day of recellularization by histological examination with hematoxylin and eosin staining.
Based on the results of this work, DCL matrices with different physical properties were obtained. Identified distinctive parameters in their architectonics were the compaction of matrix fibers and the size of matrix pores. DCL matrices were successfully recellularized with SKOV-3 tumor cells, while their population density and morphological characteristics of cells in different matrices differed, and their organ-specific localization in the preserved tissue structures was also observed. Analysis of the obtained data revealed the following correlation: a pseudoepithelial low-invasive cell morphotype was observed

during growth in matrices with a high level of fiber compaction and a small matrix pore size (≤ 5 μm²), which is typical for DCL of spleen and ovarian tissue matrices. A change in the morphotype to a highly invasive mesenchymal morphotype was observed in cells growing in DCL matrices with a low packing density of matrix fibers and large pores (10–30 μm²), which is typical for lung, liver, and kidney matrices. In the same organs, the largest number of tumor cells was observed a week after recellularization. Thus, tumor cells of the studied line, when growing in matrices with a less dense structure, demonstrate higher proliferation and invasive potential. We suggest that such features of the extracellular matrix architectonics as a large pore size and loose packing of matrix fibers can be considered as factors contributing to the formation of secondary tumor foci.

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### S2.156. Influence of the lipid composition of lipid droplets on the efficiency of their protein-free fusion

Senchikhin I.N.[1], Urodkova E.K.[1], Minkevich M.M.[1], Denieva Z.G.[1], Molotkovsky R.J.[1]*
[1]*Frumkin Institute of Physical Chemistry and Electrochemistry RAS;*
* rodion.molotkovskiy@gmail.com

The work is dedicated to the study of the fusion of lipid droplets — organelles consisting of a core of fatty acids, such as triolein, surrounded by a monolayer of phospholipids. In view of the relatively simple implementation and universality of the effect of the lipid composition of the LC membranes on the efficiency of their fusion, a targeted change in the lipid composition seems to be a convenient tool for promising therapy of diseases associated with metabolic disorders. As part of the work, we studied the protein-free fusion of lipid droplets, leading to the unification of their monolayer shells. This process requires overcoming the energy barrier E associated with the topological rearrangement of merging lipid monolayers. We have evaluated the energy barrier and studied the effect of lipid composition on the height of this barrier.

The change in lipid composition was modeled as the addition of dioleoylphosphatidylethanolamine (DOPE) to a membrane composed of dioleoylphosphatidylcholine (DOPC). The height E was calculated using the theory of elasticity of lipid membranes and molecular dynamics methods. To this end, we generalized the theory of bilayer fusion [1] to the case of monolayer fusion. In addition, the height E was determined experimentally from dynamic light scattering (DLS) data on the evolution of the average size of lipid droplet particles at different temperatures. Systems for experiments were prepared according to the procedure [2, 3], and E was estimated within the framework of the coagulation model described in [4–6].

Comparison of theoretical and experimental results indicates a general trend corresponding to bilayer fusion: an increase in the proportion of DOPE in the membrane leads to a decrease in the height of the barrier to fusion, which is recorded as an increase in the average size of lipid droplets in the experiment. The barrier to the fusion of lipid droplets with a shell of pure DOPC is high enough (more than 30 kBT) to ensure the stability of droplets in the system. At the same time, molecular dynamics data indicate that the final state of the system is energetically more stable than in the case of bilayer fusion.

The results obtained can form the basis for the creation of effective methods for the treatment and prevention of various pathologies associated with metabolic disorders, which will be based on specific diets with a strictly defined lipid composition of lipid drops.

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### S2.157. Inhibition of calcium-induced fusion of lipid membranes by plant polyphenols

Zlodeeva P.D.[1]*, Shekunov E.V.[1], Ostroumova O.S.[1], Efimova S.S.[1]
[1]*Institute of Cytology of the Russian Academy of Sciences, Saint Petersburg, Russia;*
* zlodeeva.pd@yandex.ru

Polyphenols are plant metabolites containing aromatic benzene rings with various number of hydroxyl groups. These compounds demonstrate antioxidant, anti-inflammatory, antimicrobial, antitumor and antiviral activity and are widely used in pharmacology. Due to their amphiphilic structure, polyphenols can interact to lipid membranes and change their physical properties. Since the process of membrane fusion is sensitive to changes in bilayer characteristics, we can propose that polyphenols can modulate this process.

This work is devoted to the search among polyphenols for inhibitors of calcium-mediated fusion of negatively charged small lipid vesicles (SLV) and to investigation of relation between the structure of tested compounds and their ability to inhibit membrane fusion, and to identification of possible mechanisms of their antifusogenic activity.

Calcein release assay was used to quantify the inhibitory effect of polyphenols on the fusion of SLVs composed of a mixture of phosphatidylcholine (PC), phosphatidylglycerol (PG) and cholesterol (CHOL) (40/40/20 mol%). The ability of eighteen polyphenols to inhibit lipid membrane fusion was found. Stilben piceatannol (67%), flavanonol taxifolin (37%), flavonols quercetin (85%) and myricetin (58%), and flavan-3-ol catechin (22%) demonstrated inhibitory effect. Aglycones containing 1-3 OH-groups as well as glycosides did not inhibit calcium-mediated SLV fusion. Aglycones containing 4-6 OH-groups were able to inhibit fusion of negatively charged vesicles. Analysis of the data showed that the number of hydroxyl groups in the A- and B-rings determined the antifusogenic activity of polyphenols, whereas the structure of the C-ring did not matter.

To reveal the mechanisms of the inhibitory effect of tested polyphenols, their ability to change the thermotropic characteristics of PC and PG was analyzed using differential scanning microcalorimetry. The influence of the studied compounds on the phase transition of PC was stronger than on the melting of PG. It was shown that the ability of polyphenols to inhibit the fusion of lipid membranes was associated with their disordering action.

Suggested that the antifusogenic effect of polyphenols was depended on the depth of immersion of molecules into bilayer and their orientation in the membrane. Polyphenols that are predominantly localized in the area of lipid heads and orientated perpendicular to the normal to

the bilayer surface are characterized by a significant ability to inhibit SLV fusion. Probably, such compounds induce a positive curvature of the lipid monolayers, which leads to the inhibition of lipid membrane fusion due to the increased energy of the formation of fusion intermediates characterized by negative curvature.

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### S2.158. Interaction of the glycyrrhizin and coronavirus E-protein with membrane mimetics

Kononova P.A.[1,2]*, Selyutina O.Yu.[1], Polyakov N.E.[1]
[1]*Voevodsky Institute of Chemical Kinetics and Combustion, Novosibirsk State University;*
[2]*Novosibirsk State University;*
* kononova_polina@bk.ru

Glycyrrhizic acid (GA) is the main active ingredient in licorice root (Glycyrrhiza glabra). There is much data on the antiviral activity of glycyrrhizic acid, including activity against SARS-coronavirus. One of the possible mechanisms of antiviral action is considered to be the prevention of the fusion of the virus envelope with the plasma membrane of the host cell. E-protein inhibition reduces viral pathogenicity, suggesting that E-protein is a potential antiviral target. The role of the E-protein in the functioning and pathogenesis of coronaviruses in general and SARS-CoV-2 in particular is unclear. The hypothesis about the directed effect of glycyrrhizic acid on the E-protein of the coronavirus was tested.

The interaction of GA with lipid membranes was studied using the model of liposomes (DOPC, POPC, DPPC) and DMPC/DHPC bicelles. Using different methods of NMR spectroscopy (T1 and T2 relaxation times, NOESY), evidence was obtained that GA is incorporated into the lipid bilayer. The confirmation of the presence of interaction between GA and the transmembrane domain of the SARS-CoV-2 (ETM) E-protein in the aquatic environment was obtained. The incorporation of ETM into the bilayer was assessed by the change in relaxation times T1, which are associated with changes in mobility. The relaxation times of protons and phosphorus of lipids decreased for bicelles with ETM, the addition of GA affected the relaxation time of phosphorus and protons of terminal CH3 groups, but did not affect N+(CH3)3 groups. Nuclear overhauser effect spectroscopy (NOESY) was used to determine the localization of ETM and glycyrrhizic acid molecules in the lipid bilayer. It has been directly confirmed that both glycyrrhizic acid and the E-protein transmembrane domain are inserted into the lipid bilayer. At the same time, the relaxation times for the peptide changed significantly in the presence of GA, which indicates the indirect effect of GA on ETM.

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### S2.159. Investigation of endocytosis inhibition as a possible therapeutic approach to prevent the development of reperfusion injury

Stepanov A.V.[1]*, Filippov Yu.A.[1], Novikova E.V.[1], Dobretsov M.G.[1], Kubasov I.V.[1]
[1]*Sechenov Institute of Evolutionary Physiology and Biochemistry of RAS, Saint Petersburg, Russia;*
* botanik2407@gmail.com

Necrosis and destruction of the plasma membrane of cardiomyocytes in the ischemic zone during myocardial infarction leads to the release of numerous components of damaged cells into the intercellular tissue space. DNA fragments, pro-inflammatory cytokines, apoptosis factors, many bioactive molecules synthesized in response to ischemic stress, as well as larger components of dead cells, are distributed in the intercellular environment and by the bloodstream during reperfusion to adjacent myocardial zones not damaged by ischemia. There, they can negatively affect healthy myocytes, being absorbed by these cells by endocytosis, which, in turn, can trigger cascades of cytotoxic reactions similar to those that caused cell death in the ischemic zone. Although many cellular and molecular processes that occur at the border with the ischemic zone of the myocardium are actively studied, the contribution of endocytosis to the propagation of damage signals in the intact zone remains unexplored. Thus, the aim of this work was to investigate the suppression of endocytosis as a possible therapeutic approach to prevent the development of reperfusion injury and, as a consequence, the development of extensive myocardial infarction.

The work was carried out on Wistar male rats. The model of left ventricular myocardial ischemia caused by ligation of the left coronary artery (45 min) followed by reperfusion (IR) was chosen as an experimental model. In sham-operated rats (SO), the chest was opened without coronary occlusion. To suppress endocytosis in rats with IR, the blocker chlorpromazine (CP) (0.5 mg/kg) was used.

The scar size was assessed in 2-4 weeks after IR on transverse sections (5-7 sections) of the left ventricle stained with triphenyltetrazolium chloride.

An electrophysiological study was performed on isolated hearts perfused with Tyrode's solution using the microelectrode extracellular recording method (loose patch method, microelectrode tip diameter ~ 5 μm, resistance ~ 2 MΩ).

The distribution of t-tubules of cardiomyocytes was studied using confocal microscopy (Leica TCS SP5 confocal microscope) on isolated hearts stained with Di-8-ANEPPS fluorescent dye (20 μM).

Analysis of necrosis zones on transverse sections of the heart in 2 and 4 weeks after surgery did not reveal significant differences in the proportion of scar tissue between the group of IR rats and the group of IR/CP rats (24.5±6.5% and 34.8±7.1%, respectively, at week 2 and 28.9±3.3% and 29.8±6.1% at week 4).

The study of the organization of the T-tubular system using confocal microscopy did not reveal significant differences in the length of the intervals between adjacent t-tubules and the frequency of occurrence of long (more than 3 μm) intervals both between groups of control and IR rats, and between groups of IR and IR/CP rats.

An electrophysiological study showed that there was a significant remodeling of the profiles of type 1 extracellular action potentials (EAPs) (with one negative peak) recorded in the zone of the cardiomyocyte membrane free from entrances to the t-tubules in the group of IR rats, compared to the group of SO rats. There was a gradual increase in their decay time and the formation of the afterhyperpolarization phase. Simultaneously, in type 2 EAPs (with two negative peaks) recorded in the membrane zone of subepicardial cardiomyocytes which has t-tubule entrances a significant pronounced decrease in the amplitude and duration of the second peak was observed.

The presented data suggest that these changes are associated with an increase in the activity/expression of small conductance calcium-activated potassium channels (SK channels), since the use of the selective SK channel inhibitor apamin (500 nM) led to the disappearance of the afterhyperpolarization phase.

Along with the use of CP, the characteristics of type 1 and type 2 EAPs in the group of IR/CR rats did not differ significantly from the values recorded in the SO rats. This suggests that short-term suppression of endocytosis significantly attenuates EAP remodeling after IR.

The size of the infarction zone did not differ significantly between the IR and IR/CP groups. This indicates that damage signals do not significantly affect the size of the necrotic zone, but have an effect on the processes of electrogenesis remodeling in the plasma membrane of subepicardial cardiomyocytes.

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### S2.160. Investigation of photodynamic properties of octacationic complexes of magnesium and zinc phthalocyanine on a model lipid membrane

Zykova D.D.[1,2]*, Konstantinova A.N.[1], Sokolov V.S.[1]
[1]*A. N. Frumkin Institute of Physical Chemistry and Electrochemistry RAS;*
[2]*Moscow Institute of Physics and Technology;*
* dasha_ddz1924@mail.ru

The method of photodynamic therapy is effective for combating cancer and as a way of deactivating pathogenic microorganisms resistant to antibiotics [1]. Thesearch for new effective photosensitizers (FS),requires the methods to study their adsorption and photodynamic properties in vitro in a system similar in structure to the cell membrane. In the present work, such a system was a bilayer lipid membrane, which is a good model of a cell membrane. The adsorption and photodynamic activity ofocta-cationic complexes of phthalocyanine (Pc) with magnesium ion (8bMgPc) or zinc (8bZnPc), which were synthesized at IPCE RAS, on BLM wasstudied by measuring the potential jump at the BLM-solution boundary. The molecules of these compounds have 8 positively charged groups on the periphery and are distinguished by a metal ion in the center of the macro ring.The changes in the boundary potential ΔΦb were measured by 3 methods: by the intramembrane field method (IFC) developed in the laboratory [2], by changes in the conductivity of BLM induced by nonactin, as well as by the electrophoretic mobility of liposomes.
The adsorption of FS led to a change in ΔΦb, the sign of which corresponded to the binding of positively charged molecules to BLM. The values of ΔΦb for 8bMgPc and 8bZnPc were close, which indicates a slight influence of the nature of the metal ion in the center of the molecule on their adsorption properties. The values of ΔΦb measured during the adsorption of 8bMgPc by the IFC method coincided with the values of ΔΦb determined by the change in the conductanceof BLM induced by nonactin. This indicates that 8bMgPc molecules do not penetrate through the BLM. The slopes of the dependence of ΔΦb on the concentration of Pc in solution significantly exceeded the value predicted by the modelassuming that the charges of the adsorbed compounds lie on the membrane surface, and the potential jump satisfies the Gouy-Chapman theory [2]. The values of ΔΦb measured by the IFC method significantly exceeded the values of zeta-potential. These results can beexplainedassuming that the charged groups of 8bMgPc and 8bZnPc immerse into the hydrophobic region of the BLM.
An increase in the conductanceof the membrane during the binding of 8bZnPc added to the aqueous solution of the cell, which eventually decayed to the initial value,was detected. Itwas explained by the rearrangement of lipids surrounding 8bZnPc molecules in the membrane, resulting in conductive defects in the BLM. The addition of cholesterol to DPhPC (30 mole %) did not affect the appearance of conductivity, but accelerated its decay. The increase in conductanceduring the adsorption of 8bMgPcwas not observed, allowing the conclusion that the conductance is caused by the interaction between the zinc ion in the 8bZnPc molecule and phospholipids in BLM.
The photodynamic activity of phthalocyanineswas evaluatedby measuring the rate of damageof the molecules of di-4-ANEPPS used astargets of singlet oxygen under BLM illumination. The damageof di-4-ANEPPS molecules was detected as disappearance of the dipole potential jump created by them on the surface of the BLM. The target and Pc molecules were on oppositesurfacesof the membrane to exclude their direct interaction, and the damage of di-4-ANEPPS moleculeswas due to their oxidation by singlet oxygen penetrating through the BLM. The rate R of damage of di-4-ANEPPS proportional to the stationary concentration of singlet oxygen in the membrane was determined from the kinetics of change in ΔΦb under BLM illumination and its recovery in the dark. The dependences of R on the concentration of 8bMgPc and 8bZnPc in solution were close to each other. The parameter R increases linearly with theconcentration ofall Pcin the range of 10^(-8)-10^(-6) M, and reaches a plateau at concentrations above 10^(-5) M. This decline from the linear dependence at high concentrations of Pccan be explained by the quenching of singlet oxygen by phthalocyanine molecules in the membrane [3].
Finally, one can conclude that the nature of the metal ion in the center of the molecule of the studied phthalocyanines has little effect on their adsorption and photodynamic properties, but affects the structural rearrangements of the lipid bilayer caused by the incorporation of Pc molecules into the membrane. Charged groups of the studied Pc immerse into the hydrophobic region of the BLM, and the adsorption of 8bMgPc molecules occurs without their penetration through the membrane. The rate R of damage of molecules of di-4-ANEPPS characterizing the photodynamic efficiency of phthalocyanines, increases with their concentration in solution, but ceases to grow at high concentrations due to the quenching of singlet oxygen by phthalocyanine molecules in the membrane.
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### S2.161. Investigation of the membrane activity of protein E of SARS-CoV-2 coronavirus

Denieva Z.G.[1]*, Batishchev O.V.[1]
[1]*A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russian Federation;*
* zaret03@mail.ru

COVID-19 infection, caused by the SARS-CoV-2 coronavirus, has led to the largest pandemic since the Spanish flu in 1918. In view of this, development vaccines and antiviral drugs that can stop the spread of this infection has become an acute issue. Currently, in the search for antiviral drugs against COVID-19, much attention is paid to the study of the structure of the receptor-binding domain of the surface protein S. However, the emergence of new SARS-CoV-2 coronavirus strains indicates its high variability, which reduces the effectiveness of vaccines and antiviral drugs. At the same time, the envelope protein E of this virus is membrane-active and shows a rather high conservatism. Despite the critical importance of this protein in the coronavirus life cycle, the physicochemical mechanisms of its interaction with cell membranes still remain unclear.
The aim of this work was to investigate the membrane activity of protein E of the SARS-CoV-2 coronavirus on models of giant unilamellar vesicles and lipid nanotubes. As a result, it was found that the protein forms pores in the lipid bilayer, i.e. performs the main function of viroporin. In addition, protein E is able to deform lipid membranes and form double-membrane vesicles depending on the concentration.
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### S2.162. Isolated rats heart lvp dynamics hyperpolarization activated currents blockade in acute myocardial infarction model

Kuptsova A.M.[1]*, Bugrov R.K.[1], Mosolov L.T.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University;*
* anuta0285@mail.ru

Hyperpolarization activated current present on the membrane of atypical cardiomyocytes, is the main initiator of diastolic depolarization of action potentials in the sinoatrial and atrioventricular nodes, regulating the heart rate. Some cardiac dysfunctions, sinus node dysfunction, atrial fibrillation, ventricular tachycardia, and atrioventricular block, have been associated with altered function of HCN channels present on the membrane of working cardiomyocytes. Increased ventricular HCN current is seen in hypertrophy, ischemic cardiomyopathy, and heart failure.

The study of the effect of If blockade in an isolated heart with an experimental model of acute myocardial infarction is an important and perspective direction.

The aim of this study is to investigate the effect of If blockade on the force of contraction of the Langendorff-isolated rat heart with an experimental model of acute myocardial infarction.

The model of acute myocardial infarction was reproduced by placing a ligature on the left descending coronary artery. Twenty-four hours after coronary artery ligation, the acute stage of myocardial infarction developed, confirmed on the electrocardiogram by the presence of a pathological ST wave. The control group included healthy animals. LVP (left-ventricular pressure) dynamics were studied in experiments on isolated rat hearts by Langendorff method. Hyperpolarization-activated currents were blocked with ZD7288 at concentrations of 10-9 M and 10-5 M (Sigma).

When the If blocker (10-9 mol) was added to the perfused solution in the control group of healthy animals we observed a 13% ($p<0.001$) increase in pressure developed by left ventricular myocardium, in the group with experimental model of acute myocardial infarction LVP increased by 12% ($p<0.05$). When ZD7288 (10-5 M) was added to perfusion solution in healthy animals the pressure developed by the left ventricle decreased by 13% ($p<0.001$), and in the group with acute myocardial infarction model the studied index decreased by 29% ($p<0.05$).

Thus, blockade of hyperpolarization-activated currents has a multidirectional effect on the pressure developed by the left ventricle of the isolated heart of healthy rats and with the experimental model of myocardial infarction. Possibly, 24 hours after ligation of the left coronary artery, the density and number of HCN channels change, affecting the inotropic function of the isolated rat heart with an experimental model of myocardial infarction.

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### S2.163. Kinetics and mechanisms of oxidative hemolysis of erythrocytes under the action of azo- and peroxide initiators

Sokolova E.M.[1]*, Dubenskaia N.A.[2], Psikha B.L.[1], Neshev N.I.[1]
[1]*Federal Research Center of Problem of Chemical Physics and Medicinal Chemistry RAS, Chernogolovka, Russia;*
[2]*Lomonosov Moscow State University, Moscow, Russia;*
* sem89@icp.ac.ru

When the cell antioxidant system is disrupted, the amount of active oxygen metabolites increases. It causes various oxidative damage, leading the cell to oxidative stress [1]. The most dangerous manifestation of oxidative stress is membrane lipid peroxidation (LPO). In such state, the cell ceases to perform important physiological functions. It leads to the development of a number of pathological conditions of the body, including cardiovascular diabetes mellitus, and different forms of neurodegenerative diseases. Therefore, at present one of the basic areas of chemical biology and medicinal chemistry is the search and investigation of substances with antioxidant properties and the subsequent development of pharmacological preparations based on them. This determines the relevance and practical significance of the development of biological models for effective testing of these compounds for antioxidant activity.

The effectiveness of the developed model is primarily determined by the quality of LPO initiation in a biological and by the presence of a reliable quantitative criterion, which will effectively differentiate the pro- and antioxidant components in the action of chemical compounds on a biological object. In our work erythrocytes was used as biosubstrate. An important step in the development of such a model is the selection and study of possible chemical initiators of lipid peroxidation in the cell membrane, belonging to different classes of chemical compounds. This work is devoted to the study of peroxide hemolysis of erythrocytes under the action of two initiators of lipid peroxidation, 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), belonging to the class of diazo compounds, and tert-butyl hydroperoxide (t-BuOOH), from the class of organic peroxides.

AAPH in an aqueous medium undergoes monomolecular thermal decomposition, with the formation of two alkyl radicals and the release of molecular nitrogen. The process proceeds with sufficient efficiency already at 35-40 ○C. In an oxygen environment, alkyl radicals react rapidly with oxygen to form peroxyl radicals, which attack the double bonds of membrane lipids from the outside, initiating lipid peroxidation in the membrane.

Tert-butyl hydroperoxide (t-BuOOH), unlike AAPH, is not able to spontaneously decompose into radicals at physiological temperatures. Easily penetrating through the plasma membrane of the cell due to the highly lipophilic tert-butyl fragment, t-BuOOH triggers a cascade of complex, not fully understood, reactions involving hemoglobin, during which radical products are formed that initiate the LPO process in the membrane.

We found that under the action of the studied initiators, the content of TBARS in the erythrocyte membrane increases and erythrocyte hemolysis is observed, which is consistent with the available literature data. At the same time, the kinetic patterns of hemolysis under the action of these compounds differed significantly.

In a wide range of concentrations, the kinetics of hemolysis of the 0.2% suspension of mouse erythrocytes under the action of AAPH and t-BuOOH was studied. The experimental values characterizing the change in the degree of hemolysis over time were approximated in the Origin program by the sigmoidal Boltzmann function. Both compounds caused a concentration-dependent hemolytic effect.

The hemolytic activity of AAPH and t-BuOOH was characterized by the value of the hemolysis induction period, which was determined graphically by the time to reach 10% hemolysis. In the case of AAPH, the hemolysis induction period depended linearly on the initial concentration of the initiator, while a similar dependence for t-BuOOH was significantly nonlinear and was well approximated by a biexponential function, where k1 and K2 are 2•10-2 and 65•10-2, respectively. Such a character of the dependence may indicate the presence in the system of two different factors that affect the osmotic balance of the cell in opposite ways, which can lead to a slowdown in the rate of hemolysis with an increase in the concentration of the initiator. This is directly confirmed by the earlier plateauing of hemolytic curves at high concentrations of t-Buoy, which leads to hemolysis arrest.

Although both studied initiators are capable of activating LPO in erythrocyte membranes, their hemolytic effect has some peculiarities. The linear change in the hemolysis induction period with increasing AAPH concentration is consistent with the concept of AAPH as a monomolecular generator of peroxide radicals in an aqueous medium. At the same time, the hemolytic response of the system to the action of t-BuOOH appears to be essentially non-linear. This indicates that the interaction of t-BuOOH with the cell may include processes that are not limited to peroxidation of the lipid substrate. These circumstances should be taken into account in the possible practical use of these compounds as inducers of oxidative hemolysis of erythrocytes.

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**S2.164. Lateral diffusion of lipid in single- and two-component model vesicles membranes measured using Pulsed Field Gradient 1H NMR Spectroscopy with contrasting agent**

Gimatdinov R.S.[1,2]*, Gnezdilov O.I.[2], Kambeeva D.I.[1], Melnikova D.L.[2], Petrova A.F.[2], Khaliullina A.V.[1,2]
[1]*Kazan State Medical University;*
[2]*Kazan Federal University;*
* rsg2004@mail.ru

Artificial lipid vesicles (liposomes) are widely used as model membrane systems in various biomedical studies. Among the reasons for interest in artificial liposomes is the possibility of their use as a model system for studying the features of vesicular transport and simulation of various membrane processes, active introduction into medical practice as a means of delivery in diagnostic and clinical applications.
In the liquid crystal state, the components of the vesicle envelope have a high lateral mobility. The membrane behaves as a rather liquid, fluid phase, which has the properties of a two-dimensional liquid. Explanation of the mechanisms and regularity of lateral surface diffusion of biological membrane components, their relationship with the composition, phase state of the bilayer, and various pathological processes is an important and of current interest task. When analyzing various membrane processes, the numerical values of the lateral diffusion coefficients, which characterize the translational mobility of lipid and protein molecules along the two-dimensional membrane surface, are extremely significant. Measurements of the characteristics of lateral diffusion, in addition to data on molecular mobility, make it possible to analyze the phase state of the membrane, viscosity, structure and functions, and features of the interaction between the membrane components.
Among the methods for studying the dynamic properties of biomembranes, including the lipid membranes of artificial vesicles, a special place has the method of nuclear magnetic resonance with pulsed magnetic field gradients (NMR PFG). The NMR PFG method makes it possible to obtain information about the translational mobility in the system without introducing additional macroscopic or microscopic perturbations into the system in the form of probes or labels.
In this work, the lateral self-diffusion of phospholipid molecules of one- and two-component model membranes of artificial vesicles was studied by NMR spectroscopy with a pulsed magnetic field gradient. The measurements were carried out on 1H nuclei at a frequency of 400.22 MHz on an AVANCE 400 III TM spectrometer (Bruker) equipped with a pulsed magnetic field gradient unit. To measure the self-diffusion coefficients (SDC), the pulse sequence "stimulated echo" is used. Diffusion attenuation of the intensities of individual NMR lines in the Fourier spectra of the spin echo of diffusing molecules recorded. Samples - suspensions of one- and two-component liposomes were prepared by the standard method of hydration of thin lipid films from phospholipids of soybean phosphatidylcholine (SPC) brand Lipoid S100 and water (H2O) in a mass ratio of 1:1, with the addition of a contrasting paramagnet at a concentration of 0.73 wt% and without it. Gadolinium-containing contrast agent ProHance was used as a contrast agent to reduce the water signal, with Gadoteridol molecules as the active substance in the amount of 279.3 mg per 1 ml. Cholesterol (Chol) with a molar ratio of 2:1 (SPC : Chol) acts as the second component of the membranes of two-component systems.
A new method of spectroscopic analysis was applied to depress the signal from water when measuring the lateral diffusion of lipids using the signals of 1H nuclei. A well-known method of suppressing a rapidly decaying signal from rapidly diffusing water molecules by magnetic field gradient pulses is combined with signal reduction from the aqueous phase by accelerating the nuclear magnetic relaxation of water protons with the addition of a contrasting agent, gadolinium, as a paramagnetic doping. As a result, there is a change in the ratio of the signal intensities in the 1H NMR spectrum, similar to that in the PFG magic angle spinning NMR spectroscopy method. The signal from OH-groups noticeably decreases the lines of protons of choline groups of lipids and protons of hydrocarbon chains are clearly visible on the spectrum. Thus, it is possible to study the diffusion of lipid molecules directly from the attenuation of the corresponding lines in the 1H NMR spectrum.
The diffusion decays obtained for a one-component bilayer has a simple one-exponential character in the studied diffusion time interval from 50 ms to 250 ms. The value of the lateral diffusion coefficient at a temperature of 308 K and a diffusion time of 50 ms is 6.8*10 -14 m2/sec. In a two-component membrane with the addition of cholesterol molecules, diffusion decays are complex and described by an equation for a multiphase system, which is a sum of exponential functions. The dependence of the lateral diffusion coefficients of lipids on the diffusion time is found. These facts can be explained by phase heterogeneity in the bilayer with cholesterol (formation of lipid domains - rafts) at the experimental temperature and, as indicated in [1], the non-linear relationship between the average square of molecular displacement and diffusion time due to the curvature of the bilayers in the vesicular system. At long diffusion times, in the mode of limited diffusion, the average square of molecular displacement reaches a limiting value corresponding to the size of the vesicles. The values of the radii of curvature of the lamellar bilayers of vesicles calculated based on these data agree with our data obtained by confocal microscopy.
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**S2.165. Lipid peroxidation processes involving thiosemicarbozones**

Koshman V.E.[1,2]*, Shelepova E.A.[1,2], Selyutina O.Yu.[2], Dmitryev A.A.[2], Polyakov N.E.[2]
[1]*Novosibirsk State University ;*
[2]*Voevodsky Institute of Chemical Kinetics and Combustion SB RAS;*
* kosmanvova2010@mail.ru

Thiosemicarbazones (TSCs) have a wide range of biological activities, including anticancer, and are of great interest to scientists from various fields of science. Their anticancer activity has long been attributed to their ability to inhibit ribonucleotide reductase. However, recent studies indicate a significant role of oxidative stress in the anti-tumor activity of TSCs. This aspect of their biological activity is currently very poorly understood and is of great interest to medicinal chemistry [1].
In this work, the processes of lipid peroxidation involving chelate complexes with iron and copper ions were studied using the thiosemicarbazones di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT), di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) and novel thiosemicarbazones AOBP and AODP as examples. The interaction of chelate complexes with the lipid bilayer and their role in the lipid peroxidation reaction were studied on model systems by 1H NMR methods and molecular dynamic simulation using GROMACS software.
Experiments were performed in model systems (linoleic acid micelles and DHPC/DLPC bicelles). The redox properties of Dp44mT, DpC, AOBP, and AODP complexes with iron and copper in the lipid peroxidation reaction and the role of the natural antioxidant ascorbic acid in this process were studied. The interaction of TSC complexes with the lipid membrane was also studied. It was found that the complexation of Dp44mT with iron almost completely inhibits the peroxidation reaction, while complexes with copper retain oxidative activity. At the same time, in the presence of ascorbic acid the activity of Dp44mT complexes with iron increases significantly. It was revealed that the complexation of Dp44mT with iron ions inhibits the formation of OH-radical in the Fenton reaction. OH-radical formation was observed in

the presence of ascorbic acid. The increase in the oxidative activity of Dp44mT complexes with iron ions in the presence of ascorbic acid is due to the cyclic redox reaction with Dp44mT complexes. Iron complexation with DpC also inhibits lipid peroxidation. Complex of iron with AOBP is redox active and ascorbic acid enhances its influence on the lipid peroxidation reaction. The Dp44mT molecule is located on the surface of the lipid bilayer, while the AOBP and AODP molecules can penetrate the membrane. The AODP molecule is located closer to the surface of the bilayer than AOBP.

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### S2.166. Mass-spectrometric study of the influence of the neuroprotective drug NT-1505 on structure and lipid profile of mice liver microsome membranes

Gerasimov N.Yu.[1]*, Nevrova O.V.[1], Goloshchapov A.N.[1]
[1]*Emanuel Institute of Biochemical Physics of RAS;*
* n.yu.gerasimov@gmail.com

All attempts to describe the clinical aspect of the manifestations of various forms of dementia coupled with world studies on this problematic do not give an understanding about the pathogenetic mechanisms of their development. Our work is a new attempt to obtain answers to establish of development mechanisms and shift the focus of research to changes of the lipid profile of membranes and their key role in degeneration processes. The lipid composition and structural characteristics of the membranes of mice liver microsomes were studied in the presence of the neuroptector type drug NT-1505. NT-1505 at the 10^(-4) M and 10^(-14) M concentrations was chronically intraperitoneal injected to 15 scrub mice male. Microsomes were isolated by differential centrifugation method. The qualitative and quantitative composition of lipids studied microsomes was determined by mass-spectrometry. To study the composition of lipids by mass spectrometry, lipids were isolated from liver microsomes in chloroform extract adjusted at the concentration of 0.01 M. The resulting lipid samples were diluted in the 1% acetic acid solution with the addition of 5 mM ammonium acetate to the 500 nM concentration. The microviscosity of microsomal membranes was studied by electron paramagnetic resonance of spin probes. Stable nitroxide radicals were used as probes. It was shown that NT-1505 at the 10^(-4) M increased the microviscosity of mouse liver microsomal membranes. However, it must be taken into account that the enhancement of lipid peroxidation processes can change the fatty acid composition of membrane lipids by oxidizing of unsaturated fatty acids. That, in turn, leads to disorganization of the membrane structure up to their complete destruction. Therefore, it must be taken care when using the studied drug at the 10^(-4) M concentration in the therapy of Alzheimer's type dementia. NT-1505 at the 10^(-14) M concentration of reduced the fluidity of the membranes of mouse liver microsomes. And also, there was an increase of the amount of long-chain polyunsaturated fatty acids, which are necessary for the neural development. Thus, the use of the NT-1505 neuroprotector at ultra-low concentrations may be useful at the studies of neurodegenerative processes and, in particular, in the treatment of Alzheimer's disease.

### S2.167. Mathematical model in Kolmogorov equations form of the voltage-dependent ion channels functioning with number of gate particles

Kruchinina A.P.[1]*, Kulikovskaya N.V.[1]
[1]*Moscow, Lomonosov MSU;*
* a.kruch@moids.ru

For the animal's vestibular apparatus mathematical modeling, ionic potential-dependent channels detailed description of all types was required. The hair cells morphology and electrophysiology numerous studies have shown that there is a certain set of ion potential-dependent potassium channels in the hair cell membranes basolateral part. The change in the hair cells potential in response to acting dynamic or electrical stimuli varies greatly due to the different ratio between each type channels number in the cell membranes.

In the work of Hodjkin and Huxley in 1952, the main hypothesis was formulated as: the conductivity of each individual ion channel depends on the total state of k gate particles independently switching between open and closed states at random times. Moreover, the rate (intensity) of events random flow that determine the switching depends only on the potential difference on the two sides of the cell membrane.

If all k gate particles in an ion channel are connected in series and are controlled by independent event flows, then the channel kinetics can be modeled by a «birth and death» process with k+1 states, interconnected by transition probabilities, which we will also call flows with intensities, a and b.

At each time moment, all channels that have I from k gates are open in the state Si. Each state is assigned a probability value P(i,t). When all k channels are open, the advising probability determines the time fraction when the channel is open, i.e. the ion channel conductivity is equal to the maximum value of probability that all channel's gate particles are open.

The gate particles opening and closing will be modeled by the "birth and death" process - process with a finite number of discrete states and continuous time is a Markov process if all control flows are Poisson and mutually independent. In this case, the change in the probabilities P(i,t) is described by the k-th order linear differential Kolmogorov equations system. Since we are considering the Volt-Clamp mode, we assume that the intensities of all Poisson processes are time independent, and the Kolmogorov equations become linear with constant coefficients. Such equations have a stationary stable solution - a stationary probability, and transients for switched currents are weighted sums of exponentials with negative exponents -t/Ti(V). Ti(V) are the transients time constants, t is time. For stationary probabilities and transient time constants processes, there are analytical expressions in terms a and b (the transition probabilities between a state with a different open gate particle number). Used these relationships, it is possible to determine the stationary probabilities and transient processes time constants values by the recorded change in current, to obtain experimental values of the intensities of transitions for all potential values. The simplest case is when the transitions intensities between open and closed states are the same for all gate particles in the channel, and the reverse transitions intensities also coincide.

The next step is to obtain an analytical description of the transition intensities for an arbitrary value of the cell membrane potential. This will make it possible to describe ionic potential-dependent currents in the form of the Kolmogorov equations and in cases of a time-varying potential V. Let us describe the intensities a and b by exponential functions of the form:

a(V)=f(u(V)/1-exp(-u(V))),
b(V)=g(w(V))=exp(-w(V)),

where u(V) and w(V) are linear functions of V. The approximating a and b problem is reduced to finding the coefficients of linear u(V) and w(V). For the search, the quasi-regular least squares method is used. In this work, on the basis of the experiments described in publications, the coefficients for all known potassium currents of hair cells of the vestibular organs of animals were obtained. Note that the analysis of the functioning of ion channels with many gate particles explains some significant disagreements in determining the intensity of random fluxes of individual gate particles. Hodjkin and Huxley determined the transition intensities for one gate particle by choosing the form of their analytical representation. In their calculations, they used protocols corresponding to channel activation modes, indicating that deactivation occurs in a simple exponential manner. A detailed analysis of deactivation is not given in their works.

Perhaps for a neuron, these modes are less noticeable than activation modes. However, to describe the membrane potential of the hair cells of the vestibular organs, it is essential to describe their functioning in the region of the resting potential. In this range, the time constants are maximum, and their recovery from experimental data is difficult due to small current amplitudes and large noise compared to the useful signal. If, when choosing the duration of the interval for recording a change in current in the Volt-Clamp mode, one is guided by the duration of transient processes at potentials close to the resting potential of the cell, then consideration of the final segment of the recording contains significant information. The time constant determined from the final segment of the records corresponds to the component with the largest value of the time constant T. Using this estimate of $\tau$, it is possible to find out if there are components with time constants close to T/2, T/3, etc. during the initial period of observation. The presence of such components indicates the number of gate particles of the same type, which initializes the mathematical model based on the Kolmogorov equations. If the fast components have time constants far from to T/2, T/3,…, then it can be assumed that the gate particles in the channel are inhomogeneous. Determining the times of transient processes for the potential range close to the resting potential is possible according to the data on membrane deactivation at hyperpolarized potentials.

**S2.168. Molecular dynamics method for determining the elastic parameters of lipid membranes based on measuring the dependence of the lateral pressure profiles of planar lipid bilayers on lateral tension and ambient pressure**

Kalutskii M.A.[1], Galimzyanov T.R.[1], Pinigin K.V.[2]*
[1]*National University of Science and Technology MISiS;*
[2]*Frumkin Institute of Physical Chemistry and Electrochemistry, RAS;*
* pinigin@phystech.edu

Lipid membranes represent an important structural component of living cells, forming poorly permeable shells both for cells as a whole and for cell organelles. There are a large number of cellular phenomena associated with deformations of lipid membranes: fusion, fission, poration, incorporation of membrane proteins into the membrane, membrane-mediated interaction between membrane inclusions, deformations at the phase separation boundary, etc. To analyze these deformations, lipid membranes can be considered as continuous elastic media, liquid in the lateral direction. The elastic parameters of lipid membranes play a key role in describing membrane deformations since these parameters determine the energy barriers and characteristic times of processes associated with membrane deformations. Lipid membranes of living cells are multicomponent, which allows cells to change the elastic parameters of lipid membranes by varying the lipid composition and thereby regulate the processes associated with membrane deformations. Thus, the analysis of the elastic parameters of multicomponent lipid membranes is important for studying how cells implement this regulation. In this work [1], we propose a molecular dynamics method for determining the following elastic parameters of multicomponent lipid membranes: the monolayer stretching modulus, monolayer bending modulus, monolayer spontaneous curvature, local Poisson's ratio, and position of the neutral surface. The method is based on measuring the dependence of the lateral pressure profiles of planar lipid bilayers on lateral tension and ambient pressure. The use of planar lipid bilayers excludes the redistribution of lipids in regions with different curvatures, which complicates the determination of the intrinsic elastic parameters of lipid mixtures in other methods [2] that employ curved lipid membranes. The proposed method is a generalization and significant revision of the method introduced in Ref. [3]. The necessity of a significant correction to the theory of Ref. [3] is shown, the neglect of which can lead to a systematic error of up to ~25% in the values of the elastic parameters. Instead of finding the local stretching modulus according to Ref. [3], a significantly simplified procedure for determining the elastic parameters is proposed, based on calculating the derivatives of the local tension moments with respect to stretching. From the assumption of global incompressibility of lipid monolayers, the expression for the local Poisson's ratio is derived, which permits a more precise determination of the elastic parameters. In the case of the local incompressibility assumption and quadratic elastic energy law, a relation is obtained between the Gaussian curvature modulus as a function of stretching and the bending modulus. The method is applied to lipid membranes composed of dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC), and their 50:50 mixture. It is shown that the bending modulus of the mixture of these lipids does not follow the classical Reuss averaging.

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**S2.169. Molixan modulates Na+ transport in frog skin epithelium**

Melnitskaya A.[1]*, Krutetskaya Z.[1], Badulina V.[1], Antonov V.[2], Krutetskaya N.[1]
[1]*Saint-Petersburg State University;*
[2]*Saint-Petersburg State Pediatric Medical University, Saint-Petersburg, Russia;*
* a.melnitskaya@spbu.ru

The study of the mechanisms of transepithelial transport of substances is an intensively developing area of modern biophysics, physiology and medicine. Classical model objects for studying ion transport mechanisms across biological membranes are the skin and bladder of amphibians.

Na+ transport in osmoregulatory epithelium is a complex, multicomponent system, which involves various Na+ transporting proteins and signaling cascades localized in different cell membranes, which are the target for oxidative stress. Numerous cysteine residues localized in different segments of these proteins determine their redox sensitivity and are a target for the action of intra- and extracellular oxidizing and reducing agents.

Pharmacological analogs of oxidized glutathione (GSSG) glutoxim® (disodium salt of GSSG with nanoconcentration of d-metal, PHARMA-VAM, St. Petersburg) and molixan® (complex of glutoxim and inosine nucleoside, PHARMA-VAM) are used as immunomodulators and cytoprotectors in the complex therapy of bacterial, viral and oncological diseases. These drugs have a complex effect on the processes of redox regulation in cells, but the subtle biophysical mechanisms of their action are far from being fully understood.

Previously, we found that GSSG and glutoxim modulate Na+ transport in frog skin. Application of these agents from the apical side of the skin was found to inhibit Na+ transport, while when added from the basolateral side of the skin, GSSG and glutoxim mimic the effect of insulin and stimulate Na+ transport. In this regard, the aim of this work was to study the effect of another disulfide-containing drug, molixan, on Na+ transport in the frog skin epithelium.

To measure the electrical parameters of the frog Rana temporaria skin, we used an automated device for voltage-clamp and recording

current-voltage characteristics (I–V characteristics). To measure I–V characteristics, a linearly varying voltage (ramp) was applied to the skin at a rate of 20 mV/s. In the intervals between I–V characteristics measurements, the transepithelial potential (VT) of the skin was maintained at 0 mV (short - circuit mode) or at the open - circuit potential VOC (VOC = VT at transepithelial current IT = 0). From the I–V characteristics, the skin electrical parameters were determined: short - circuit current ISC (ISC = IT at VT = 0), VOC, and transepithelial conductance gT. Na+ transport was evaluated as amiloride-sensitive ISC. At the end of each experiment, amiloride (20 μM), amiloride-sensitive epithelial Na+ - channel blocker (ENaC), was added to the solution washing the skin apical surface. Statistical analysis was performed using Student's t-test. Data are presented as x ± sx. Differences were considered significant at $p \leq 0.05$.

It has been shown that the treatment of the frog skin apical surface with molixan inhibits Na+ transport. On average (here and below, n (number of experiments) = 10), after application of 100 μg/mL molixan to the frog skin apical surface, ISC decreases by 15.76 ± 4.32 %, and VOC decreases by 16.42 ± 6.02 %; no changes in gT were observed. At the same time, after application of 100 μg/mL molixan from the skin basolateral surface, ISC increases by 39.24 ± 7.17 %, VOC increases by 41.21 ± 10.08 %; the value of gT also does not change.

The inhibitory effect of molixan may be associated with its ability to interact with functionally significant cysteine residues of Na+ - transporting proteins, which leads to inhibition of their activity and suppression of Na+ transport. The obtained results are consistent with the literature data, according to which ENaC and other Na+ - transporting proteins are rapidly and reversibly inhibited by agents that oxidize SH - groups of cysteine residues. At the same time, molixan, applied from the skin basolateral surface, mimics the action of insulin and stimulates Na+ transport. The results obtained are consistent with our previous data, as well as with the literature data on the ability of GSSG and glutoxim to have a receptor-mediated effect on cellular processes. It may be assumed that GSSG and its pharmacological analogs glutoxim and molixan may interact with cysteine-rich extracellular domains of the α-subunits of the insulin receptor and cause receptor transactivation, which leads to the activation of Na+ - transporting proteins and stimulation of Na+ transport.

It is known that the key Na+ - transporting proteins (ENaC, Na+-K+ - ATPase and Na+ /H+ - exchangers) contain numerous cysteine residues, which are targets for the effect of intra- and extracellular oxidizing and reducing agents. However, the addition of the ENaC blocker amiloride (20 μM) to the solution washing the frog skin apical surface caused a complete suppression of Na+ transport. It may be assumed that the effect of molixan on Na+ transport is mainly due to the modulation of ENaC activity.

Thus, the present work shows the modulating effect of molixan on Na+ transport in the frog skin epithelium. The results obtained also indicate that molixan, GSSG, and glutoxim unidirectionally modulate Na+ transport in frog skin. It can be assumed that the effect of these disulfide-containing oxidants on Na+ transport is mediated by similar regulatory mechanisms.

### S2.170. NADPH-oxidase regulation via formyl peptide receptors on bone marrow granulocytes in obesity resistant mice

Tikhonova I.V.[1], Dyukina A.R.[1]*, Shaykhutdinova E.R.[2], Safronova V.G.[1]

[1]*Institute of Cell Biophysics of Russian Academy of Sciences, Pushchino, Russia;*

[2]*Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of Russian Academy of Sciences, Pushchino, Russia;*

* Dyukina@rambler.ru

It is known that obesity is a precursor of various pathologies (type 2 diabetes mellitus, cancer, arthritis, hypertension, etc.) and is characterized by low grade chronic inflammation. The inflammatory cascade in obesity is initiated mainly by polymorphonuclear neutrophilic granulocytes (neutrophils), the most reactive cells of innate immunity with a high cytotoxic potential, including the production of reactive oxygen species. A change in the functional activity of neutrophils in obesity was shown, however, neutrophil functioning s, including the generation of reactive oxygen species, has not been studied under conditions of resistance to obesity.

The aim of the work was to investigate NADPH-oxidase-dependent generation of reactive species initiated thought membrane formyl peptide receptors (Fpr1, Fpr2) in bone-marrow granulocytes of obesity-resistant mice (ORM). As long-term high fat diet (HDF) consuming is a stressor factor modifying immune profile in obesity-prone mice, we assumed that in obesity-resistant mice it would also modify immune cell functions including production of reactive species initiated by membrane formyl peptide receptors.

Male C57BL/6j mice were used in the study and kept under barrier conditions on HFD (total calorie content of 516 kcal/100 g) for 16 weeks. A group of animals kept on a standard diet (calorie content 306 kcal/100 g) served as a control. The mice included in the experiment, which received HFD and did not differ from controls in body length and weight. The mice fed HFD and having significantly higher body weight gain than controls were not taken in the experimental group.

Then four groups were formed: (I) controls; (II) controls with acute inflammation; (III) ORM; (IV) ORM with acute inflammation. An acute inflammation was caused by intraperitoneal injection of zymosan suspension in Hanks' solution (5 mg/ml, 150 μl) 12 hours before the experiment. Controls (I) and ORM (III) were injected by 150 μl Hanks' solution intraperitoneally.

A biochemical analysis was carried out, the concentration of glucose level in the whole blood of animals was determined and the relative mass of hematopoietic organs with actively proliferating tissue (thymus, spleen, liver) was measured. Granulocytes were isolated from the bone marrow by centrifugation in a percoll density gradient. Chemiluminescent analysis was used to assess the intensity of reactive species generation, which was initiated by 1 μM N-formyl-MLF (fMLF, activation of Fpr1) and 1 μM WKYMVM (synthetic agonist Fpr2). Inhibitors of PLC (U73122, 0.2 and 2 μM), PKC (GF109203X, 1 μM), p38MAPK (SB202190, 10 μM), ERK1/2 (FR180204, 10 μM), JNK (SP600125, 10 μM), were used to establish the role of indicated enzymes in the signal transduction from FPRs to NADPH oxidase in ORM. Response amplitude and production of reactive species were estimated. The effect of the inhibitors was calculated as the ratio of the parameter obtained from the cells treated with the inhibitor to the parameter of the intact cells taken as 100%.

A higher level of spontaneous RS production in ORM cells was shown. Half maximal effective concentration (EC50) for N-formyl-MLF responses was higher in ORMs with and without inflammation compared to the same controls, indicating a insignificant role of Fpr1. Increased responses to WKYMVM (Fpr2 agonist) was in controls with acute inflammation, but they were similar in other groups. Possibly Fpr2 were partially inactivated in ORM owing their inflammatory state. HFD and acute inflammation led to increase of the positive regulation of NADPH-oxidase activity by PLC. Our results demonstrated decreased role of PKC in regulation of NADPH-oxidase activity initiated by formyl peptide receptors in ORM granulocytes, and also in the cells of controls with inflammation activated via Fpr2.

Weakened Fpr1 and Fpr2 signaling via MAPKs was revealed in ORM granulocytes using specific inhibitors for p38, ERK1/2, JNK. P38 signaling via Fpr2 was lower in ORM with inflammation. Thus, HFD modified the role of formyl peptide receptors and suppressed MAPKs signaling in NADPH-oxidase regulation in ORM.

The data obtained may be useful to understand immunological features of obesity resistance and open the possibility of using of formyl peptide receptors as potential therapeutic targets to inhibit obesity-related inflammation and develop strategies against obesity-related metabolic disorders.

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**S2.171. Nanomechanical signatures of early passage glioma culture cells depend on the expression of the CD44 marker in the absence of a mutation in the IDH1 gene, and do not depend on the presence of an IDH1R132H mutation**

Shmelev M.E.[1]*, Farniev V.M.[1], Shved N.A.[1,2], Kumeiko V.V.[1,2]
[1]*Far eastern Federal Univercity;*
[2] *A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences;*
* shmelev.m.e@gmail.com

Atomic force microscopy (AFM) is a relatively new technique in biomedicine, procuring an assessment of the morphological and functional characteristics of cancer cells and their microenvironment responsible for tumor invasion and progression.
Materials and methods:
In the course of this work, high-resolution AFM mapping of a large number of cells was used. We analyzed the nanomechanical properties of cell cultures of early passage gliomas with different IDH1 R132H mutational status. During the analysis, each cell culture was additionally clustered by the presence of the CD44 marker to identify possible nanomechanical features that differentiate cell phenotypes that differ in proliferative activity and a characteristic surface marker.
Results:
Mutant IDH1 R132H cells compared with wild-type IDH1 (IDH1wt) cells are define by a significant cell hardening. CD44+ IDH1wt cells were twice as harder than CD44- IDH1wt cells. In contrast to IDH1 wild-type cells, CD44+ IDH1R132H and CD44- IDH1R132H did not show nanomechanical patterns that provide statistically significant differentiation of these subpopulations. Median cell stiffness values depend on the presence of IDH1 R132H mutation and CD44 labeling: IDH1R132Hmt, 4.7 mN/m; CD44+/IDH1wt, 3.7 mN/m; CD44-/IDH1wt, 2.5 mN/m. The obtained results indicate that quantitative nanomechanical mapping becoming a promising method for rapid analysis of the cell population, suitable for detailed diagnosis and target treatment of gliomas.

**S2.172. Nanoparticles with lipoic acid and glutathione**

Inshakova A.M.[1]*, Shchelkonogov V.A.[1,2,3], Evstratova A.Yu.[1], Al Nissafi L.[1], Shastina N.S.[1], Baranova O.A.[2,3], Chekanov A.V.[2,3], Solov'eva E.Yu.[2], Fedin A.I.[2]
[1]*MIREA – Russian Technological University;*
[2]*Russian National Research Medical University (RNRMU);*
[3]*Kotelnikov Institute of Radioengineering and Electronics;*
* aminshakova@yandex.ru

The blood-brain barrier (BBB) maintains homeostasis in the brain through selective transport of metabolic compounds, which prevents many high-potential drugs from freely penetrating the BBB. Glutathione receptors in the brain are mainly localized in neuroglial cells. It has been proven that the transport of glutathione into brain cells is carried out using a specific mechanism of adsorption-mediated endocytosis. Endocytic pathways are activated within cells using a targeting fragment or ligand that is used as a vector. Nanoscale drug delivery systems are widely used as a strategy for penetrating the BBB and improving drug transport into the central nervous system (CNS). Nanoparticles with glutathione (liposomes, micelles, etc.) have shown great potential, both in targeting and in improving the penetration of drug components into the brain. It is known that glutathione (GSH) is an unstable tripeptide; however, despite its widespread use, its stability has not yet been studied to a large extent [1, 2]. As a result, the aim of the work is to develop nanodispersed forms of glutathione and lipoic acid for improved delivery of antioxidants to brain cells.
Homogeneous (PDI<0.3) nanoemulsions with LA and glutathione based on phosphatidylcholine and Pluronic F68 with a particle size of 106 to 148 nm were obtained by spontaneous emulsification. A heterogeneous nanodispersion with LA and GSH based on F68 was also developed, consisting of 2 particle fractions: 40–60 nm (15%) and 120–280 nm (85%). The resulting nanodispersions were electroneutral. The resulting nanoparticles are characterized by high dispersion stability during long-term storage (more than 3 months) at room temperature and are promising candidates for further evaluation of their antioxidant and antiplatelet effects in vitro experiments.
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**S2.173. Neurotransmitters secretion in the taste bud**

Rogachevskaya O.A.[1]*, Cherkashin A.P.[1], Khokhlov A.A.[1], Kolesnikov S.S.[1]
[1]*Institute of Cell Biophysics of RAS;*
* o.rogachevskaja@gmail.com

The first stage in the formation of taste sensations that convey information about the quality and value of food is the responsibility of taste receptor cells that come into contact with substances dissolved in saliva. Taste cells are grouped into dense associates - taste buds, and according to morphological and functional criteria are divided into four types: basally-situated, immature type IV cells, and 3 types (I-III) of mature elongate receptor cells, which apical membrane reaches the taste pore. In type II cells (or "receptor" cells, since they express bitter, sweet, and umami receptors along with other components of the taste transduction), a non-canonical vesicular-free chemical synapse functions, where the neurotransmitter ATP is secreted through channels in a calcium-independent manner. The type II cell and adjacent nerve ending are surrounded by an astrocyte-like type I cell, forming an intercellular compartment similar to the synaptic cleft. It is supposed that type I taste cells detect salty and, through the generation of an action potential, directly activate the adjacent nerve ending. Type III cells (mediate sour and possibly some salty taste), aka "synaptic cells", are the only cells that form classical chemical synapses with nerve endings, quantumly releasing serotonin in a calcium-dependent manner in response to taste stimulation. Thus, type II and III cells convert the received information about the taste stimulus into the secretion of neurotransmitters (ATP and serotonin mainly) that activate nerve endings.
Since in vitro physiological experiments often require registration of the neurotransmitters release from a single taste cell, which means the need to detect nanomolar concentrations of the neurotransmitter in a local area of the experimental chamber, one of the main approaches used in the study of taste reception is the method of sensor cells expressing the receptor of the secreted molecule and generating a cellular response to the appearance of a neurotransmitter.
In particular, to study the regulation of serotonin secretion in type III taste cells, we created a serotonin sensor based on CHO cells and a 5-HT2C receptor coupled to the intracellular calcium mobilization system, which makes it possible to record its signals using Ca2+-probes and microphotometry. However, the resulting biosensor generated Ca2+ signals according to the "all-or-nothing" principle, which can

only display the fact of neurotransmitter secretion, but not its quantity. Therefore, we created a serotonin sensor based on HEK293 cells, 5-HT4 serotonin receptor coupled to the adenylyl cyclase cascade, genetically encoded cAMP-sensor, fluorescent protein Pink Flamindo. This biosensor had a gradual sensitivity to serotonin, which made it possible to study the mechanisms of regulation of serotonin secretion from type III taste cells.

To study ATP secretion we used CHO cells expressing P2X2/P2X3 heterodimeric ion channel and COS cells expressing endogenous P2Y receptors as ATP-sensors, which allowed us not only to demonstrate the fact of ATP-release by type II taste cells, but also investigate the detailed mechanism of secretion.

Taste stimulation of type III cells did not lead to the generation of responses of ATP sensor cells, however, using a confocal microscope and the fluorescent dye quinacrine, which specifically stains ATP-containing vesicles in the cells, we showed that, in response to depolarization of type III cells, a decrease in vesicular structures at the basement membrane, which can be considered as evidence of the exocytosis of ATP as a neurotransmitter by these taste cells.

Using the biosensor method to study the stimulus-dependent secretion of neurotransmitters, it is almost impossible to stimulate only the apical membrane of cells, and there is always the possibility of non-specific effects of taste compounds. Therefore, for on-line monitoring of ATP secretion, we developed a unique methodology based on the Ussing approach and the luciferin-luciferase method. For this, a fragment of the lingual epithelium containing a grooved taste bud was fixed in a modified Ussing chamber, which, due to the physical isolation of the apical and basolateral parts, made it possible to stimulate the apical part of the gustatory epithelium separately and to detect ATP secreted from the basal part of the epithelium using a mixture of luciferin-luciferase. This technique made it possible to visualize the release of ATP by the lingual epithelium ex vivo in response to stimulation with gustatory substances and preserve the viability of gustatory tissue and its ability to respond to bitter substances for several hours.

The described approaches significantly expand the tools used in the study of neurotransmitter secretion and can be used for different cell types.

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### S2.174. Nonlocal electrostatic aspects of electroosmotic coupling between water flow and electric current in tight junctions between epithelial cells

Rubashkin A.A.[1]*, Iserovich P.[2], Reinach P.S.[3]
[1]*Institute of Cytology RAS, St. Petersburg, Russia;*
[2]*SUNY Downstate Medical Center, Brooklyn, NY, USA;*
[3]*Wenzhou Medical University Ophthalmology and Optometry Department, Wenzhou, China;*
* andrey.rubashkin@gmail.com

Epithelial tissues are an interface between organs and the environment. The electroosmotic nature of the paracellular water transport in leaky epithelial was demonstrated by J. Fischbarg and co-workers in experiments with rabbit corneal epithelium in-vitro model [1, 2]. In the same paper, the important role of tight junctions (TJ) between cells in the generation of electroosmosis was experimentally proven. These works have shown that electroosmosis in tight junctions differs from electroosmosis described by the classical theory of Helmholtz-Smoluchowski (HS). The foundations of the theory of this phenomenon were laid in [3, 4]. In them, to calculate the change in the solvation energy W during the transition of ions to TJ, the classical solvation theory based on the Born formula was used. However, for such a structured medium as water, the application of the Born formula is incorrect, since the calculation based on it gives strongly overestimated values for W ions [5]. In this communication, the development of previous theoretical works [3, 4] is presented, and for calculating W, the nonlocal electrostatic theory is used, the foundations of which are outlined in the monograph [5]. To analyze the electroosmotic binding in TJ, we used a system of two equations. The first is the Stokes-Brinkman hydrodynamic equation, which relates the distribution of the water velocity over across section the TJ with the strength of the applied electric field and with the electric current in the TJ. The second is the Poisson equation, which relates the distribution of the electrostatic potential in a TJ with the concentrations of fixed and mobile electric charges. Both of these equations include Na+ and Cl- concentrations that are proportional to the respective ion distribution coefficients (n). Therefore, the change in W of ions during their transition to TJ determines the nature of the electroosmotic binding between water and ion flows. We performed a nonlocal electrostatic calculation of the change in W ions and distribution coefficients between TJ and free solution. The calculation showed that an increase in the correlation length (L) of water in TJ reduces the value of n ions and electrical conductivity in TJ, and the coupling coefficient between the water flow and electric current through TJ increases with increasing L. The calculation also showed that the theoretical value of the coupling coefficient coincides with the experimental value previously determined in [1] if L of water in TJ increases by 20% compared to its value in a free solution. Note that the increase in L in TJ is due to the presence of claudin protein molecules in them, and the existence of this effect was substantiated in [6-7]. The analysis carried out shows that there are two factors that influence the emergence of water transport through the TJ and its direction. The first is the increase in L in TJ compared to its value in a free solution. The second is the presence of both electric charges of claudin protein molecules (distributed inside TJ) and charges on the membranes that limit TJ. Let us list a number of changes in the TJ, which are the result of an increase in L in the TJ. 1) an increase in the Na+/Cl- charge selectivity in TJ, 2) a decrease in the electrical conductivity in TJ compared to its value in a free solution, 3) an increase in the coupling coefficient between water flow and electric current in TJ compared to its calculated value according to the classical formula of electroosmosis HS. These changes are qualitatively explained as follows. An increase in L leads to an increase in the absolute value of changes in W of Na+ and Cl- ions during their transition from a free solution to TJ. In this case, changes in W retain their negative values, so the values of n ions are less than one. It follows from this that the concentration of ions in TJ is lower than in a free solution. The latter effect leads to a decrease in electrical conductivity compared to its value in a free solution containing NaCl. The decrease in electrical conductivity, together with an increase in the Debye length in TJ, leads to an increase in the coupling coefficient between the water flow and electric current in TJ compared to its value calculated using the HS electroosmosis formula, since the electrical conductivity is in the denominator. These effects in tight junctions determine the difference between electroosmosis in TJ and electroosmosis described by the classical HS theory.

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**S2.175. Parameters of the action potential of atrial cardiomyocytes of newborn rats against the background of AT1 receptor blockade**

Iskakov N.G.[1,2]*, Anikina T.A.[1], Nikolaev T.I.[1], Nasartdinova R.R.[2], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University;*
[2]*The Volga Region University of Sports and Tourism;*
* nikitaiskakov1992@mail.ru

A large group of chemicals called comedians has been found in the cardiovascular system, through which an impulse is transmitted from one cell to another. In the sympathetic nervous system, the main mediators are ATP, neuropeptide Y. Cotransmitters play an important role in the processes of age-related development of the heart. Neuropeptide Y was discovered in 1982, and immediately after its discovery, this peptide was synthesized and defined as a neurotransmitter regulating various functions of the body. Neuropeptide Y has been identified as the most common peptide in the mammalian central nervous system. Neuropeptide Y has been found in the heart, vessels of the central nervous system, digestive system and other tissues of the body. According to the authors, neuropeptide Y is associated with a number of physiological processes. The central effects of this peptide include the regulation of respiration, hypotension, hypothermia, and endocrine functions. Peripheral effects include regulation of the cardiovascular and respiratory systems. One of the objects in which neuropeptide Y can play an important role is the heart. In the rat heart, the effects of NO are realized by activating 1R, 2R and 5. Neuropeptide Y has been shown to affect the frequency of spontaneous activity and the contractile function of the heart. Neuropeptide Y causes a multidirectional change in heart rate. An increase in heart rate was observed in porpoise preparations. In dogs and cats, NPY injection caused a decrease in heart rate. The Langendorf isolated heart preparations in rabbit and dogs did not show a change in the frequency of spontaneous activity during the application of neuropeptide Y.
The study was conducted on 7-day-old white mongrel rats (n=18). The electrical activity of cardiomyocytes was studied using intracellular microelectrode abduction on a right atrial myocardium preparation with preserved sinus node and spontaneous activity. The membrane potential (MP) and action potential (PD) were recorded using glass microelectrodes (tip diameter <1 mm, resistance 30-80 MΩ), which were manufactured on the day of the experiment on a horizontal puller P-1000 (Sutter Instruments). The obtained recordings of myocardial electrical activity were analyzed in the original program "Elph 3.0". We analyzed the following amplitude-time parameters of the registration of the action potential: the frequency of PD generation, the amplitude of the action potential, the duration of the PD depolarization phase, the duration of the PD repolarization phase at the level of 20, 50 and 90%. Statistical processing of the results was carried out using the program calculated according to the Student's paired criteria ($p < 0.05$). All used chemical reagents of the Sigma company. The effects of the selective Y1-receptor blocker BIBP 3226 (10-6M) and the effect of the Y1.5-receptor agonist [Leu,31 Pro34] NPY (10-6M) on the background of the selective Y1-type receptor antagonist BIBP 3226 (10-6M) were studied on one drug.
The aim of our study was to study the effects of a selective blocker and the effect of a Y1.5-receptor agonist on the background of a selective Y1-type receptor antagonist.
The selective blocker BIBP 3226 10-6M caused changes in the duration of the repolarization phase. The duration of the action potential at the level of 20%, 50% and 90% repolarization (DPD20, DPD50, DPD90) increased from 25.8±4.7 to 28.1±4.8 ms, from 78.2±8.6 to 84.6±8.2 ms, from 175.9±9.1 to 192.4±7.2 ms, which is 9%; 8%; 10%, respectively ($p<0.05$; n=8). The combined use of the blocker and agonist caused an increase in the duration of repolarization at the DPD20 and DPD50 levels from 17.5±2.0 to 19.5±2.3, from 43.7±8.4 to 46.6±8.6, which is 11% and 7.5%, respectively ($p<0.05$; n=10).
Thus, the combined administration of a selective blocker and a Y1 receptor agonist to newborn animals led to the preservation of the blocker effect. It is possible that Y5 receptors are involved in changes in the duration of the PD repolarization phase in 7-day-old animals, since [Leu,31 Pro34] NPY is an agonist of Y1 and Y5 receptors.

**S2.176. Participation of alpha2-adrenoreceptors in the regulation of bioelectrical parameters of newborn rat atrial cardiomyocytes**

Galieva A.M.[1]*, Ziyatdinova N.I.[1], Zefirov A.L.[2], Iskakov N.G.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University, Kazan, Russia;*
[2]*Kazan State Medical University, Kazan, Russia;*
* galieva_alina94@mail.ru

Adrenergic receptors, also called adrenoceptors, are proteins composed of 400 to 600 aminoacids, with seven membrane-spanning domains, and belong to the superfamily of G protein-coupled receptors (GPCR). They are membrane receptors that activate G proteins following the binding of a ligand, thus causing a change in the cell membrane permeability to one or more ions and/or activating an enzyme attached to the receptor. Gs and Gi proteins activate and inhibit the enzyme adenylylcyclase respectively, while Gq proteins activate phospholipase C. [1] According to a number of authors, in rat ventricular and guinea pig and human atrial myocardium, activation of alpha2-adrenoreceptors leads to Gi-protein activation, which decreases adenylate cyclase activity, cAMP level, and thus protein kinase A activity, which eventually blocks norepinephrine exocytosis. Furthermore, it is not excluded that the Gq protein-protein kinase C pathway is also involved in this process. it is believed that alpha2-adrenoceptor subtypes (a-, c-, and c-) are associated with Gi protein, i.e., they implement a single signaling system. [2]
The study was performed on one-week-old white mongrel rats. Narcotized animals had their thorax opened and a multicellular preparation with the auricle of the right atrium of the heart was made. Electrical activity of cardiomyocytes was studied using intracellular microelectrode lead with an imposed rhythm at a frequency of 5 Hz. The α2-AR agonist clonidine hydrochloride solution (10-6 M) was applied for 20 min.
In one-week animals clonidine hydrochloride in the studied concentration did not cause significant changes in the value of membrane potential, depolarization phase duration, and action potential amplitude. However, it increased the repolarization phase of the action potential by 50% ($p<0.05$), 90% ($p<0.05$).
Thus, stimulation of α2-adrenoreceptors by a nonselective agonist in the cardiomyocytes of one-week-old rats with an imposed rhythm in the concentration studied led to an increase in the duration of the repolarization phase of the action potential, which may be related to age-related features of intracellular mediator cascades of this type of heart adrenoreceptors.
The study was supported by the Russian Science Foundation Grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/.
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### S2.177. Participation of membrane mechanisms in the regulation of electrokinetic properties of erythrocyte populations under stress

Zverev A.A.[1]*, Shamratova V.G.[2], Dautova A.Z.[1], Isaeva E.E.[2]
[1]*The Volga Region University of Sports and Tourism;*
[2]*Bashkir State Medical University;*
* Alekcei5@rambler.ru

The most important parameter of erythrocytes, which ensures their unhindered movement along the bloodstream, is the negative charge of the surface. The magnitude of the cell charge is usually judged by their electrokinetic potential (EKP) and the experimentally measured speed of cell movement in an electric field - electrophoretic mobility (EPM) (Elblbesy MA, 2017). A decrease in the surface potential of erythrocytes, reducing the forces of electrostatic repulsion of cells, enhances their aggregation, changes blood viscosity, and initiates the process of thrombus formation (Sheremet'ev IuA, 2013). Therefore, maintaining the optimal value of the charge of erythrocytes both at rest and during functional loads is of paramount importance for maintaining suspension stability and the necessary rheological characteristics of moving blood. Naturally, information about the regularities and mechanisms of ensuring such stability is not only of theoretical, but also of practical interest.

Our studies of the electrophoretic mobility of erythrocytes (EPME) in normal and pathological conditions, physical and emotional stress of the body showed that a differential approach to the study of the erythrocyte population can contribute to the solution of these problems (Matyushichev V.B., 2007). From these positions, peripheral blood erythrocytes were considered not as a homogeneous mass of functionally identical cells, but as a cell population, the composition and dynamics of which reflect the membrane functions and metabolism of individual cells, the influence of plasma factors, the activity of erythrocyte production and destruction organs. With this approach, it was possible to establish that the bioelectrical homeostasis of erythrocytes is achieved mainly due to the redistribution of their individual subpopulations in proportions that can ensure the preservation of the optimal total level of EKP.

The aim of the work was to study the contribution of membrane mechanisms to the regulation of the electrokinetic properties of erythrocyte populations under stress.

Methods. A group of students (n=20) aged 18-19 have been examined being under examination stress and in a state of emotional and physical rest. Electrophoretic mobility of erythrocytes was determined by microelectrophoresis in autoplasma diluted in Ringer's medium. The parameters of the EPME distribution shape were used for a quantitative assessment of the qualitative features of the structure of populations. Histogram parameters were taken into account in addition to average values: asymmetry (As) and excess (Ex) coefficients, which make it possible to identify individual erythrocyte subpopulations in the general population and assess the degree of their heterogeneity. The activity of Na, K-ATPase was assessed by means of adding strophanthin 10-5 M.

Results. The primary processes underlying the change in the balance of erythrocyte subpopulations with different charges can be implemented at different levels, from the membrane to the systemic. Taking into account that, along with the relatively passive (surface charge), active component, which reflects the permeability of membranes and the operation of ion pumps, participates in the formation of the EKP of the cell, it can be assumed that ion transport membrane systems are involved in the dynamics of the redistribution processes of erythrocyte subpopulations along their EKP (Krylov VN, 2014). The Na-pump, K-pump are a universal link that performs metabolic self-regulation of the receptor and electrical properties of membranes. The influence of the Na-pump, K-pump on the electrokinetic properties of erythrocytes is evidenced by the presence of correlations between the EPME value and the Na, K-ATPase activity of erythrocyte membranes. EPME of the students under stress increases significantly due to increase of the proportion of cells with increased EKP. EPME decreases due to the inhibition of the pumps of the subpopulation of cells with increased EKP under the influence of strophanthin. In a state of emotional balance the students have no changes in the average values of EPME and distribution parameters under the influence of strophanthin. The ability of strophanthin to suppress the operation of the pump extends only to homogeneous subpopulations (positive Ex), which are characterized by a higher EKP than the population average (negative As). With emotional and physical stress of the body, the proportion of such subpopulations in the total pool increases, indicating an increase in the functions of the Na-pump, K-pump. As for the predominance of cells with a reduced level of EPM in the population, there is both a lack of response to the action of the inhibitor and a slight increase in EKP, indicating a change in the state of the membrane, in particular, its permeability. Among the factors causing destructive changes in the membranes, obviously, one can include the activation of free radical processes, the negative influence of the plasma environment, the pH of the medium, etc.

Thus, under conditions of relative dynamic equilibrium of the erythrocyte production and destruction processes, the autoregulation mechanisms are involved in the redistribution of the balance of subpopulations, acting mainly at the membrane-cellular level. During emotional, physical stress or pathology, additional regulatory elements of a plasma or systemic nature are actively connected to them, the share of which in the control of EPME and the spectrum of influence on the state of the erythrocyte population under specific conditions can vary within fairly wide limits. Our experiments with the use of the Na-pump, K-pump inhibitor strophanthin (10-5 M) in vitro showed that the effect of strophanthin is determined only in relation to the subpopulation of cells with increased EPME.

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### S2.178. Peculiarities of AP repolarization in cardiomyocytes of three-week-old rats during application of clonidine hydrochloride

Galieva A.M.[1]*, Ziyatdinova N.I.[1], Shakirov R.R.[1], Biktemirova R.G.[1], Zefirov T.L.[1]
[1]*Kazan (Volga Region) Federal University, Kazan, Russia;*
* galieva_alina94@mail.ru

Activation of the sympathetic nervous system is responsible for the body's "fight or flight" reaction. The physiological responses to the activation of the sympathetic nervous system and adrenal medulla are mediated through the action of the endogenous catecholamines

norepinephrine (or noradrenaline) and epinephrine (or adrenaline) on adrenergic receptors. Adrenergic receptors belong to the superfamily of G protein-coupled receptors (GPCR). Adrenoceptors are divided into alpha1, alpha2, beta1, beta2 and beta3 receptors. [1] α2 receptors are coupled to inhibitory Gi proteins, that inactivate adenylyl cyclase, decreasing cyclic adenosine monophosphate (AMP) production. In addition to the endogenous ligands epinephrine and norepinephrine, they may be activated by several agonist drugs, including clonidine, brimonidine, and moxonidine. [2]
Purpose . To study the effect of α2-adrenoreceptor stimulation on cardiac electrical activity in three-week-old rats at 10-6 M concentrations.
Material and Methods. The investigation was carried out on three-week-old white mongrel rats. The anesthetic was 25% urethane solution at the rate of 1.2 g/kg of animal weight, which was injected intraperitoneally. The anesthetized animal's chest was opened, the heart was quickly extracted and placed in a petri dish with oxygenated Tirode's solution. The heart was dissected and a multicellular preparation with the auricle of the right atrium, transverse scallop, and fragments of the superior and inferior vena cava were made. Electrical activity of cardiomyocytes was studied using intracellular microelectrode lead on the right atrium preparation at the imposed rhythm with a frequency of 5 Hz. External stimulation was performed through platinum electrodes. The obtained records of myocardial electrical activity were analyzed using the original Elph 3.0 program. The solution of α2-adrenoreceptor agonist clonidine hydrochloride (10-6 M) was applied for 20 min.
Results. In three-week-old animals clonidine hydrochloride in the studied concentration did not cause significant changes in the values of membrane potential, depolarization phase duration, and action potential amplitude. However, application of α2-adrenoreceptor agonist at a concentration of 10-6 M resulted in prolongation of the repolarization phase of the action potential by 50% and 90%.
Conclusion. We have revealed that application of clonidine hydrochloride at a concentration of 10-6M has a positive effect on the amplitude-time parameters of cardiomyocyte electrical activity in 3-week-old rats.
The study was supported by the Russian Science Foundation Grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/.
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### S2.179. Peroxiredoxin 6 reduces damage to kidney nephrons in the early reperfusion period

Gordeeva A.E.[1]*, Kurganova E.A.[1,2]
[1]*Institute of Cell Biophysics RAS, Pushchino, Russia;*
[2]*Pushchino State Natural Science Institute, Pushchino, Russia.;*
* gordeeva1310@yandex.ru

Nephrons are highly sensitive elements of the kidney, which react sharply to hypoxia, leading to the development of pathological changes. Reperfusion saves the cell from hypoxia, but leads to an aggravation of pathological changes due to the activation of free radical reactions. It is at the initial moment of reperfusion that a cascade of pathological processes is triggered. These reactions are detrimental to nephrons, and it is advisable to use antioxidant enzymes to reduce their effect. In this work, the antioxidant enzyme peroxiredoxin 6 (Prx6) is used to protect nephrons from reperfusion injury. Prx6 neutralizes a wide range of hydroperoxides, has good bioavailability and is able to penetrate into cells, increasing their antioxidant status, in addition, it effectively reduces the severity of damage in various free radical pathologies.
The aim of this work is to study the effect of exogenous Prx6 on the state of nephrons in the initial reperfusion period after ischemia. In the experiments, male Wistar rats were used to reproduce the model of ischemia-reperfusion of the right kidney with left-sided nephrectomy. The period of ischemia is 45 minutes, reperfusion - 2, 5 and 24 hours. Prx6 was administered intravenously 15 minutes before ischemia. Recombinant Prx6 was obtained at the Reception Mechanisms Laboratory of the Institute of Cell Biophysics. The peroxidase activity of the exogenous protein is 200 nmol/mg/min for H2O2 and 100 nmol/mg/min for tert-butyl peroxide.
It was shown that structural damage to nephrons occurs after 2 hours of reperfusion: an increase in Bowman's space and expansion of convoluted tubules. The maximum lesion was noted after 24 hours of reperfusion: focal-diffuse dystrophic and necrotic changes in the epithelium of the convoluted tubules are pronounced. During this period, there is a maximum increase in the concentration of urea and creatinine in the blood relative to the control values (by 6 and 3 times, respectively), which indicates a violation of the filtration capacity of the kidney. A restructuring of the nephron apparatus was noted, which is expressed in an increase in the area of the renal corpuscles, the area of the vascular glomeruli, and the area of the Bowman space. Nephrocyte dystrophies and parenchymal foci with an immunosignal for the KIM-1 kidney lesion molecule were noted. When exogenous Prx6 was used, no restructuring of the nephron apparatus was noted already with the onset of reperfusion, and its components were not increased. Reduced immunosignal area for KIM-1 and minimization of nephron dystrophy. The decrease in nephron damage with the onset of the reperfusion period against the background of the use of Prx6 was reflected in an improvement in the functionality of nephrons. The use of Prx6 led to a decrease in the concentration of urea and creatinine already in the early reperfusion period and maintaining at this level for 24 hours. Thus, exogenous Prx6, when administered intravenously before renal ischemia, reduces nephron damage with the onset of reperfusion. This contributes to the improvement of the compensatory-adaptive properties of nephrons during the reperfusion period and the preservation of their functionality. The implementation of Prx6 of its protective properties with the onset of reperfusion is associated with its powerful antioxidant properties, mainly with peroxidase activity, which makes it possible to neutralize the hyperproduction of reactive oxygen species.
The work is done in the framework of the state assignment of Pushchino Scientific Center for Biological Research of RAS (No 075-01512-22-00).

### S2.180. Phase transitions in chimeric antigen receptor systems

Prikhodko I.V.[1], Guria G.Th.[1,2]*
[1]*National Research Center for Hematology, Moscow, Russia;*
[2]*Moscow Institute of Physics and Technology;*
* guria@blood.ru

The creation of chimeric antigen receptors is one of the most promising technologies for the treatment of oncological diseases [1]. Currently, modifications of chimeric antigen receptors are being actively developed, which aim to increase not only the sensitivity, but also the specificity of recognition of cancer cells [2].
A number of recent works state that the specificity of chimeric antigen receptors is associated with their ability to form clusters [3,4]. The mechanisms of receptor clustering are being actively studied [5, 6]. It was found that, in a number of cases, the mechanism of cluster nucleation has a threshold character.
The ability of chimeric antigen receptors to threshold cluster formation can be assessed from the standpoint of nucleation theory. Due to this, opportunities open up at in vitro stages of development of chimeric antigen receptors to compare their eventual specificity. Currently, specificity is assessed at the stage of in vivo testing [7]. The development

of the ideas of the nucleation theory [8] makes it possible to estimate the specificity of recognition based on the analysis of the ability of chimeric antigen receptor systems to undergo phase transitions. Corresponding parametric diagrams are constructed.

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## S2.181. Pore-forming ability of nisin and perspectives for its anticancer utilization

Tyulin A.A.[1]*, Efimova S.S.[1], Ostroumova O.S.[1]
[1]*Institute of cytology RAS, Saint Petersburg;*
* atyulin530@gmail.com

Even though there is noticeable improvement in cancer therapy in recent years, oncology-related diseases remain a serious global health problem. New developed drugs become ineffective due to multidrug resistance development, which reduces positive effect of chemotherapeutic agents and plays a crucial role in tumor metastasis [1,2]. Given that, there is a high demand of new strategies and specific chemical compounds for cancer treatment improvement. Antimicrobial peptides are becoming more appealing in this regard, since fundamental differences in membranes lipid composition of normal and malignant cells [3,4] might be used for their targeting on cancer cells.

Nisin is non-toxic lantibiotic composed of 34 amino acids, which has been approved by Food and Drug Administration (FDA) and World Health Organization (WHO) for human consumption as a food preservative [5]. Its anticancer activity was shown on blood [6,7], gastrointestinal [6], hepatic [6,8], and other cancer cell lines. Additionally, nisin did not reveal such an activity in case of non-malignant cells [8,9]. Even though there are many works dedicated to nisin's anticancer activity, its mechanisms of action remain unclear. However, it is known that nisin activates intrinsic apoptosis pathway [10], the process in which cardiolipin, mitochondrial lipid species, plays important role [11]. Thus, it might be crucial to study nisin activity in cardiolipin-contained membranes.

The aim of the current study was to investigate molecular mechanisms of the nisin action on phospholipid planar bilayers and malignant cell cultures, as well as to assess perspectives for nisin utilization in combination with small molecules, which enhance its membrane activity towards malignant cells.

The study revealed that nisin do not form transmembrane pores in the model membranes composed of dioleoyl phosphatidylcholine (DOPC), or dioleoyl phosphatidylethanolamine (DOPE) in concentration up to 2 mM. Introduction of the dioleoyl phosphatidylserine (DOPS) into membrane (DOPC/DOPS, or DOPE/DOPS (50/50 mol%)) did not affect the pore formation probability, but it decreased nisin's threshold detergent activity from 2 mM to 600-700 uM. Further replacement of DOPS with tetraoleoyl cardiolipin (TOCL) led to step-like current fluctuation appearance, nisin concentrations were no more than 10 uM. According to the obtained results, introduction of phloretin in concentration 20 uM into nisin-modified membranes composed of DOPC/TOCL led to dipole potential decline by 100 mV, which caused five-fold elevation of the steady-state nisin-induced transmembrane current. Additionally, ability of phloretin to potentiate nisin-induced mitochondrial membrane depolarization was shown on HepG2 cell line. The combination of compounds led to a more noticeable decline in depolarization in comparison with their single effect.

Taking into consideration that PS externalizes during carcinogenesis, nisin's threshold detergent concentration decrease in DOPC-contained bilayers might facilitate its penetration into malignant cells, where it interacts with its intracellular targets. The noticeable pore-forming activity of nisin in presence of TOCL might indicate that nisin causes apoptosis via mitochondrial pathway by forming pores in the inner mitochondrial membrane. Finally, phloretin can be considered as a nisin potentiator in mitochondrial membrane depolarization process.

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## S2.182. Quenching of NBD-PC fluorescence in DOPC, DOPA and TOCL containing membranes with the addition of cytochrome c

Konyukhova S.[1]*, Volkov V.[1], Stepanov G.[1], Osipov A.[1]
[1]*N.I. Pirogov Russian National Research Medical University, Moscow, Russia;*
* sopfia.k.2000@gmail.com

Nowadays in molecular medicine one of the promising approaches to the treatment of pathologies is the use of specific intracellular mechanisms of programmed cell death[1]. One of the most studied types of cell death is apoptosis and ferroptosis [2,3], in the development of which the oxidation of membrane phospholipids plays an important role. Moreover, if the oxidation of cardiolipin is a marker of apoptosis, the oxidation of phosphatidylethanolamine with arachidonic acid is specific for ferroptosis. Such specificity of oxidation of various phospholipids in various processes of cell death gave rise to a new scientific direction – regulatory lipidomics.

Despite this, still not all elements of the mechanism of development of programmed cell death are clear. For example, the interaction of cytochrome C (CytC) with cardiolipin-containing membranes leads to an increase in CytC peroxidase activity and triggers apoptosis [4,5]. But it remains unclear why in the presence of cardiolipin and CytC in the mitochondria of normal cells they do not die. This may be due to the fact that not only cardiolipin (TOCL), but also other phospholipids participate in the interaction with CytC. One of these phospholipids may be phosphatidic acid (DOPA), which is structurally very similar to cardiolipin (cardiolipin consists of two phosphatidic acid molecules). At the same time, the amount of DOPA is regulated by phospholipase D, and phospholipase D is present on the outer sheet of mitochondrial membranes.

In this paper, the ability of CytC to form complexes with DOPA and with TOCL was investigated. The materials used in the work were phosphatidylcholine liposomes with 20% DOPA or TOCL content, as well as with 1% admixture of fluorescently labeled phosphatidylcholine (NBD (C6) PC). The interaction of CytC with membranes containing a fluorescent label leads to a sharp quenching of fluorescence due to a nearby heme iron. Using NBD(C6) PC spectrofluorometry (excitation λ 460 nm, emission λ 480-590 with a maximum of 536 nm), the interaction of phospholipid membranes with CytC was shown.

Thus, the fluorescence intensity of NBD(C6) PC in DOPA and TOCL containing membranes decreases by 30.5% and 17.8%, respectively, with the addition of CytC (20:1). All comparisons were made to membranes consisting entirely of phosphatidylcholine.

In conclusion we would like to say it is clearly shown that not only TOCL, but also DOPA-containing membranes can interact with cytochrome C, which means that this interaction can play an initiating role in the development of apo- and ferroptosis.

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## S2.183. Redox state of human red blood cells and hemoglobin glutathionylation under stress conditions

Zaripov P.I.[1,2]*, Kuleshova I.D.[1], Melnikova E.V.[1], Poluektov Y.M.[1], Anashkina A.A.[1], Mitkevich V.A.[1], Makarov A.A.[1], Petrushanko I.Yu.[1]
[1]*Engelhardt Institute of Molecular Biology;*
[2]*M.V. Lomonosov Moscow State University, faculty of Biology, department of Biophysics ;*
* aglaepanchina@yandex.ru

The condition of red blood cells is critical to the vitality of the entire organism. The lifetime and functional activity of red blood cells is determined by the conditions of their formation in the bone marrow and the level of stress to which they are exposed during their life. Assessing the nature of stress exposure and developing methods to reduce damage to red blood cells under stress and prolong their life are highly relevant. The solution will allow for increasing the shelf life of donor blood, better treatment of pathological conditions (anemia) as well as increasing human lifespan. Today it is obvious that the aging of red blood cells and changes in their functional properties are closely related to the redox state of cells, which depends on the ratio of reduced and oxidized forms of molecules. Oxidation of protein molecules, primarily hemoglobin (Hb), is a key factor of damage and, consequently, premature aging of erythrocytes. Glutathionylation of hemoglobin is the attachment of glutathione through a disulfide bridge to the thiol group of protein. It leads to the increasing affinity of hemoglobin for oxygen, which can contribute to the adaptation of cells to various stress conditions.

The aim of our work was to characterize the redox state of red blood cells under physiological stress influences and establish the role of Hb and its glutathionylation in these processes.

We initiated stress conditions typical for erythrocytes circulating in the bloodstream - hypoxia, mechanical, hypoosmotic, and metabolic stress. Flow cytometry method was used to assess the changes in the redox parameters of cells [1]. The parameters of forward-scatter and side-scatter were recorded, reflecting, respectively, changes in the size and shape of cells. The intracellular content of free glutathione (GSH), reactive oxygen species (ROS), NO, and Ca2+ was characterized. We also assessed the level of GSH in erythrocyte lysates using Ellman's reagent - 5,5'-dithiobis-(2-nitrobenzoic acid) - DTNB [2]. Western blotting method was used to assess the glutathionylation of Hb.

Under hypoxia conditions (oxygen partial pressure decrease up to 1%) change in the intracellular Ca2+ has occurred first. Changes in Ca2+ play an important signaling role, followed by changes in parameters characterizing the redox state of red blood cells: ROS, NO, and intracellular glutathione levels.

After three hours of incubation under hypoxic conditions, there is a significant increase in NO and GSH levels, and a decrease in ROS. The increase in NO content is associated with de novo synthesis through the triggering of Ca2+-dependent NO synthases [3]. On the other hand, the increase in GSH level is caused by the release of glutathione molecules from the noncovalent complex with GB [4]. Under hypoxic conditions glutathionylation of Hb does not change.

Mechanical stress arising in vivo during the passage of erythrocytes through the small capillaries was modeled by passing cells through a column of a mixture of α- and micro-cellulose [5]. Mechanical stress leads to the development of significant oxidative stress - an increase in the level of ROS, NO, decrease in intracellular GSH and Ca2+. The cells shrink, while the shape of the cells does not change significantly. There is a great increase in glutathionylation of Hb due to the development of oxidative stress.

Acute osmotic stress caused by a decrease in osmolarity from 330 to 220 mOsm leads to an increase in intracellular Ca2+, while NO levels do not change. The decrease in GSH and the increase in ROS, in this case, is less pronounced than under mechanical stress and causes a

slight decrease in glutathionylation of Hb. After 24 hours of incubation, almost all intracellular parameters return to normal and the level of glutathionylation corresponds to the control group. Thus, over time, the erythrocytes adapt to hypoosmotic stress.
Metabolic stress was induced by 24-hour incubation of red blood cells in the glucose-free buffer. Cell size increases, probably as a result of disruption of ion pumps due to ATP deficiency. GSH content decreases against the background of the absence of ROS growth, which may be due to the lack of NADP-H required for GSSG reduction by glutathione reductase, as well as to the termination of GSH de novo synthesis. In addition, a deficiency of NADP-H, required for NO synthase function, may be responsible for the decrease in NO levels. Metabolic stress causes a significant increase in Hb glutathionylation. This is due to a decrease in GSH levels and an accumulation of GSSG.
Thus, an increase in Hb glutathionylation under stressful conditions can be induced by oxidative stress or lack of ATP. Continued research will allow to reveal the molecular mechanisms of cellular defense and adaptation of erythrocytes to stress and in the future to suggest of defence from processes of red blood cell aging.
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### S2.184. Registration of a paroxysmal depolarization shift using the patch-clamp method in the outside-out configuration

Laryushkin D.D.[1,2]*, Kritskaia K.A.[1], Kosenkov A.M.[1], Gaidin S.G.[1], Zinchenko V.P.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences;*
[2]*NATIONAL RESEARCH NUCLEAR UNIVERSITY MEPHI Moscow Engineering Physics Institute;*
* mr.ldp@yandex.ru

Epilepsy is one of the most common neurological diseases characterized by sudden seizures. It is believed that epilepsy is based on disturbances in the balance of inhibition and excitation of neuronal networks, leading to a complex spatio-temporal structure of synchronization and desynchronization of large neuronal ensembles. The cellular correlate of epileptiform activity recorded by EEG is a paroxysmal depolarization shift (PDS). PDS is a positive shift in the membrane potential of a neuron, reaching — 15 mlV and lasting up to 400 ms, against which one to several action potentials may occur.
To date, several mechanisms have been proposed that could explain the phenomenon of hypersynchronization of neurons during epilepsy, such as: a high concentration of extracellular potassium, a decrease in extracellular space and/or ephaptic connections. Along with this, there is evidence that hypersynchronization can occur due to an endogenous electric field, which is confirmed by several mathematical models, but no experiments have been conducted on the role of the electric field in the occurrence of PDS. Thus, we assumed that if the power of the endogenous electric field is sufficient to depolarize the membrane, then we would be able to register such a field using the patch-clamp method. For the experiments, we used a mixed neuroglial culture of the hippocampus of a Wistar rat. Activation of epileptiform discharges was induced by bicuculline at a concentration of 10 μmol . To register the signal, the patch-clamp method was used (with the pClamp10 software.2) in the whole-cell configuration, and after the benchmarks were recorded (two clusters of PDS), we switched to the outside-out configuration, recorded two clusters and removed the 1.25 μm electrode, etc., while the amplitude of the PDS was distinguishable against the background noise. Data analysis was performed using the python3 programming language using the numpy, scipy.signal, and pyABF packages.
As a result of our experiments, we have shown that using the patch-clamp method in the outside-out configuration, it is possible to register the PDS of the neuronal network in the rat neuroglial culture caused by bicuculin. The results obtained can be used in the study of epilepsy at the cellular level.
This study was conducted in the framework of the State assignment of PSCBR RAS № 075-01512-22-03 «New generation neuroprotective drugs» № 1022080100047-5-1.6.4.

### S2.185. Relationship between structural-mechanical properties of the extracellular matrix and its ability to repopulate by tumor cells on the example of decellularized organs

Pospelov A.D.[1]*, Kutova O.M.[1], Efremov Y.M.[4], Nekrasova A.A.[4], Trushina D.B.[3], Gefter S.D.[1], Cherkasova E.I.[1], Timofeeva L.B.[1,2], Zvyagin A.V.[1,4,5], Timashev P.S.[4], Balalaeva I.V.[1]
[1]*Lobachevsky state university, Nizhniy Novgorod, Russia;*
[2]*Privolzhsky Research Medical University, Nizhniy Novgorod, Russia;*
[3]*Federal research center crystallography and photonics,RAS, Moscow, Russia;*
[4]*Sechenov First State Medical University, Moscow, Russia;*
[5] *Macquarie University, Sidney, Australia;*
* eso103163@gmail.com

Decellularized (DCL) organ matrices are a promising platform for creating tumor models alternative to monolayer cell cultures and artificial three-dimensional scaffolds. The ability of native matrix to mimic cellular microenvironment enables to study the influence of physical and chemical properties of the matrix on invasion, metastasis and development of tumor drug resistance, as well as to reveal mechanisms of tissue specificity of different tumor types and tumor-organism interaction. The aim of the work was to study the influence of biomechanical parameters of the extracellular matrix on the morphology and invasive potential of different breast cancer cell lines.
We developed an original protocol for decellularization of murine organs based on sodium deoxycholate, sodium dodecyl sulfate and Triton X-100. To test the assumption of a relationship between matrix stiffness/porosity and its ability to be colonized by tumor cells, we recellularized DCL matrices with human breast cancer cell lines MDA-MB-231 and SKBR-3, which were distributed within the matrix by direct injection. The choice of these lines was based on such factors as the difference in the degree of differentiation and invasive potential. MDA-MB-231 line belongs to triple-negative type of breast cancer, characterized by extremely low degree of differentiation, high invasive potential and high proliferative activity. The SKBR-3 line, in turn, is a highly differentiated cancer, morphologically not much different from the squamous epithelium, with a low growth rate and low invasive potential (links). Such a difference will theoretically make it possible to assess the relationship between the stiffness/porosity of the matrix and their effect on the growth and morphotype of tumor cells.
Scanning electron microscopy (Hitachi TM 4000Plus) was used to analyze the matrix microstructure. Macroindentation (Mach-1 TM v500csst) and nanoindentation (Bioscope Resolve microscope) were

used to determine biomechanical properties. Standard histomorphological analysis and DNA content determination were performed to determine the degree of repopulation.
Efficient removal of cells from murine organs using the developed protocol with preservation of matrix microstructure was shown. It was determined that the matrices differ significantly in pore shape and size, fiber thickness and stiffness, making each organ unique in terms of the potential for cell-matrix interactions. A significant negative correlation and a similarly strong trend was found between matrix density as measured by nanoindentation and its degree of repopulation by MDA-MB-231 and SKBR-3 cells, respectively. At the same time, no relationship was observed between the degree of repopulation and matrix density measured by macroindentation or porosity. This strongly suggests that the characteristics of matrix stiffness, manifested at the level of interaction with individual cells, are critical for cell adaptation, while stiffness and other biomechanical properties of the tissue as a whole are not essential. Based on this, we can assume that cells require a certain stiffness range for optimal growth, which leads to the possibility of determining potential niches for metastasis
The work was carried out in the course of the NCMU "Photonics Center" project with the financial support of the Ministry of Science and Higher Education of the Russian Federation, Contract No. 075-15-2020-927, and with the use of the unique Transgenebank scientific equipment.

**S2.186. Restoration of ion channels in human cardiomyocytes after operations with cardioplegic solutions Normacor and Custodiol**

Kovalenko S.G.[1,2]*, Frolova S.R.[1,2], Berezovsky A.V.[1], Agladze K.I.[1,2]
[1]*laboratory of experimental and cellular medicine MIPT;*
[2]*laboratory of molecular and cellular diagnostics MRRCI named after M.F. Vladimirsky;*
* sandaara.romanova@phystech.edu

The study is aimed at establishing the patterns of arrhythmias that occur after coronary bypass surgery using cardioplegic solutions Custodiol and Normacor, at the level of voltage-gated ion channels.
Normacor has an advantage over Custodiol in that it is used for normothermy.
The electrophysiological properties of fast sodium channels, L-type calcium channels and slow potassium channels in patients' cardiomyocytes before and after coronary bypass surgery were compared. The aim of the study was to compare the restoration of ion currents of cardiomyocytes after cardioplegia with Normacor and Custodiol.
Primary human cardiomyocytes were isolated from a biopsy of the right atrium using an optimized protocol in our laboratory (patent RU 2749986). The average age of patients was 63 years. All experiments were carried out by the patch-clamp method at a physiological temperature of 37 °C. Current of fast sodium channels (INav), L-type calcium channels (ICa, L) and slow potassium channels (IKs) have been studied. In the first part of the research, currents in human cardiomyocytes from a bioptate specimen excised at the following moments were compared (in the example of Custodiol): 1) bioptate sampling before the administration of cardioplegic solution into the heart – the control group "before surgery"; 2) bioptate sampling after washing from cardioplegic solution. Washing from cardioplegia was carried out in two ways: either in laboratory conditions by external washing in a calcium-free buffer, or in the operating room until the heart rate was restored. It turned out that for the task at hand, where the restoration of ion channels after surgery is being investigated, it is more correct to use a bioptate excised after washing in the operating room, since depending on the quality of washing, there is a different degree of channels' inactivation .
In the second part of the study, we compared two cardioplegic solutions Custodiol and Normacor in terms of their effect on the restoration of ion channels of human atrial cardiomyocytes (INav, ICaL, IKs) after surgery. The control group consisted of cardiomyocytes from a bioptate excised "before surgery", and the effect of cardioplegic solution was observed in bioptates "after surgery" with washing in the operating room. It was found that after washing the Custodiol, fast sodium channels are quickly restored, but the IKs currents retain an inactivated state by 30%. For ICa,L, there is a decrease in amplitude to the same extent both after the operation with Normacor and after Custodiol. After Normacor washing, Na-channels recover worse than after Custodiol, while Ks-channels after Normacor recover completely.
Postoperative arrhythmias in most cases occur during the first 48 hours and may occur within two weeks after surgery, most likely due to suppression of potassium currents.

**S2.187. Rianodine and SN-6 inhibitor cause a positive chronotropic effect in pacemaker cells of embryonic chick heart**

Lebedeva E.A.[1]*
[1]*IPhys FCR Komi SC UB RAS;*
* Mirestel@ya.ru

Introduction. To date, it is assumed that the mechanism of generation of spontaneous impulses heart in adult animals is based on the spontaneous release of Ca2+ from the sarcoplasmic reticulum through ryanodine receptors. This process is associated with the of the membrane ionic currents, in particular with the operation of the Na+/Ca2+ exchanger [Lakatta et al., 2010; Lyashkov, et al., 2018]. At the same time, the cellular mechanisms which underlie spontaneous activity in embryonic heart cells are unclear and the available experimental data are contradictory [Opthof, 2007]. One of the important questions is whether the activity of the embryonic myocardium is determined by the same mechanisms as in adult animals heart [Goenezen et al., 2012]. The aim of this study was to evaluate the contribution evaluate the contribution of the Na+/Ca2+ exchanger and ryanodine receptors to the generation of electrical activity in the of embryonic chick right atrium cells.
Materials and methods. Experiments were performed using standard microelectrode technique and pharmacological analysis on spontaneously contracting preparations of the right atrium embryonic chick (HH36, m=3.6±0.3 g). We used SN-6 (4-Thiazolidinecarboxylic acid) as an inhibitor of Na+/Ca2+ exchanger. To study the role of ryanodine receptors, we used ryanodine. Values are presented as means ± SE (M±σ) and were analyzed by the unpaired U- test Mann–Whitney ($p<0.05$).
Results. In the control saline solution (31±1 ºC) in preparations from the of embryonic chick right atrium the spontaneous rate of action potential (AP) was 145±15 imp/min, and the maximal upstroke velocity (dV/dtmax) was 103±41 V/s (n=18). Action potentials had a phase of slow diastolic depolarization (DD, phase 4). Inhibition of the Na+/Ca2+ exchanger with SN-6 (10 μM, n=11) resulted in an increase in the spontaneous rate by 15% due to a shortening of the duration of of slow diastolic depolarization by 18% compared with the control ($p<0.05$). Other electrophysiological parameters of AP did not change significantly. When we added ryanodine (1 μM, n=10) to the perfusion solution, we obtained a similar effect: the duration of the phase of slow diastolic depolarization was shortened. This caused an increase in the spontaneous rate by 17% ($p<0.05$). We recorded this effect throughout the entire exposure of ryanodine (60 minutes). Cessation of electrical activity in preparations of the embryonic chick right atrium under of ryanodine was not registered.
Conclusion. An increase in the intracellular concentration of Ca2+ as a result of the addition of ryanodine or inhibition of the Na+/Ca2+ exchanger in chick embryo pacemaker cells did not slow down, disrupt, or stop the generation of electrical impulses. In contrast to adult animals, where disruption of the functioning of these

ion channels leads to a negative chronotropic effect [Lakatta et al., 2010], an increase in the generation of the rhythmic action potentials occurred in the embryonic heart. The data obtained allow us to conclude that at this stage of embryonic development, the Na+/Ca2+ exchanger and ryanodine receptors do not play a key role in the mechanism of the formation of electrical impulses in the embryonic chicken heart.

**S2.188. Role of free fatty acid receptors in the effects of sodium butyrate on mouse colon contractility in a model of irritable bowel syndrome**

Shaidullov I.[1]*, Sorokina D.[1], Bouchareb D.[1], Sitdikov F.[1], Sitdikova G.[1]
[1]*Kazan Federal University;*
* ilnarshaidullov@rambler.ru

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract, characterized by a variable combination of chronic or recurrent abdominal pain and changes in bowel function in the absence of any abnormalities. Recent studies have shown that chronic changes in the immune system at the molecular level are associated with abnormal intestinal fermentation of short-chain fatty acids (SCFAs), which are key products of the fermentation of indigestible carbohydrates by commensal bacteria living in the gastrointestinal tract. SCFAs may be involved in the regulation of peristalsis and have an excitatory or inhibitory effect on the motility of the gastrointestinal tract. It has recently been shown that the effects of SCFAs may be mediated through free fatty acid type 2 (FFA2) and type 3 (FFA3) receptors, although their role in the development of IBS is unclear. The aim of our study was to analyze the role of FFA2 and FFA3 in the effects of butyrate on the spontaneous activity of the mouse colon.
The experiments were carried out on mice aged 45 days. The force of contraction of segments of the proximal mouse colon recorded under isometric conditions. During the whole experiment, the preparation washed with an aerated Krebs solution. Sodium butyrate was used at a concentration of 10 mM.
In the control, the mouse proximal colon showed spontaneous activity starting approximately 45 minutes after sample insertion. The use of sodium butyrate caused a decrease in tonic tension, amplitude and frequency of spontaneous contractions. Activation of FFA3 receptors - AR420626 at a concentration of 10 μM for 10 minutes did not change the parameters of spontaneous segment activity. Under these conditions, the inhibitory effects of sodium butyrate on tonic tension, amplitude, and frequency of spontaneous contractions were preserved and did not differ from its effects in the control.
The FFA2 receptor inhibitor GLPG0974 (100 μM) also did not change the parameters of the colonic contractility in the control group. Under these conditions, the inhibitory effect of sodium butyrate on tonic tension and amplitude was preserved, but its effect on the contraction frequency was less than in the control.
In the IBS model group, the inhibitory effects of sodium butyrate on spontaneous activity induced by AR420626 and GLPG0974 also persisted. At the same time, the frequency of spontaneous contractions against the background of the action of GLPG0974 was less pronounced than in the control group ($p>0.05$).
These results suggest that the inhibitory effect of butyrate on spontaneous contraction activity is not related to the activation of FFA3 receptors, but that FFA2 receptors may mediate the inhibitory effect of sodium butyrate on spontaneous contraction rate.
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**S2.189. Role of glutathione tripeptide in redox-dependent regulation of protein functions**

Petrushanko I.Yu.[1]*
[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Science;*
* irina-pva@mail.ru

The report consider the role of intracellular glutathione in the redox-dependent regulation of protein functioning in various cells under physiological and pathological conditions. Tripeptide glutathione is the main low-molecular-weight thiol of animal cells. Normally, the reduced form of glutathione (GSH) predominates in cells. The content of GSH in cells is 5-10 mM, and the concentration of the oxidized form of glutathione (GSSG) is 100 times lower than GSH. However, under conditions of oxidative stress, the GSH/GSSG ratio can decrease to 1. The redox potential of this redox pair determines the intracellular redox potential [1]. Glutathione is involved in many redox-dependent processes, being a substrate for glutathione peroxidases, and also directly participating in the reactions of radical neutralization and protein modification. Glutathionylation of protein is the addition of glutathione to the thiol group of a protein to form a disulfide bridge. This redox-dependent modification protects thiol groups from irreversible oxidation and can change functioning of protein, which plays an important role in cell adaptation to various conditions.
The features of the regulation of the functioning of proteins by glutathione are considered using the Na,K-ATPase, which maintains the transmembrane gradient of sodium and potassium, and hemoglobin, the main protein of erythrocytes that transports oxygen.
We have shown that the catalytic subunit of Na,K-ATPase undergoes regulatory and basal glutathionylation [2–4]. Regulatory glutathionylation is realized in response to changes in the redox status of cells, under oxidative stress and hypoxia, and leads to inhibition of transport activity or changes in the receptor function of the enzyme [2,3]. The regulatory cysteine residues that glutathionylation are responsible for changes in the transport and receptor functions of the protein was found [3]. When normal redox conditions are restored, deglutathionylation of regulatory cysteine residues occurs, and the protein restores its functionality. It has been shown that the cause of inhibition of Na,K-ATPase during glutathionylation is a violation of ATP binding. It has been established that glutathionylation is the cause of inhibition of Na,K-ATPase during hypoxia and plays an important role in cell adaptation to oxygen deficiency, preventing ATP depletion of cells [2,3]. It was found that the induction of Na,K-ATPase glutathionylation using the glutathione derivatives reduces cell damage and prolongs the time of normal contractility of cardiomyocytes [5]. Violation of the receptor function of Na,K-ATPase to cardiotonic steroids under hypoxia [3] is associated with the disruption of the interaction interface between glutathionylated Na,K-ATPase and Src-kinase. The availability of cysteines for regulatory glutathionylation depends on the conformation of Na,K-ATPase [6]. Basal glutathionylation of Na,K-ATPase, i.e., glutathionylation of cysteine residues that are inaccessible in the native enzyme, occurs during protein synthesis and is observed during long-term changes in redox conditions, for example, under conditions of prolonged hypoxia [4]. Cysteine residues capable of undergoing basal glutathionylation have been found. The role of basal glutathionylation is not completely clear. It can probably change the stability of the protein and serve as a kind of marker of the redox conditions under which it was synthesized. In the case of the oxygen-transporting protein hemoglobin (Hb), both glutathionylation of cysteine reduces and the formation of a non-covalent complex with GSH are played important role. Glutathionylation is known to increase the affinity of Hb for oxygen [7]. The level of Hb glutathionylation increases under various stress conditions, and this modification is probably adaptive and makes it possible to partially compensate for

the lack of oxygen during stress. Recently, we found that Hb is able to form the non-covalent complex with GSH, the formation of this complex depends on Hb oxygenation [8]. The oxy-Hb has a high affinity for glutathione and can bind four GSH molecules, while the deoxy-Hb has only two GSH molecules. It was found that partial dissociation of GSH from deoxy-Hb underlies the observed increase in the level of GSH in erythrocytes under hypoxic conditions [8]. This increase in GSH levels can play an important role in protecting erythrocytes from oxidative stress that develops in tissues with a lack of oxygen. Since the affinity of Hb for oxygen increases in the complex with GSH, it can be assumed that the formation of this complex also play a regulatory role [8].
It was found that the formation of the GSH:Hb complex leads to structural destabilization and changes in the heme part of the protein, while Hb glutathionylation affects the secondary structure of hemoglobin.
It was also considered the role of glutathione in the redox-dependent regulation of erythrocytes under various stress conditions, in malignant cells, blood-brain barrier cells, neuronal cells, and bacteria.
The obtained data indicate that glutathione is an important regulator of the cell redox status and can significantly change the functioning of proteins due to protein glutathionylation or by forming a non-covalent complex with proteins, which plays an important role in cell adaptation to external influences.
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### S2.190. Searching for synergists of the pore-forming activity of polymyxin B

Martynyuk V.A.[1]*, Grekhnev D.A.[1], Zakharova A.A.[1], Efimova S.S.[1], Vigont V.A.[1], Ostroumova O.S.[1]
[1]*Institute of Cytology Russian Academy of Science;*
* ve08ra@mail.ru

Polymyxin B (PMB) is an amphipathic cyclic lipopeptide antibiotic currently used to treat infections caused by multiresistant gram-negative bacteria. It is believed that the PMB mechanism of action is a violation of the integrity of both the external, lipopolysaccharide, and internal, phospholipid, membrane of the bacterial cell and an increase of its permeability [1]. A significant limitation in the clinical use of PMB is its high toxicity, as a result of which an urgent problem is the search for synergists of its activity in order to reduce side effects and increase the efficacy of this compound.
Recently it was shown that PMB can form toroidal pores in model phospholipid bilayers, which is confirmed by an increasing pore-forming activity of the lipopeptide in response to the introduction of lipids characterized by a conical shape or low molecular weight compounds capable of inducing a positive curvature of the lipid monolayer. Moreover, an increase in PMB-induced membrane conductivity was demonstrated in the presence in the membrane-bathing solution of agents which are able to reduce the boundary potential of the bilayer, in particular, the plant polyphenol phloretin increased the pore-forming activity of PMB by about 30 times [2].
Using model cell systems and the method of diffusion into agar from paper disks, it was found that the introduction of 1 mM of floretin into agar leads to a statistically significant increase in the zone of inhibition of E. coli growth by PMB at a dose of 75 mcg. To further search for compounds that potentiate the pore-forming activity of PMB, a method to register currents flowing through model lipid bilayers was used. Lipid membranes mimicking the outer membrane of gram-negative bacteria were formed by the method of Montal and Muller [3]. It was found that the introduction of plant polyphenols butein or resveratrol into 0.1 M solution of KCl (pH 7.4), bathing the lipid membrane of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylglycerol and Kdo2-lipid A (49.5:49.5 + 1 mol %), up to 20 μM caused an increase in the conductance of the membrane modified by PMB by more than 10 times. The introduction of piperine alkaloid into a membrane-bathing solution up to 400 μM leads to an increase in the macroscopic PMB-induced current by about 30 times. The role of changes in the boundary potential and packaging of membrane lipids in the potentiating effect of butein and resveratrol is discussed. The work was supported by RNF grant №. 22-15-00417.
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### S2.191. Sigma-1 receptor ligand haloperidol modulates Ca2+ responses in macrophages

Milenina L.S.[1]*, Krutetskaya Z.I.[1], Antonov V.G.[2], Krutetskaya N.I.[1], Badulina V.I.[1], Simonyan A.O.[1]
[1]*Saint-Petersburg State University;*
[2]*Saint-Petersburg State Pediatric Medical University;*
* l.milenina@spbu.ru

Sigma-1 receptors are ubiquitous multifunctional ligand-regulated molecular chaperones in endoplasmic reticulum membrane with a unique history, structure, and pharmacological profile. Sigma-1 receptors modulate a wide range of cellular processes in health and disease, including Ca2+ signaling.
To elucidate the involvement of sigma-1 receptors in Ca2+ signaling processes in macrophages, we studied the effect of sigma-1 receptor antagonist neuroleptic haloperidol on Ca2+ responses induced by endoplasmic Ca2+-ATPase inhibitors thapsigargin (TG) and cyclopiazonic acid (CPA), as well as disulfide-containing immunomodulators glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA-VAM, St. Petersburg) and molixan® (complex of glutoxim and inosine nucleoside, PHARMA-VAM) in rat peritoneal macrophages.
The experiments were carried out on cultured resident peritoneal macrophages of Wistar rats using an automated setup for measuring the intracellular Ca2+ concentration, [Ca2+]i, based on Leica DM 4000B fluorescent microscope (Leica Microsystems, Germany). [Ca2+]i was measured using Fura-2AM fluorescent probe. Statistical analysis was performed using Student's t test. Differences were considered significant at $p \leq 0.05$.
The effect of haloperidol on Ca2+ responses induced by endoplasmic Ca2+-ATPase inhibitors. In control experiments, it was found that the addition of 0.5 μM TG to macrophages in a calcium-free medium causes a slight increase in [Ca2+]i, reflecting Ca2+ mobilization from intracellular Ca2+ stores. The average increase in [Ca2+]i during the mobilization phase was $31 \pm 9$ nM ($n = 7$; $p < 0.05$). With the subsequent addition of 2 mM Ca2+ into the external medium, store-dependent Ca2+ entry into the cytosol was observed. On average, the

increase in [Ca2+]i during Ca2+ entry was 152 ± 20 nM (n = 7; p < 0.05). Similar results were obtained using 10 μM CPA. On average, the [Ca2+]i increase during Ca2+ mobilization phase, induced by CPA, was 26 ± 9 nM (n = 7; p < 0.05), and during Ca2+entry phase it was 141 ± 22 nM (n = 7; p < 0.05).

It was shown that macrophage preincubation with 30 μg/mL haloperidol for 20 min prior to the addition of 0.5 μM TG resulted in suppression of both phases of TG-induced Ca2+ response. On average, haloperidol suppressed Ca2+ mobilization phase by 23.2 ± 7.9% (n = 7; p < 0.05), and the subsequent store-dependent Ca2+entry - by 42.3 ± 13.6% (n = 7; p < 0.05). Similar results were obtained using 10 μM CPA. On average, haloperidol suppressed Ca2+ mobilization from the stores by 25.9 ± 8.0% (n = 7; p < 0.05) and suppressed store-dependent Ca2+ entry by 43.8 ± 12.5% (n = 7; p < 0.05), induced by CPA. This indicates the involvement of sigma-1 receptors in the activation of store-dependent Ca2+ entry induced by TG or CPA in macrophages.

It was also found that the addition of 30 μg/mL haloperidol against the background of the developed Ca2+ entry, induced by TG or CPA, causes a significant suppression of store-dependent Ca2+ entry into macrophages. Thus, the suppression of Ca2+ entry was 48.5 ± 17.1% (n = 7; p < 0.05) for TG and 48.1 ± 16.9% (n = 7; p < 0.05) for CPA. This suggests the involvement of sigma-1 receptors not only in activation, but also in maintenance of store-dependent Ca2+ entry into macrophages.

The effect of haloperidol on Ca2+ responses induced by glutoxim and molixan in macrophages. In control experiments, it was shown that macrophage incubation for 20 min with 100 μg/mL glutoxim or 100 μg/mL molixan in calcium-free medium causes a slowly increasing [Ca2+]i increase, reflecting Ca2+ mobilization from intracellular Ca2+ stores. 20 min after the addition of agents, [Ca2+]i increased on average from a baseline level of 90 ± 18 to 135 ± 18 nM (n = 7; p < 0.05) for glutoxim and 134 ± 20 nM (n = 6; p < 0.05) for molixan. When 2 mM Ca2+ was introduced into the external medium, a further [Ca2+]i increase was observed, reflecting store-dependent Ca2+ entry into the cytosol. On average, the [Ca2+]i increase during Ca2+ entry was 223 ± 22 nM (n = 7; p < 0.05) and 202 ± 20 nM (n = 6; p < 0.05) for glutoxim and molixan, respectively.

It was found that macrophage preincubation with 30 μg/mL haloperidol for 6 min prior to addition of 100 μg/mL glutoxim resulted in a significant suppression of both Ca2+ mobilization from the stores (by 50.3 ± 8.4%, n = 7; p < 0.05) and subsequent store-dependent Ca2+ entry into the cell (by 54.5 ± 9.5%, n = 7, p < 0.05), induced by glutoxim. Similar data were obtained in experiments on the effect of 30 μg/mL haloperidol on Ca2+ responses elicited by 100 μg/mL molixan. On average, haloperidol caused suppression of Ca2+ mobilization from the stores by 49.3% (n = 7; p < 0.05) and suppression of Ca2+ entry into the cell by 47.6% (n = 7; p < 0.05), induced by molixan. This indicates the involvement of sigma-1 receptors in the activation of store-dependent Ca2+ entry, induced by glutoxim or molixan, in macrophages. It was also found that the addition of 50 μg/mL haloperidol against the background of the developed Ca2+ entry, induced by glutoxim or molixan, causes a significant (by 51.4 ± 9.0%, n = 12; p < 0.05) suppression of store-dependent Ca2+ entry into macrophages.

Thus, we have shown for the first time on rat peritoneal macrophages that sigma-1 receptor antagonist neuroleptic haloperidol significantly suppresses both phases of Ca2+ responses induced by immunomodulators glutoxim and molixan, as well as endoplasmic Ca2+-ATPase inhibitors, TG and CPA, in peritoneal macrophages. The data obtained indicate the involvement of sigma-1 receptors in complex signaling cascade induced by glutoxim or molixan and leading to [Ca2+]i increase in macrophages, as well as sigma-1 receptors participation in store-dependent Ca2+ entry regulation in macrophages. The results also indicate that the combined use of glutoxim or molixan and the neuroleptic haloperidol in clinical practice is undesirable.

**S2.192. Slow changes of electrostatic potentials caused by photoinduced release of protons on the surface of lipid membrane**

Sokolov V.S.[1]*, Tashkin V.Yu.[1], Kharitonova Yu.V.[1], Zykova D.F.[1], Galimzyanov T.R.[1]
[1]*Institute of Physical Chemistry and Electrochemistry of RAS;*
* sokolovvs@mail.ru

The investigations of the mechanisms of proton transport in membranes the attention was paid to the protons bound on the membrane surface. Their transfer to bulk water solution is assumed to delay due to a potential barrier in a layer of the oriented molecules of water, and the movement of protons between the donor and acceptor molecules in the membrane proceed inside this layer. To verify this mechanisms, the height of the barrier should be evaluated be measuring the rate of the exchange of protons between the membrane and water. To eject the protons on the membrane surface, the photoactivating compounds are used, the molecules of which release the protons on the membrane surface by exciting by light. This study requires the methods of detection of the protons bound with the membrane, the most popular among them use the membrane bound pH sensitive fluorescent probes. However, the protons bound to the membrane can be detected directly by measuring the electrostatic boundary potential (BP) on the membrane/water interface. Recently we studied the effect of the release of protons from the photoactivation of 2-methoxi-5-nitrosulfate sodium (MNPS) on the electric properties of bilayer lipid membrane (BLM). Its molecule under exciting by UV light releases the proton together with sulfo group. It was shown that the illumination of BLM with adsorbed MNPS molecules by UV light flash leads to change of the membrane capacitance and the BP [1]. The restoration of the potential in the dark was very slow and took about minute. To understand the mechanism of these slow processes, the changes of the BP and the membrane capacitance caused by the photoactivated release of protons on the membrane surface during the illumination by the continue light have been studied. The changes of BP were measured by the method of Inner Field Compensation [1]. The illumination of BLM from phosphatidylcholine with adsorbed MNPS anions (by light emitted diode, wavelength 375 nm, electric power varied from 0.1 to 0.8 W) led to reversible changes of BP and the membrane capacitance. The changes of BP consisted of two contributions: disappearance of the MNPS anions as well of binding of the protons on the membrane. To determine the contribution due to protons binding, the theoretic model of the process has been developed taking into account the damage of the MNPS molecules on the membrane coupled with the release of protons as well as the exchange of the MNPS molecules and protons between the membrane and bulk water solution. The steady-state change of the BP caused by the protons depended on the light intensity, the concentration of MNPS, buffer and pH of the solution. The highest change of BP was observed at high pH about 9, it decreased with pH and disappeared at pH less than 6. The effect of pH on the change of the membrane capacitance was opposite: it decreased with increase of pH. The typical time of change of BP during illumination and its restoration in the dark exceeded 30 s. The kinetics of the PB change depended on the rate of stirring of the solution in the cell. The changes of BP caused by illumination considerably increased if to incorporate into the BLM the molecules, the charge of which depends on pH. We incorporated the molecules of styryl dyes di-4-ANNEPS or RH-421, which adsorb on BLM in neutral form changing the dipole membrane potential. This molecule can also bind the proton transforming into a charged form, which does not adsorb on BLM. The illumination of BLM containing both the molecules of MNPS and styryl dyes led to change of BP due to desorption of the dyes molecules accepting the protons.

These observations indicate that the kinetics of the BP change caused by binding of protons on the membrane surface is determined by the change of the protons concentration in the unstirred layer near the membrane, and the value of the BP change - by dependence of the

surface charge of BLM on pH in the solution. The highest dependence of the surface charge of BLM from phosphatidylcholine on pH was in the pH range between 6 and 9, where the highest BP changes were observed. The evaluation of relative change of the concentration of the protons released on the BLM surface under the illumination of the BLM with bound MNP molecules yields a value about two orders.
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### S2.193. Structural and physiological studies of potassium channelopathies

Li B.[2], Zhang H.[2], Mischenko A.[2], Glukhov G.[1,2], Pashkov A.[1], Mai L.[2], Karlova M.[1], Novoseletsky V.[1,2], Abramochkin D.[1,2], Zaklyazminskaya E.[3], Sokolova O.S.[1,2]*
[1]*Moscow Lomonosov University, Faculty of Biology;*
[2]*MSU-BIT University, Shenzhen, China;*
[3]*Petrovsky Russian Scientific Center for Surgery, Moscow, Russia;*
* sokolova184@gmail.com

Potassium currents IKs, IKr, It0, IK1, ISS, and IK2P all contribute to repolarization in cardiomyocytes in normal and failing hearts. Mutations in genes encoding the alpha and beta subunits of the potassium channels conducting these currents lead to several arrhythmic disorders, such as Long QT syndrome (LQTS), Short QT syndrome (SQTS), familial atrial fibrillation (FAF), Brugada syndrome (BrS), and early repolarization syndrome (ERS), and can also be found in cardiac sudden death (SCD) victims (OMIM). Loss-of-function mutations (LoF) usually lead to LQTS, whereas gain-of-function (GoF) mutations have a more variable appearance (SQTS, BrS, ERD, and FAF). Presumably, the clinical phenotype and the changes in ECG in patients with mutations in these genes correlate with ion permeability defects. On the other hand, there are many mixed and overlapping phenotypes, resulting from complex molecular pathways of channel dysfunction. Modern new-generation sequencing (NGS) technologies provide a unique opportunity to simultaneously test multiple genes in patients with suspected channelopathies and to identify the genetic cause of the disease. However, the large volume of performed genetic testing reveals many rare/unique genetic variants of unknown clinical significance. The correct interpretation of these genetic findings is crucial for correct genetic counselling and clinical care, including the selection of the best choice of anti-arrhythmic treatment, SCD risk estimation, and decision-making about anti-arrhythmic device implantation. Hence, functional studies of newly discovered variants are critical to classify the variant as pathogenic or non-pathogenic. Here we present results of the genetic screening of the KCNJ2, KCNQ1 and KCNH2 genes in a Russian cohort of LQTS and BrS patients, and discuss the structural background for interpretation of genetic variants. Work was supported by RSF (22-14-00088), Shenzhen Municipal Government and Shenzhen MSU-BIT University.

### S2.194. Structural changes of the erythrocyte membrane and cytoskeleton under the influence of hormones

Mokrushnikov P.V.[1]*, Rudyak V.Y..[1,2,3]
[1]*Novosibirsk State University of Architecture and Civil Engineering(Sibstrin);*
[2]*Institute of Thermophysics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia ;*
[3]*Novosibirsk State University, Novosibirsk, Russia ;*
* pavel.mokrushnikov@bk.ru

The literature discusses in detail the cascade of biochemical reactions in the interaction of hormones and cells [1], which change the functions of membranes and cells. Nevertheless, the structural changes of membranes that occur during their interaction with stress hormones and androgens are still poorly studied. A change in the structure (conformation) of plasma membranes is understood as a change in the secondary, tertiary and quaternary structures of membrane proteins, phases of the lipid bilayer, redistribution of proteins and lipids along the bilayer, a change in the morphology of membranes.The subsequent changes in the functions of membranes and cells that occur at the same time remain unclear. The aim of this work is to experimentally study the structural changes in the erythrocyte membrane and cytoskeleton that occur under the influence of stress hormones (cortisol, adrenaline, norepinephrine) and androgens (androsterone, testosterone, DEA, DEAS), hereinafter simply hormones.
Atomic force microscopy methods have shown that when erythrocyte membranes interact with hormones, the membranes are covered with quasi-periodic folds. They appear due to increased longitudinal and transverse mechanical stresses in the membrane caused by a change in the conformation of membrane proteins and the formation of protein-lipid domains around them. The wavelength of the folds of plasma membranes is equal to or a multiple of 100 nm. This suggests that it is around the proteins associated with the cytoskeleton that protein-lipid domains arise, since the cell size of the spectrin-actin-ankyrin network is 100 nm. The results of studies obtained by fluorescent methods and IR spectroscopy confirm our assumption. By fluorescent methods, it was found that when hormones bind to the plasma membrane, the conformation of membrane proteins changes. Using IR spectroscopy, it was found that this increases the intensity of the bonds between the active groups of proteins and lipids in the membrane, increases the ordering of proteins and the lipid bilayer. Using fluorescent methods using the pyrene probe, it was found that when hormones were exposed to the membrane, with the exception of DEAS, the microviscosity of the lipid bilayer increased more strongly in the protein-lipid interaction region than in the lipid-lipid interaction region. When cytochalazine B, which causes inhibition of polymerization of actin filaments of the spectrin-actin-ankyrin network, was added to the suspension of erythrocytes with noradrenaline, folds on the membrane surface were not observed [2]. This means that without changing the conformation of membrane proteins and cytoskeleton proteins, folds in the membrane are not created. Thus, it is shown that when interacting with hormones, structural changes occur in the membrane, a fixed quasi-periodic network of protein-lipid domains associated with the cytoskeleton appears in it.
The following explanation of the results obtained can be given. It is known that stress hormones and androgens, when interacting with plasma membranes, bind to adrenoreceptors, changing their conformation [1]. Adrenoreceptors interact with the spectrin-actin-ankyrin network, change its conformation, and through it change the conformation of membrane proteins associated with the cytoskeleton. In the protein-lipid domains formed near these membrane proteins, which have changed their conformation after the interaction of the membrane with hormones, the lipid bilayer transitions from the liquid-disordered state to the gel phase Ld→Lß or to the liquid-ordered phase Ld→Lo. The lipid bilayer is deformed. It is not free in the cytoplasmic membrane, this is hindered by the spectrin-actin-ankyrin network, to which protein-lipid domains are attached. Mechanical longitudinal stresses of alternating compressions and stretches occur in the membrane [2, 3]. With small changes in the conformation of membrane proteins, the folds protrude 2-3 nm above the membrane surface. This corresponds to the difference in the heights of the lipid layer in the liquid-ordered Lo and liquid-disordered Ld state. With a further increase in the change in the conformation of adrenoreceptors, the conformation of the spectrin-actin-ankyrin network changes, and this network is compressed. This compression creates transverse and longitudinal forces in the membrane applied to the attachment points of the network to the membrane.

With an increase in these mechanical stresses and stresses created by the non-free deformation of the lipid bilayer, the membrane loses stability and becomes covered with folds up to 50 nm high.
Thus, in the erythrocyte membrane, when hormones (androgens, catecholamines) are exposed to it, a fixed quasi-periodic network of protein-lipid domains is formed. Domains are formed around membrane proteins associated with the cytoskeleton. The formation of this network in membranes can affect the transfer of gas molecules through the membrane by kinks-solitons [4], lateral diffusion of lipids in the membrane [5], the activity of its Na+,K+-ATPases, the plasticity of membranes and the possibility of passage of erythrocytes through microcapillaries [2].
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### S2.195. Study of the effect of a new cardioprotective compound 3-hydroxy-6-methyl-2-ethylpyridinium nitroxysuccinate on the ion channels of cardiomyocytes

Frolova Sh.R.[1,2]*, Kovalenko S.G.[1,2], Kramkova V.K.[1], Mishchenko D.V.[3], Agladze K.I.[1,2]
[1]*Moscow institute of physics and technology;*
[2]*M. F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow 129110, Russia;*
[3]*Institute of Problems of Chemical Physics, Russian Academy of Sciences;*
* isheydi02@gmail.com

Currently, the study of the occurrence of arrhythmias and ways to deal with them is still a very relevant area. One of the causes of arrhythmias is cardiac ischemia. Oxidative stress often leads to ischemia, in which free radicals oxidize cardiomyocyte membrane lipids. Thus, damaging the membranes, oxidative stress leads to cell hypoxia, and consequently to cardiac ischemia. It is also known that nitric oxide NO plays an important role in the regulation of vascular tone, but oxidized lipids reduce NO synthesis in the body. For the prevention of diseases associated with oxidative stress, drugs with antioxidant activity can be used. A new compound synthesized at the Institute of Problems of Chemical Physics (IPCP) of the Russian Academy of Sciences, 3-hydroxy-6-methyl-2-ethylpyridinium nitroxysuccinate [1], a derivative of pyridoxine, has antioxidant properties, it increases the production of nitrogen monoxide in heart cells and protects the iron-sulfur centers of the respiratory chain of heart, brain, and liver mitochondria in animal tissues from oxidative stress [2]. The hybrid compound 3-hydroxy-6-methyl-2-ethylpyridinium nitroxysuccinate consists of two components: nitrosuccinate and hydroxypyridine, therefore, it leads to the generation of nitric oxide production in cells, and also protects the FSC of the respiratory chain of mitochondria of the heart, brain and liver in composition of animal tissues from oxidative damage.
Because in the cardiovascular system, ion channels play a major role in generating action potentials for conducting excitation in the heart, this study was aimed at studying the effect of a new antioxidant compound 3-hydroxy-6-methyl-2-ethylpyridine nitroxysuccinate on voltage-gated ion channels of cardiomyocytes that play the main role in the formation of the action potential. Voltage-gated fast sodium channels Nav1.5 and fast calcium channels Cav, L-type were studied under the action of this compound.
The aim of our study was to understand whether this compound affects the functioning of ion channels and in what concentrations.
The study was carried out by the patch-clamp electrophysiological method in the "perforated whole cell" configuration on isolated neonatal cardiomyocytes of newborn rat pups.
It was found that, at a concentration of up to 1 mM, 3-hydroxy-6-methyl-2-ethylpyridine nitroxysuccinate does not affect the operation of fast sodium channels Nav1.5 and fast calcium channels Cav, L-type. At concentrations above 1 mM, suppression of fast sodium currents INav1.5 and fast calcium currents ICav, L-type was observed.
As a result, it was shown that at concentrations in which 3-hydroxy-6-methyl-2-ethylpyridine nitroxysuccinate exerts antioxidant properties (20–160 μM L–1) [3], it does not affect the functioning of fast Nav1 sodium channels. 5 and fast calcium channels Cav, L-type. And in concentrations starting from 1mM, it suppresses these ton currents in a dose-dependent manner.
Research on voltage-gated potassium ion channels is ongoing.
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### S2.196. Study of the role of VDAC in the development of mitochondrial dysfunction in primary cell cultures under conditions of hyperglycemia

Belosludtsev K.N.[1]*, Starinets V.S.[2], Serov D.A.[3,4], Ilzorkina A.I.[2], Karagyaur M.N.[5], Dubinin M.V.[1], Belosludtseva N.V.[1,2]
[1]*Mari State University, Yoshkar-Ola, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
[3]*Prokhorov General Physics Institute RAS, Moscow, Russia;*
[4]*Institute of Cell Biophysics RAS, Pushchino, Russia;*
[5]*Moscow State University, Moscow, Russia;*
* bekonik@gmail.com

Diabetes mellitus is a metabolic disease associated either with a violation of insulin secretion by pancreatic cells (type I diabetes mellitus) or with insulin resistance of organs and tissues of the human and animal body (type II diabetes mellitus). As a result, hyperglycemia develops, which is accompanied by a violation of protein and lipid metabolism. These disorders lead to pathological changes in the organs and tissues of the body [1].
It is generally accepted that mitochondrial dysfunction is one of the processes involved in the development of diabetes mellitus at the

cellular level. Indeed, for many organs and tissues, as well as cell lines, it has been demonstrated that diabetes mellitus or hyperglycemia leads to increased generation of reactive oxygen species by mitochondria, disruption of oxidative phosphorylation processes, and a drop in membrane potential. It is believed that this is due to a violation of the cellular quality control of mitochondria, the intracellular system responsible for mitophagy, mitochondrial biogenesis, and mitochondrial dynamics [2].

Mitochondrial metabolism requires efficient exchange of ions and metabolites between mitochondria and the cytoplasm across the outer mitochondrial membrane. This exchange is carried out with the help of voltage-dependent anion channels (VDAC). In this regard, it is not surprising that VDAC may be involved in a wide range of pathologies associated with mitochondria [3]. Thus, recent studies have shown that during the development of diabetes, there is an overexpression of the VDAC1 protein [2,4]. This suggests its possible role in the pathogenesis of this disease. In this regard, the aim of this work was to investigate the effect of pharmacological and genetic inhibition of VDAC on the development of mitochondrial dysfunction under conditions of hyperglycemic stress in cell cultures.

In the first part of the work, we studied the effect of VBIT4, a VDAC inhibitor, on the development of mitochondrial dysfunction in a primary culture of mouse lung endotheliocytes under conditions of hyperglycemia. It was established that the exposure (36 h) of the primary culture of mouse lung endothelial cells in a medium with a high glucose content (30 mM) leads to a significant decrease in cell viability. 5 μM VBIT-4 abolishes the cytotoxic effect of hyperglycemia. Hyperglycemia led to a significant increase in the formation of reactive oxygen species by cells, an increase in the activity of spontaneous formation of MPT pore in mitochondria, a drop in membrane potential, and an increase in colocalization of mitochondria and lysosomes (indicating the possibility of mitophagy activation). Incubation of cells under these conditions with 5 μM VBIT-4 resulted in a decrease in the generation of reactive oxygen species, suppression of the opening of the MPT pore, and restoration of colocalization of mitochondria and lysosomes.

In the next part of the work, a culture of human skin fibroblasts with a reduced expression of VDAC1 was obtained. It has been demonstrated that cells with decreased VDAC1 expression were practically insensitive to an increase in the level of glucose in the medium up to 30 mM. It was found that under conditions of hyperglycemia, there is a significant increase in DCF fluorescence in fibroblasts (1.97 fold). The decrease in VDAC1 expression was also accompanied by an increase in fluorescence, but it was less pronounced (1.67 fold). This may indicate that a decrease in VDAC1 expression may be a protective mechanism in the development of hyperglycemia. It has been demonstrated that the relative decrease in calcein fluorescence in the presence of cobalt, induced by hyperglycemia, in cells with reduced VDAC1 expression is less pronounced (1.18 fold) than in the case of unmodified cells (1.35 fold).

Based on the obtained results, it can be assumed that pharmacological or genetic suppression of VDAC1 activity in primary cell cultures is a factor preventing the development of mitochondrial dysfunction under conditions of hyperglycemic cell stress.

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## S2.197. Studying the mechanism of caspofungin effect on model membranes imitating target cell membranes

Andriianov V.S.[1]*, Zakharova A.A.[1], Efimova S.S.[1], Ostroumova O.S.[1]
[1]*Institute of Cytology of the Russian Academy of Science;*
* vladimir.andriyanov.99@gmail.com

Caspofungin (CF) is a semi-synthetic cationic lipopeptide of the echinocanides group currently used in medicine as highly effective first-line antifungal drug for the treatment of candidiasis and aspergillosis, including invasive ones. The main mechanism of the antifungal action of caspofungin is the inhibition of an enzyme built into the fungal membrane, 1,3-β-D-glucan synthase, which produces a specific component of the cell wall of sensitive fungi. Taking into account the amphiphilic properties of the lipopeptide and its detergent effect on the lipid bilayer [1], it can be assumed that CP has a complex antimycotic activity, also affecting the plasma membranes of target cells. Our goal was to study the effect of CF on model lipid membranes.

In achieving this we have utilized the method of recording currents flowing through flat lipid bilayers obtained by the Montal and Muller method [2] and containing lipid components characteristic of the plasma membranes of fungal cells. The introduction of caspofungin into a 0.1 M KCl solution (pH 7.4), bathing the membrane made of an equimolar mixture of dioleoylphosphatidylcholine (DOPC), dioleylphosphatidylserine (DOPS) and ergosterol (erg), in concentration ranging from 40 to 100 μM increase the membrane conductivity to 400 pS (transmembrane voltage equals 50 mV). With the replacement of DOPC with dioleylphosphatidylethanolamine addition of lipopeptide in the same concentrations decreases current membrane conductivity to 300 pS. A twofold increase of the negatively charged DOPS lipid decreases the membrane conductivity up to 40 pS. The results obtained may lead to an assumption that the inclusion of a conical lipid in the bilayer and an increase in the concentration of a negatively charged lipid in the membrane is weakly related to the change of CF membrane activity. The work is supported by the Russian Science Foundation grant No. 22-74-10023.

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## S2.198. Testing toxicity of mitochondrial uncouplers in molluscan neurons

Popova L.B.[1]*, Kirsanov R.S.[1], Krasnov V.S.[1,2], Khailova L.C.[1], Korshunova G.A.[1], Kotova E.A.[1], Antonenko Y.N.[1]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University;*
[2]*Faculty of Chemistry, Lomonosov Moscow State University;*
* lala@belozersky.msu.ru

Uncouplers of oxidative phosphorylation "dissipate" the proton gradient by transporting protons back to the mitochondrial matrix bypassing ATP synthetase, thereby uncoupling respiratory electron transport from ATP synthesis. Moderate uncoupling was shown to be therapeutically beneficial. Uncouplers could serve as the basis for anti-obesity drugs and medicines against pathologies associated with oxidative stress. However, many conventional protonophores are highly toxic compounds. There is an urgent need in new mitochondrial protonophoric uncouplers, which have a large window between concentration that causes uncoupling and concentration that is already toxic to cells.

Toxicity tests are of key importance for practical application of new protonophores. Here, we report measurements of electrical activity of an isolated molluscan nervous system, as a test system for studying the toxicity of mitochondrial protonophore-type uncouplers. Advantages of this test system are as follows: 1) Neurons are in their natural environment, surrounded by glial cells and nerve sheaths. 2) Ganglia placed in the saline solution retain their functions. For example, isolated pedal ganglia continue to generate motor rhythm, and the effect of sensory inputs is negligible. 3) Ganglia have big neurons that can be identified from mollusk to mollusk. This allows us to explore the properties of individual neurons in a large number of various experiments. 4) Molluscan neurons have the entire basic set of receptors and ion channels, which does not qualitatively differ from those of mammals. The experiments were carried out on neurons of isolated ganglia of gastropod Lymnaea stagnalis. The ganglia were extracted and split at the bottom of a bath filled with Lymnaea saline. We have defined parameters of neuronal activity, showing universal changes with all studied uncouplers of oxidative phosphorylation. The addition of an uncoupler to the surrounding solution causes depolarization of the neuron plasma membrane, an increase in spike frequency, and a decrease in spike amplitude. The rate of spike depolarization and repolarization decreases that leads to spike broadening. Over time, the cell completely stops spike generation. In this test system, we studied both classical mitochondrial protonophoric uncouplers, such as CCCP, FCCP, and DNP, and new uncouplers synthesized at our institute. Uncouplers differ in effective concentration and in the time of the effect development. A weaker effect on the membrane potential and neuron spike activity corresponds to a lower toxicity of the uncoupler. The classical protonophores CCCP and FCCP at a concentration of 2–10μM changed the neuronal spike activity in 2–10 minutes. Complete suppression of spike activity was observed after 20-40 minutes. By contrast, DNP, which had been used for a long time as an anti-obesity drug, exhibited an effect at concentrations of 200–500μM. Recently, several new mitochondrial protonophore-type uncouplers were synthesized at our institute. The first new uncoupler tested in our system was mitofluorescein (mitoFluo), which is a conjugate of fluorescein with decyl(triphenyl)phosphonium [1,2]. Bright fluorescence of mitoFluo allowed us to track the accumulation of mitoFluo in cells. Qualitatively, the effect of mitoFluo on neurons was the same as that of CCCP, but it occurred at higher concentrations (10–20μM) and with a much longer exposure (2–2.5 hours). We obtained very interesting data with CMTPP-C10, also a newly synthesized protonophoric uncoupler [3]. At concentrations of 10 - 20μM, CMTPP-C10 affected neurons much softer and slower than CCCP. Within an hour after the addition, the neuronal activity returned to control. Another new group of uncouplers, 7-hydroxycoumarin derivatives, UB-3-COOCn, showed almost no effect on neuronal activity despite the fact that in experiments on liposomes and isolated mitochondria, they exhibited properties similar to classical uncouplers [4]. About 10 years ago, the protonophore BAM15, a derivative of oxadiazol pyrazine, was synthesized. In the first paper describing BAM15, it was declared that it is not toxic as it does not affect the plasma membrane [5]. In our test system, we have shown that BAM15 at micromolar concentrations (5μM) causes the same irreversible suppression of neuronal electrical activity as CCCP, but the reaction develops more slowly, within an hour. Therefore, BAM15 is toxic even at low concentrations [6]. We also studied the protonophoric effect of triclosan, an antimicrobial drug widely used in everyday life [7]. In our test system, triclosan and CCCP have similar effects on neuronal activity. However, the effect of triclosan is detected with a longer exposure (40–60 min.) and requires higher concentrations (10–20μM), which corresponds to the difference in the uncoupling effect of triclosan and CCCP on isolated mitochondria. We believe that the changes in neuronal activity are associated with changes in calcium ions concentration in neuron cytoplasm, since protonophoric uncouplers induce calcium release from mitochondria, and operation of the main neuronal channels is regulated by changes in the intracellular calcium ions concentration. The weak toxic effect of the newly synthesized protonophoric uncouplers on neurons might be due to interaction of these uncouplers with cellular enzymes. For example, we have shown that 7-hydroxycoumarin derivatives, UB-3-COOCn, are degraded by mitochondrial aldehyde dehydrogenase (ALDH2) [4].
Abbreviations: CCCP-carbonyl cyanide-m-chlorophenylhydrazone; FCCP - p-trifluoromethoxyphenylhydrazone carbonyl cyanide; DNP - 2,4-dinitrophenol; UB-3-COOCn – alkyl esters of umbelliferone-3-carboxylic acid.
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**S2.199. The antioxidant activity of plant materials extracts used in traditional Chinese medicine**

Parshina E.Yu..[1]*, Yusipovich A.[1], Baizhumanov A.[1]
[1]*M.V.Lomonosov Moscow State University;*
* parshinae@gmail.com

The basis of most traditional Chinese medicine (TCM) treatments is herbal medicine. It is known that many medicinal plants used in TCM have antioxidant activity [1]. They are usually used in the form of soup, infusion or tea. Previously, we studied aqueous extracts of some TCM preparations [2] and found that extracts of E. ulmoides, C. deserticola, and C. officinalis have the highest total values of total antioxidant activity among other studied extracts. TCM preparations are also used in the form of alcoholic tinctures and extracts, and the therapeutic efficacy of aqueous and alcoholic extracts of the same herbs may vary [3]. In addition, the antioxidant effect of extract components may manifest itself differently in different cellular compartments.
For this reason, in this work, we studied the antioxidant activity in the aqueous and lipid phases of alcoholic extracts of nine types of plant materials used in traditional Chinese medicine: Dendrobium officinale, Ganoderma lucidum, Gastrodia elata, Cornus officinalis, Eucommia ulmoides, Cistanche deserticola, Astragalus membranaceus, Panax quinquefolius and Codonopsis pilosula.
Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was used to identify the active substances and functional groups in the extracts. The total antioxidant activity (TAA) of extracts in the aqueous phase was determined by the formation of a colored complex of reduced iron with 2,4,6-tripyredyltriazine. To assess the ability of extracts to inhibit lipid peroxidation in liposomes induced by the Fenton reaction, the amount of TBA-active products was determined. The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent.
According to the ATR-FTIR data, polysaccharides and terpenoids were found in all the studied samples, phenolic compounds were identified in all extracts, exept of G. lucidum.
Extracts of E. ulmoides, C. officinalis, and C. deserticola showed the highest TAA values. TAA of the extracts correlated well with TPC ($r=0.817$, $p=0.011$), but not with the amount of TBA-active products. Extracts of E. ulmoides, C. officinalis, and G. lucidum had the maximum effect on lipid peroxidation, despite the fact that G. lucidum extract had the lowest TAA value. We assume that non-phenolic substances, such as terpenoids, which are also present in the studied extracts, played the main role in protecting against lipid peroxidation.
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**S2.200. The deviation from the rule "one neuron - one receptor" in the expression of chemoreceptor genes in olfactory neurons of vertebrates: occasional or determined phenomenon**

Kopylova E.E.[1]*, Kabanova N.V.[1], Kovalitskaya Y.A.[1], Kovalenko N.P.[1], Masulis I.S.[1], Bystrova M.F.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences, Pushchino, Russia;*
* ikopylov76@gmail.com

The neuron population of the vertebrate olfactory epithelium may be considered as a matrix of chemosensory elements capable of detecting a wide range of compounds. The ability to differentiate substances diverse in chemical nature is provided by the fine turning of sensory cells at the level of olfactory receptors (OR) expression predetermining in fact correct afferent projections in corresponding bulbar zone. It remains generally accepted that the rule “one neuron – one receptor” lies in the basis of selective recognition of odorant stimuli. Mechanisms regulating the expression of unique gene from the set of multiple genes encoding olfactory receptors (1360 for mouse, 816 for human, 811 for the dog) are still not known in details being one of the most intriguing problems regarding managing of genome functions during maturation of neurons. Few ways are employed for OR selection and switching: heterochromatin modification by means of metilase, recruitment of transcription factors Lhx2 and Ebf , formation of inter- and intra-chromosomal contacts making certain genetic loci available for transcription. Nevertheless, the scRNAseq data allow to suppose the expression of 2 - 9 OR genes in mature olfactory neurons. These facts incompatible with dominating hypothesis could be considered as a consequence of automatic cell sorting, resulting in capturing of mRNA originated from neighbouring cells during sample preparation. Special efforts allowing avoiding the implication of concomitant RNA in the reactions could substantially improve the results. In present work we refined the procedure of isolation of single olfactory neurons from mouse olfactory epithelium. Mature neural cells were selected by the presence of OMP marker, subjected to preparation and total cDNA was synthesized from single cell lysate. cDNA was used as a template for PCR with degenerated oligonucleotide primers specified to broad range of OR.PCR fragments obtained from each cell were cloned in pJET1.2 vector used for transformation of E.coli, so that plasmid library is thought to contain protein-coding regions of all OR species expressed in given cell. Plasmids purified from 20 to 40 colonies containing the insert, were sequenced and the type of OR gene was determined using BLAST in the genome of Mus musculus (C57BL/6J, RefSeq GCF_000001635.27).
Totally 10 individual olfactory neurons were taken into analysis and three types of transcript distribution was observed. In the first case the expression of the singular OR was detected unambiguously. In the second one two OR transcripts appeared to be expressed with obvious prevalence of the major one. In the third case from two to four OR transcripts were detected. For the latter type major multiple transcripts were present in the following combinations: Or8k35 and Or4c109 (60,7 and 32,14 % correspondently); Or6z6 and Or4c116 (57,7 and 34,6%); Or4p8 and Or6z6 (68,4 and 21,1%); Or4e1 and Or4c113 (48 and 36%). In those cases when one prevalent OR gene was detected, it may belong to the group of highly expressed genes according to populational RNAseq available from Ibarra-Soria and co-authors, - Or6p1, Or4c117, Or4e1, Or6z7, as well as tends to possess only trace expression in the case of Or4c113. The detection of weakly transcribed mRNA confirms rather high sensitivity of the approach used in this study. Or6z6 transcript, being co-expressed with Or4c116 in single cell, exceeds it’s counterpart 2-fold whereas in mixed population it’s level is shown to be 25-fold higher. For the pair Or4p8/Or6z6 the opposite ratio is observed: Or6z6 being more active in mixed population, upon concomitant expression with Or4p8 is presented as less abundant product. In both cases transcription of Or6z6 located on Chromosome 7 is obviously coupled with activity of genes Or4c116 or Or4p8 nested in Chromosome 2. The latter is enriched by OR genes subgroupped in 3 remote clusters. One cannot exclude that locus of Chromosome 7 bearing Or6z6 is able to form physical contacts with Chromosome 2 in the area of long cluster 38 containing 269 OR genes including Or4c116 and Or4p8. These genes separated by 213 927 bp may participate in the formation of the same structural domain by means of inter-chromosomal contacts between Chromosome 2 and Chromosome 7 and due to spatial proximity may be expressed along with Or6z6. Individual pattern of OR co-expression observed in given cell is determined by DNA structural features in the vicinity of inter-chromosomal nodes and alternative modes of concomitant expression with the prevalence one or another OR gene may arise in different cells. When more than one OR gene is involved in transcription in certain cell the ratio of corresponding mRNA species depends likely on transcriptional status of simultaneously expressing genes rather than on individual properties of each one per se. This effect may be relayed on transcription-driven dynamical rearrangements of chromatin structure in the regions of inter-chromosomal contacts and regulatory factor’s binding. The results of this study allow to suggest that olfactory neurons are capable to express one as well as few OR genes and in multy-receptor cells the choice of the partner gene seems to be not occasional.
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**S2.201. The difference between the responses of premotor interneurons to serotonin and the precursor of its synthesis 5-HTP in intact and sensitized snails**

Bogodvid T.K.[1,2]*, Andrianov V.V.[2], Muranova L.N.[2], Gainutdinov K.L.[2]
[1]*Volga Region University of Physical Culture, Sports and Tourism;*
[2]*Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan;*
* tat-gain@mail.ru

One of the manifestations of long-term memory is long-term sensitization (LTS). Sensitization is a form of non-associative learning in which the animal experiences a significant increase in the magnitude of the evoked response to a previously neutral stimulus following the application of a strong (damaging) stimulation [1,2]. If a single strong stimulus causes short-term sensitization lasting minutes, then the repetition of such stimulation causes LTS lasting days and weeks [3]. It has been shown that the developed LTS persists from 2 weeks to 1 month [1]. The long-term nature of the phenomenon is also proved by the fact that LTS is not produced when blockers of protein biosynthesis and transcription blockers are used. These results demonstrate that LTS, despite the non-associative nature of its production, needs protein synthesis; it is a form of long-term memory. It was found that when Aplysia receives a dangerous (strong) stimulus, for example, a tail electric shock, the network of serotonergic neurons of the animal releases endogenous serotonin [4]. This released serotonin (5-HT) induces a series of cellular changes that lead to an increase in the defensive reflex. Evidence

of the need for 5-HT for the formation of LTS came from experiments using a neurotoxin that depletes serotonin.
In addition to the well-known role of 5-HT as a mediator in synaptic transmission, it was shown that it can perform integrative functions when released into the extracellular environment [5]. These results provided the basis for the application of 5-HT bathing solution as a reinforcer to create cellular analogues of learning. It is known that the application of 5-HT causes effects similar to the facilitation of dehabituating and sensitizing stimuli on the neural network underlying the defensive response. By means of applications of 5-HT in the solution bathing the central nervous system, it is also possible to reproduce the electrophysiological correlates of plasticity [6]. Previously, we found that applications of 5-HT and 5-hydroxytryptophan (5-HTP) into a solution washing the preparation of the nervous system caused a decrease in the membrane potential of premotor interneurons in both intact and trained snails [7]. At the same time, in trained snails, in contrast to intact ones, applications of 5-HT and 5-HTP caused an increase in the threshold potentials of LPa3 and RPa3 premotor interneurons. In this work, we studied changes in the excitability of premotor interneurons in response to the application of 5-HT and 5-HTP in preparations of intact snails and snails after LTS.
The experiments were carried out on isolated preparations of the nervous system of the mollusk Helix lucorum. To develop the LTS of the defensive reflex, the animals were presented with electrical stimuli in the head area 4 times a day for 4 days at an interval of 1.5-2 hours. Registration of electrical characteristics was carried out on the premotor interneurons of the defence reflex LPa3 and RPa3; to evoke an action potential, a rectangular current pulse with a duration of one second was applied through the recording electrode. Membrane potential (Vm) and action potential generation threshold (Vt) values were analyzed in response to the application of 5-HT and 5-HTP solutions in preparations of intact snails and snails after LTS. It was found that the application of 5-HT and 5-HTP significantly reduced the membrane potential in the groups of both intact and sensitized snails (by 4 mV). The action potential generation threshold, on the contrary, increased insignificantly. The results obtained indicate changes in the properties of various 5-HT receptors during the formation of LTS.
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## S2.202. The effect of acute phase inflammatory proteins, C-reactive protein, serum amyloid A, alpha-1-acid glycoprotein, fibrinogen and ceruloplasmin, on the activity of peripheral blood neutrophils

Fedorova N.D.[1]*, Sumbatian D.A.[1], Sokolov A.V.[2], Filatov M.V.[1], Trashkov A.P.[1], Varfolomeeva E.Yu.[1]
[1]*Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre «Kurchatov Institute», Gatchina, Russia;*
[2]*Federal State Budgetary Scientific Institution "Institute of Experimental Medicine", Saint Petersburg, Russia;*
* fedorova_nd@pnpi.nrcki.ru

Neutrophils are the leading cells of the innate immune system and the main population of leukocytes responsible for the primary reaction of the body to various infectious particles. The latter are destroyed by neutrophils due to the processes of phagocytosis and a cascade of reactions, including the respiratory burst reaction (RBR). As a result of RBR, neutrophils produce reactive oxygen species (ROS) and reactive halogen species, powerful cytotoxic agents that destroy particles in the phagolysosome. All of these processes require regulation, since excessive activation of neutrophils can lead to ROS-mediated damage to the tissues surrounding the focus of inflammation, and proteins of the acute phase of inflammation (APP) claim to be regulators of inflammation. We have previously shown the participation of ceruloplasmin in inhibiting RBR of neutrophils in blood samples [1], and fibrinogen, on the contrary, increased the intensity of RBR [2]. The effect on neutrophil functions has not been studied in detail for all APPs and especially their combinations. In this paper, for the first time, the effect of a number of APPs, C-reactive protein (CRP), serum amyloid A (SAA), alpha-1-acid glycoprotein (a1AGP) and fibrinogen on the ability of peripheral blood neutrophils to RBR using flow cytometry with registration of ROS production in cells as part of peripheral blood samples was investigated [3]. Significant changes in the ability of neutrophils to produce ROS were found for a number of combinations of the studied APPs. The study of the interaction of ceruloplasmin and fibrinogen with peripheral blood neutrophils on a confocal microscope revealed their membrane localization. It seems promising to identify receptors for these APPs on the neutrophil membrane, as well as to study their influence on the biomechanical characteristics of peripheral blood neutrophils.
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## S2.203. The effect of combined magnetic fields on the growth of NCTC clone L929 cells

Trubitsyna T.A.[1]*
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
* TTA-pro@yandex.ru

The aim of this work is to study the combined magnetic fields tuned to parametric resonance for Ca2+ (Ca2+-CMF), K+ (K+-CMF) and Mg2+ (Mg2+-CMF) ions on the growth of cells of the NCTC clone L929 fibroblast line.
Several series of experiments were conducted to select optimal conditions for the cultivation of selected cells. During the experiments, the cells of the NCTC clone L929 line were in thermostatically controlled

chambers either without maintaining 5% CO2, or with its maintenance. One camera was located in the Earth's magnetic field (control), the second was placed in a Helmholtz coil pair (in which a combined magnetic field was generated or not generated).
Field parameters: Ca2+-CMF: BDC = 48.7 mcT, BAC = 89.6 mcT, $f$ = 37.2 Hz; K+-CMF: BDC = 48.7 mcT, BAC = 89.6 mcT, $f$ = 57.2 Hz; Mg2+-CMF: BDC = 48.7 mcT, BAC = 89.6 mcT, $f$ = 61.2 Hz. The growth dynamics, morphology and viability of cells were evaluated every day by fluorescent staining and further microscopy.
The results of the first series of experiments, according to the assessment of cell culture conditions, showed that short-term exposure (2 hours) cultivation, without exposure to CMF, can be carried out without maintaining 5% CO2. Under such conditions, the dynamics of cell growth coincided in both chambers and showed no significant changes in comparison with the control group, which was cultured under standard conditions of a CO2 incubator. Similar results were shown by prolonged exposure (3 days) cultivation without exposure to CMF and with the maintenance of 5% CO2. DMEM/F-12 was determined to be the optimal culture medium for cell culture under such conditions. The results obtained allow the further use of the described setup to assess the effect of CMF on various "physical" targets of substrate-dependent cells. It has been shown that prolonged exposure to Ca2+-CMF does not change the normal morphology and good viability, and a change in the growth rate of NCTC clone L929 cells, in comparison with the control cameras located in the Earth field and the control group in a CO2 incubator.
Prolonged exposure to K+-CMF showed a slowdown in growth, preservation of normal morphology and good viability of NCTC clone L929 cells, in comparison with the control cameras located in the Earth field and the control group in a CO2 incubator.
With prolonged exposure to Mg2+-CMF, the preservation of normal morphology, good viability is observed, and a change in the growth rate of cells of the NCTC clone L929 line, in comparison with the control cameras located in the Earth field and the control group in the CO2 incubator. But the effect was lower than when exposed to a CMF tuned to parametric resonance for Ca2+ or K+ ions.
The results obtained by us show the possibility of the influence of the described fields on human cells, which will be used for further development of magnetotherapy methods.

### S2.204. The effect of methoxamine on the action potential of newborn rats cardiomyocytes

Mansour N.[1]*, Ziyatdinova N.I.[1], Zefirov A.L.[2], Krylova A.V.[1], Zefirov T.L.[1]
[1]*Kazan (Volga region) Federal University, Kazan, Russia;*
[2]*Kazan State Medical University, Kazan, Russia;*
* nourm94@mail.ru

Background: α1-Adrenoceptors are seven transmembrane domain GPCRs involved in numerous physiological functions controlled by endogenous catecholamines, noradrenaline and adrenaline, and targeted by drugs useful in therapeutics. Three separate genes, whose products are named α1A-, α1B-, and α1D- adrenoceptors, encode these receptors. Although the existence of multiple α1-adrenoceptors has been acknowledged for almost 25 years, the specific functions regulated by each subtype are still largely unknown. This work aimed to study the role of methoxamine in the regulation of electrical activity of the myocardium of the right atrium of rats in early postnatal ontogenesis.
Materials and Methods: The study was carried out on white rats (n =7). Membrane potential (MP) and action potential (AP) of the imposed rhythm were recorded using glass microelectrodes. The stimulus duration of the imposed (1ms) and repetition rate (3Hz). The phases of AP were analyzed: the duration of depolarization, the duration of repolarization at the level of 20%, 50%, 90% (APD 20, APD 50, APD 90). Statistical significance was assessed using Student's t-test.
Results: Methoxamine at a concentration of 10-8 M lengthened the repolarization phase of the action potential of working atrial cardiomyocytes, while there was no change in the duration of the depolarization phase. Methoxamine increased APD20 by 57% APD50 by 54% APD 90 by 41% ($P < 0.05$). Methoxamine did not cause significant changes in membrane potential (MP). In addition, the values of the amplitude of the action potential, and overshoot did not change.
Conclusions: Methoxamine causes changes in the pattern of the electrical activity of the myocardium of the atria in newborn rats by increasing the repolarization phase of the action potential. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

### S2.205. The effect of a1-adrenoreceptors stimulation on AP frequency of cardiomyocytes in different ages rats

Mansour N.[1]*, Ziyatdinova N.I.[1], Zverev A.A.[2], Bilalova G.A.[1], Zefirov T.L.[1]
[1]*Kazan (Volga region) Federal University, Kazan, Russia;*
[2]*Volga State University of Physical Culture, Sports and Tourism, Kazan, Russia;*
* nourm94@mail.ru

Background: α1-Adrenergic receptors (ARs) are catecholamine-activated G protein-coupled receptors (GPCRs) that are expressed in rat and human myocardium and vasculature,and play essential roles in the regulation of cardiovascular physiology. Though α1-ARs are less abundant in the heart than β1-ARs, activation of cardiac α1-ARs results in important biological processes such as hypertrophy, positive inotropy, ischemic preconditioning, and protection from cell death. This work aimed to study the role of α1-adrenergic receptor agonist methoxamine (10-8 M) on the frequency of generation of the action potential in the heart of rats at different ages of postnatal ontogenesis.
Materials and Methods: The study was carried out on newborn, 3- and 20- week-old white rats using the microelectrode technique. A preparation of atrial myocardium with preserved sinus node and spontaneous activity was prepared. Methoxamine was immersed in a special tank, where a thermostatically controlled working solution "Tyrode" was supplied (which contains 7.54 g/l NaCl; 0.3 g/l KCl; 0.134 g/l; CaCl12; 0.06 g/l MgSO4; 0.14g/l NaH2PO4; 1.68 g/l NaHCO3; 0.9 g/l of glucose), which was concentrated by a gas mixture consisting of 95% oxygen and 5% carbon dioxide (37±1ºC). The results were processed by the Elph 3.0 program. The samples were tested for normal distribution. Statistical processing was carried out using paired Student's t-test. The effect of the α1-adrenergic receptor agonist methoxamine was studied at a concentration of 10-8M.
Results: Methoxamine at a concentration of 10-8M in newborn animals caused an increase in the frequency of occurrence of the action potential by 42% ($p < 0.05$), and in 3-week-old animals caused an increase in the frequency of spontaneous activity by 24% ($p < 0.05$). In 20-week-old animals caused an increase in the frequency of spontaneous activity by 10% ($p < 0.05$).
Conclusions: the results revealed that the stimulation of α1-adrenoreceptors in newborn, 3- and 20- week-old rats led to an increase in the frequency of action potential generation. However, the maximum effect was expressed in newborn rats, and the minimum effect was observed in adult animals. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

### S2.206. The penetration of low-melting agarose molecules in the liquid state through cellular and nuclear membranes is the reason for the variability in the results of Comet assay

Sirota N.P.[1]*, Kolmanovich D.D.[1]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
* sirota@iteb.ru

Comet assay (Comet test) is the best test widely used to demonstrate the absence of damage in the DNA molecule. It is considered an excellent screening tool for detecting DNA damage, which is of great practical importance. Despite the high applicability of this method, there is a large variability and low convergence of results in various experimental studies conducted both within the framework of one and in interlabotatory work, which makes it difficult to compare studies. It was believed that this was the result of using different protocols. However, a number of studies have already been conducted using agreed protocols to clarify the causes of variability in the interlaboratory results (1,2,3)
In these studies, it was shown that the results of the comet test can be influenced by the concentration of agarose, the density of comets formed, the duration of the alkaline incubation period of nucleoids before electrophoresis, the pH of the buffer solution for electrophoresis and the lysis time (4).
There is no data in the literature on studies of the interaction of fusible agarose with intracellular genetic material. We found that when preparing preparations with cells immobilized in fusible agarose, agarose penetrates into the cell nucleus, where its steric interaction with genomic DNA chains occurs. When agarose is gelled, spiral structures are formed in the form of strands of several agarose molecules (5). A structure with tension along the formed alpha helices in such strands arises. Additional tension may occur during the spreading of the agarose droplet under the influence of the gravity of the cover glass during the formation of the drug. In the process of cell lysis, cell and nuclear membranes disintegrate and genomic DNA molecules deproteinize. After the completion of lysis, the tension in the alpha helices leads to a reduction of the agarose filaments. And as a consequence, there is a movement of double-stranded DNA sterically closed with agarose strands on the drug. Analysis of preparations subjected to staining with ethidium bromide (intercalator into double-stranded DNA) under a fluorescent microscope showed the presence of DNA comets with tails oriented in accordance with the direction of flow of fusible agarose.
The treatment of preparations (after lysis and staining) with DNase 1 demonstrated that DNA is present in the structure of the tails of DNA comets. Similar images were observed on preparations with Ehrlich ascitic carcinoma cells, isolated mouse splenocytes and mouse EMT6/P breast carcinoma cells.
Literature:

**S2.207. The proton transport through planar bilayer lipid membranes induced by precursors of stabilized triphenylphosphonium ylides is increased upon the introduction of methyl groups into the phenyl rings**

Rokitskaya T.I.[1]*, Kirsanov R.S.[1], Kotova E.A.[1], Antonenko Y.N.[1]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University;*
* rokitskaya@genebee.msu.ru

We have recently shown that precursors of stabilized phosphonium ylides, such as (decyloxycarbonylmethyl)triphenylphosphonium bromide (CMTPP-C10) and its analogs with different alkyl lengths, carry hydrogen ions through artificial lipid membranes, as well as membranes of mitochondria and chloroplasts [1]. The proton transport results from the cyclic movement of the cationic and neutral (phosphorus ylide) forms of the phosphonium derivative in lipid membranes.
It is known that the rate constant of flip-flop of an alkyltriphenylphosphonium cation through lipid membranes increases significantly upon the introduction of methyl groups into the phenyl rings [2]. The mechanism of action of protonophores, being weak acids, suggests that proton transport increases linearly with an increase in their adsorption coefficient [3]. We assumed that the introduction of methyl groups into the phenyl rings of triphenylphosphonium would increase the rate of membrane permeation of the cationic form of the compounds, while reducing the length of the alkyl by the same number of methylene groups would preserve the lipophilicity of the compounds and the coefficient of their adsorption on the membrane surface. We synthesized the precursors of stabilized triphenylphosphonium ylides with one or two methyl groups in the phenyl rings: (heptyloxycarbonylmethyl)tri(p-tolyl)phosphonium bromide (CMTTP-C7) and (butyloxycarbonylmethyl)tri(3,5- dimethylphenyl)phosphonium bromide (CMTPP-diMe-C4), and studied them on planar bilayer lipid membranes. At low pH (pH=2.2), the addition of the phosphonium salts to the planar bilayer lipid membrane (BLM) formed from diphytanylphosphatidylcholine led to the appearance of relaxation of the current through the membrane after application of a voltage jump. The relaxation kinetics accelerated with increasing voltage. The values of the characteristic relaxation time upon the voltage switching off were 2.3±1.0 s, 8.2±9.0 s and 31.4±0.1 s, for CMTPP-diMe-C4, CMTTP-C7, and CMTPP-C10, respectively. As the pH of the aqueous solution increased, the current relaxation kinetics accelerated, and the stationary current through the BLM increased for all compounds. The dependence of the stationary current on pH was bell-shaped with a maximum at pH=5.0 for CMTPP-C10, pH=7.0 for CMTTP-C7, and pH=8.0 for CMTPP-diMe-C4. The values of the stationary BLM current at pH=8.0 increased significantly with an increase in the number of methyl groups in the phenyl rings. For all compounds, the current through the membrane was due to the transport of protons, since the shift in the current-voltage characteristics of the BLM observed upon the creation of the proton concentration gradient was close to the theoretical value.
In the case of BLM formed from the lipid with ester bonds (diphytanoylphosphatidylcholine), the steady-state conductance caused by the addition of carboxymethyltriarylphosphonium derivatives decreased tenfold compared to BLM from the lipid with ether bonds. This is explained by the retardation in the translocation of lipophilic cations through the BLM due to an increase in the membrane dipole potential. The subsequent addition of the lipophilic tetraphenylborate anion to BLM from diphytanoylphosphatidylcholine at concentrations two orders of magnitude lower than the concentration of phosphonium cations led to a significant increase in the stationary proton current.
Our data confirm the assumption that the translocation of the cationic form of compounds is the limiting stage in the transport of protons through bilayer lipid membranes. An increase in the translocation rate constant of the charged form of protonophores leads to an increase in the proton flux through the membranes.
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**S2.208. The pulsing-activities of Triticum aestivum mitochondria depend on their mobility: cold acclimation influence**

Abdrakhimova Y.R.[1]*, Abdrakhimov F.A.[2]
[1]*Institute of fundamental medicine and biology, Kazan (Volga Region) Federal University, Kazan, Russia;*
[2]*Kazan Institute of Biochemistry and Biophysics of RAS, Kazan, Russia;*
* yoldez.abdrahimova@kpfu.ru

Key words: T.aestivum, confocal laser microscopy, mitochondria, transmembrane potential (Δψm) pulsing, ROS mitoflashes, mobility, cold acclimation
Mitochondria as well-known fulfill a multifunctional role in a maintenance and regulation of the cell processes mainly due to the unique properties of the inner membrane, first of all the ability to generate transmembrane potential (Δψm). Along with energy supply, fluctuations of Δψm might be involved into cell signaling systems because of their transitory

high-amplitude sharp and short-time (in the second range) character. Such dynamic events termed as 'pulsings' or 'flickerings' of Δψm were revealed using tetramethyl rhodamine derivates (TMRM) at the end of 20th century [1]. Often coupled with these, reactive oxygen species (ROS) flashes or 'mitoflashes' were detected recently [2]. To date, nature of the dynamic phenomena, including triggering mechanisms, remains mainly obscure.
We researched the dependence the frequency of mitochondrial dynamic events on the motility of the organelles in the coleoptile cells of wheat seedlings (T.aestivum) that were grown in the dark (23-25oC, 3d) and then cold acclimated (0-4oC, 5d). Samples of tissue slices were dyed by 0.5 μM TMRM or/and 10 μM DCFH2DA, and their vital microscopy was conducted by the use of LSM510 META (Carl Zeiss MicroImaging) with subsequent multi-tracking analysis (ImageJ (Fiji), Track-Mate v6.0.1) [3]. For quantitative estimation of mitochondrial mobility parameters (traffic velocity, direct and topography of translocation, morphology transformation), we applied a frame time-lapse series received by real-time monitoring for 3 min in 2000-3000 μм2 (ROI) with temporal resolution being 0.8 мs/pixel (500 мs/frame).
According to single-organelle tracking analysis, the chondriome was relatively divided into 3 subpopulations, namely ′running′ (more than 10 μm distance traveled for 3 min), ′walking′ (1-10 μm) and ′sitting′ (less than 1 μm) ones. The high pulsing activity (80% from the total events) was inherent mainly for non-mobile mitochondria (′sitting′) which percentage was 55% from the organelle total number in ROI . It should be stressed that at the moment when the pulsing events were happen, velocity speeds of the mobile mitochondria declined sharply and became common with those of 'sitting' ones. Interestingly, mitochondrial 'immobilization' by the anti-cytoskeletal agent latrunculin (300 nm) also increased the pulsing activity. These facts allow to propose being of the cell sub-cortex sites of reorganizing and/or modifying of mitochondrial membrane components to result in a formation of the transient pore channels that detected in a sudden and reversible manner of TMRM emission due to Δψm dissipation. The latter was often, but not at every turn, accompanied by flashing of intramitochondrial oxidation of DCFH2, widely applicable ROS-detecting indicator.
After long-term cold acclimation, TMRM fluorescence intensity in mitochondria increased up to 2 times evoked by ΔΨm-dependent accumulation of this dye with simultaneous decline of the pulsing-activity. Moreover, morphological heterogeneity of the chondriome increased, and complex irregular-shape mitochondria were characterized by mainly amoeboid-type of motility behavior which dynamic parameters were difficult to quantitatively estimate.
Thus, the pulsing-activity decreasing under cold acclimation reflects, on our opinion, inhibition of life- support system reactivity in response to perturbing factors that could be critical for cell survival. Detected pulsing induction under the stopping of mitochondrial traffic (growth optimal temperature), also the total reduction of the flash events (long-term hypothermia) indicate on more systemic then strictly stochastic as postulated in literature nature of the dynamic phenomena.
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### S2.209. Ultrastructural changes of heart mitochondria in rats in adaptation to hypoxia of different severity

Khmil N.V.[1]*, Pavlik L.L.[1], Germanova E.L.[2], Lukyanova L.D.[2], Mironova G.D.[1]
[1]*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia;*
[2]*Institute of General Pathology and Pathophysiology, Russian Academy of Sciences, Moscow, Russia;*
* nat-niig@yandex.ru

Mitochondria, as primary consumers of cellular oxygen, are closely integrated into the pathways of oxygen perception, as well as adaptation to its deficit, through their structural or functional modifications. This work investigated ultrastructural features of heart mitochondria in response to single and multiple exposures to various regimens of hypobaric hypoxia (HBH; FiO2=14–10–8%). Investigated rats had different initial resistances – low (LR) and high (HR) – to oxygen deficit. Proceeding from the character of their localization in the cell, cardiomyocyte mitochondria are divided, as a rule, into three subpopulations: interfibrillar (IFM), subsarcolemmal (SSM) and perinuclear (PNM).
The work established that LR and HR animals had initial differences in the structure of all three types of heart mitochondria:
1. Normally, IFM of the LR group had medium-density matrices, while in those of the HR group they were more electron-dense. Besides, IFM in LR animals are, as a rule, localized in one row between myofibrils, while in HR rats they are often arranged in two or more rows.
2. SSM in LR animals featured one organelle each in invaginations of the sarcolemmal membrane, which gave it a tortuous appearance, whereas the same mitochondria in the HR group were arranged in small clusters under the sarcolemmal membrane.
3. PNM in both groups of animals with normoxia were characteristically arranged at the poles of the nucleus, and the nuclei themselves were localized in the middle of the cell. Herewith, the average density of PNM organelles, as the average number of small mitochondria, was significantly higher in HR animals.
Thus, the reduced density of the matrix, a less dense packing of cristae, a smaller total number and the number of small mitochondria in IFM and PNM subpopulations, as well as the character of mitochondrial localization in the subsarcolemmal zone in LR, as compared with HR, animals apparently determined the reduced resistance to hypoxia in LR rats.
A weak and moderate single 30-min exposure to HBH did not lead to destructive changes in the ultrastructure of all three subpopulations of mitochondria in LR and HR animals. However, a severe hypoxia in LR animals was observed to cause, in addition to adaptive changes, small destructive disorders characteristic of pathological conditions (chaotic arrangement and lysis of cristae, vacuolization, diffuse lysis of myofibrillar bundles). In the SSM area, especially in LR animals with severe hypoxia, processes of mitochondrial fusion intensified. Mitochondria sharply enlarged, stretching out in length. Herewith, the cell membrane closely encircled the mitochondria, increasing the area of their communication with blood oxygen. A characteristic distinction for mitochondria in the PNM area in LR, in contrast with HR, animals was a sharp increase in the number of small organelles.
A distinctive feature in the morphology of cardiomyocytes in severe hypoxia in both LR and HR rats was a change in the localization of nuclei and their emergence near the sarcolemma. The movement of the nuclei to the sarcolemma is probably a mechanism of adaptation to hypoxia.
Long-term adaptation was formed as a result of 1-h repeated (within 12 days) hypoxic exposure to three different HBH regimens.
Ultrastructural analysis of IFM showed that weak and moderate hypoxic effects did not lead to large changes in both types of animals. A characteristic feature of IFM ultrastructure in LR rats was the emergence, after a weak HBH exposure, of electron-dense formations resembling micro-mitochondria previously described in the literature and by us.
Pronounced changes as a result of weak and moderate hypoxia were noted in SSM and PNM of only LR animals. Although the matrix density, crista packing and organelle shape did not change significantly, the total numbers of both SSM and PNM increased almost twofold in the LR group. SSM were arranged in invaginations of the sarcolemmal membrane not by one organelle each, but in clusters of 3 to 15 organelles. Translocation of mitochondria into the subsarcolemmal area was observed.

Changes caused by severe hypoxia in all mitochondrial subpopulations of both LR and HR animals were comparable to the effects of previous hypoxic regimens. Organelle shape, matrix density, crista packing and inter-crista space did not significantly change. Nevertheless, the total number of both SSM and PNM increased compared to the control in the LR group, and did not change in HR rats.
Thus, the translocation and rearrangement of mitochondria under the sarcolemmal and nuclear membranes, observed at some exposures, can be considered as an adaptive, compensatory adaptive, reaction of the cell's mitochondrial apparatus to changes in the physiological state of the organism as a whole. Emergence of micro-mitochondria, which are considered to be precursors of small mitochondria, is also attributed to adaptation processes. Adaptation processes occurred in both animal phenotypes, but were more pronounced in LR animals. This is consistent with our earlier obtained data that hypoxic training leads to a significantly greater increase of lifetime in extreme hypoxia (3% O2) in LR than HR animals. Taking into account all the data obtained, a 12-day exposure to a 30–60 min hypoxia of moderate severity can be recommended for the treatment of patients in an altitude chamber, since optimal conditions for the adaptation of animals to hypoxia develop within this period.
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**S2.210. Why opening-closing of two adjacent coupled gramicidin A channels in the lipid membrane synchronous?**

Antonenko Y.N.[1]*, Rokitskaya T.I.[1], Kotova E.A.[1], Novoderezhkin V.I.[1]
[1]*Lomonosov Moscow State University;*
* antonen@belozersky.msu.ru

The pentadecapeptide gramicidin A (gA) is known to form an ion channel via head-to-head transmembrane association of two monomers. Earlier, dimers of synthetic gA analogues, with monomers either covalently linked [1,2] or connected via the binding of their biotin tags to the same avidin or streptavidin molecule [3] were studied. The conductance of these channels was found to be approximately twice the conductance of a single channel. The dual channels were long-lived, with the lifetime (minutes) being approximately one or two orders of magnitude longer than the lifetime of the original channel (several seconds, depending on the conditions), which was explained by the cooperative influence of neighboring, i.e., adjacent channels, on the elastic deformation of the lipid bilayer [1-4].
However, another property, namely, the synchronous switching on and off of doublechannels formed as a result of the interaction of two single gramicidin channels, was not fully understood. The current through the membrane was measured with a time resolution of about 1 ms. With such accuracy it can be said that double-conductance channels are formed simultaneously without a visible intermediate step. More precisely, such steps were sometimes observed [1-3], however, in 40% of events, the acts of opening-closing of double channels were simultaneous. Here, we used the ideas of the theory of excitons to hypothesize on a possible reason for such synchronization. We assume that the synchronization of the two channels is due to the existence of common vibrational modes associated with conformational mobility in these two closely spaced channels. To make it more clear, we are talking about the analogy with two harmonic oscillators with a strong coupling between them. We consider a model in which two conducting channels can interact with a conformational mode, which creates coupling between them and thus promotes their mixing. If we assume that the coupling constant depends on a slowly changing conformational coordinate, then we can explain (1) the synchronous switching on of the two channels; (2) the long-lived nature of the double-conductance state; (3) the formation of short-lived states with single conductance before the opening of the double channel; and (4) closing of the double channel via the same short-lived single states.

It can be proposed that the phenomenon of synchronous channel opening could take place not only with gA analogues, but also with some other channel-forming peptides, such as alamethicin and syringomycin, which are known for their ability to form channels of minimal conductance along with large (high-conductance) channels [5,6]. It is generally accepted that the conductance of the large channels is higher because of the involvement of an increased number of peptide monomers in the formation of the channel wall [5], thereby suggesting an increase in the internal pore dimensions. However, evaluation of the channel dimensions from water-soluble polymer exclusion revealed almost the same pore size for small and large channels of both alamethicin [7] and syringomycin [6]. These data could be explained by simultaneous opening of a number of single channels forming a cluster, similar to gA dual channels. It can be assumed that strong coupling of alamethicin single channels of minimal conductance with neighbouring ones leads to formation of collective open states, which may have different conductances depending on the number of single channels involved in the cluster.
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**S2.211. α1-Adrenergic Receptors play a role in the electrical activity of the rats' heart**

Mansour N.[1]*, Ziyatdinova N.I.[1], Mosolov L.T.[1], Zefirov T.L.[1]
[1]*Kazan (Volga region) Federal University, Kazan, Russia;*
* nourm94@mail.ru

Adrenoceptors have played a crucial role in the history of pharmacology. They were essential parts of the work that led to the Nobel Prize in Physiology or Medicine in 1988 and 1994 and the Nobel Prize in Chemistry in 2012. These prizes highlighted the roles adrenoceptors have played in our understanding of how GPCRs work and in the evolution of rational drug discovery. The almost ubiquitous expression of adrenoceptors and their pleiotropic responses has led to successful drugs for a myriad of diseases. In our research, we aimed to study the effect of the α1-adrenergic receptor agonist methoxamine (10-7 M) on the myocardial electrical activity of adult rats.
The study was carried out on adult rats (n=7), using the microelectrode technique. A preparation of atrial myocardium with preserved sinus node and spontaneous activity was prepared. Methoxamine was immersed in a particular solution "Tyrode". The results were processed using the Elph 3.0 program. The samples were tested for normal distribution. Statistical processing was carried out using paired Student's t-test. We examined the effects of the α1-adrenergic receptor agonist methoxamine at a concentration of 10-7M.
Methoxamine at a concentration of 10-7M decreases the area under the curve of the peak, and also the action potential duration at the level of 20% (APD 20%), 50% (APD 50%) and 90% (APD 90%) of repolarization ($p < 0.05$), while there was no changing in the duration of depolarization phase. Also, the values of the amplitude of the action potential, membrane potential and overshoot did not change. Methoxamine at a concentration of 10-7 M in adult rats caused an increase in the frequency of action potential.
Thus, it was found that stimulation of α1-adrenergic receptors affects the electrical activity of the heart of adult rats, by changing the duration of repolarization. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

# S3. Energy transformation mechanisms. Bioenergetics. Molecular motors

### S3.212. Artificial photosynthesis - a promising energy solution

Allakhverdiev S.I.[1,2]*
[1]*K.A. Timiryazev Institute of Plant Physiology, RAS, Moscow;*
[2]*Institute of Basic Biological Problems, RAS, Pushchino;*
* suleyman.allakhverdiev@gmail.com

Fossil fuels are non-renewable, inefficient, and environmentally unfriendly. The use of alternative renewable energy sources is increasing. Natural photosynthesis is a proven for thousands of years example of alternative energy efficiency, producing organic compounds and O2, and under certain conditions H2, from water and CO2 at the expense of solar energy. Burning H2 provides the maximum energy among other fuels and water, an environmentally friendly product. Production of H2 by artificial photosynthesis systems is a promising and high priority. Solar energy can be converted into electricity (in solar cells) or used in systems generating H2 from water. We are investigating the creation and operation of solar energy converters based on phototroph components to produce environmentally friendly energy. We created an original setup to analyze the operation of solar cells based on photosynthetic systems in a wide range of temperatures and light intensities. We have obtained original data on the "work" of solar cells capable of generating photocurrents, based on different components of the photosynthetic apparatus, including thylakoids and photosystem II membranes immobilized on the surface of titanium dioxide under different conditions. Particular attention is paid to the search for efficient catalysts for water oxidation, since such catalysts are key components of solar cells producing molecular hydrogen from water in the light. We found that the most effective catalyst for water oxidation under artificial photosynthesis conditions is a manganese-containing complex. To produce photohydrogen, we modified photosystem I (PSI) in which the secondary electron acceptor, vitamin K, was replaced with platinized naphthoquinone (PtNP), which increases the efficiency of electron transfer to the electrode. With this modification of PSI, it was possible to create an artificial system capable of efficiently generating molecular hydrogen at the expense of light energy.

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### S3.213. Changes in mitochondrial membrane potential levels in respiratory chain dysfunction caused by mtDNA mutations

Popov D.Y.[1]*, Pogonyalova M.Y.[1], Vinokurov A.Y.[1]
[1]*Orel State University named after I.S. Turgenev;*
* rennda@yandex.ru

Introduction

Mutations of mitochondrial DNA can have various effects on cell bioenergetics, in particular on the mitochondrial membrane potential (ΔΨm), decrease or increase of which negatively affects cell viability and can cause pathology development [1]. A decrease in ΔΨm leads to matrix condensation and cytochrome c entry into the intermembrane space, triggering apoptosis [2]. An increase in ΔΨm leads to the hyperproduction of reactive oxygen species, which cause damage not only in mitochondria but in the cell as a whole [3].

The aim of the present study was to determine the effect of mitochondrial DNA mutations on the change and mechanism of ΔΨm maintenance.

Materials and methods

The objects of the study were cytoplasmic hybrid cell lines based on THP-1 cells, each with 5 to 10 mtDNA mutations.

The ΔΨm value as well as the state of mitochondrial FAD were studied using a ZEISS LSM 900 confocal microscope. To assess ΔΨm levels, cells were incubated in 25 nM TMRM solution in Hanks' medium for 45 min at 37 °C without washing. TMRM fluorescence intensity over time was recorded by recording the baseline signal followed by addition of CCCP (2 μM). The value of ΔΨm was estimated from the change in fluorescence intensity. Total mitochondrial content, FAD reduction rate, and FADH2/FAD ratio were estimated from autofluorescence using an excitation wavelength of 488 nm. CCCP (2 μM) and sodium azide (10 mM) were used to convert the coenzyme to a fully oxidized or reduced form, respectively. The state of NADN was investigated using a wide-field fluorescence microscope with a fluorite x20 immersion objective using excitation radiation from a xenon arc lamp. Autofluorescence was recorded in the wavelength range of 430-480 nm using excitation radiation at 340 nm. CCCP (10 μM) to maximize respiration and rotenone solution (10 mM) to block respiratory chain complex I were used to estimate mitochondrial content, NAD reduction rate, and NADH/NAD ratio. Numerical data are presented as (median [Q1;Q3], number of cells analyzed). The % values given are normalized to the median THP-1.

Results

The decrease in ΔΨm relative to THP-1 (100% [86%; 119%], N=45) in mutant cells of HSM1 (76% [55%; 108%], N=48) and MAM3 (72% [56%; 91%], N=60) lines may be the result of increased heteroplasmy mutation in the tRNALeu gene (44 and 32% respectively). The decrease in ΔΨm is explained by the fact that mutations in the tRNALeu gene disrupt tRNA conformation and stability and the efficiency of the aminoacylation reaction. Mutations of tRNAs can lead to disruptions in the translation mechanism and, consequently, to changes in mitochondrial protein synthesis, which leads to a decrease in the activity of respiratory chain complexes I and V [4-6]. However, MAM1 (120% [95%; 156%], N=44) and MAM2 (117% [76%; 257%], N=54) lines showed increased ΔΨm levels with a heteroplasmy tRNALeu gene mutation degree of 23 and 25%, respectively. This can be explained by the activation of compensatory mechanisms of ΔΨm maintenance, which may differ depending on the set of mutations and the level of heteroplasmy. In the case of the MAM2 line such a mechanism for maintaining ΔΨm is represented by a more active ETC complex II, as evidenced by a reduced FADH2/FAD ratio (0.66 [0.44; 1.99], N=48) and a high FADH2 formation rate (253% [121%; 372%], N=99). The cybrid line MAM1 showed a high FADH2/FAD ratio (2.65 [1.28; 3.91], N=36), reduced mitochondrial FAD content (70% [58%; 91%], N=59), and a low FADH2 formation rate (110% [56%; 156%], N=49), indicating reduced ETC complex II function. In this lineage, the mechanism of ΔΨm maintenance may be associated with a large contribution of ETC complex I (NADH/NAD (0.28 [0.18; 0.38], N=102)), which may be explained by a high heteroplasmy (68%) mutation in complex I subunit 5, which, according to literature, is negatively correlated with the development of atherosclerosis [7, 8].

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### S3.214. Direct participation of hydrogen ions in ATP synthase F1-factor functioning

Nesterov S.V.[1,3]*, Yaguzhinsky L.S.[2,3]
[1]*NRC "Kurchatov institute", Moscow, Russia;*
[2]*Belozersky Research Institute of MSU, Moscow, Russia;*
[3]*Moscow institute of physics and technology, Moscow, Russia;*
* semen.v.nesterov@phystech.edu

An analysis of the experimental data closely related to the work of our research team shows that transmembrane-transferred hydrogen ions are involved in the work of the F1 factor of mitochondrial and chloroplast ATP synthases. After transmembrane transfer through the Fo protons are transferred to the F1-factor via the gamma subunit, which also transmits torque to the catalytic centers of ATP synthase. The existence of kinetic barriers for proton transfer on both sides of the inner mitochondrial membrane is also shown. Mechanical mixing of the interface during rotation of the ATP synthase rotor ensures lowering of the kinetic barrier for proton transfer from the membrane surface to F1-factor. A model has been proposed and discussed, according to which, during the operation of F1-Fo ATP synthase the mechanical movement of the gamma subunit in parallel with the conformational rearrangement of the active center of the enzyme (Boyer's scheme) provides the replacement of magnesium ion by hydrogen ions ensuring the effective displacement of the ATP molecule.

### S3.215. Electrogenesis in the root environment of various lettuce varieties

Kuleshova T.E.[1]*, Gasieva Z.A.[1], Galushko A.S.[1], Panova G.G.[1]
[1]*Agrophysical Research Institute;*
* www.piter.ru@bk.ru

The occurrence of a potential difference in living systems is due to a complex of physicochemical processes that ensure the maintenance of an uneven distribution of ions at the cellular, tissue and organism levels. In the process of plant development, an electrical potential gradient arises along the entire organism, due to ion diffusion, concentration effects, and differences in the intensities of biochemical processes.
Bioelectrochemical systems based on electroactive processes in the root environment of plants and associated microorganisms - plant-microbial fuel cells - are a new promising environmentally friendly source of renewable energy. Although the possibility of practical use of bioenergetic resources has already been shown in many studies, the nature of electrogenesis, including its dependence on the genetically determined physiological characteristics of plants and their state during development, has yet to be revealed.
The purpose of this research was to study the dynamics of the potential difference formation in the root environment of various lettuce varieties.
Measurement of electrical characteristics was carried out by placing biocompatible corrosion-resistant electrode systems in the root environment, which provided surface electrical contact with the root and root zone. Potential difference changes were monitored using the Arduino hardware platform every 15 minutes during the entire growing season (28-32 days). The experiments were carried out under controlled conditions of the agrobiopolygon.
To reveal the role of plants in the formation of electrogenic reactions in the root environment, changes in the potential difference in a bioelectrochemical system containing a nutrient solution without plants (control) and with plants were measured using the lettuce variety Typhoon as an example. At the initial stage of the experiment, a potential difference of the order of 70–100 mV was observed in the nutrient solution, apparently due to differences in the concentrations of the nutrient solution components at the upper and lower electrodes. We can say that the nutrient solution acts as an analogue of the electrolyte in a galvanic cell. Over time, the voltage in the control cell decreased, most likely due to the leveling of concentrations. When growing plants, the potential difference, on the contrary, increased to ~200 mV and was stable throughout the entire growing season for lettuce. Probably, the increase in voltage in the bioelectrochemical system when plant objects are placed in it is associated with the development of the root system, the vital activity of rhizosphere microorganisms, the transport of mineral substances and, as a result, the intensification of diffusion processes.
To select the most promising plants in terms of obtaining electricity during the cultivation of plant products, a study was made of the electrophysiological properties of the following lettuce varieties, which differ in the efficiency of the photosynthetic apparatus: Solos F1, Chinese curly, Chinese red-green, Mercury, Dubrava, Ballet, Robin, Cockade. The plants were grown in peat soil (Agrobalt C, Russia) in the vegetative and irradiation installations developed by us under controlled conditions of the ARI agrobiopolygon.
The dynamics of the potential difference for the studied varieties was similar: an increase in values from ~200 mV from the beginning of the vegetation cycle to more than 300 mV by the 15th day and then stabilization was observed. The average value of the potential difference in the root environment-plant system was 281±32 mV for Solos F1, 221±42 mV for Chinese curly, 206±47 mV for Chinese red-green, 306±32 mV for Mercury, 291±35 mV for Dubrava, 289±27 mV for Ballet, 286±31 mV for Robin, 272±37 mV for Cockade.
The highest value of the potential difference in the root environment-plant system was typical for lettuce of the Mercury variety – it reached 430 mV. At the same time, the electrical characteristics of plants did not directly correlate with biomass indicators. The mass of the aerial parts of plants in one cell was 47.8±8.6 g for Solos F1, 48.1±13.5 g for Chinese curly, 58±15.3 g for Chinese red-green, 71±9.3 g for Mercury, 42.6±13.3 g for Dubrava, 113.1±25.8 g for Ballet, 54.6±11.6 g for Robin, 82±26.4 g for Cockade.
On the basis of the data obtained, ideas about the electrical characteristics in the root environment of various lettuce varieties were formed and the design of a phytofuel cell, a bioelectrochemical system based on electrogenic processes in the root environment-plants system, was proposed.
Thus, the possibility of using electrogenesis in the root environment-plant system as a new green source of electricity was shown. The potential for using the bioelectrochemical systems described above includes the provision of power supply to environmental sensors, light sources, wireless sensor networks, the Internet of things, phytomonitoring systems in natural conditions and protected ground, remote areas, partial power supply of plant life support devices in artificial agroecosystems.

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### S3.216. Hydrogen electrode with imobilized hydrogenase with high current density

Tsygankov A.A.[1]*, Starodubov A.S.[1], Melnikova M.S.[1]
[1]*Institute of Basic Biological Problems RAS;*
* ttt-00@mail.ru

Application of enzymes in fuel cells instead of noble metals would allow construction of cheap fuel cells. Furthermore, such fuel cells are biodegradable. The possibility of direct electron transfer from hydrogen to electrode by hydrogenase was demonstrated for the first time in USSR (doi:10.1016/0302-4598(84)87009-9). For a long time currents of such electrodes around 1 mA/cm2 were accounted as high density currents. However, in 2014 the hydrogenase from Citrobacter sp S-77 immobilized on dry electrode (gas-breathing electrode) surpassed platinum in electrocatalitic activity (doi:10.1002/anie.201404701). Last years hydrogenase immobilized on air-breathing electrode actively studied in different laboratories. In some reports currents as high as 14 mA/cm2 were shown. Different groups immobilized hydrogenase on electrode using conductive matrix without orientation of hydrogenase (doi: 10.1002/chem.202000750), using electrodes activated by conductive viologen polymers due to electrostatic interaction of hydrogenase and electrode surface (doi: 10.1038/s41467-018-07137-6; 10.1002/cssc.20200099), or using activated nanotubes with covalent attachment of hydrogenase (doi: 10.1021/acscatal.8b00708).
HydSL hydrogenase of Thiocapsa bogorovii is thermostable enzyme and that is why it is very attractive for practical application in fuel cells. In accordance with homology modeling C-terminus of small subunit, HydS, is transmembrane alpha helix (doi: 10.1016/j.bbabio.2021.148492). We developed an approach of HydSL oriented immobilization based on hydrophobic interaction of HydS C-terminus and carbon nanocarbon powder Vulkan R72. Electrodes with immobilized HydSL hydrogenase were installed in gas-breathing fuel cell and developed up to 12 mA/cm2 current at power more than 7 mW/cm2.
In the presentation the peculiarities of different methods of immobilization as well as ways for current density improvement are discussed.
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### S3.217. Interaction of protonophoric uncouplers with mitochondrial enzymes

Kotova E.A.[1], Khailova L.S.[1], Krasnov V.S.[1], Kirsanov R.S.[1], Firsov A.M.[1], Korshunova G.A.[1], Antonenko Y.N.[1]*
[1]*Lomonosov Moscow State University;*
* antonen@belozersky.msu.ru

Uncouplers disrupt energy storage in energy-transducing cell membranes, namely, the inner membrane of mitochondria, the thylakoid membrane of chloroplasts and the plasma membrane of bacteria, in the form of a transmembrane difference of the electrochemical potentials of protons, called the proton-motive force. Uncouplers attracted attention as possible anti-obesity drugs even before the discovery of the main mechanism of their action, namely, their protonophoric activity, i.e. the ability to carry out cyclic transfer of protons through a bilayer lipid membrane, leading to the dissipation of the proton-motive force. In the last 10–15 years, uncouplers boosted high interest as promising compounds for creating medicines against many diseases, including anticancer, antiviral, neuro-, nephro-, and cardioprotective drugs. The search for and study of the mechanism of action of new uncouplers remains an urgent and interesting task. The present study of 7-hydroxycoumarin derivatives originates from our previous successful research series on the design and synthesis of fluorescent uncouplers based on the well-known synthetic dye fluorescein. This compound has a hydroxyl group that can be deprotonated at physiological pH and a carboxyl group where alkyl substituents can be attached to increase lipophilicity. Actually, to be an effective protonophoric uncoupler, a compound needs to be lipophilic. Lipophilic derivatives of fluorescein, namely, its octyl and dodecyl esters, proved to be quite effective uncouplers with moderate toxicity [1].
Uncouplers based on 7-hydroxycoumarin were synthesized according to the same scheme. Unlike fluorescein, it is a natural pH-dependent fluorophore found in medicinal plants of the umbrella family (hence its second name, umbelliferon) and a number of other families. Its hydroxyl group has a pK of 7.5. The problem of attaching lipophilic substituents was solved by searching for derivatives of this compound with a carboxyl group. Two series were synthesized: esters of umbelliferone-3-carboxylic and umbelliferone-4-acetic acids. The compounds of both series had a pronounced uncoupling activity, but this activity surprisingly disappeared on a minute scale, in contrast to the classical uncouplers DNP and CCCP, as well as the mentioned fluorescein octyl ester. Since nothing of the kind occurred on model membranes, it was logical to assume that the activity of umbelliferone derivatives in mitochondria disappears due to the enzymatic hydrolysis of the ester bond. Indeed, the analysis performed using thin layer chromatography (TLC) showed that after 10 minutes of incubation in the presence of isolated rat liver mitochondria (RLM), the esters of both series were almost completely converted into the acids, while nothing similar happened with fluorescein octyl ester. We assumed that ALDH2, whose esterase activity is well known, is the enzyme catalyzing the hydrolysis of ester derivatives of umbelliferone. Moreover, this activity is sensitive to the same inhibitors as the main activity of the enzyme, acetaldehyde oxidation. The well-known inhibitor of aldehyde dehydrogenase disulfiram suppressed the decline in the uncoupling activity of esters of umbelliferone-containing acids. In parallel, the TLC data showed the suppression of the hydrolysis of these esters. We tested the action of another inhibitor of mitochondrial aldehyde dehydrogenase, daidzin, and concluded that the disappearance of the uncoupling activity of umbelliferone-containing acid esters is associated with the esterase activity of ALDH2. This conclusion is also supported by the data on the interaction of various coumarin derivatives with aldehyde dehydrogenase [2].
It is known that liver contains ALDH2 in excess compared to other organs. Therefore, it could be expected that in mitochondria isolated from other organs, the uncoupling activity of esters of umbelliferone-containing acids would not quickly disappear. Experiments on heart and kidney mitochondria confirmed this assumption. Therefore, esters of umbelliferone-containing acids turned out to be tissue-specific uncouplers. The stability of the uncoupling activity of these esters in heart mitochondria allowed us to compare their effect on the rate of respiration. It turned out that umbelliferone-3-carboxylic acid esters exhibit an order of magnitude higher uncoupling activity than umbelliferone-4-acetic acid esters.
We also studied dependence of the uncoupling activity of 7-hydroxycoumarin derivatives on the activity of the adenine nucleotide translocase ANT1. It turned out that the decrease in the membrane potential of rat heart mitochondria in the presence of esters of 3-carboxylic and 4-acetic acids is partially reversed by the addition of the specific ANT1 inhibitor carboxyatractyloside [3,4]. It was concluded that, like fatty acids, 7-hydroxycoumarin derivatives perform an uncoupling effect in mitochondria with the participation of ANT1.
This work was financially supported by the Russian Science Foundation (Russian Science Foundation grant 21-14-00062).
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### S3.218. Magnetic Isotope Effects and Nuclear Spin Catalysis in Living Cells and Biomolecular Motors: Recent Advances and Future Outlooks

Koltover V.K.[1]*
[1]*Federal Research Center of Problems of Chemical Physics and Medical Chemistry, RAS, Chernogolovka, Moscow Region, Russian Federation;*
* koltover@icp.ac.ru

Some chemical elements have two kinds of stable isotopes: magnetic and nonmagnetic ones. In physics and chemistry, magnetic isotope effects (MIE) have long been known for a number of chemical elements, including magnetic isotopes of carbon, oxygen, sulfur, germanium, mercury, and uranium [1]. Not long ago, MIE were discovered in living nature. In experiments with the cells E. coli, enriched with different isotopes of magnesium, the activity of superoxide dismutase in stationary phase of the cells, grown on the medium enriched with the magnetic isotope of magnesium, Mg-25, was found to be 40 % lower than it was in the cells, enriched with the nonmagnetic isotope, Mg-24 [2]. Besides, the adaptation kinetics of cells to the growth media, enriched with magnetic Mg-25, proceeds essentially faster as compared to the media enriched with nonmagnetic Mg-24 [3]. The MIE were also found in experiments with the yeast cells, S. cerevisiae, enriched with different isotopes of magnesium. The rate constant of the post-radiation recovery of the cells irradiated by X-rays or short-wave UV is two times higher if the cells are enriched with Mg-25, by comparison with the cells enriched with Mg-24 [4]. Furthermore, the catalytic effects of the magnetic magnesium isotope were revealed in the reaction of ATP hydrolysis driven by myosin, the important molecular motor of bioenergetics utilizing the chemical energy of ATP to perform the mechanical work. The rate of the enzymatic hydrolysis of ATP with magnetic Mg-25, as the enzyme cofactor, is twice higher than the rates of the reactions with nonmagnetic Mg-24 or Mg-26 [5, 6]. A similar effect of the nuclear spin catalysis was detected in experiments with zinc as the myosin cofactor. The rate of the ATP hydrolysis with the magnetic isotope, Zn-67, increases by 40–50 % compared to that with the nonmagnetic isotopes, Zn-64 or Zn-68. Besides, the catalytic effects of the nuclear spin of Mg-25 were found in the experiments with ATPase isolated from the yeast mitochondria and Mg-dependent ATPase from the myometrium plasma membranes. MIE unambiguously indicates that, in the chemo-mechanical process catalyzed by the molecular motor, there is a limiting step which depends on the electronic spin state of the reagents, and this step is accelerated by the nuclear spin of the magnetic isotope. It can be assumed that, under the condition of electron-conformational excitation of macromolecule, the electron density is transferred, for example, from the hydroxyl group of the water molecule, bound inside the active center of the enzyme, onto ADP or Mg(II). It yields a radical ion pair. After that, the oxy-anion of ADP nucleophilically attacks the inorganic phosphate to give ATP. The stable spin state of Mg(Zn)-ATP as the product of this inverse reaction must be singlet (S = 0). Meanwhile, the nuclear spin of Mg-25 (Zn-67) via the hyperfine interaction with the unpaired electron of the radical ion pair, converts this pair into the triplet state (S = 1), thus creating the spin barrier. It hinders the undesirable reverse reaction of ATP synthesis, thereby facilitating the reaction of ATP hydrolysis. The hypothesis about the virtual radical ion pair in the synthesis of ATP via oxidative phosphorylation was proposed about 50 years ago [7]. The alternative explanations for the nuclear spin catalysis in the reactions of ATP hydrolysis catalyzed by the molecular motors were offered in [8-10]. The energy released from ATP hydrolysis (~0.54 eV) is not enough for the electron-conformational transition of the enzyme macromolecule to the singlet excited state. It is sufficient to create the lower triplet state (S = 1) but such a transition from the ground state (S = 0) is prohibited by the spin conservation law. The nuclear spin of the isotope, Mg-25 or Zn-67, removes this ban, thereby accelerating the chemo-mechanical cycle in the enzymatic reaction, Alternatively, the nuclear spins of Mg-25 or Zn-67 may act onto the nuclear spins of hydrogen of molecules of water, thereby accelerating the inter-conversion of the ortho-isomers (with the parallel orientation of the hydrogen proton spins) and the para-isomers (the anti-parallel proton spins) of the water molecules, thus facilitating the necessary conformation rearrangement of the macromolecule. Although detailed mechanisms of the ability of biomolecular motors to perceive the nuclear magnetism require further investigations, the recent developments in this new field highlight promising venues for future research of the nuclear spin catalysts in biophysics with possible applications of the magnetic isotopes in medical physics, including radiation medicine and biomedical effects of magnetic fields.

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### S3.219. Mitochondrial energetics based on monovalent cation transport

Zorov D.B.[1]*
[1]*A.N.Belozersky Institute of Physico-Chemical Biology Lomonosov Moscow State University;*
* zorov@belozersky.msu.ru

The chemiosmotic theory of oxidative phosphorylation in coupling membranes, including the inner membrane of mitochondria, was primarily based on cyclic proton transfer, for which two Nobel Prizes were awarded (1978 and 1997). In accordance with this concept, proton pumps representing at least three supercomplexes of the respiratory chain (complexes I, III and IV) transfer protons from the mitochondrial matrix into the extramitochondrial medium, leading to the generation of the transmembrane potential of hydrogen ions, represented by electrical and concentration components. This potential is the driving force for the rotation of the components of ATP synthase, (complex V) with subsequent conformational rearrangements in this complex associated with ATP synthesis. Such cyclic proton transport was recognized as exceptional and probably the only one in the organization of bioenergetics, which was called protonic. It should be noted that the combination of chemical and osmotic components in the name was not clear, because when examined in detail, osmotic rearrangements were not assumed.

But the main dissonance of the global principle of protonic energetics was the discovery of the existence of another type of energetics, i.e., sodium energetics, essential for bacteria living in a high content of

sodium ions, and both sodium pumps and sodium ATP synthase have been found there.
These and other logical and experimental arguments served as the theoretical basis for the assumption of the presence in mitochondria of a different type of bioenergetics than protonic, namely, based on the transport of potassium ions, coupled with the synthesis of ATP in complex V. This assumption followed from the enormous prevalence of potassium ions in the cytosol of the cell over protons. Indeed, concentration of protons in the cytosol is ≈100 nM (pH 7.2–7.4), while of potassium ions is millions of times more (≈ 140 mM), and taking into account the high selectivity of the Fo channel for the proton, it was quite logical to admit the possibility of transport of potassium ions through it.
These arguments required experimental confirmation, which followed. To do this, a number of studies were conducted, which included work on bilayer phospholipid membranes, liposomes with reconstituted ATP synthase, isolated mitochondria and intact cardiomyocytes.
Using bilayer membranes, the baseline permeabilities of the ATP synthase channel for proton and K+ (PH+ and PK+) were respectively estimated as 5.2±0.9×10-11 and 8.7±2.9×10-17 m3/s, and which increased approximately 3.5 times after the addition of diazoxide, an activator of mitochondrial ATP-dependent K+-channel respectively, thus maintaining the selectivity of the Fo channel ~106:1 with a strong preference for proton transport over potassium ions.
After the reconstitution of ATP synthase into liposomes and the application of a pH and potassium ions gradients, a directed transport of potassium ions sensitive to ATP synthase inhibitors was detected. Quantitative evaluation showed that at physiological concentrations of potassium ions for each transported H+, mitochondrial ATP synthase conducts 3.7 K+, and both of these processes were coupled with the synthesis of ATP. It was also important that diazoxide equally enhanced the transport of K+ and H+ through synthase, which was one of the proofs that proton and K+ follow the same root in the ATP synthase complex.
One of the most important questions was on the driving force for the directed transport of K+ through ATP synthase, given that there is practically no K+ gradient in mitochondria on the inner membrane. It turned out that under such conditions, with an efficiently operating proton pump, the driving force is the presence of an electric field through the membrane, that is, it is the membrane potential that is the driving force for the transport of potassium ions. It was assumed that the induced increase in the K+ levels in the matrix is discharged due to the activation of the K+/H+ antiporter. However, a slight mismatch between the two multidirectional flows of K+ leads to a small and temporary accumulation of this ion in the matrix. In this case, the most important difference between hydrogen and potassium ions is that potassium ions, unlike protons, are osmotically active, as a result of which the accumulation of K+ in the matrix is accompanied by the entry of water into the matrix yielding mitochondrial swelling. Considering that a small (regulatory) swelling of mitochondria, not accompanied by a drop in transmembrane potential, leads to activation of mitochondrial respiration, this leads to an increase in coupled ATP synthesis, as a result of which switching from protonic to potassium energy leads to an increase in ATP synthesis, which has been demonstrated using both reconstituted systems and isolated mitochondria.
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**S3.220. Spectral changes S. paucimobilis rhodopsin in the presence and absence of Zn2+ as a function of pH**

Zagryadskaya Y.A.[2], Tsybrov F.M.[2], Chizhov I.V.[1], Okhrimenko I.S.[2]*
[1]*Institute for Biophysical Chemistry, Hannover Medical School, Hannover, Germany;*
[2]*Research Center for Molecular Mechanisms of Aging and Age-related Diseases, Moscow Institute of Physics and Technology, Dolgoprudny, Russia;*
* ivan.okhrimenko@phystech.edu

Members of the genus Sphingomonas have a flexible metabolism and thus can process a wide variety of both natural organic compounds and synthetic ones, such as environmental pollutants. This has generated extensive scientific interest in the metabolic pathways of these organisms, the properties of the enzymes involved in these pathways, and the genetics of their catabolic processes (Balkwill, 2006). Due to their unique biodegradable and biosynthetic abilities, Sphingomonas have a number of biotechnological applications, such as bioremediation or the processing of environmental pollutants (Cheng, 2021), and plant growth promotion (Saeed, 2021) as zinc solubilizing bacteria (Kamran, 2017; Saxena, 2015). Some sphingomonas (especially Sphingomonas paucimobilis, in whose genome the rhodopsin SpaR was found (Okhrimenko, 2016, 2017, 2018)) can also be the cause of human diseases (Woo, 2014; Hardjo, 2016).
The maximum of absorption of retinal Schiff base (RSB) of S. paucimobilis rhodopsin (SpaR) solubilized in n-dodecyl-β-D-maltopyranoside corresponds to 540 nm at pH 7.5, and does not depend on pH in a range from 2,5 to 11. At pH values lower than 2.5 maximum of the absorption spectra is shifted to 570 nm due to the titration of proton acceptor group Asp 73 (corresponds to Asp 85 in H. salinarum bacteriorhodopsin). The pK of proton-acceptor group of SpaR was estimated as ~ 1,03. This value is much lower than in known proteorhodopsins (Friedrich, 2002; Mowery, 1979), for instance the pK of H. salinarum bacteriorhodopsin is ~ 2.7. SpaR has three histidine residues and chelates zinc ions but the changes of the absorption spectrum of RSB during titration at low pH and pKD73 in the presence of 10 or 5 mM Zn2+ is almost the same as in the absence of Zn2+, namely, in the presence of zinc pK(D73) ~ 0.98. The spectral changes with decreasing pH are reversible, but slight denaturation of SpaR at low pH is observed upon incubation of the SpaR at the lowest pH values. The maximum changes of OD in response of change of pH were observed at 515 nm and 615 nm, global fitting of OD changes at these wavelengths was done, then the results were normalized and plotted as fractions of protonated states. Fitting the dependence describing the bimolecular reaction (proton+D73) gave the above pK values at the inflection point of the sigmoidal curve. These values reflects the pK of proton acceptor group Asp 73 which is spatially close to RSB. The pKD73 of SpaR is notably low and do negligible depends on Zn2+ concentration. The study was supported by the RSF project 23-14-00160.
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### S3.221. Structure of proton half-channels of FoF1-ATP synthase in various types of model membranes

Ivontsin L.A.[1]*, Mashkovtseva E.V.[1,2], Nartsissov Y.R.[1,3]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Moscow, Russia;*
[2]*Pirogov Russian National Research Medical University, Moscow, Russia;*
[3]*Biomedical Research Group, BiDiPharma GmbH, Siek, Germany;*
* ivontsin@icmph.ru

Adenosine triphosphate (ATP), a high-energy compound is a universal source of energy for many biochemical processes and plays an important role in the metabolism and energy transmission in living organisms. In a cell, the ATP formation is carried out by the protein complex FoF1-ATP synthase using an electrochemical gradient of hydrogen ions. Proton translocation through the Fo membrane factor along two non-coaxial half-channels is one of the crucial processes in the catalytic cycle of the enzyme. However, despite the presence of a large number of high-resolution pdb-structures, the location and structure of the half-channels are still the subject of investigation, and parameters of proton transport had not been finally established yet.
To analyze the possible areas of proton motion, as well as to investigate the structural dynamics of amino acid side chains and protein hydration, we performed molecular dynamics simulation of the membrane part of the E. coli FoF1-ATP synthase [PDB ID: 6VWK] embedded in the lipid bilayer and water environment. The versatility of the molecular mechanism of the enzyme operation makes it possible to obtain information about the process when studying proteins from any organisms. We considered membranes with different lipid compositions, in particular, various levels of cardiolipins, which play an essential role in energy transducing processes maintaining the structure and functional activity of the respiratory complexes.
The inlet half-channel was a complex structure with two entrances in the form of water cavities and a highly conservative proton transfer chain near Asp61 of c-subunit including amino acids residues. However, a direct transition between amino acids was not always possible due to their distance from each other. The localization of three clusters of structural water molecules (W1-W3) critical for proton transport, necessary for the transport chain continuity, were established by analyzing the geometry of the mutual arrangement of the protein amino acid residues and the solvent. Wherein, the presence of cardiolipins in the membrane had a significant effect on the half-channels hydration, and an increase in the probability of direct proton transfer between some pairs of polar amino acids was also observed.
We found stable spatial positions (SP) of some amino acids side chains of the a-subunit, characterized by a constant set of parameters and called SP1, SP2, SP3, in all types of membranes. It has been established that aAsn214 in SP1 was oriented to aHis245, and in SP3 to cAsp61, change-over between which resembled the operation like a switcher between elements of an electric chain. Thus, the proton transfer chain is always unclosed, and switching between positions SP1 and SP3 of aAsn214 determines the time of proton transport [1].
While, the outlet half-channel was a water cavity and contained a high number of hydrophilic amino acid residues, which together with water molecules formed large networks of hydrogen bonds through which a proton can move from the key cAsp61 to the cytoplasm.
Thus, the obtained results give an idea of the membrane phospholipid composition impact on the location and structure of both half-channels in ATP synthase. This possible network of polar amino acids residues and water molecules allows us to simulate the possible proton trajectories through the membrane part of the protein, as well as to estimate the parameters of proton transport hardly detectable experimentally.
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### S3.222. Study of the effect of chronic treatment with uridine on the development of mitochondrial dysfunction in the myocardium of C57BL/6 mice with experimental diabetes mellitus

Belosludtseva N.V.[1,2]*, Starinets V.S.[1], Mikheeva I.B.[1], Dubinin M.V.[2], Mironova G.D.[1], Belosludtsev K.N.[1,2]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
[2]*Mari State University;*
* nata.imagination@gmail.com

Diabetes mellitus (DM) is a group of endocrine and metabolic disorders of different etiologies, which are associated with insufficient secretion of insulin from the β-cells of the pancreatic islets of Langerhans and variable degrees of peripheral insulin resistance resulting in hyperglycemia. The search for new approaches to the treatment of DM is one of the most serious global challenges facing modern science and the healthcare system around the world. Complications of diabetes include damage to many target organs, mainly the heart as one of the body's most energy-demanding organs. Diabetic cardiomyopathy manifests itself in significant abnormalities of both the structure and functions of cardiomyocytes, which may ultimately lead to the development of myocardial ischemic injury, heart attack, and death of diabetic patients. The cellular and molecular mechanisms contributing to the development of diabetic cardiomyopathy involve damage to cardiomyocytes from glycated end products and ROS, cardiomyocyte

glucotoxicity and lipotoxicity, altered substrate utilization and energy metabolism, impaired ion homeostasis, and mitochondrial dysfunction in the heart tissue.
Uridine is a pyrimidine nucleoside that plays an important role in maintaining cellular function and energy metabolism. As the UTP precursor, uridine can activate glycogen synthesis. Uridine metabolism is closely associated with glucose homeostasis, lipid metabolism, and amino acid exchange by regulating key enzymes and their reaction products, such as UTP, dihydroorotate dehydrogenase, and uridine phosphorylase, which are then involved in systemic metabolism. Uridine and its derivatives have been widely used to reduce cytotoxicity, suppress drug-induced hepatic steatosis, and improve neurophysiological functions. Our previous studies showed that uridine administration prevents myocardial injury in rat models of acute ischemia and ischemia/reperfusion by restoring redox balance and activating the mitochondrial ATP-dependent potassium channel. Recent data suggest that uridine can regulate the functioning of the mitochondrial respiratory chain. Therefore, the control of uridine levels in plasma and tissues can be coupled to mitochondrial function and systemic energy homeostasis. However, the effects of uridine in diabetes mellitus and insulin-resistant states remain poorly understood.
In this study, the antidiabetic potential of uridine and its effect on mitochondrial homeostasis in heart tissue was examined in the C57BL/6 mouse model of a high-fat diet/streptozotocin-induced diabetes. It was found that the chronic administration of uridine (30 mg/kg/day, i.p.) to diabetic animals resulted in a significant reduction in plasma glucose and triglycerides, as well as an increase in the rate of glucose utilization according to the intraperitoneal glucose tolerance test. Analysis of TEM micrographs showed that uridine preserved the ultrastructure and number of mitochondria in the cardiomyocytes of diabetic mice. The quantification of the expression level of the Ppargc1a, Pink1, Parkin, Drp1 and Mfn2 genes by RT-PCR revealed that uridine treatment restored mitochondrial biogenesis and stimulated mitophagy in the diabetic heart. This may lead to a reduction in the number of dysfunctional mitochondria and maintenance of mitochondrial health in the cardiomyocytes of diabetic mice treated with uridine. In parallel, uridine prevented diabetes-induced oxidative damage to the heart mitochondria and decrease in the capacity of oxidative phosphorylation, but had no significant effect on the mitochondrial calcium retention capacity and potassium ion transport. Taken together, the data obtained allow us to characterize the action of uridine as a metabolic drug capable of reducing the systemic consequences of diabetes and preventing the disruption of mitochondrial homeostasis coupled with bioenergetic defects and oxidative stress in the heart of mice with experimental diabetes.
The work was supported by the RSF grant No. 20-15-00120.

### S3.223. Study of the mechanisms of mitochondrial uncoupling by butyl(triphenyl)phosphonium analogs with substituents in phenyl rings

Rokitskaya T.I.[1]*, Khailova L.S.[1], Korshunova G.A.[1], Antonenko Y.N.[1]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow ;*
* rokitskaya@genebee.msu.ru

In recent years, conjugates of biologically important molecules with lipophilic cations have been actively synthesized and studied to increase the electrophoretic accumulation of such compounds in cell mitochondria. One of the best known mitochondria-targeted compounds are conjugates of triphenylphosphonium with the antioxidants ubiquinone or plastoquinone covalently cross-linked with a hydrocarbon decyl linker, MitoQ and SkQ1, respectively. It was found that in addition to the antioxidant action of MitoQ, SkQ1 and quinone-free alkyl(triphenyl) phosphonium cations cause uncoupling of respiration and oxidative phosphorylation on mitochondria due to the protonophoric action of the ion pair: lipophilic cation - fatty acid anion [1] or induction of nonspecific permeability of the inner mitochondrial membrane [2].
Previously, we showed that the flip-flop rate constant of dodecyl(triphenyl)phosphonium analogs [3] and the permeability of butyl(triphenyl)phosphonium analogs (C4TPP-X) depend significantly on substituents in phenyl rings [4]. Therefore, one can expect significant differences in the protonophoric activity of the ion pair (analogue of the butyl(triphenyl)phosphonium and a fatty acid) in model and biological lipid systems.
On isolated rat liver mitochondria C4TPP-X cations led to an increase in the rate of respiration and a decrease in the potential of the inner membrane. The efficiency of these processes increased significantly in the presence of palmitate and correlated with the partition coefficient in the octanol-water for lipophilic cations. The ability of C4TPP-X cations to induce proton transport across the lipid membrane of liposomes loaded with the pH-sensitive fluorescent dye pyranine also increased with their lipophilicity and depended on the presence of palmitic acid in the membrane-forming composition. Of all the studied cations, only butyl [tri(3.5-dimethylphenyl)]phosphonium (C4TPP-diMe) was able to induce proton transport through planar bilayer lipid membranes and liposomes by the mechanism of ion pair formation (cation–fatty acid). The rate of oxygen consumption by mitochondria in the presence of C4TPP-diMe increased to the maximum values corresponding to conventional uncouplers; for all other cations the maximum uncoupling rates were significantly lower. The most lipophilic cations of the C4TPP-X series at high concentrations led to swelling of isolated rat liver mitochondria in media containing potassium chloride or sucrose. We assume that the studied cations of the C4TPP-X series, with the exception of C4TPP-diMe at low concentrations, cause nonspecific leak of ions through lipid model and biological membranes which is significantly enhanced in the presence of fatty acids. Thus, the uncoupling of respiration and mitochondrial phosphorylation occurs mainly by a mechanism similar to the detergent one.
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### S3.224. Study of the molecular mechanism of proton pumping in heme-copper respiratory oxidases in real time

Siletsky S.A.[1]*
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University;*
* siletsky@genebee.msu.ru

To elucidate the organization and mechanism of coupled proton transfer in heme-copper terminal oxidases, a prestationary study with adequate temporal resolution of a type aa3 cytochrome oxidase from R.sphaeroides with a mutation of the key residue in the proton-conducting D channel was carried out. The studied mutant oxidase retains functional oxygen-reductase activity to a significant extent in stationary measurements. The study of the kinetics of membrane potential generation by a mutant enzyme embedded in proteoliposomes indicates a slowdown in the stages of proton transfer, while maintaining the ability to pump protons through the membrane as a whole. In the kinetics of the generation of the membrane potential of the mutant enzyme, a

number of important differences from the wild type were revealed, indicating a different articulation of the stages of coupled proton pumping in the catalytic cycle, despite the preservation of functional activity. The study allows us to draw conclusions about significant changes in the electrogenic mechanism of proton pumping in the mutant enzyme, indicates a significant stability of the organization of elements in the mechanism and provides new information about the organization of proton transfer in the D-channel in the mutant oxidase and in the wild type.

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### S3.225. The effect of mitochondrial DNA mutation complex on the content and production of ATP in cells

Kazakov M.S.[1]*, Shitikova E.Yu.[1], Vinokurov A.Yu.[1]
[1]*Oryol State University named after I.S. Turgenev;*
* kms898@mail.ru

Kazakov M.S.(1), Shitikova E.Yu.(1), Vinokurov A.Yu.(1)
(1) Laboratory of Cell Physiology and Pathology of the STC Biomedical Photonics Orel State University named after I.S.Turgenev,

The number of diseases associated with mitochondrial DNA (mtDNA) mutations is approaching 400 [1]. The possibility of pathology manifestation depends on the localization, the level of heteroplasmy, and the combination of mutations. Mutations in mtDNA can negatively affect ATP synthesis due to disturbances in the electron transport chain (ETC), initiating the appearance and development of a number of diseases [2]. Therefore, the aim of this work is to investigate the effect of mtDNA mutation combinations on ATP content and synthesis.

Materials and methods

The lines of cytoplasmic hybrids (cybrids) (TCP, TCN, TCI, HSM1, HSM2, LSM1, LSM2, MAM1, MAM2, MAM3, 520, 521, 522) based on THP cells, each having from 5 to 10 mtDNA mutations with different heteroplasmy levels, affecting genes one (m.3336 T>C) (depending on the lineage, the level of heteroplasmy varies from 0% to 37%), the second (m.5178C>A) (0% to 22%), the fifth (m.13513G>A) (10% to 68%), the sixth (m.14459 G>A) (0% to 61%) subunits of complex I, cytochrome b (m.15059G>A) (0% to 38%), (m.14846 G>A) (0% to 55%), 12S rRNA (del652G) (0% to 44%), (m.1555A>G) (0% to 28%), and tRNA(Leu) (m.c3256C>T) (0% to 50%), (m.12315G>A) (0% to 44%). To analyze the physiological level of ATP, we used a luciferase method using an ATP determination kit (LifeTechnologies, USA). Luminescence levels were monitored using a FLUOstar Omega fluorimeter. Depletion time of cellular ATP was measured by fluorescence microscopy using magFura-2 probe at excitation wavelengths of 340 nm (Mg-bound form) and 380 nm (free form). Before the study, cells were incubated in 3 μM probe solution. ATP synthesis was blocked by adding oligomycin A (2 μg/ml) and iodoacetic acid (100 μM). The moment of a sharp increase in the fluorescence ratio of 340 nm/ 380 nm was a signal of ATP depletion. To assess the conjugation of oxidative phosphorylation, we performed a polarographic respiration study using an Oxytherm+R respirometer. HBSS with 10 mM glucose content was used as measuring medium. During the study, baseline oxygen consumption rate was analyzed and after adding the ATP synthase inhibitor oligomycin A (2 μg/ml).

Results and discussion.

A statistically significant decrease in ATP content relative to the THP line (from 1.9-fold for TCN to 19-fold for LSM1) was observed in almost all cybrid lines. This change may be a consequence of both impaired ATP synthesis and increased macroerg consumption.

To confirm the theory of increased ATP consumption, studies were performed using the ratiometric fluorescence probe magFura-2. The results showed that most of the lines studied had no less ATP depletion time than THP (from 3.8 h. in 522 to 7.3 h. in LSM2 and 4.9 h. in THP). This parameter does not correlate with the data on ATP content. One reason may be the high level of cell dissociation, which was assessed by a cell respiration rate study. All cybrid lines had reduced respiration rates compared with THP (from 33 ng(O2)/(min*10^6 cells) in LSM1 to 54 ng(O2)/(min*10^6 cells) in MAM2 and 64 ng(O2)/(min*10^6 cells) in THP). A statistically significant response to oligomycin A was observed for THP, MAM1, and MAM2 lines (23%, 15%, and 18%, respectively). Thus, the rate of oxygen consumption associated with ATP synthesis is relatively low in the case of all cell lines (mean change of about 14%) because of possible uncoupling, which may be a tool to reduce the negative effects of mitochondrial dysfunction associated with mutations of the rRNA (del652G), tRNA (m.3256 C>T, m.12315G>A) and ETC complexes (m.15059G>A, m.14846G>A, m.5178C>A) in mtDNA [3]. At the same time, data from the MAM1 and MAM2 lines show a higher level of mitochondrial function despite a significant mutational load. In our opinion, this may be due to a high level of heteroplasmy mutations of complex 5 subunit I (m.13513G>A) and 12S rRNA (m.1555A>G), for which a number of studies have shown a negative correlation with the development of atherosclerosis [4, 5]. This is probably due to the fact that the m.1555A>G mutation prevents the synthesis of defective ETC proteins due to ribosome disruption, while the m.13513G>A mutation leads to an increase in Complex I functionality.

Thus, disruption of ATP metabolism and further development of the pathology is the result not only of the heteroplasmy of individual mutations, but also of their mutual influence, which can have both negative and compensatory character.

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### S3.226. The effect of short-term protein-carbohydrate deficiency in nutrition on memory indicators

Bakhshaliyeva A.Ya.[1]*
[1]*Institute of Physiology n. a. academician Abdulla Karayev of the Ministry of Science and Education of the Republic of Azerbaijan, Baku.;*
* afetfarm@mail.ru

It is known that the energy necessary for the vital activity of the body is the substrate of the processes of decomposition of metabolites entering the blood during digestion, which provides metabolism, structural and functional activity of the cell. Metabolites entering the bloodstream during digestion, acting as stimuli, accelerate energy production by intensifying the functions of intracellular metabolism, which in turn activates the genetic apparatus and ensures its functional activity. Along with proteins, carbohydrates are also important as the most

important factors of genetic expression, which, as a signaling molecule, regulate a set of genes and stimulate the implementation of various physiological processes.
Considering that cellular plasticity depends on the interaction of proteins and carbohydrates, it seems appropriate to study changes in the adaptive properties of the body, reflex activity and memory functions against the background of a deficiency of both proteins and carbohydrates in the diet. The purpose of our research work was to observe changes in learning and memory processes, as well as the general behavior of experimental animals against a background of 60% protein and 40% carbohydrate deficiency in feed for 20 days.
The studies were carried out on 15 white male rats of 3 months of age. Experimental animals, 5 rats each, were divided into 3 groups: I - intact group fed under vivarium conditions; II - fattening group with 60% protein deficiency (P/D) according to Nikonorov's recipe (Nikonorov M. et al., 1973); III - fattening group with 60% protein deficiency and 40% carbohydrate deficiency according to Nikonorov's recipe (P/D + CH/D). On the 20th day of feeding, all three groups of animals were taught and tested the conditioned reflex of passive avoidance (CRPA) (Jarvik M.E., Koop A. 1967). During the studies, the following behavioral indicators were recorded: training time, latency period, grooming, vertical motor activity, horizontal motor activity and the number of defecation acts.
Behavioral results showed that the training time of rats fed with protein deficiency (P/D) decreased by 20.2% ($p<0.05$) compared to the intact group. But in the group fed with a deficiency of proteins and carbohydrates (P/D + CH/D), this indicator increased by 11.7% ($P<0.01$).
After the development of a conditioned reflex in rats of both groups there was a decrease in the latency period compared to the intact group: in P/D rats, the decrease was 20.8% ($p<0.01$), and in P/D+CH/D rats 41.5% ($p<0.001$). In comparison with the intact group (100%), the P/D group has vertical motor activity by 16.7% ($p<0.05$) and grooming by 22.9% ($p<0.01$) higher, which indicates an active state and preservation of the reflex. At the same time, a decrease in horizontal motor activity by 19.5% ($p<0.01$) and an increase in defecation by 28.1% ($p<0.01$) indicate emotional stress in rats.
With protein-carbohydrate deficiency nutrition, there is a decrease in the activity of vertical movements of rats by 22.9% ($p<0.01$) and grooming by 25% ($p<0.01$) compared with the intact group. Against the background of these indicators, a decrease in horizontal motor activity by 63% ($p<0.001$) is considered an indicator of emotional stress and immobility in rats. In addition, a 12.5% decrease in the number of bowel movements in rats ($p<0.05$) may indicate a relative decrease in overall metabolism.
From a comparative analysis of the behavior results, it can be concluded that the indicators of rats fed with 60% protein deficiency for 20 days differ imperceptibly from the indicators of the intact group. However, in rats receiving 60% protein deficiency and 40% carbohydrate deficiency, mild delays in memory functions were observed, and in the general behavior of animals, sedentary activity was manifested due to mild emotional stress.
It is known that neurotransmitters, enzymes, hormones of protein and peptide nature are involved in the implementation of the main mechanisms of complex neurohumoral functions of reflex activity, learning and memory processes. On the other hand, metabolic homeostasis is always under the control of transcription, and the regulation of transcription can be influenced by metabolites of intermediate metabolism (glucose, amino acids, lipids, cholesterol). In conditions of prolonged protein starvation, reversible and irreversible changes occur in all oxidative processes of the central nervous system, which sometimes lead to the destruction of neurons. In this case, the signals of transcription factors are integrated and use alternative regulatory pathways. And also, with insufficient provision of the body with polysaccharides, hypoglycemia has a negative effect on the nervous tissue, causing destructive changes in neurons and related nerve dysfunction and the occurrence of glycemic encephalopathy.
If we take into account that glucose is a plastic and energy substrate of nerve cells, then against the background of protein deficiency, the negative impact of carbohydrate-deficient nutrition and the associated occurrence of hypoglycemia on the violation of metabolic homeostasis, general metabolic processes and energy supply of neurons in the nervous tissue is inevitable. A sharp decrease in the energy supply of a nerve cell may eventually lead to dysfunction of neurons and the associated violation of conditioned reflex activity.

### S3.227. The terminal oxidase cytochrome bd-II from Escherichia coli serves as the hydrogen peroxide scavenger

Borisov V.B.[1]*, Nastasi M.R.[2], Forte E.[2]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory, 119991 Moscow, Russia;*
[2]*Department of Biochemical Sciences, Sapienza University of Rome, I-00185 Rome, Italy;*
* viborborbor@yahoo.com

Cytochrome bd-II is a terminal oxidase of the electron transport chain of Escherichia coli. The enzyme reduces molecular oxygen to water using electrons derived from quinol and couples this redox reaction with generation of the proton motive force. The latter is used by bacteria to produce ATP and perform other types of work. Cytochrome bd-II is composed of subunits AppB, AppC and AppX. AppB carries the site for quinol binding and three hemes, the low-spin b-558 and the high-spin b-595 and d [1]. The bd-II oxidase still remains poorly characterized. Its physiological roles are not clear but seem to differ from those of other E. coli terminal oxidases such as cytochrome bd-I and cytochrome bo. Intriguingly, in the presence of hydrogen peroxide (H2O2), cytochrome bd-II confers a fitness advantage during anaerobic growth to E. coli in the inflamed murine intestine and to Salmonella in the streptomycin-treated gut [2]. We decided to establish whether the bd-II oxidase plays a role in H2O2 metabolism and tolerance, in addition to its contribution to conservation and transformation of energy by the E. coli membranes. By using high-resolution respirometry and spectrophotometry we showed that preparations of the detergent-solubilized cytochrome bd-II isolated from E. coli are capable of rapid degradation of H2O2. The reaction proceeds with generation of half a mole of O2 per mole of H2O2. The reaction rate increases with the increase of [H2O2] up to 0.5 mM, however at higher [H2O2] it tends to saturate. The observed activity is insensitive to N-ethylmaleimide that excludes participation of the protein thiol groups in the reaction. The lack of inhibitory effects of antimycin A and ubiquinone-1 suggests that the quinol binding site of the enzyme is also not involved in the reaction. CO and NO targeting the reduced heme d do not affect the activity as well. Furthermore, the addition of H2O2 in the presence of dithiothreitol and ubiquinone-1 does not inhibit the O2 reductase activity of cytochrome bd-II and does not lead to its inactivation. These data indicate that heme d, at which the four-electron reduction of O2 occurs, hardly takes part in the H2O2 degradation. In contrast, the reaction is inhibited by cyanide ($IC50 = 4.5$ μM) and azide which usually target a high-spin oxidized heme. Since the bd-type oxidase has two high-spin hemes, b-595 and d, but the latter is unlikely to participate in the observed reaction, heme b-595 could serve as the site in the enzyme responsible for the catalytic decomposition of H2O2. The ability of the bd-II oxidase to efficiently scavenge H2O2 may play a role in bacterial physiology by conferring resistance to oxidative stress.
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**S3.228. Variability of H+/O stoichiometry of electron transport chain complexes in different functional states of mitochondria. Research methods, modifying agents, and possible physiological significance**

Samartsev V.N.[1], Semenova A.A.[1], Belosludtsev K.N.[1,2], Dubinin M.V.[1]*
[1]*Mari State University, Yoshkar-Ola, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* Dubinin1989@gmail.com

The transport of electrons by complexes of the electron transport chain (ETC) in mitochondria is associated with the transfer of H+ from the matrix to the intermembrane space. This leads to the storage of energy in the form of proton-motive force (Δp) expressed in volts. The Δp energy is spent on the synthesis and transport of ATP and is partially dissipated due to passive leakage of protons [1]. In the absence of ATP synthesis (in state 4), passive proton leakage is considered as one of the mechanisms of oxygen consumption by mitochondria. This so-called free respiration (oxidation) is of great physiological importance [1]. To characterize the generation of Δp by mitochondrial ETC complexes, the stoichiometric H+/O (H+/2e–) ratio is used [1, 2]. Methods for determining the H+/O ratio (H+/2e–) for ETC complexes in isolated mitochondria are based on recording the amount of protons transferred across the membrane in exchange for a penetrating cation (K+ in the presence of valinomycin) [2]. Such energy-dependent transport of K+ makes it impossible to directly determine the values of the H+/O ratio (H+/2e–) during oxidative phosphorylation and free respiration. It is theoretically substantiated that the values of the H+/2e– ratio during oxidative phosphorylation are 4, 2, and 4 for complexes I, III, and IV, respectively [1]. In contrast to this, as we assumed earlier [3], during free respiration in mitochondria, the values of the H+/O ratio are 4, 4, and 2 for complexes I, III, and IV, respectively.
To determine the values of the H+/O ratio (H+/2e–) of complexes III and IV during free respiration of mitochondria, a new method based on the selective shutdown of one of them is proposed. Thus, during succinate oxidation by mitochondria, selective deactivation of complex III will lead to a decrease in the H+/O (H+/2e–) ratio to the value characteristic of complex IV. In this work, we used N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) capable of shunting ETC complex III [4] and α,ω-hexadecanedicarboxylic acid (HDA), switching this complex to idle mode [5].
The experiments were carried out on mitochondria isolated from the liver of white rats by the conventional method of differential centrifugation. Mitochondrial respiration was recorded by the polarographic method. The protonophoric activity of the studied compounds was determined by inducing swelling of deenergized mitochondria in an isotonic potassium acetate solution in the presence of valinomycin.
We have found that TMPD and HDA, stimulating mitochondrial respiration, a) do not significantly affect the efficiency of ATP oxidative synthesis, b) do not increase passive proton leakage, and c) do not affect energy transformation by complex IV (cytochrome c oxidase). Consequently, when succinate is oxidized by liver mitochondria under conditions of free respiration, TMPD and HDA selectively disable ETC complex III from energy transformation. The rate of passive leakage of protons in state 4 can be determined as the product of the respiration rate and the H+/O ratio [6]. We have theoretically substantiated that under these conditions the H+/O coefficient can be determined from the ratio of respiration rates in the absence and presence of TMPD and HDA. Based on this model, the change in the H+/O ratio depending on the stimulation of mitochondrial respiration by TMPD and HDA in state 4 decreases and reaches a minimum value – 2. The H+/O ratio reaches a similar value at the maximum stimulation by TMPD in the presence of HDA and by HDA in the presence of TMPD. Consequently, the action of TMPD and HDA, when their maximum effects are reached, is non-additive. It is quite probable that under the combined action of TMPD and HDA up to the maximum level, complex III is completely switched off from the generation of Δp. Under these conditions, only complex IV will take part in the generation of Δp, and the H+/O ratio characterizing the operation of this complex is 2.
Thus, in liver mitochondria during free respiration, in contrast to the oxidative synthesis of ATP, the values of the H+/O ratio are 4 and 2 for complexes III and IV, respectively. It is known that during the synthesis and transport of ATP, the movement of protons from the intermembrane space into the matrix is associated with the performance of work [1]. In contrast, the free respiration of mitochondria is carried out by simple diffusion of protons through the inner membrane without doing work. Obviously, the rate of such diffusion depends on the total number of protons released by the ETC complexes into the intermembrane space. Induction of free respiration in liver mitochondria by disabling ETC complex III from energy transformation is considered as one of the "ways of salvation" for hepatocytes in various pathological conditions accompanied by impaired carbohydrate and lipid metabolism and increased oxidative stress.
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## S4. Biomechanics. Biological mobility

**S4.229. A combination of multiscale methods for studying the biomechanical properties of three-dimensional cell constructs**

Efremov Y.M.[1]*, Presniakova V.P.[1], Zurina I.M.[1], Koteneva P.I.[1], Kosheleva N.V.[1], Timashev P.S.[1]
[1]*Institute for Regenerative Medicine, Sechenov University;*
* yu.efremov@gmail.com

Three-dimensional multicellular constructs obtained in vitro can be considered as an intermediate level of organization between individual cells and complex tissue structures presented in vivo. Multicellular structures are increasingly used for both fundamental research and practical applications [1, 2]. For example, cell sheets are multilayer and flat, while spheroids are spherical, and both are self-organizing multicellular structures and the simplest models of cell aggregates that have developed intercellular interactions and paracrine signaling, and also partially recreate the structural complexity of native tissues containing an extracellular matrix (ECM). Both spheroids and cell sheets can be used in tissue engineering as scaffold-free constructs, material for bioprinting, or in combination with various scaffolds [1, 2].
Many previous studies have focused on the biology of multicellular structures; however, very little data is available on their mechanical properties [3]. Understanding the mechanical behavior of such structures is a necessary step to establish the fundamental principles of tissue biomechanics, which are closely related to the mechanisms of new tissue formation, regeneration, and development of pathologies. Mechanical interactions affect the formation of cell aggregates, the processes of their rearrangement and fusion, as well as the viability and functioning of individual cells in their composition. Mechanical property tuning is a promising way to control these processes.
One of the available methods for studying the mechanical properties of cells and ECM is atomic force microscopy (AFM) [4], which is used to

evaluate the local viscoelastic properties of materials on a nanoscale. However, the AFM method is limited to the surface layer of cells, while the assessment of the contribution of ECM elements to the mechanics of the formed cell constructs requires methods with a greater degree of material deformation. Such macroscopic methods include squeezing or stretching the construct as a whole. Combining data obtained by different scale methods requires the use of certain mechanical models that can describe the biomechanical behavior of constructs at different scales.

In this work, we used mouse and rat fibroblast lines, as well as human mesenchymal stromal and epithelial cells. With the help of simultaneous monitoring of morphology and mechanics at the level of single cells and multicellular constructs using AFM, data were obtained indicating the relaxation of stresses in the cytoskeleton during the detachment of cells from the substrate and formation of the cell sheet. The method of microindentation was also used, which showed that the role of ECM can increase with an increase in the time of cultivation of the cell sheet.

To study spheroids, in addition to AFM, the method of compression between plates was used. Comparison of the data of these two methods made it possible to establish the effective surface tension of the spheroid, as well as the role of the ECM in macroscopic deformation. To describe the biomechanics of spheroids, mechanical models of an elastic body with surface tension, viscoelastic and poroelastic bodies were used, whose combination made it possible to describe the difference in the mechanical behavior of spheroids from two different types of cells, mesenchymal and epithelial. The spheroids from mesenchymal cells had a higher surface tension and a denser packing of the ECM in the inner part, which manifested itself in a higher surface rigidity according to AFM data and longer relaxation times during compression between the plates [5]. The results obtained will contribute to a more detailed description of the biomechanics of cell sheets, spheroids, and tissues, and can also be used in modeling the processes of cell sheet formation, fusion of spheroids, and to control their mechanical properties.

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### S4.230. A decrease in AMPK activity accelerates the differentiation of primary myoblasts isolated from atrophied rat soleus

Vilchinskaya N.A.[1]*, Mirzoev T.M.[1], Shenkman B.S.[1]
[1]*Institute of Biomedical Problems of RAS;*
* vilchinskayanatalia@gmail.com

Functional unloading of skeletal muscles leads to the development of atrophic processes and a decrease in the total number of satellite cells that regenerate these muscles. Under mechanical unloading conditions was observed acceleration of rat soleus myoblasts differentiation and a decrease in the activity of AMP-activated protein kinase (AMPK). It can be assumed that a decrease in AMPK activity contributes to the acceleration of myoblast differentiation and enhanced formation of myotubes after mechanical unloading. The main goal of the study was to estimate the involvement of AMPK in the regulation of differentiation in myoblasts derived from atrophied rat soleus muscle.

To test this hypothesis, a specific AMPK activator, AICAR, was used to prevent a decrease in AMPK phosphorylation during differentiation of myoblasts isolated from rat soleus after a 7-day functional unloading. Real-time PCR was used to evaluate the level of expression of MRF, myoblast fusion factors, and the expression of various MyHC isoforms. Using immunocytochemistry methods, the myotube differentiation index was determined. The level of AMPK activity was measured by Western blotting.

In differentiating myoblasts derived from the atrophied rat soleus, there was a decrease in the content of phospho-AMPK, phospho-ACC and phospho-rpS6 compared to control myoblasts; when such myoblasts are incubated with AICAR, the content of these proteins does not differ from the level of the control group. The use of AICAR at differentiation of the myoblasts isolated from the soleus after functional unloading prevents an increase in the expression of mRNA of MyoD, Miogenin, Mymx and fast isoforms of MyHC IIb and IIdx, and promotes to maintain the mRNA expression of slow myosin isoformsMyHC I and IIa. In differentiating myoblasts obtained from rat soleus, after 7 days of mechanical unloading, an increased index of myotube differentiation is observed; under AICAR treatment on the myoblasts, the level of myotube differentiation returns to the values of the control group.

Thus, the accelerated differentiation of myoblasts isolated from atrophied rat soleus muscle is compensated by maintaining a control level of AMPK activity using AICAR. At the same time, increased expression of MRF, myoblast fusion factors, and MyHC isoforms is normalized. The work is supported by the Russian Science Foundation grant 20-75-10080.

### S4.231. About the topological structure of multicellular animals

Antonets V.A.[1,2]*
[1]*Institute of Applied Physics of RAS;*
[2]*Lobachevsky University;*
* antonetsva@gmail.com

The report considers the impact of well-known physical laws of motion of atoms and small molecules on the emergence and formation of mechanisms for the trophic supply of plastic and energy processes in multicellular organisms.

According to modern views, multicellular organisms have arisen and returned to single-celled existence multiple times during evolution. Rare, intermediate forms of life between single-celled and multicellular are observed even now.

Upon the emergence of multicellular organisms, the breathing mechanism of each cell remained diffusive, just as it was in single-celled organisms. The feeding mechanism changed and became diffusive, as the cells incorporated into a single organism cannot consume similar cells, as occurs in single-celled organisms.

However, the energy and plastic supply of each cell of a macroscopic organism, due to direct diffusion absorption of substances from the surrounding environment through its external shell, is not possible for two reasons.

Firstly, the time of substance diffusion delivery to a certain distance R is proportional to the square of that distance. So it can be stated that there is a lethal size at which all necessary for nutrition and respiration simply will not arrive in time in the internal cells of a macroscopic multicellular organism.

Secondly, as the number of cells in a hypothetical multicellular organism increases, the area of its outer surface increases more slowly than its volume. The number of cells in a multicellular organism objectively characterizes its need for substances (plastic supply) and energy. The surface area determines the maximum possible diffusion delivery of substances. And since the requirements grow faster than the possibilities, an incompatible with life deficit inevitably arises.

Most multicellular animals became viable by switching to a different type of nutrition. Not being autotrophs, they became consumers, consuming comparable in size fragments of other multicellular organisms – plants and animals, to the point of consuming prey whole. This adaptation occurred due to the synchronous formation in the body of the first multicellular animals of a system for the destruction of ingested food to the molecular level (digestion), as well as structures with developed surfaces that occupy a small physical volume but provide sufficient contact area for:

- gas exchange between the organism and the environment,
- absorption of food broken down to the molecular level,
- transportation to each cell of the body of oxidant and food molecules.

For humans, these are easily recognizable light organs with alveoli, a delicate intestine with numerous absorbing villi and a circulatory system with a network of active arteries and veins connected by capillaries. All three systems have a fractal character. The lungs and the vascular network have a branching tree-like structure with merging "crowns", where oxygen exchange occurs. The inner surface of the thin intestine has a brush-like structure. Through the villi, proteins and carbohydrates enter the venous network, and fats into the lymphatic.

It seems that speaking further is like to knock at an open door. Nevertheless, the question about the structure and transport function of the circulatory system remains unanswered. Following an old tradition, in textbooks and anatomical atlases, the circulatory system is considered as two loops (circles) providing oxygen exchange. Meanwhile, the transport of food molecules is almost not discussed or discussed briefly. Accordingly, blood is marked as arterial and venous, i.e. oxygen-rich and oxygen-deprived.

If we index the blood and the molecule saturation of food, then the blood circulation looks significantly different. The consumer of transportable molecular nutrition remains the same - the cells of the organism, and the source becomes the small intestine.

Meanwhile, if the oxygen input and carbon dioxide emission are concentrated in the lungs, then the transition from chyme of carbohydrates and proteins to blood, and fats to lymph, occurs in the small intestine. This can be a starting point for the circulation of carbohydrates, proteins, and fats in the organism. And this circulation begins not through the arterial vessel, but through the portal vein. In the discharge of processed products, several systems are always involved. First of all, this is the liver, kidneys, and large intestine. In the case of heavy physical exertion, the skin is involved in the discharge, and in pathological cases, the lungs.

The geometric structure of the capillary network in the lungs and small intestine is well understood, with merged "tree crowns". However, the embedding of the capillary network in the continuum of tissues such as muscle, liver, and kidney is not as clear.

Molecule absorption in capillaries occurs quite quickly, causing the concentration of molecules to drop rapidly from the entrance to the exit of the capillary, limiting the effective length of transport. The author was unable to find any information on how this embedding is structured geometrically. However, the constraints on the effective length of capillaries suggest that it represents an embedding of layers with a thickness equal to the length of a Krogh's cylinder.

With the proposed view, there arises not only a problem of the geometric embedding of a branching vascular network into the continuum of tissues, but also a problem of the stoichiometric balances of oxygen, protein, and fat flows, as well as the removal of heat flows generated by physical work and chemical reactions that sustain the functioning of the organism, into the surrounding space.

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**S4.232. Analysis of the postural balance of badminton athletes in the realisation of the positonic reflex on head turns**

Egorova V.K.[1]*, Baltin M.E.[1,2], Fedyanin A.O.[1,2], Yafarova G.G.[1]
[1]*Kazan federal university;*
[2]*Volga Region State University of Physical Culture, Sport and Tourism;*
* veronikatsyupa@gmail.com

Positonic reflexes ensure the preservation of balance when changing the position of the body. In an adult, these reflexes are under inhibitory control from the supra-stem structures. The study of the reaction of the postural system in the implementation of position reflexes in professional athletes is relevant. The aim of the work is to determine the change in plantar pressure during the realization of the reflex to head turns in badminton athletes.

The study involved 12 athletes and 12 subjects not involved in any sport (control group), aged 19 to 23 years. A plantographic study was carried out with a duration of 20 seconds in a standard rack, as well as when turning the head to the right and left. The coefficient of lateral asymmetry (Cl) was calculated by the ratio of the average plantar pressure of the right foot to the left; a test was also conducted to determine functional motor asymmetry.

In the group of athletes, 83% (n=10) revealed a cross motor asymmetry: the leading leg is the left, the leading arm is the right. In the control group, only 25% of the subjects (n=3) had cross-motor asymmetry; 58% (n=7) had right-sided motor lateralization, and 17% (n=2) had left-sided lateralization.

According to the Cl in the standard rack (head straight), each group was divided into 3 categories according to the severity of the support lateralization: 1) right support lateralization (RSL), Cl >1.15; 2) left support lateralization (LSL), Cl <0.85; 3) ambidextrous support asymmetry, Cl = 0.85 - 1.15. In the group of athletes, RSL was registered in 3 subjects, their leading motor limb was the left; 2 out of 3 athletes with LSL leading leg had the left and 1 had the right. 6 athletes were ambidextrous in reference lateralization, while 5 of them had left–sided motor asymmetry, and only 1 had right-sided.

In the control group, 3 subjects had RSL, the leading motor limb in 2 of 3 was the right, 1 was the left; a similar distribution was in subjects with LSL (n=3, 2 with right-sided motor asymmetry, 1 – left-sided). 6 people in the control group were ambidextrous in reference lateralization, while 4 of them had right–sided motor asymmetry, 1 - left-sided, 1 subject was ambidextrous in motor asymmetry.

In the group of athletes with RSL, when turning towards the supporting limb (to the right), there was a tendency to increase the pressure of the contralateral limb (Cl decreased by an average of 2%). In athletes with LSL, a left turn caused an increase in the pressure of the ipsilateral limb by an average of 6%. Turning in the opposite direction from the supporting limb in athletes with RSL did not lead to a change in Cl, and in subjects with LSL, turning the head to the right side led to a slight decrease in the pressure of the supporting limb, Cl increased by an average of 11% and amounted to 0.78 ± 0.07. In athletes with ambidextrous lateralization, turning the head both to the right and to the left caused an increase in pressure of the left limb by an average of 9 and 6%, respectively.

In the control group, who have RSL, head turns also led to a redistribution of plantar pressure in the subjects from the floor: however, when

turning towards the support, the Cl increased by an average of 4%, which indicates an increase in the pressure of the ipsilateral limb; and when turning the head to the left, the pressure of the right foot also increased in these subjects (Cl increased by an average of 8%) whereas in athletes, we did not observe a change in plantar pressure when turning the head in the opposite direction from the supporting limb. In the subjects of the control group with a predominance of LSL, head turns also led to increased pressure of the right limb: when turning towards the supporting foot, Cl increased by an average of 16%, when turning to the right – by 14%. In this group, a similar pattern was observed in ambidextrous: the increase in pressure of the right leg when turning right and left averaged 6 and 5%, respectively.

Thus, in the group of athletes, cross-motor and support asymmetry prevailed (10 out of 12 subjects), whereas in the control group, subjects with cross-asymmetry accounted for only 25%. In athletes with LSL, when turning towards the support, there was a tendency to increase pressure on the ipsilateral limb, and in non-sports subjects, when turning to the left, a transfer of support pressure to the contralateral limb was observed, which indicates that athletes with LSL retain support lateralization when turning towards the support. The subjects with RSL (both athletes and the control group) retained right-sided support lateralization when turning towards the support.

Turning in the opposite direction from the supporting limb in athletes with RSL did not cause any changes, whereas in control subjects, turning to the left led to an increase in the pressure of the ipsilateral limb. Both in athletes and in the control group, in subjects with LSL, turning the head in the opposite direction from the support caused an increase in the pressure of the contralateral limb.

Thus, the predominance of cross-motor/support asymmetry in athletes may allow them to improve their capabilities when initiating a motor act of the lower extremities. In athletes, a more pronounced preservation of the supporting lateralization is revealed when turning the head, whereas in the control group, destabilization of the supporting lateralization is more common in similar conditions. These results may indicate the restructuring of the motor system in professional athletes, including those manifested in the preservation of postural balance during the implementation of cervical-tonic reflexes.

The work was carried out within the framework of the program "Strategic academic Leadership of Kazan Federal University" (PRIORITY-2030).

**S4.233. Application of computational fluid dynamics to solution of actual cardiovascular tasks**

Kuchumov A.[1]*
[1]*PNRPU;*
* targs2@gmail.com

The development of non-invasive diagnostic methods in modern surgery and mathematical and computer models makes it possible to describe the biomechanical processes occurring in the body with an increasing degree of accuracy. This increases the possibility of their use in the improvement of existing and the development of new personalised methods of diagnosis and treatment prognosis. Computational fluid dynamics is a dynamic tool for engineering and interdisciplinary problems. Medicine is one of the fields where the application of computational methods and technologies is essential. One of the important aspects is the consideration of the application of biomechanics and computational fluid dynamics methods. In the present work the results of computational fluid dynamics methods application in solving cardiovascular surgery problems (blood flow modeling to estimate the efficiency of bypass at aortopulmonary anastomosis in children with congenital heart disease, estimation of hemodynamic parameters in aortic valve in norm and pathology, modeling of blood flow in stenting) are presented.

Blood flow modelling to assess the effectiveness of bypass for aorto-pulmonary anastomosis in children with congenital heart disease

Based on magnetic resonance imaging, an aorta-pulmonary artery-shunt system was constructed to analyse haemodynamics in children with congenital heart disease. Blood was treated as a Newtonian fluid (density, 1060 kg/m3; density, 0.0035 Pa-s). Boundary conditions were obtained from ultrasonic measurements.

In this paper, an aorta-shunt-pulmonary artery blood flow model was considered. A set of geometric images in four patients was obtained for subsequent import into the ANSYS CFX finite element solver to solve the haemodynamics problem. Three variants of modified Blalock-Taussig shunt placement were analyzed using common hemodynamic indices (wall shear stress, time-averaged wall shear stress, oscillatory shear index). It was revealed that the variants of shunt formation should be individual, i.e., take into account anatomical and physiological features of a particular patient. The asymmetry of blood flow in the pulmonary arteries in different locations of the shunt implantation was noted. Hemodynamic performance was also compared to assess the effectiveness of the modified Blalock-Taussig shunt. An objective and personalised approach to the specific treatment of each individual patient will significantly reduce pediatric mortality and improve the quality of rehabilitation.

Assessment of hemodynamic parameters in the aortic valve in normal and abnormal conditions

This paper analyses the use of two approaches to simulate turbulent processes: using the large vortex method and based on turbulent viscosity models. The axisymmetric problem was solved on an idealised three-dimensional geometry constructed on the basis of ultrasonic image data and literature review. The problem was solved within the FSI approach using the COMSOL Multiphysics software package. Blood flow is modelled as an incompressible Newtonian fluid with constant density and viscosity.

The Holzapfel-Hasser-Ogden anisotropic hyperelasticity model is used to simulate the biomechanical behavior of aortic valve leaflets in normal conditions. The pathological state of aortic valve cusps is described by a linear elastic model.

The mathematical formulation includes Navier-Stokes equation with incompressibility condition, equations to describe turbulence patterns. The equation of motion for solids is also written. The system is closed by initial and boundary conditions as well as fluid-solid coupling conditions. A velocity profile is given at the inlet to the computational domain. To determine the pressure at the outlet of the computational domain, a two-element Windexcel model is used, where the velocity profile is taken as an input.

The results obtained describe changes in the main haemodynamic indices: velocity, pressure, near wall tangential stresses and tangential stress fluctuation index. Results are also compared for kinetic and turbulent kinetic energy values between the two turbulence models and the normal and abnormal condition.

Simulation of blood flow during stenting

The purpose of this work is to evaluate the influence of artery, plaque and stent mechanical parameters on the effectiveness of stenting by analyzing both the stress-strain state and haemodynamics parameters. In this study, an idealised model of artery - plaque - stent system is considered. The geometric model consists of several layers: adventitia - outer layer, media - middle layer, plaque and stent. The mechanical parameters of the arterial layers were described using the three-parameter Ogden model. Hemodynamic parameter distributions were found as a result of one-way and two-way fluid-structure interaction algorithms.

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## S4.234. Assessment of the postural balance of badminton athletes after functional load

Baltin M.E.[1,2]*, Fedyanin A.O.[1,2], Mavliev F.A.[2], Baltina T.V.[1]
[1]*Kazan Federal University;*
[2]*Volga State University of Physical Culture, Sports and Tourism;*
* tvbaltina@gmail.com

Introduction. Postural and core stability is critical for almost all movements in sports [1], especially when maintaining balance on uneven ground or when responding to sudden disturbances [2]. Balance training improves joint stability, jumping ability, speed, and muscle contraction strength. Excellent body balance is critical for developing badminton skills, athletic performance [3], and injury prevention [4].
Materials and methods. The study involved 12 badminton players (age 20.91±2.03, time spent playing badminton 11.5±3.7 years) and 8 non-athletes (age 21.34±1.87 years). To assess postural stability, the "Tolerance control" test was carried out before and after the functional load (45 squats per 1 minute) The "Tolerance control" test consisted of 3 stages: Romberg test with open eyes (OE), Romberg test with closed eyes (CE) and the "Target" test. In the MedStat program, intragroup differences were determined by the Wilcoxon T-test and intergroup differences by the Mann-Whitney U-test. The level of statistical significance $p < 0.05$.
Results. In nonathletes, the total length of center of pressure displacements after exercise in the OE and CE samples was greater in the frontal plane than before exercise. In athletes, after the load, the displacement length increased in the sagittal plane more than in the frontal one. The shift of the center of pressure in athletes was significantly less after exercise in the OE sample and the "Target" test, and significantly more in the CE sample. Thus, we have shown that in athletes the leading analyzer in maintaining balance is the visual one. After exercise, non-athletes showed an increase in body displacement along the front, which indicates a change in the ankle balance strategy to a less effective femoral one. All participants showed a decrease in KFR in the CE test and in the "Target" test. In the "Target" test, badminton players showed high productivity, an effective strategy for maintaining the center of gravity in a given zone before and after the load test. It has been shown that, in general, regular physical activity improves postural control due to the structural adaptation of the extensor muscles of the lower limb, which allows athletes to use anticipatory strategies in maintaining balance.
Conclusion. The visual analyzer is the leading one in maintaining postural balance in badminton players. We assume that the current active neuromuscular regulation of the posture as a result of training in athletes leads to stabilization in the sagittal plane with the complication of postural tasks.
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## S4.235. Biomechanical aspects of shoulder girdle muscle power assessment of wrestlers

Zverev A.A.[1]*, Mavliev F.A.[1], Abdrachmanova A.S.[1]
[1]*The Volga Region University of Sports and Tourism;*
* Alekcei5@rambler.ru

Muscle power is an important indicator that is needed in many sports where a demonstration of strength and speed is required, in particular in wrestling. The Wingate test is often used to assess muscle power, it allows to assess the anaerobic performance of muscles and evaluate the results with existing standards [Popadic, 2009]. The results of this test can be presented both in absolute (W) and relative (W/kg) values, which allows a more accurate assessment of the athlete's physical performance. According to researchers, other indicators calculated during the test are less reliable and not always reproducible. The unresolved question remains that power, as a combination of speed and strength, during the test, will also depend on the length and diameter of the limbs, which can contribute to the final result. In this case, the diameter of the limbs, with the same level of subcutaneous fat and bone diameter, will indirectly determine the size of the working muscles, while the length of the limbs will determine the length of the levers through which this power will be realized. Therefore, with the same length of the ergometer handles, there may be a significant difference between the test results of the subjects only due to differences in limb lengths.
Wrestlers engaged in local ethnic Kuresh, judo, freestyle wrestling, sambo and having 1 category and above were tested to assess the possible impact on the power of anthropometric parameters. Measuring tape, weighing machine were used to anthropometric measurements viz. height, arm diameter and length, forearm length (cm) and weight (kg) for each of these athletes. The body length of the subjects was 176.9 ± 8 cm, weight 76 ± 7.8 kg, arm circumference 30 ± 2 cm, arm length 32.8 ± 2 cm, forearm 27.6 ± 1.4 cm. Age of wrestlers at the time of testing was 19 .8±1.5 years. Monark Cycle Handheld Ergometer with Wingate testing software (891 E) lasting 5 seconds was used to measure an absolute and relative peak power (W, W/kg), as well as the rotational speed of the ergometer handle (rpm). All the subjects did a warm-up and a trial test before testing to level the technical aspects of the test. Testing was carried out 3 times for 5 seconds with a rest between attempts. The load on the ergometer was dosed as 3.75% of body weight.
The analysis shows that the circumference of the shoulder doesn't correlate with the indicators of both absolute and relative power. Perhaps this is determined by the fact that power is achieved by the total activity of not only the shoulder muscles, but also the muscles of the body (pectoral muscles and the latissimus dorsi), which are actively involved in the test by bending and unbending the arms in the shoulder joints, as well as the consistency of their work.
Shoulder length, as well as forearm length, had positive correlations with the test results: with absolute peak power r=0.61-0.068 (p<0.006-0.044), forearm length also correlated with absolute peak power r=0.61-0.74 (p <0.025-0.008), in addition, the length of the shoulder and forearm correlated with the speed of rotation of the ergometer handle - r=0.68-0.73 (p<0.01-0.02). Correlations with relative powers weren't found.
These data indicate the need to take into account not only the mass of the subject, but also the parameters of limb lengths, which, other things being equal, can affect the power indicators of athletes. The influence of the length of the shoulders and forearms on the demonstrated power is apparently realized through a higher speed of rotation of the ergometer handle of athletes with longer limbs. These influences can significantly distort the indicators of the power of the muscles of the shoulder girdle, in particular in adolescent children, whose biological maturity, and, accordingly, the body length as a whole, will be different at the same passport age.

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### S4.236. Comparison of the effect of tropomodulin1 on actin-myosin interaction in cardiac and skeletal muscles

Kochurova A.[1]*, Beldiia E.[1,2], Sazonova E.[1,2], Shchepkin D.[1], Kopylova G.[1]
[1] *Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences;*
[2]*Ural Federal University, Yekaterinburg, Russia;*
* kochurova.a.m@mail.ru

Introduction. The contraction of striated muscles requires a strictly ordered structure of the contractile apparatus, including a certain length and stability of thin filaments, which are controlled by a number of proteins, including tropomodulin (Tmod). Tmod interacts with the slowly growing minus end of the actin filament and two molecules of tropomyosin (Tpm). The Tmod1 isoform is expressed in the contractile apparatus of the myocardium and skeletal muscles. It has been shown that Tmod affects the force generating capacity of skeletal muscles. Knockout of the TMOD1 gene resulted in the appearance of the Tmod3 isoform at the minus end of the actin filament and a decrease in the force generated by the muscles. We have previously found that Tmod is involved in actin-myosin interaction in the myocardium, and its effect depends on Tmod isoforms [Kopylova et al. 13th International Multiconference on "Bioinformatics of Genome Regulation and Structure/Systems Biology" – BGRS/SB-2022. Novosibirsk, 04-08 of July, 2022. P. 46-47], which can be explained by the difference in the amino acid sequence in the N-terminal part of the Tmod isoforms containing Tpm binding sites. Here, we compared the effect of Tmod1 on actin-myosin interaction in the left ventricle of the heart and fast skeletal muscles.
Materials and methods. Myosin was obtained from the left ventricle of the sheep heart and the fast skeletal muscle psoas of the rabbit. Actin and skeletal troponin (Tn) were isolated from m. psoas of the rabbit. Human cardiac troponin (Tn), alpha tropomyosin (Tpm), and human Tmod1 were expressed in E. coli. To study the molecular mechanism of the influence of Tmod1 on the actin-myosin interaction, an in vitro motility assay (IVMA) was used. Regulated thin filaments were reconstructed in a flow cell from fluorescently stained F-actin, Tpm and Tn. With cardiac myosin, filaments containing cardiac Tn were used, with skeletal myosin, skeletal Tn.
Results. It was found that Tmod1 differently influences the actin-myosin interaction in the myocardium and skeletal muscle. Tmod1 dose-dependently reduced the sliding speed of actin–Tpm filaments containing actin and Tpm over cardiac myosin in the IVMA: the concentration of Tmod1 at which the filament velocity decreased twofold was 59.4±2.9 nM. Tmod1 had no effect on the speed of actin-Tpm filaments with skeletal myosin. The effect of Tmod1 on the calcium regulation of actin-myosin interaction was studied by analyzing the dependence of the sliding speed of thin filaments on myosin on the calcium concentration in the IVMA. The addition of 500 nM Tmod1 reduced by 0.15 pCa the calcium sensitivity (calcium concentration at which the filament speed is half maximum) of the sliding speed of thin filaments on cardiac myosin and did not affect the characteristics of the calcium dependence of the speed with skeletal proteins. One of the mechanisms of regulation of actin-myosin interaction is the activation of thin filaments by the crossbridges of myosin, crossbridge-crossbridge cooperativity (Xb-Xb cooperativity). We studied the effect of Tmod1 on Xb-Xb cooperativity by analyzing the dependence of the actin–Tpm filament speed on the concentration of myosin loaded into the flow cell in IVMA. Tmod1 improved Xb-Xb cooperativity with cardiac myosin by decreasing the concentration at which the speed reaches half of the maximum, but did not affect it with skeletal myosin.
Conclusion. To compare the Tmod1 effect on actin-myosin interaction in the myocardium and fast skeletal muscles, we used protein isoforms (myosin, Tn, and Tpm) expressed in these muscles. Tmod1 reduced the calcium sensitivity of the actin-myosin interaction and enhanced Xb-Xb cooperativity with myocardial contractile proteins but did not affect the haracteristics of the interaction of skeletal myosin with actin. Thus, the effect of tropomodulin on actin-myosin interaction depends on the isoform composition of contractile and regulatory proteins.
The experiments were performed with the equipment of the Shared Research Center of Scientific Equipment of Institute of Immunology and Physiology and supported by the RSF grant no. 22-24-00729.

### S4.237. Development of muscle fatigue in tennis players of various qualifications

Chershintseva N.N.[1]*, Bartova Yu.D.[1], Tarasova E.V.[1], Fazleev N.Sh.[1], Zverev A.A.[1]
[1]*Federal State Budgetary Educational Institution of Higher Education "Volga Region State University of Physical Culture, Sport and Tourism";*
* chersinceva@mail.ru

To date, tennis is among the top popular sports around the world, which every year contributes to an increase in the number of people involved. In long-term draws, when the racket and the ball interact, the muscles of the hand are particularly susceptible to stress, and this, in turn, can affect the quality of the game: untimely strikes with a lower ball flight speed and altered movements on the court. Studying the effect of muscle fatigue on qualified results in tennis is a serious problem from the point of view of foreign researchers. The grip strength test is often used to evaluate complex muscle performance by determining the maximum grip strength that can be achieved in one muscle contraction, which further serves as a marker of overall muscle strength.
The purpose of the study is to evaluate and analyze the indicators of grip strength and the development of muscle fatigue in tennis players of various qualifications.
Methods and organization of research. The study was conducted on the basis of the Volga Region GUFKSIT University. The study involved 10 students of tennis players with a sports category not lower than the second adult. The average age of the subjects was 19.0±1.8 years. The registration of grip strength indicators was performed at the PowerLab installation (ADInstruments, Australia). The experiment consisted of the following stages: single maximum compression of the dynamometer with the leading hand, rest for 30 seconds, fatigue development – 15 seconds (5 approaches). The subject did not see the screen with the results of the experiment. All stages were performed with an outstretched arm from a standing position. The analysis was carried out in the universal module «Peak Analisys», where the following indicators were evaluated: Width (msec), Height (newton), APeak (newton), TRise (sec), TFall (sec), the area of the curve of the indicators of the wrist dynamometer. The obtained data were analyzed in the Microsoft Excel program. The normal distribution of the sample, the mean values and the standard deviation (M±δ) were determined.
The results of the study and their discussion. The tennis player's hand is in constant tension associated with the specifics of the sport, and prolonged tension of the muscles of the hand and forearm can lead to the rapid development of muscle fatigue, and consequently to the loss of a point. In the first series of the experiment, we determined the amplitude-time characteristics of isometric muscle tension in one short compression of the dynamometer. When assessing the relationship of the studied indicators with the level of sportsmanship of tennis players, it was revealed that the maximum compression force of the dynamometer was 251.7 ± 58.0 N for athletes with the title of Candidate master

of sports of Russia (CMS) - 359.1 ± 43.9 N. The time to reach the maximum strength (0.5 and 0.6 sec) and the rate of contraction (497.5 and 544.1 N/sec) in the dischargers was less than in the athletes of the CMS, which may be due to the difference in muscle fibers in athletes of different qualifications. Apparently, white muscle fibers predominate in CMS, which are characterized by high strength, but rapid fatigue, and in dischargers – red, capable of less strength, but with slow fatigue. The next series of experiments was aimed at the development of fatigue in athletes. We used the classical scheme of fatigue development using a dynamometer. The values of the development of the first 15-second fatigue were taken as control. When evaluating the results obtained in the studied tennis players, we found differences in all the studied indicators. The main indicator of muscle contraction is the amplitude, which was greater in the CMS with all five approaches to the dynamometer. On average, this value was 32%. The area under the fatigue development curve was also larger in the CMS and amounted to 37% with all approaches. The development of this dynamics may indicate the same energy reserves in the muscles. During the data analysis in subsequent approaches, the amplitude and area of the muscle fatigue curve decreased, both for dischargers and candidates for master of sports, but the indicators were higher for CMS. By the fifth development of fatigue, Candidates master of sports registered an increase in amplitude due to a decrease in the time to reach the maximum compared to other approaches, while it decreased for dischargers. With the fifth approach, the area of the muscle fatigue curve increased in the dischargers due to an increase in the time to reach the minimum contraction. The change in the area was accompanied by a decrease in the fatigue gradient time by 6.5%, the fatigue amplitude by 30%, the strength at the end of the fatigue development approach by 32%, the speed before fatigue by 37%, while the reduction rate decreased until the third approach of fatigue development, after which it increased for dischargers, and the time to reach maximum strength was higher the CMS has only the first and third approaches. The higher the level of sports qualification of tennis players, the higher the indicators of the compression force of the dynamometer. In all approaches to the development of fatigue, except for the first, the dischargers observed a faster time to achieve maximum fatigue indicators than the tennis players higher in rank.

The dischargers held the average dynamometer values for a longer time than the Candidates for the master of sports of Russia. Apparently, this is due to the formation of cortical and subcortical motor acts that activate the work of muscles, as well as significant improvement of intermuscular and intramuscular coordination mechanisms of movement control in athletes of higher qualifications.

### 7S4.238. Evaluation of postural stability of healthy people during transcutaneous electrical stimulation of the lumbar and cervical spinal cord at a frequency of 1 and 5 Hz

Zheltukhina A.F.[1]*, Bikchentaeva L.M.[1], Baltina T.V.[1]
[1]*Kazan (Volga Region) Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russia;*
* angelina7385@yandex.ru

Relevance.

One of the ways to modulate the neural circuits of the spinal cord was presented by a non-invasive method of transcutaneous electrical spinal cord stimulation (TSCS). The TSESM method is applicable both for studying the principles of regulation of locomotor functions in people with no motor disorders, and for selecting rehabilitation methods for patients with impaired motor function [1]. It was shown that with the help of the TSESM method, the regulation of locomotor functions in apparently healthy individuals becomes possible [2].

Objective.To evaluate the effectiveness of the impact of transcutaneous spinal cord stimulation at the lumbar (Th11-Th12) and cervical (C5-C6) levels with a frequency of 1 and 5 Hz on the indicators of postural stability in apparently healthy individuals.

Materials and methods.In the process of work, 67 people (7 men and 60 women) were examined, aged from 20 to 40 years. All studies were conducted with the informed voluntary consent of the participants in accordance with the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee of the Federal State Autonomous Educational Institution of Higher Education KFU (protocol No. 34 dated January 27, 2022).

The assessment of postural stability of the subject before and after TSCS was carried out using the Stabilan-01 stabilographic platform.

At the first stage, a control test (K) was carried out, stabilometric testing in a calm stance, with open eyes without stimulation, lasting eleven minutes.

After a 10-minute break, stabilometric testing was performed in a free stance, with stimulation - an 11-minute examination, according to the scheme:

1st minute: recording without stimulation in order to adapt the subject to the stand on the stabilographic platform;

From the 2nd to the 6th minute (total difficulty 5 minutes): with stimulation;

From 7th to 11th minutes (total difficulty 5 minutes): no stimulation, recording after stimulation.

Each subject underwent a series of 11-minute examinations, consisting of 3 samples: control, TSCS of the cervical spinal cord at the level of C5-6 cervical vertebrae and lumbar stimulation at the level of Th11-12 thoracic vertebrae.

Spinal cord stimulation. TSCS at the T11-12 level was performed using the Neurosoft MVP-8 stimulator (RF). The cathode stimulating the self-adhesive electrode was placed between the spinous processes of Th11–Th12 vertebrae.

A five-channel BIOSTIM-5 stimulator (Cosyma Ltd., Russia) was used to perform TSCS at the level of the cervical spinal cord. A stimulating skin round electrode (cathode) with an adhesive layer 32 mm in diameter was placed on the skin between the spinous processes of C5 and C6 vertebrae, rectangular electrodes (anode) with an adhesive layer 45 × 80 mm in size were placed symmetrically on the clavicles.

Stimulation was carried out by rectangular bipolar pulses with a duration of 1.0 ms, the stimulus intensity varied in the range from 50 to 70 mA. The duration of stimulation was 5 minutes. During the study, the indicators of the state of the cardiovascular system (heart rate, blood pressure) were monitored.

Statistical processing and analysis of the obtained data were carried out using the SigmaPlot 12.0 program.

Results and discussion.

When performing a control test, it was found that in a calm stance with visual control when standing on a hard surface, the subjects demonstrated the ability to maintain balance. So, after eleven minutes, compared with the first minute, the displacement of the CP along the frontal axis remained at the initial level, along the sagittal axis it decreased, the length of the trajectory of the CP along the frontal and sagittal axes, as well as the angular average velocity did not change.

TSCS at the level of the cervical and lumbar spinal cord revealed that stimulation at a frequency of 5 Hz had a positive effect on the change in stabilographic parameters: the average angular velocity increased, the area of the ellipse decreased, the length of the CP trajectory along the sagittal axis decreased, while stimulation at the level of Th11- 12 was more efficient. As one of the possible explanations for the improvement in postural stability during TSCS, an increase in synaptic conduction, as well as an increase in the excitability of afferent inputs, is given [3]. TSCS with a stimulation frequency of 1 Hz of both the cervical and lumbar spinal cord worsened the function of maintaining a vertical posture in a person.

The results of our research suggest that using the TESCM method it is possible to positively influence the functioning of spinal neural

networks in people with impaired motor functions, thereby increasing the quality of motor abilities.
Source of financing.The work was carried out within the framework of the program "Strategic Academic Leadership of the Kazan Federal University" (PRIORITET-2030).
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### S4.239. Expression of heat shock protein 90 on the plasma membrane of human fibrosarcoma HT1080 cells under different physiological conditions

Zhmurina M.A.[1]*, Vrublevskaya V.V.[1], Skarga Yu.Yu.[1], Petrenko V.S.[1], Morenkov O.S.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences, PSCBR RAS, Pushchino, Russia;*
* mariya100694@gmail.com

Cell migration is a complex biophysical process, which is the directional movement of one or a group of cells in response to a number of biochemical (cytokines, chemokines, growth factors) and biophysical signals, both extracellular and intracellular. Cellular migration, based on the functioning of the actin-myosin complex, plays a critical role in many physiological and pathological processes: in the embryonic development of organisms, in wound healing, metastasis of tumor cells, tissue remodeling. Heat shock protein 90 (Hsp90), performing important intracellular functions associated with its chaperone activity, is known to be actively secreted into the external environment and expressed on the cell surface. Extracellular Hsp90 functions as a motogen, stimulates the processes of cell migration and invasion in vitro, participates in the processes of wound healing and metastasis of tumor cells in vivo. There are two isoforms of Hsp90: the inducible isoform Hsp90α and the constitutive isoform Hsp90β. Hsp90α is considered to be a more effective stimulator of cell migration and invasion than Hsp90β. The mechanism of action of extracellular Hsp90 is based on receptor-dependent activation of signaling pathways that provide cell motility. The main Hsp90 receptors are LRP1 and HER2. It has been shown that in addition to protein receptors, surface cellular heparan sulfate proteoglycans (HSPG) play an important role in the binding of Hsp90 on the plasma membrane. The role of HSPG interaction with membrane-associated Hsp90 in the processes of cell migration/invasion is currently unclear; however, desulfation and degradation of HSPG leads to a significant loss of membrane-associated Hsp90 from the plasma cell membrane, which correlates with reduced cell motility. Yet, the expression of Hsp90 on the cell membrane in different cell types and under different physiological conditions has not been sufficiently studied.
The purpose of the study was an analysis of the membrane expression of two Hsp90 isoforms in human fibrosarcoma HT1080 cells under different physiological conditions. Immunofluorescence combined with registration using flow cytofluorimetry was used to study the membrane expression of Hsp90. Membrane-associated Hsp90s of HT1080 cells were stained using Hsp90α- and Hsp90β-specific antibodies, secondary anti-species Alexa488-labeled antibodies, with subsequent detection of the results using a CytoFLEX flow cytometer (Beckman Coulter). Cells were treated with heparin, a competitive inhibitor of Hsp90 binding to HSPG, to discriminate Hsp90s associated with protein receptors from those bound with HSPG.
Hsp90 plays an important role in cell migration, despite the low level of Hsp90 on the plasma membrane of HT1080 cells (the amount of membrane-associated Hsp90 is 500 – 1000 times lower compared to intracellular Hsp90), since the treatment of cells with Hsp90-specific polyclonal rabbit antibodies resulted in a decrease in migration and invasion of HT1080 cells in vitro. Membrane-associated Hsp90α and Hsp90β differed significantly in the affinity of interaction with HSPG: Hsp90β was significantly more sensitive to heparin compared to Hsp90α. Dissociation of Hsp90β from HSPG was observed already at 20 μg/ml heparin, while dissociation of Hsp90α started only at 50 μg/ml. In cells at exponential and stationary growth phases, the portion of Hsp90a associated with HSPG was 30-50%, while the portion of HSPG-associated Hsp90β was 60-80%. During the transition from the exponential to the stationary phase of cell growth, a 30-40% decrease in the level of Hsp90α and Hsp90β on the cell surface was observed. At the same time, the reduced expression of Hsp90α and Hsp90β on the membrane was due to a decrease in the number of HSPG-bound Hsp90, while the level of Hsp90 associated with protein receptors was virtually independent from the phase of cell growth.
It was shown that 24 h cultivation of HT1080 cells in the absence of serum leads to a slight decrease in the membrane expression of Hsp90α (by about 10%), while about 75% of Hsp90α was associated with protein receptors. Cells cultured in a medium with serum, had 60-70% receptor-bound Hsp90α. In contrast to Hsp90α, the level of Hsp90β on the plasma membrane of cells after "serum starvation" decreased sharply (by about 70%) mainly due to the loss of HSPG-associated Hsp90β, while the level of receptor-bound Hsp90β remained virtually the same in cells cultured in serum-free medium and medium with serum.
Following 2 h after culture medium replacement or simple medium stirring without replacement led to a 40-50% increase in the level of membrane-associated Hsp90α and Hsp90β compared with cells in still culture flasks. We believe that these manipulations mediated changes in the cellular microenvironment, which significantly affected the expression of Hsp90α and Hsp90β on the cell surface.
Thus, we found that the membrane expression of two isoforms of Hsp90, which play an important role in a complex biophysical process of cell migration, significantly depends on the conditions of cell culture. At the same time, the greatest changes in the membrane expression were observed in Hsp90β, while Hsp90α was less susceptible to changes. In less favorable physiological conditions (stationary phase growth of cells, absence of serum), the level of HSPG-associated Hsp90α and Hsp90β decreased. At present, the dependence of cell proliferation and migration from the levels of membrane-associated Hsp90α and Hsp90β is unclear. Further research is also needed to clarify the mechanism of Hsp90 translocation to the membrane and the role of HSPG in this process.

### S4.240. Features of postural balance of young badminton players

Nazarenko A.S.[1], Chershintseva N.N.[1], Zverev A.A.[1]*
[1]*The Volga Region University of Sports and Tourism;*
* Alekcei5@rambler.ru

Introduction. Badminton refers to situational sports, where during the game the athlete has to constantly move around the court and simultaneously monitor the actions of the opponent and the shuttlecock, which undoubtedly places high demands on the balance function of the athlete. The balance function is the sum of actions that implement postural control through various sensory systems, and the reliability of the functioning of these components will to a certain extent depend on the

degree of effectiveness of each, both during a short period of adaptation to a new body position, and after long-term adaptation processes as a result of sports activities. [one]. At the same time, various disturbances in maintaining the balance function caused by physical and sensory fatigue not only affect sports performance, but can also increase the risk of injury in athletes. In turn, the most qualified athletes have the best postural characteristics, both in specific and non-specific postural conditions [2]. At the same time, the scientific studies carried out to date have only marginally examined the influence of specific sports exercises on the control of postural balance by its underlying mechanisms. At the same time, most of the scientific research in the assessment of postural balance is carried out on adult athletes who already have formed mechanisms for long-term adaptation of the body to muscle load and completed processes of age development. However, the dynamics of improving the postural function of young athletes in the process of long-term adaptation to sports training remains not fully understood. The aim of this study was to study the features of postural balance in young badminton players in a state of relative rest and after exercise.
Organization and research methods. The studies involved badminton players (n=12) aged 8-10 years and non-athletes (n=11). All subjects were healthy and did not have any restrictions on sports. Postural balance was assessed using the Stabilan 01-2 stabilographic hardware-software complex (SJSC 'Ritm', RF) by analyzing oscillations of the center of pressure. The subjects performed the Romberg stabilographic test (test with open and closed eyes). Next, the subjects performed physical activity in the form of 20 squats, after which the level of postural balance was again assessed in the Romberg test with open eyes. To analyze the postural balance of young badminton players, the most informative stabilographic indicators of the fluctuation of the center of pressure were chosen: EllS, mm2 – area of the confidence ellipse; VSR, mm/s – average speed of movement of the center of pressure; KFR, % - the quality of the equilibrium function. All studies were carried out in compliance with the basic bioethical rules and norms for conducting experimental work. The sample was tested for normal distribution, and the statistical significance of the effect compared to control values was identified using paired and unpaired Student's t-test and ANOVA.
Results and discussion. According to the Romberg test (open eyes), the level of postural balance in young athletes and those not involved in sports did not differ and was in the range of the age norm. However, the area of confidence ellipse in young athletes was slightly lower (187 mm2, p˃0.05) than in non-athletes (231 mm2), reflecting a trend towards higher balance control with a smaller area of support and dispersion of the center of pressure.
In the Romberg test (closed eyes), there was an increase in the rate of oscillation of the center of pressure in all subjects, which affected the growth of stabilographic indicators, a decrease in the integral indicator "quality of the balance function" (up to 57-67%) and, in general, the level of postural control. However, there were also no intergroup differences in postural balance among the subjects.
It is important to note that the absence of significant differences in the level of postural balance in the Romberg test (test with open and closed eyes) can be explained, on the one hand, by the genetic determinism of the ability to maintain a high level of body balance, and on the other hand, by the non specificity of the test.
After exercise, in all subjects, as well as in the absence of vision, most of the stabilographic indicators of postural balance increased ($p<0.01$), which inevitably led to a decrease in the integral indicator "balance function quality" (up to 59-69%) , underlying the concept of the minimum rate of change in the center of pressure: the higher the value of this indicator, the higher the ability to postural control. At the same time, it was found that the postural response to physical activity depends on its type, intensity, duration, duration of proprioceptive stimulation, the form of muscle contraction and the degree of activation of muscle fibers. In turn, short and intense general exercises increase the effect on postural control by increasing hyperventilation rather than local fatigue.
Conclusion. Thus, the systematic nature of the training process already in childhood is an important determinant of an adequate adaptive effect to improve postural balance. In this case, one should take into account the initial level of postural control, which may be due to a genetic predisposition that determines the corresponding adaptive shifts in response to systematic training.
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### S4.241. In vitro study of the structure of titin aggregates from skeletal and smooth muscles

Uryupina T.A.[1]*, Bobylev A.G.[1], Bobyleva L.G.[1], Vikhlyantsev I.M.[1]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
* bobylev1982@gmail.com

Protein aggregation is a rather widespread process in cells of living organisms. It is known that, due to various reasons, a native protein can change its conformation to form non-functional aggregates. During aggregation, there occur changes in the structure of the protein, as well as the redistribution of bonds from intramolecular to intermolecular. The object of our research is the giant muscle protein titin (connectin) discovered in the late 1970s. To date, it has been shown that alternative splicing of the titin (ttn) gene leads to the formation of different-length isoforms of this protein with molecular weights of ~2000–3900 kDa in striated animal muscles; in smooth vertebrate muscles, titin isoforms with molecular weights of 500–2000 kDa have been found.
An in vitro study of titin preparations isolated from smooth (MW 500 and 1500 kDa) and skeletal (MW 2000–2200 kDa) muscles revealed the ability of different isoforms of this protein to form oligomers and aggregates. In particular, according to electron and atomic-force microscopies, all investigated titin isoforms formed amorphous aggregates at 0.15 M glycine–KOH, pH 7.0–7.5. The skeletal muscle isoform of titin also formed bundles of linear fibrils in a solution of 0.1 M KCl, 10 mM imidazole, pH 7.0. Titin aggregates bound the dye thioflavin T, which was indicative of their amyloid nature. By X-ray diffraction, titin aggregates were found to have reflections at ~10 and ~4.8 Å to imply the presence of a quaternary cross-β structure characteristic of all amyloid fibrils. For this reason, aggregates of different isoforms of titin can be called amyloid aggregates. A distinctive feature of amyloid aggregation of titin is the absence of changes in the secondary structure of the protein during aggregation as revealed by circular dichroism and Fourier-transform infrared spectroscopy. The results obtained indicate minor changes in the structure of titin during the formation of amyloid aggregates by this protein.
The results of this research expand the views about the features of amyloid aggregation of different proteins and the structure of amyloid aggregates.
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### S4.242. Indicators of badminton players morphofunctional with account anaerobic capabilities

Chershintseva N.N.[1]*, Nazarenko A.S.[1], Fedinin A.O.[1], Zverev A.A.[1]
[1]*Volga state university of physical culture, sports and tourism;*
* chersinceva@mail.ru

The effectiveness of sports activity depends on the realization of the physical qualities of strength, speed, muscle endurance or their combination in competitions. The manifestation of these physical qualities depends not only on the degree of perfection of the control of the neuromuscular apparatus and vegetative functions by the central nervous system, but also on the maximum use of power, capacity, efficiency of one or another mechanism of energy supply of muscle activity [4].
Strength abilities are characterized by high muscle tension and are manifested in overcoming, yielding and static modes of muscle work. They are determined by the physiological diameter of the muscle and the functionality of the neuromuscular apparatus [5].
The magnitude of the maximum muscle strength depends on many anatomical, morphological, nervous, humoral, bioenergetic, biomechanical and other factors. It has been shown that muscle strength is directly proportional to the area of its physiological diameter, i.e. the thicker the muscle, the stronger it is. Therefore, the power capabilities of a person largely depend on his muscle mass [3].
The muscle strength also depends on their reactivity, elastic properties of muscle tissue and viscosity, on the structure of muscle fibers, their morphological and functional properties. The study of the topography of the strength of various muscle groups helps to identify the role of various indicators in the development of fitness, the formation of a rational movement technique and the achievement of high results. Morphological status has a significant impact on the manifestation of strength, speed, endurance, reactivity of the body and its adaptation to environmental factors, and is also a marker of fitness [1]. The badminton players show various correlations reflecting the mutually regulating influence, both in a state of relative rest and after an active load [2].
The study using the Wingate test was aimed at determining fairly objective indicators of speed-strength tests, indicating the actual individual anaerobic power, with maximum energy expenditure due to the alactic mechanism. In addition to fixing standard ergometry indicators, a body composition analyzer of body composition was carried out, which allows you to adequately assess the applied physical activity and predict results of sports.
The testing of badminton players aged 14-22 years (n=27) was carried out on the Monark Ergomedic 894 E foot bicycle ergometer and the Monark Ergomedic 891 E hand bicycle ergometer. Weight, for the foot and hand erg was calculated as 7.5% and 3.5% of the athlete's body weight. The participants of the experiment had no medical contraindications to stress testing. Body composition analysis was performed on Tanita (MC-980). Statistical processing of the results was carried out using the SPSS 20 program with a critical level of significance $p \leq 0.05$.
The level of anaerobic performance of badminton players is one of the leading indicators that determines the performance of athletes. The functionality of both the upper and lower body belts of badminton players varies depending on the stages of training, the size of human muscle fibers and other factors. In our experiments, the peak power of the legs and arms was 12.7 and 9.1 W/kg, the time to reach the maximum power was 1.8 and 1.5 seconds, the relatively average power was 11.5 and 7.2 W/kg, and the maximum speed was 143.1 and 149.9 rpm. We have identified strong correlations between peak power, as well as the time to reach maximum power of the legs with the muscles of the trunk, arms, legs, basic metabolism and water content in the body. In arms strong correlations with Tanita parameters were found only with the time to reach maximum power.
Thus, in our experiments, strong relationships were demonstrated between various morphofunctional parameters of badminton players, which can be taken into account when assessing the fitness of athletes and improve physical fitness indicators at different stages of the training process.

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**S4.243. Influence of medium viscosity on the occurrence of open states in the DNA molecule**

Dorohova A.A.[1,2]*, Drobotenko M.I.[2], Svidlov A.A.[1,2], Dzhimak S.S.[1,2]
[1]*Federal Research Center the Southern Scientific Center of the Russian Academy of Sciences;*
[2]*Kuban State University;*
* 013194@mail.ru

The stability of the DNA molecule is ensured by the dissipation of thermal energy, due to two main factors:
1. Occurrence of open states and denaturation bubbles.
It is known that open states (OS) are necessary for the implementation of transcription and replication processes, but their role in dissipating the energy of mechanical movements is also important.
2. Another factor that makes it possible to dissipate the energy of mechanical movements of the DNA molecule is the interaction with the surrounding aquatic environment.
The noted factors 1 and 2 are associated with mechanical deformations of DNA; therefore, for a quantitative analysis of their influence on the dynamics of the DNA molecule, it is natural to use mechanical models of DNA. Modeling mechanical deformations of DNA is a powerful research method for describing its nonlinear dynamics.
In our work, the influence of the viscosity of the external medium on its internal dynamics and stability is studied by the method of mathematical modeling of mechanical deformations of DNA. Previously [1], studies were carried out for small values of external force actions that did not lead to the occurrence of open states (OS). In this paper, we consider the force effects that lead to their appearance.
The mathematical model of the angular motions of nitrogenous bases is based on the analogy between a DNA molecule and a mechanical system consisting of two chains of interconnected pendulums. At the same time, nitrogenous bases correspond to the rotating pendulums, and the sugar-phosphate chains of the DNA molecule correspond to the elastic thread to which these pendulums are attached; the hydrogen bond of a pair of complementary nitrogenous bases corresponds to the elastic bond of the corresponding pair of pendulums [2-4]. Studies were carried out on the example of the gene encoding interferon alpha 17 [5].
Numerical studies carried out in our work show that the occurrence and dynamics of OS in a DNA molecule depends not only on the magnitude of the external action (in our case, this is the torsion moment), but also to a large extent on the viscosity of the environment.
It has been established that the dynamics of OS zones can have an abrupt character with a small change in the value of the torsion moment.

When a torsion moment is applied to all 980 base pairs of the interferon alpha 17 gene, the following effect is observed: an increase in the viscosity of the medium leads to an increase in the value of the torsion moment necessary for the occurrence of OS and DNA unwinding, i.e. viscosity plays an important stabilizing role in DNA dynamics.
It is known that the external effect on the DNA molecule, as a rule, has a local character, therefore, in this work, we studied the influence of a localized torsion moment on different (by the content of A-T and G-C pairs, as well as by location) regions of the interferon alpha 17 gene. It was found that under localized action, the value of the external torsion moment necessary for the occurrence of OS at all calculated values of viscosity depends on the nucleotide composition.
When a torsion moment is applied to areas close to the gene boundaries, there is a clear dependence of the torsion moment required for OS onset on viscosity. At the same time, the significance of the end effect, which weakens DNA, decreased with increasing viscosity of the medium.
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### S4.244. Involvement of PI3K and IP3R in the regulation of atrophic processes in skeletal muscles during functional unloading

Zaripova K.A.[1]*, Belova S.P.[1], Shenkman B.S.[1], Nemirovskaya T.L.[1]
[1]*Institute of Biomedical Problems RAS;*
* katsu.no.himitsu@gmail.com
It was shown that IP3R-dependent slow Ca2+ signals may be involved in the activation of specific transcriptional programs in muscle fibers. Objective: to test the hypothesis about the involvement of IP3R and PI3K in the development of atrophic processes in skeletal muscles at early stages of functional unloading. We also investigated for the first time whether in vivo ATP-mediated muscle signalling during functional unloading affects the activation of calcium-dependent signalling pathways and the expression of inositol 1,4,5 triphosphate receptors (IP3R). We conducted an experiment with 3-day suspension of rats and PI3 kinase (PI3K) inhibition. PI3K catalyzes the phosphorylation of phosphatidylinositol diphosphate (PIP2), giving PIP3 a highly charged residue that recruits phospholipase C (PLC) to the membrane, triggering the hydrolysis of PIP2 into diacylglycerol and IP3. IP3 binds to IP3Rs, which present in the nuclear envelope and in the sarcoplasmic reticulum, causing a weak calcium release signal, both in the cytosol and nucleoplasm, which promotes the activation of transcription factors leading to changes in expression of genes involved in the phenotype of muscle. With functional unloading of muscles, the concentration of calcium in the myoplasm significantly increases. If our hypothesis is correct, then affecting IP3R by inhibiting PI3K during functional unloading will prevent or significantly reduce the expression of key muscle E3 ligases MuRF1 and MAFbx and will prevent the activation of transcription factors that affect the muscle phenotype.
For the experiment, 24 male Wistar rats were divided into 3 groups of 8 animals each: control group with placebo (C); 3-day hindlimb suspension with placebo (HS); group of 3-day hindlimb suspension with the administration of the PI3K inhibitor LY294002 (LY, 30 mg/kg, intraperitoneally). Experiments were performed at the Institute of Biomedical Problems, RAS, Russia. The experiments were approved by the Committee on Bioethics of the Russian Academy of Sciences (protocol No. 617). The study was conducted in accordance with the internationally accepted regulations and rules of biomedical ethics.
Soleus muscles of rats suspended without drug administration underwent significant atrophy compared with the control group (by 33%, $p<0.05$). The weight of the muscles in the group with the administration of the LY was significantly higher than in HS group. The ATP level in the LY inhibitor group did not differ from group C, while it was increased by 24% in the HS group ($p<0.05$). The level of pAMPK in m.soleus in the LY group also did not differ from the control group, while it was reduced by 37% in the untreated (HS) group. The content of IP3R increases by 41% when suspended compared to the control ($p<0.05$), and the administration of the LY prevents these changes. We measured the content of markers of calcium-dependent signaling –CaMKII and CaN. Phosphorylation of CaMKII was increased by 59% ($p<0.05$) in unloaded soleus muscle (gr. HS) compared with the control group, however, administration of a PI3K inhibitor prevented these changes. Similar results were obtained for calcineurin. Its expression in the m.soleus of LY group was 16% lower than in the group suspended with placebo ($p<0.05$).
In HS group, the level of mRNA expression of E3-ligases MuRF1 and MAFbx, as well as ubiquitin, was significantly higher (by 82, 137 and 158%, respectively, $p<0.05$) than in the control group. The administration of the PI3K inhibitor completely prevented the increase in the expression of MuRF1 mRNA in LY group, and significantly reduced the expression of MAFbx and ubiquitin in it (by 22 and 119%, respectively, relative to HS group, $p<0.05$). In the group with the administration of the LY inhibitor during suspension, the level of protein synthesis markers IRS-1, 4E-BP, phosphorylation of ribosomal protein S6 did not differ from the level of the control group, while in HS group these parameters were significantly reduced (by 34, 15 and 55 (for pS6(Ser235/236) and 83% (for pS6(Ser240/244) respectively), $p<0.05$).
Thus, inhibition of phosphoinositide-3-kinase during functional unloading of m.soleus prevents the accumulation of ATP, slows down atrophy of m.soleus, as well as the expression of E3 ligases and ubiquitin, prevents an increase in the content of IP3 receptors, regulates the activity of calcium-dependent signalling pathways – reduces CaN mRNA expression and CaMKII phosphorylation; affects the regulation of markers of anabolic signaling – prevents a decrease in phosphorylation of 4E-BP, ribosomal protein S6; and also prevents a decrease in the rate of elongation processes by preventing eEF2 phosphorylation.
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### S4.245. Mathematical model of eyeball control implemented by the oculomotor muscles

Minyaylo Y.U.[1], Kruchinina A.P.[1]*
[1]*Moscow, Lomonosov MSU;*
* a.kruch@moids.ru
Annotation.

The calculations were carried out on the basis of constructing a geometric three-dimensional model of each muscle separately based on data on the coordinates of the muscle beginning and the point of muscle attachment to the eye. The paper presents an algorithm for restoring the six oculomotor muscles (OEM) force moments based on oculographic information. A three-dimensional geometric model is constructed for each muscle, based on muscle beginning coordinates the and the point of muscle attachment to the eye. The calculations performed were applied to construct a mathematical model of the vestibulo-ocular reflex.

Introduction

The eyeball rotations are provided by six oculomotor muscles: lateral, medial, upper and lower straight, upper and lower oblique. The lateral and medial muscles provide rotation of the eye in the horizontal plane. The other four OEMs work in pairs straight and oblique. The lower straight and upper oblique provide downward rotation of the eyeball, and the upper straight and lower oblique provide upward rotation. In addition, the oblique muscles are responsible for the torsion rotations of the eye. The combined contraction of the oculomotor muscles implements the eyeball rotation in the right direction.

Description and eye movements analysis is an important task for many studies. Modern technologies allow us to track eye movements with high accuracy. Commonly video or electro-oculography are used for this. However, with the help of this equipment, only the result is recorded: direction and amplitude the eyeball turned. Another issue remains open and important: which oculomotor muscles provided the observed movement.

The purpose of this work is to construct a mathematical algorithm for describing the control of the eyeball, implemented by the oculomotor muscles. Comparisons with vestibular information and visual tasks can already be applied to the received assessment. The model is useful for closing the mathematical description of the vestibular-ocular reflex.

Methods

The eye and oculomotor muscles are a mechanical system. In order to build a mathematical model we have introduced a number of simplifications:

- the eyeball is an absolutely solid body, a perfect ball with a constant radius R = 12.43 mm;
- the eye rotation center coincides with its geometric center and remains a fixed point during any eyeball movements;
- OEMs are represented by strings that can actively contract.

In these terms the task is to describe sphere rotations with a fixed center, as a result of the tangential forces applied to its surface. To set rotations it is most convenient to use the rotation axis and the rotation angle.

The force application point to eyeball is muscle guiding force vector contact point and eyeball surface directed along the muscle fibers towards its eye socket attachment. According to muscles represented by strings, force vector is directed along the string. The radius vector is directed from the coordinate system origin associated with the eye to the contact point. The vector product of the force vector by the radius vector gives the force moment, which is directed along axis rotation. Therefore, the force moment unit vector coincides with the unit vector defining the rotation axis. So, the contact point radius vector and the force guiding vector for each OEM, allow us to calculate the muscle force moment and the rotation axis it provides.

The geometric parameters of OEMs presented in paper [1] formed the basis for calculating the moments. For each muscle the origin coordinates (x, y, z) and the attachment to the eyeball coordinates are given, relative to the origin located in the fixed sphere geometric center.

For each muscle we individually solved three calculating sub-tasks:

1) the coordinates of a unit radius vector;
2) the unit force vector coordinates;
3) the muscle force moment.

To test the model, a series of experiments was conducted with the subjects rotations in three planes corresponding to the semicircular channels functional pairs. Rotations were carried out on specialized centrifuge chairs according to the sinusoidal law. During the rotations, the head angular velocity vector and the reciprocal eye movement oculogram were recorded. The subject's vision field was blocked in order to exclude the visual information influence on eye movements. Thus, vestibular information was mainly involved in the eye control.

It is known [2] that each semicircular channel activates exactly one OEM of each eye. In this case, the greater the head angular acceleration projection on the channel sensitivity axis, the greater the corresponding muscle contribution to the eyeball movement. From experimental data we can estimate the angular velocity projection onto semicircular channels, form oculomotor muscle control and find the resulting eye rotation axis as an activated muscles axis linear combination. By calculating the axis at each time of recording, we can simulate the eyeball movement and compare it with the experiment eye recording.

Results

The unit force moment vector coordinates are obtained for six oculomotor muscles. They are also the rotation axes guiding vectors. A model of eyeball control in the form of rotation axes linear combination of activated OEM is proposed. Model application to describe the vestibulocular reflex makes it possible to reliably estimate the linear component in the slow phases of nystagmus and restore the coefficients of the degree of muscle activation. The muscle activation coefficient is assumed to be equal to normalized unit vector projection of the head angular velocity onto the channel activating this muscle.

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**S4.246. Mechanical and signal responses of functionally unloaded rat's m.soleus in response to a chronic increase in β-myosin activity**

Sergeeva K.V.[2]*, Nikitina L.V.[1], Tyganov S.A.[2], Zaripova K.A.[2], Sharlo K.A.[2], Shenkman B.S.[2]
[1]*Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences;*
[2]*Scientific Center of Russian Federation – Institute for Bio-medical Problems of the Russian Academy of Sciences;*
* sergeeva_xenia@mail.ru

It is well known that atrophy develops as a result of a reduction in the mechanical activity of mammalian skeletal muscles, while the most pronounced changes are observed in the postural soleus muscle [1, 2], with a predominant expression of the slow isoform of myosin heavy chains type I(β). At the same time, in a number of studies, the presence of autonomous neuromuscular muscle activity was detected, recorded after 3 days of functional unloading [3, 4]. In this work, it was assumed that pharmacological potentiation of spontaneous contractile activity of the soleus muscle with omecamtiv mekarbil (OM) will activate the anabolic signaling pathways leading to the preservation of muscle mass, strength and intrinsic stiffness of the unloaded rat's hindlimbs. OM is a selective activator of slow β-myosin. Being localized near the interface of several key conservative structural elements of myosin, OM stabilizes the lever arm in a primed position preceding the power stroke, increasing the number of myosin heads that can bind to the actin filament [5]. This kinetic effect, in turn, promotes accelerated release of phosphate and inhibits the rotation of the lever arm, prolonging the time that myosin spends in a strongly actin-bound state [5, 6, 8]. To achieve this goal, animals of the following experimental groups were used in the experiment: control group (C); control group with the administration of OM for 10 days

(C+OM); a group subjected to hindlimb suspension for 14 days (H); and a hindlimb suspension group combined with OM administration starting from 4th day of unloading (H+OM) (after the appearance of spontaneous electromyographic activity).

We found that injections of OM kept the muscle protein synthesis rate at the level of control values, illustrated by the partial prevention of muscle fibers atrophy of both fast and slow types. Presumably, this effect is a reflection of the positive effect of the drug on the translational efficiency of mRNA (the rate of protein synthesis per ribosome). In the H+OM group we observed inactivation of GSK-3ß and subsequent dephosphorylation of its target initiation factor eIF2B-ε, activation of signaling proteins p90RSK, p70S6K, as well as a higher IRS-1 content compared to the hindlimb suspension group without administration of OM. In addition, OM was found to prevent a decrease in the strength and intrinsic stiffness of the soleus muscle isolated after two weeks of disuse. Meanwhile, administration of OM did not prevent the activation of proteolysis: in particular, a significant increase in the expression of ubiquitin ligase MuRF-1, ubiquitin and calpain occurred in both groups of hindlimb unloading, and also had no effect on markers of translational capacity (45S pre-rRNA, 18S rRNA and 28S rRNA). Thus, a chemically-induced increase in the power and duration of spontaneous contractions of the soleus muscle under unloading conditions creates prerequisites for protein synthesis. At the same time, it should be assumed that the use of OM is advisable with pharmacological drugs that inhibit the expression of ubiquitin ligases.

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### S4.247. Metamodel construction algorithm for predicting aortic hemodynamics in children with congenital heart disease

Kuchumov A.G.[1,2], Golub M.V.[1]*, Rakisheva I.O.[2], Doroshenko O.V.[1]
[1]*Kuban State University;*
[2]*Perm National Research Polytechnic University;*
* m_golub@inbox.ru

Obstructive lesions of the right ventricular outflow tract, isolated or combined with other congenital heart defects, account for 25-30% of congenital heart anomalies. Biomedical engineering is a relatively new and rapidly advancing area in which biomechanical models can enhance the efficiency of decision-making in treatment. About half of patients with congenital heart disease in their first year of life require surgical treatment. A breakthrough solution in the surgical treatment of cyanotic congenital heart defects, such as the anomaly of tetralogy of Fallot, pulmonary valve atresia and some others, was the creation of an inter-system shunt (in particular, the modified Blalock-Taussig shunt). However, the installation of a modified Blalock-Taussig shunt has high risks (mortality varies from 2.3% to 16%). The major complications of the modified Blalock-Taussig shunt are related to thrombosis in small diameter shunt and small circle hypervolemia in large diameter shunt. Nowadays, the selection of the diameter and the location of a shunt is purely empirical. The selection of the optimal shunt diameter also remains a challenging problem, which has not been solved to date and needs to be solved in the daily work of the surgeon.

One of the effective approaches to solving this problem can be the construction of a personalized blood flow model, which will allow analyzing the effectiveness of surgical treatment. The modern computational fluid dynamics tools are efficient for studying physiological flows, and it may allow for comprehensive noninvasive evaluation for diagnosis and treatment of disease, as well as serve to design new devices for clinical trials. However, due to the unique characteristics of each patient, such personalized modeling is time-consuming (analysis for one patient may take from several hours to several days or even weeks), cumbersome, and computationally expensive (three-dimensional computational fluid dynamics problems must be solved). Moreover, determining model parameters is another challenging issue. In addition, processing and evaluating a large volume of collected data is not only labor-intensive and time-consuming but also quite subjective (e.g., when analyzing medical images).

A potential solution to the problem described above is the application of machine learning algorithms bot to speed up computations and for effective decision-making. In this communication, we propose an algorithm for constructing a metamodel, i.e., a model based on a limited number of simulations using a complex three-dimensional fluid dynamics model, to describe hemodynamics in the aorta of a child with congenital heart disease using data from flow models with personalized geometries. It should be also noted such a metamodel for children with congenital heart disease is constructed for the first time.

In the first stage of the algorithm, data on aortic valve geometry has been collected from multi-slice computed tomography images. The latter allows the slices to be imported into ITK-SNAP and then into the ANSYS finite element analysis system to create a three-dimensional computational fluid dynamics model. The hemodynamic problem is solved using Ansys Fluent to find the main hemodynamic parameters (flow velocity).

The data on the diagnosis and blood flow volume flow distribution of a particular patient, which are used as boundary conditions when calculating hemodynamics in the aortic valve, are associated with each model. Based on the three-dimensional geometric model, the main geometrical characteristics of the aorta (distances between arterial exit sites, diameters, curvature, etc.) have been identified, which are further required for metamodel tuning and neural network training. Currently, the database of aortic models consists of more than 500 three-dimensional models. This makes it possible to classify characteristic aortic species by key geometric characteristics and hemodynamic features using data analysis and machine learning methods.

Taking into account the aortic geometry as input data, trained neural networks will be able to output the required distributions in a short time, which is to be much faster than directly employing three-dimensional simulation. Various algorithms will be tested at the next stages, including convolutional neural networks that can simulate complex, nonlinear relationships between input and output variables. Thus, with the accumulation of sufficient adequate data for training, including anatomical models and hemodynamic data, real-time, patient-specific neural networks will suggest potential surgical solutions based on expert decisions and simulations of aortic hemodynamics. More than 1000 different geometries and simulation results (distribution of

hemodynamic parameters) are planned to be analyzed and classified at the final stage.
The research is carried out with the financial support of the Kuban Science Foundation in the framework of the scientific project №20.1/12.

### S4.248. Method of obtaining and analyzing the functional features of cardiomyocytes of the myocardium of the pulmonary veins

Simonova R.A.[1]*, Butova X.A.[1], Myachina T.A.[1], Kopylova G.V.[1], Khokhlova A.D.[1,2], Shchepkin D.V.[1]
[1]*Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences, Yekaterinburg;*
[2]*Ural Federal University named after the first President of Russia B.N. Yeltsin;*
* raisa.simonova@mail.ru

It is known that cardiomyocytes of pulmonary veins can show spontaneous activity, be a source of ectopic and trigger activity. In this regard, the study of the function of the myocardium of the pulmonary veins plays an important role in understanding the development of atrial fibrillation. Cardiomyocytes of the pulmonary veins penetrate the thickness of the vessels, and the extracardial myocardium differs in functional characteristics from the myocardium of the atria and ventricles [1]. The electrophysiological features of cardiomyocytes of the pulmonary veins are mainly studied in comparison with cardiomyocytes of the left atrium [2]. The aim of the study was to optimize the method of isolation of mechanically active single cardiomyocytes of pulmonary veins and to analyze the characteristics of the contraction of their sarcomeres.
The manipulations were performed on guinea pigs in accordance with Directive 2010/63/EU and approved by the Ethics Committee of the IIP UB RAS.
Single cardiomyocytes were obtained by a standard technique of Langendorff perfusion with modifications [3]. Before isolation of single cardiomyocytes, animals were injected intramuscularly with sodium heparin (5000 IU/kg) to prevent the development of coronary artery thrombosis. After euthanasia, the heart in the open chest was washed with a cooled (15-16° C) solution A, then placed on a Langendorff, where it was subjected to retrograde perfusion (4.0–4.5 ml/min) by successive change of three solutions at 35° C. At the first stage, the heart was washed for 5 minutes with a Solution A containing heparin sodium to cleanse the coronary vessels of blood and stabilize heart contractions in vitro. Further, to reduce excitability and inhibit contractile function, the heart was perfused with a solution with a high K+ content and a low Ca2+ content for 10 minutes from the moment of complete cessation of contractions. To successfully isolate single cardiomyocytes from the myocardial "sleeves" of the pulmonary veins, the optimal perfusion time and enzyme concentrations were selected. The intercellular framework was enzymatically cleaved by collagenase II (0.5 mg/ml, Worthington, USA) and protease XIV (0.05 mg/ml, Sigma-Aldrich, USA). When signs of intermediate cleavage of the intercellular framework were reached in the form of progressive pallor of the epicardial surface of the heart and the appearance of viscous droplets, retrograde perfusion was stopped. The atria and myocardium of the pulmonary veins were subjected to additional injections with a solution with a high collagenase content (0.5 mg/ml, 6-7 ml/min) for 20 minutes, after which the procedure for obtaining cells was similar to that described [3]. The finished suspension of isolated myocytes was stored in a HEPES-containing buffer Tyrod at 22-24 ° C and used for 6-8 hours.
Measurements of the contractile function of isolated cardiomyocytes were performed at 35-37ºC and an electrical stimulation frequency of 1 Hz on specialized equipment (IonOptix Corporation, USA). It is shown that the amplitude and time characteristics of the contraction of sarcomeres of cardiomyocytes of the pulmonary veins differ from the characteristics of the contraction of cardiomyocytes of the left atrium. As a result of the work, the method of isolation of mechanically active single cardiomyocytes from the myocardial "sleeves" of the pulmonary veins of the heart of guinea pigs was optimized and the first data on the characteristics of contraction of cardiomyocytes of the extracardial myocardium were obtained.
The experiments were carried out on the equipment of the Central Research Institute of the IIF of the Ural Branch of the Russian Academy of Sciences with the support of the RNF grant No. 23-24-00356.
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### S4.249. Modulation of AMPK Activity Affects the Expression of Apoptotic Markers in Differentiating Myoblasts Isolated from Atrophied Skeletal Muscle

Mirzoev T.[1]*, Rozhkov S.[1], Turtikova O.[1], Shenkman B.[1], Vilchinskaya N.[1]
[1]*Institute of Biomedical Problems of RAS;*
* tmirzoev@yandex.ru

Various environmental and pathophysiological stimuli can induce a significant loss of skeletal muscle tissue. Regrowth of atrophied myofibers depends on muscle satellite stem cells (SCs) that exist outside the myofiber plasma membrane. It was shown that disuse-induced atrophy appears to result in deficits in the SCs of rodents, manifested by an inability of muscle tissue to grow or to regenerate. It is also known that denervation-induced muscle atrophy can increase susceptibility of SCs to apoptosis. Since we recently found a decrease in AMP-activated protein kinase (AMPK) activity during enhanced differentiation of primary myoblasts derived from atrophic rat soleus muscle, we hypothesized that there may be a potential link between AMPK activity and susceptibility of differentiating myoblasts to apoptosis. Hence, the aim of the study was to estimate the effect of AMPK activation on the expression of apoptotic markers in differentiating myoblasts derived from atrophied rat soleus muscle.
All experimental procedures were approved by the Biomedicine Ethics Committee of the Institute of Biomedical Problems of the RAS. Thirty male Wistar rats weighing 190-210 g were randomly assigned to the following 2 groups: control (C, n=10) and 7-day hindlimb suspension (HS, n=20). Under anesthesia, soleus muscles from control and HS rats were surgically excised from both hindlimbs using standardized dissection methods and then used for isolation of the pool of muscle SCs. After muscle excision, the rats were sacrificed by decapitation under isoflurane anesthesia. Myoblasts derived from the soleus muscles of HS rats were divided into two parts: AICAR-treated cells and non-treated cells. Primary myoblasts isolated from the soleus muscles of the control rats were not treated with AICAR. AMPK and acetyl-CoA carboxylase (ACC) phosphorylation levels were assessed by Western-blotting. mRNA expression of the apoptotic markers was evaluated by RT-PCR. In differentiating myoblasts derived from the atrophied soleus muscle there was a significant decrease ($p<0.05$) in AMPK (Thr172) and ACC (Ser79) phosphorylation compared to the control cells. This reduction in AMPK activity was accompanied by a significant upregulation of pro-apoptotic markers Caspase-9, BAX, p53 and downregulation of

anti-apoptotic BCL-2. Treatment of atrophic muscle-derived myoblasts with AICAR during differentiation prevented a reduction in AMPK and ACC phosphorylation. Moreover, AICAR treatment attenuated an increase in the expression of Caspase-9, BAX, p53 and prevented a decrease in BCL-2 mRNA expression.
Thus, the maintenance of AMPK activity with AICAR treatment suppresses the enhanced mRNA expression of apoptotic markers in differentiating myoblasts derived from atrophied rat soleus muscle. The study was supported by the RSF grant # 20-75-10080.

### S4.250. Regional variances of the contractile function disturbance of atrial cardiomyocytes in rats with paroxysmal atrial fibrillation

Butova X.A.[1]*, Mikhryakova P.P.[2], Myachina T.A.[1], Simonova R.A.[1], Shchepkin D.V.[1], Kochurova A.M.[1], Kopylova G.V.[1], Khokhlova A.D.[1,2]
[1]*Institute of immunology and physiology UrB RAS;*
[2]*Ural Federal University named after the First President of Russia B. N. Yeltsin;*
* x.butova@gmail.com

Atrial fibrillation (AF) is the most common arrhythmia and is characterized by very rapid and uncoordinated electrical and contractile atrial activity. AF often presents first in a paroxysmal form (defined by episodes that last <7 days and terminate spontaneously), then in persistent forms (duration of episodes >7 days) [1]. Unlike persistent AF, in which structural and functional changes in the heart are actively investigated, atrial contractile dysfunction in paroxysmal AF remains unclear. The left and right atria (LA, RA) have structural and functional features so that playing different roles in the onset and maintenance of AF [2]. Therefore, a differential assessment of the effect of paroxysmal AF on atrial contractility is required.
The aim of this study is to estimate disturbances in LA and RA contractile function and intra-atrial functional heterogeneity in paroxysmal AF of rats at the single cell and molecular levels of myocardial organization.
All experiments were performed using male Wistar rats (aged 10 weeks) according to Directive 2010/63/EU. Paroxysmal AF in rats was induced by daily injections with AChCl (60 μg/mL) and CaCl2 (10 mg/mL) via the tail vein at 1.3 mL/kg for 7 days [3]. To detect paroxysmal episodes, ECG was recorded using a three-channel electrocardiograph (ECG300G-VET, China).
Single cardiomyocytes from LA and RA were isolated using a standard technique of Langendorff perfusion with author's modifications. Sarcomere shortening-relengthening during mechanically non-loaded contractions and auxotonic cell force under mechanical load produced by 4 carbon fibers [4] were measured using the MCSYS-02 system (IonOptix, US). Intracellular calcium ([Ca2+]i) transients were recorded using Ca2+-sensitive fluorophore Fluo-8AM (AAT Bioquest, US) and LSM 710 scanning confocal system (Carl Zeiss, Germany). All experiments on single cardiomyocytes were carried out at 1 Hz and 30 °C.
Detection of the sarcomere shortening-relengthening alternans in LA and RA cardiomyocytes (defined as the visible beat-to-beat maximum (MAX) and minimum (MIN) alternations in sarcomere shortening amplitudes lasting more than 5 shortening-relengthening cycles from the amplitude value near to the steady-state) and analysis of their parameters were performed using the IonWizard software (IonOptix, US). Morphometric parameters of isolated LA and RA cardiomyocytes (cell length and width) were assessed using the FIJI ImageJ software (US National Institutes of Health, USA).
The interaction of myosin with native thin filaments (NTF) and F-actin, extracted from the LA and RA was studied in an in vitro motility assay. Phosphorylation proteins was analyzed by Pro-Q Diamond Phosphoprotein Gel Stain (Thermo Fisher Scientific, US) and SYPRO Ruby (Thermo Fisher Scientific, US).
Paroxysmal AF significantly increased cell length (without changing their width) for both LA and RA cardiomyocytes. In LA cardiomyocytes, AF decreased the amplitude of sarcomere shortening-relengthening as well as the maximal rates of sarcomere shortening and relengthening. In RA cardiomyocytes, AF decreased end-diastolic sarcomere length and maximal rate of sarcomere shortening. Alternans of the sarcomere shortening-relengthening were more common in LA and had larger MIN and MAX alternations in sarcomere shortening amplitudes compared to those in RA. In AF, the amplitudes of LA and RA sarcomere shortening did not differ, in contrast to the control group.
In LA cardiomyocytes, AF decreased an amplitude of [Ca2+]i transient. In RA cardiomyocytes, AF provoked a decrease in the total duration and time to a 50% decline of [Ca2+]i transient in addition to a depression in the [Ca2+]i transient amplitude.
Paroxysmal AF decreased the auxotonic force of cell contraction only in RA cardiomyocytes that led to the appearance of differences in auxotonic cell force between LA and RA, which were absent in the control group.
In the in vitro motility assay, the sliding velocity of F-actin and NTF did not differ between the AF and control groups. However, AF led to the disappearance of the intra-atrial differences in the sliding velocity of F-actin, which were found in the control group. In LA, AF decreased the phosphorylation levels of cMyBP-C and TnI. In RA, AF increased the RLC phosphorylation. These intra-atrial features in phosphorylation level in AF could explain the higher vulnerability of the sarcomere shortening-relenghtening parameters to paroxysmal AF in LA cardiomyocytes.
Thus, paroxysmal AF induced pronounced disturbances in the mechanics and phosphorylation levels of sarcomeric proteins in LA, and in parameters of [Ca2+]i transient and auxotonic cell force in RA. The revealed changes in the cell geometry of LA and RA cardiomyocytes indicated the presence of eccentric hypertrophy of the atria in paroxysmal AF. Moreover, paroxysmal AF caused a change in the initial pattern of functional heterogeneity detected in a healthy heart. These regional variances in the atrial functional remodeling in AF suggest a difference in the possible ways to compensate for the contractile disturbances in LA and RA.
This work was performed using the equipment of the Shared Research Center of Scientific Equipment of IIP UrB RAS and supported by the Russian Science Foundation #22-75-10134.
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### S4.251. Structural and functional features of tropomyosin with mutations associated with impaired myocardial development

Shchepkin D.[1]*, Kochurova A.[1], Beldiia E.[1,2], Koubasova N.[3], Tsaturyan A.[3], Levitsky D.[4], Bershitsky S.[1], Yampolskaya D.[4], Matyushenko A.[4], Kopylova G.[1]
[1]*Institute of Immunology and Physiology, of the RAS, Yekaterinburg, Russia;*
[2]*UrFU, Yekaterinburg, Russia;*
[3]*Institute of Mechanics of Moscow State University, Russia;*
[4]*A.N. Bach Institute of Biochemistry of the RAS, Moscow, Russia;*
* cmybp@mail.ru

It was shown that tropomyosin (Tpm) is involved not only in the regulation of muscle contraction but also in the heart development, namely, in the "maturation" of the ventricular myocardium, which consists of thickening of myocardial fibers network and narrowing of

the intertrabecular lacunae (McKeown et al., Dev. Dyn., 2014). Mutations in the TPM1 gene encoding cardiac alpha tropomyosin (Tpm1.1) associated with non-compact left ventricular cardiomyopathy (LVNC) and congenital heart defects (CHD) have been found (Chang et al., Mol. Genet. Metab., 2011; England et al., J. Mol. Cell Cardiol., 2017). We investigated the structural and functional properties of Tpm1.1 with LVNC and CHD mutations L113V, I130V, D159N, R160H, and S229F. Using differential scanning calorimetry, we found that all mutations affect the domain structure of Tpm1.1. The interaction of the N- and C-termini of neighboring tropomyosin molecules on the actin filament (F-actin) is necessary for performing the regulatory function. We evaluated the effect of mutations on this interaction by measuring the viscosity of the Tpm1.1 solution. The R160H substitution weakened the interaction between the N- and C-ends of tropomyosin molecules, while all other mutations enhanced it.

We analyzed the effect of mutations on the interaction of Tpm1.1 with F-actin. Mutations I130V and D159N reduced the affinity of Tpm1.1 for F-actin, while the others did not affect it. We also studied the effect of mutations on the thermal stability of the F-actin-Tpm complex by measuring the light scattering of the F-actin solution with Tpm1.1. The R160H mutation reduced the thermal stability of the complex, while the D159N substitution increased it.

To study the effect of mutations on the calcium regulation of actin-myosin interaction in the atria and ventricles, we investigated the calcium dependence of the sliding velocity of thin filaments reconstructed from F-actin, troponin, and Tpm1.1 on atrial and ventricular myosin in an in vitro motility assay. The effects of mutations depended on the isoforms of myosin. Mutations D159N and R160H significantly reduced the maximum velocity of thin filaments at a saturating calcium concentration on ventricular myosin. Mutations L113V and D159N decreased the maximum filament velocity on atrial myosin, while I130V and S229F increased it. Mutations I130V, D159N, and R160H decreased the calcium sensitivity of the filament velocity on atrial myosin.

Modeling of molecular dynamics showed that the L113V mutation causes changes in the structural properties of Tpm1.1, including those in the regions of the molecule remote from the site of amino acid substitution. Mutations D159N and R160H had no significant effect on the flexural rigidity of the Tpm molecule. The absence of changes in the bending rigidity of the Tpm molecule with the D159N and R160H mutations indicates that the functional changes in the regulation of the actin-myosin interaction caused by these mutations are apparently not directly related to changes in the mechanical properties of the Tpm molecule.

Thus, tropomyosin mutations associated with LVNC and CHD affect the characteristics of the actin-myosin interaction in the ventricular and atrial myocardium.

The experiments were performed on the equipment of the Collective Use Center of the Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences and supported by the state programы 122022200089-4, 122041100022-3 и AAAA-A19-119012990119-3.

### S4.252. The autonomous activity of the disused postural muscle: The compensation of the destructive muscle remodeling or its deepening?

Shenkman B.S.[1]*, Sharlo K.A.[1], Tyganov S.A.[1], Sergeeva K.V.[1], Kalashikov V.Y.[1], Turtikova O.V.[1], Lvova I.D.[1]
[1]*Institute of Bio-medical Problems;*
* bshenkman@mail.ru

Skeletal muscle atrophy accompanied by a phenotypic transformation that is characterized by the downregulation of slow-twitch myosin heavy chain isoform MyHCI (β) and upregulation of fast-twitch MyHCIId/x and MyHCb isoforms is often observed in rodent soleus muscle during functional unloading using the hind limb-suspension model [Booth et al, 1990]. These changes are usually considered as the consequences of the cessation of contractile activity. However, if muscle is unloaded for more than 3 days it cannot be considered to be in a state of complete disuse. In 1987, Alford et. al. showed that, starting from 3 days of simulated weightlessness electromyographic (EMG) activity had been elevated and continued to increase up to 14 days of exposure. This phenomenon, which can be called "autonomous" neuromuscular activity, has been reproduced in similar conditions by other authors [Kawano et al, 2002, 2004; and others] but, contrary to expectations, did not prevent the development of atrophy and changes in the mRNA expression pattern of slow and fast MyHC isoforms.

At the same time, using spinal cord isolation it was shown that electrical activity recorded in some rodent muscles was the result of a significant decrease in the expression of the potassium chloride co-transporter KCC-2 in neurons of the lumbar spinal cord with a subsequent change in the direction of the chloride current and inversion of the resting membrane potential. These changes lead to a sharp increase in the excitability of motor neurons [Boulangez et al., 2010]. A decrease in the expression of KCC-2 can be prevented by the administration of the neuroleptic prochlorperazine. In our laboratory, a significant decrease in the content of KCC-2 in the spinal cord of rats subjected to 7-day functional unloading has recently been shown [Kalashnikov et al., 2021]. Using chronic electrode implants, autonomous electrical activity of soleus muscle was registered after 2 days of hind limb suspension. Administration of prochloperazine prevented a decrease in the content of KCC-2 in the spinal cord and significantly reduced the autonomous activity of the muscle. These findings give us the opportunity to further investigate the intramuscular and spinal consequences of decreased autonomous muscle activity under functional unloading. We investigated the effects of prochlorperazine administration, which is known to reduce sufficiently the soleus EMG autonomous activity during unloading, on the myosin phenotype and signaling markers of the mitochondrial biogenesis and muscle proteostasis.

A significant decrease in the autonomous activity of soleus muscle together with functional unloading led to a natural deepening of the already reduced expression of the regulators of mitochondrial biogenesis (COX1, COX2, COX4, PGC1α) when using prochlorperazine. This fact indicates that mitochondrial signaling is directly dependent on muscle activity. The profoundly reduced expression of the markers of the mitochondrial biogenesis in unloaded rats administered with prochlorperazine was accompanied with the reduced superoxide ions accumulation (according to data of dihydroethidium fluorescence) and reduced phosphorylation of MAPkinases p38 and JNK.

At the same time, such a decrease in the autonomous activity of the soleus muscle accompanied by functional unloading and prochlorperazine administration allowed the preservation of the mRNA expression of MyHC isoforms at the control level. This effect was concomitant with the absence of NFAT export from muscle nuclei. This phenomenon can be associated with a decrease in the level of phosphorylation of MAPK p38, which can phosphorylate NFAT and promote its export from nuclei [Sharlo et al., 2019]. The maintaining of the vivarium control levels of MHC isoforms expression in prochlorperazine administered unloaded animals corresponded well with the preserved slow and fast fiber type ratio as compared to unloaded placebo rats with the ratio shifted to the fast fibers. When analyzing markers of anabolic and catabolic signaling pathways the unloading-induced decrease in the main markers of anabolic pathways (including the parameters of ribosomal biogenesis) and increase in the expression markers of the muscle-specific E3-ubiquitine-ligases was completely or partially prevented by the administration of prochlorperazine.

Thus it could be supposed that the autonomous tonic activity of the rat soleus muscle facilitating the accumulation of the reactive oxygen species and possibly the other signaling messengers (e.g. Ca2+ ions) during unloading leads to destruction of the proteostatic balance and the fiber type transformation in the postural muscle.

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### S4.253. The cytoskeleton of the soleus muscle under critical illness myopathy conditions

Tyganov S.A.[1]*, Zaripova K.A.[1], Turtikova O.V.[1], Skiteva E.N.[1,2], Kondratiev S.A.[2], Zabrodskaya Y.M.[2], Shenkman B.S.[1]
[1]*Institute of Biomedical Problems RAS;*
[2]*Department of Anesthesiology and Intensive Care of the Russian National Institute of Chemistry;*
* sentackle@yandex.ru

The functional unloading state of skeletal muscles is observed during immobilization, in prolonged bed rest, with spinal injuries and musculoskeletal disorders. This condition leads, among other effects, to the destruction and destabilization of the muscle cytoskeleton. Critical care myopathy (CIM) is a consequence of modern treatment in anesthesiology and intensive care, including pharmacological interventions and the use of life-sustaining devices. CIM conditions create a unique situation where there is a complete loss of mechanical stimuli in skeletal muscle, i.e., loss of external strain, which is determined by body weight, and internal strain, which depends on muscle contractions (Rebeca C. Kalamgi and Lars Larsson; 2016). Muscle damage in an intensive care patient is non-specific in nature and is the result of several processes: an adaptive response to severe damage to the body, focal or total damage to the central nervous system, damage to the spinal nerves, neuromuscular synapses, muscle damage, non-specific action of drugs. For this reason, understanding the molecular mechanisms that regulate skeletal muscle state is important to develop effective rehabilitation programs and possible pharmacological interventions that can prevent or alleviate loss of skeletal muscle mass and function in patients with chronic impairment of consciousness.
The aim of this study was to investigate the impact of critical illness myopathy on the cytoskeleton of skeletal muscle. To achieve this goal, we conducted a detailed analysis of a large number of cytoskeletal proteins and extracellular matrix in patients with chronic impairment of consciousness. Incisional muscle biopsies from the soleus muscle were taken from 8 patients treated in the Department of Anesthesiology and Intensive Care of the Russian National Institute of Chemistry. Muscle biopsies taken from healthy men using the Bergström needle biopsy were used as controls. Evaluation of the severity of paresis in patients with CNS is a difficult task due to the absence or pronounced decrease in the volume and spectrum of purposeful reactions, the execution of commands. All patients included in the study had deep tetraparesis, which was predominantly diffuse in nature, lateralization of paresis was detected in two patients in both cases, the etiological factor of CNS was a traumatic brain injury. Changes in muscle tone in patients with CIM are mainly represented by spasticity with a few of the most common patterns. Muscle biopsies taken from patients were frozen in liquid nitrogen immediately after taking. The cytoskeletal protein content was analyzed by Western blotting, real-time PCR, and histochemical analysis.
A morphological study of the soleus muscles in patients with CIM showed a colossal atrophy of muscle fibers and replacement of their volume with connective tissue. We observed a decrease in the cross-sectional area of muscle fibers by 73%, expression of embryonic myosins, indicating denervation, disruption of the structure of cytoskeletal proteins, fibrosis, lipid deposition, and a decrease in glycogen stores, which can have a negative impact on the quality of life of the patient, and also significantly complicate the rehabilitation stage. Further study of the cytoskeleton using PCR and Western blotting, in addition to the obvious confirmation of atrophic changes, showed a shift in the myosin phenotype towards "fast" (glycolytic) muscle fibers. This is evidenced by both the change in the expression of the myosin heavy chains themselves and the auxiliary structural proteins such as actinins, talin, and tropomyosin. The growth of the extracellular matrix, according to our data, was due to an increase in the expression of collagen 1a and collagen 6a. Based on the results of the first year of the Project, for the first time we have identified specific changes in skeletal muscles that develop under CIM conditions. It is believed that these changes may depend on many factors, such as systemic inflammatory response syndrome and sepsis. However, the use of mechanically ventilated animal models made it possible to exclude the influence of these systemic factors on skeletal muscle, while maintaining similar atrophic changes (Rebeca C. Kalamgi and Lars Larsson; 2016). In addition, it has also been shown that sepsis per se does not lead to the development of a muscle phenotype in CIM (Friedrich et al., 2015). This means that there is a need to determine the triggers on which CIM-specific atrophy depends.
This work was supported by RSF grant № 22-25-00615.

### S4.254. The effect of change in estradiol levels on the contractile function of atrial and ventricular cardiomyocytes

Myachina T.A.[1]*, Butova X.A.[1], Simonova R.A.[1], Kochurova A.M.[1], Kopylova G.V.[1], Shchepkin D.V.[1], Khokhlova A.D.[1,2]
[1]*Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences;*
[2]*Ural Federal University named after the first President of Russia B.N. Yeltsin;*
* myachina.93@mail.ru

Estradiol is the primary form of estrogen produced by the ovaries, adrenal gland and also peripheral tissue. Estradiol balance is very important for normal heart function. Elevated estradiol levels are the cause of the development of atrial fibrillation and ventricular arrhythmias. Estradiol deficiency during menopause is associated with a higher risk of developing cardiovascular disease compared with premenopausal women. It was shown that estradiol deficiency significantly affected the ventricles contractility [1]. However, the influence of estradiol on the atrial mechanical function is still poorly understood.
Therefore, this work aimed to study the influence of high and low estradiol levels on atrial and ventricular myocardial contractility at cellular and molecular levels.
The study was conducted on female Wistar rats in accordance with the Directive 2010/63/EU.
An ovariectomized (OVX) animal model was used to evaluate the effect of estrogen deficiency on the myocardium. Rats aged 17-18 weeks were divided into 2 groups. In the OVX group, the animals were anesthetized and then both ovaries were removed. In the Sham group, surgery included of anesthesia, visualization of the ovaries and closure of the wounds and then the group served as a control group. Six weeks after surgery, single cardiomyocytes from the left ventricle (LV) and the left atrium (LA) were obtained using the Langendorff technique with modifications [2].
The effect of high estradiol level on the myocardium was evaluated in an acute experiment. Cardiomyocytes from the atria and the ventricles were incubated in solution containing 10 nM 17-β estradiol for 10 min. Cardiomyocytes from the control group were exposed to the same protocol in a Tyrode solution.
Measurements of cytosolic calcium and sarcomere shortening were performed from cardiomyocytes during unloaded shortening. For measurements of cytosolic calcium, cardiomyocytes were loaded with Fluo-8.
The measurements were carried out at the electric stimulation frequence of 1Hz and a solution temperature of 36 – 37°C.
To study actin-myosin interactions and phosphorylation of sarcomeric proteins, proteins were obtained from a suspension of atrial and ventricular cardiomyocytes incubated in a solution containing 17-β-estradiol,

or from the LV and LA myocardium of OVX and Sham groups. Protein phosphorylation was analyzed using Pro-Q Diamond phosphoprotein staining (Invitrogen, Eugene, OR, USA). SYPRO Ruby (Invitrogen, Eugene, OR, USA) staining was used to estimate the amount of total proteins. The movement of actin filaments over myosin was measured in an in vitro motility assay [3].

In the Sham group, LV cells had longer end-diastolic sarcomere lengths (EDSL) and greater sarcomere shortening compared with LA. In OVX rat, EDSL and sarcomere shortening amplitudes were greater in LA, but did not change in LV compared with the control group. In control group, the [Ca2+]i transient amplitude was greater in LA myocytes than in LV. OVX increased significantly the [Ca2+]i transient amplitude in LV cells. In Sham rats, LA cardiomyocytes had shorter time to peak shortening (TTP) and time to 50% relaxation (TR50), and shorter [Ca2+]i transient decay compared with LV cardiomyocytes. In OVX group, TTP and TR50 were smaller in LV myocytes only.

Changes in the mechanical characteristics of cardiomyocytes were accompanied by changes in protein phosphorylation and sarcomeric protein functioning. In the in vitro motility assay, filaments demonstrated a higher sliding velocity on atrial myosin than on ventricular one. OVX increased the sliding velocity of filaments on both LA and LV myosin.

An acceleration of the cross-bridge cycle can contribute to the acceleration of LA cardiomyocyte contraction. The impairment of the contractile function of LV cardiomyocytes after OVX may be explained by decreased Ca2+ sensitivity of the thin filament and acceleration of the [Ca2+]i transient in LV cells.

17-β-estradiol did not affect EDSL in the atria and ventricles. In 17-β-estradiol group, the sarcomere shortening amplitudes were smaller in ventricles, but not in atria compared with the control group. The sarcomere shortening amplitudes were greater in ventricles, but remained unchanged in atria after incubation. 17-β-estradiol prolonged the time course of [Ca2+]i transient in atria, but not in ventricles.

A decrease the ventricles contractility may be explained by decrease of phosphorylation levels of cardiac myosin-binding protein-C.

Thus, female sex hormones regulate cardiac contractile function at the cellular and molecular levels, having different effects on the contractility and its Ca2+ regulation in the atrial and ventricular myocardium.

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### S4.255. The role of delayed tonic activity of m.soleus in the restructuring of key molecular signaling processes in the development of muscular hypogravitational atrophy

Sergeeva K.V.[1]*, Sharlo K.A.[1], Tyganov S.A.[1], Kalashnikov V.E.[1], Turtikova O.V.[1], Lvova I.D.[1], Shenkman B.S.[1]

[1]*The Institute of Biomedical Problems RAS, Moscow, Russia;*

* sergeeva_xenia@mail.ru

Previous studies clearly demonstrated that there is a sharp decline in rat soleus EMG activity at the initial stage of hindlimb suspension (HS) (1-2 days) [1, 2]. However, soleus muscle electrical activity starts increasing after 48 h of exposure to HS, and after 3 days of HS the soleus muscle EMG activity significantly differs from the values recorded immediately after the onset of HS [2]. Furthermore, it has been shown that rat soleus muscle EMG activity progressively increases from day 2 to day 6 of HS [2]. We assumed that autonomous activity is the result of a significant decrease in the expression of the potassium chloride co-transporter KCC-2 in neurons of the lumbar spinal cord, followed by a change in the direction of the chloride current and inversion of the membrane potential, leading to a sharp increase in the excitability of motor neurons [3] that was observed in our previous study following 7-day HS [2]. Indeed, daily prochlorperazine administration, decreasing KCC2, attenuated the autonomous soleus muscle activity [2]. The aim of this study was to examine the signaling events during prochlorperazine administration that significantly reduces the tonic autonomous electrical activity of rat soleus muscle under conditions of 3-day simulated gravitational unloading. The animals were divided into the following groups: control group – C; hindlimb suspension group for 3 days – 3HS, hindlimb suspension for 3 days with daily intraperitoneal administration of prochlorperazine – 3HS+P.

During the 3-day HS the administration of the prochlorperazine did not prevent a decrease in isometric strength, but contributed to the preservation of intrinsic stiffness of the soleus muscle. When analyzing markers of anabolic signaling pathways after 3 days of exposure, it was found that the level of phosphorylation of the translation initiation factor 4E (4E-BP1) was maintained at the control level when using prochlorperazine. Phosphorylation of ribosomal protein S6 (S6RP) was increased by 85% in 3HS+P group and, at the same time, decreased by 58% in 3HS group. Thus, the administration of prochlorperazine prevented a decrease in the phosphorylation levels of two mTOR targets. It can be assumed that on the 1-3 day ofunloading, blocking spontaneous tonic muscle activity led to a more pronounced decrease in the activity of AMP-activated protein kinase (AMPK). Since AMPK is in reciprocal relations with mTOR [4], its suppression could cause activation of mTOR. Another possible mechanism for the effect of blocking spontaneous tonic muscle activity due to the introduction of prochlorperazine on mTOR-dependent signaling pathways may be the prevention of an increase in the content of reactive oxygen species (ROS). It is known that starting from the earliest periods of functional unloading the number of ROS increases [5] and can lead to inactivation of the mTOR/Akt signaling pathway, as well as contribute to dephosphorylation and activation of the proteolytic regulator FOXO3 [6]. In the present study, the activity of the transcription factor FOXO3 was significantly reduced in 3HS+P group compared to 3HS group. It should be noted that the ROS-dependent activation of MAP kinases p38 and JNK plays a key role in these effects. We found that after 7 days of HS, the administration of prochlorperazine was accompanied by a significant decrease in the content of superoxide anions (according to dihydroethidium fluorescence) and a reduced level of phosphorylation of MAPkinases p38 and JNK in the muscle compared to animals receiving saline solution. Thus, preventing the accumulation of ROS with prochlorperazine administration can contribute to both inhibiting the proteolysis and preventing the inactivation of the mTOR signaling pathway.

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### S4.256. The role of heat shock proteins HSP90α and HSP90β in the migration of HT1080 human fibrosarcoma cells

Petrenko V.S.[1]*, Vrublevskaya V.V.[1], Zhmurina M.A.[1], Skarga Y.Y.[1], Morenkov O.S.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences", Pushchino, Russia;*
* 79182797935@yandex.ru

Cell migration is a complex, finely regulated biophysical process involved in various stages of both normal and pathological functioning of eukaryotic organisms. Cell migration ensures the maintenance of tissue homeostasis and morphogenesis, participates in the development of the immune response, the process of wound healing, as well as in the invasion and metastasis of cancer cells. Studying cell migration is important for understanding the mechanisms of metastasis of malignant tumor cells and the development of antimetastatic anticancer drugs.
Cell migration is realized via reorganization of the cytoskeleton, in which actin and myosin II are involved. Polymerization of actin filaments at the cell periphery leads to the growth of lamellipodia. Actin is also involved in the formation of stress fibrils, which ensure the sliding of actin filaments relative to each other due to the action of motor proteins, mainly myosin II. The mechanical tension that results from actin polymerization and contraction of stress fibers is ultimately transferred to the extracellular substrate through adhesion sites, thereby providing cell movement. The process of cell migration is regulated by the intracellular signaling system involving receptors, cytoskeleton-modifying proteins, kinases, various adapter and signaling proteins, etc. Heat shock protein 90 (Hsp90) is a highly conserved chaperone protein that mediates folding, stabilization, and degradation of many intracellular proteins (more than 700 client proteins), including proteins associated with cell motility. Hsp90 plays an important role in cellular homeostasis maintenance, cell survival and differentiation, proliferation, carcinogenesis, cell migration, and other cellular processes. Two isoforms of Hsp90 are known: stress-induced Hsp90α and constitutively expressed Hsp90β, encoded in humans by the HSP90AA1 HSP90AB1 genes, respectively. The Hsp90 isoforms have a high amino acid sequence homology (~86%), and therefore the Hsp90α and Hsp90β isoforms demonstrate both similar and differing functional properties in the cell. To date, the specific functional role of Hsp90α and Hsp90β in cell migration has not been elucidated. The aim of our study was to determine the role of individual Hsp90 isoforms in the processes associated with cell motility.
To study the role of one Hsp90 isoform in cellular processes, it is necessary to suppress the activity of the other isoform. To date, specific inhibitors of Hsp90α and Hsp90β have not been described. We have chosen an approach to generate cell lines with knocked-out genes encoding different isoforms of Hsp90. Based on the CRISPR/Cas9 nickase vector AIO-GFP, two constructs were created for mutagenesis of exon 1 of the HSP90AA1 and HSP90AB1 genes in HT1080 human fibrosarcoma cells. The correctness of the generated plasmids was confirmed by sequencing. The cells were transfected by electroporation and 3 HT1080 Hsp90α-null mutant lines and 3 Hsp90β-null mutant lines were obtained, as confirmed by immunoblotting and flow cytometry using Hsp90α- and Hsp90β-specific antibodies. Sequencing of the clone libraries generated for all monoclones revealed the presence of deletions and insertions in exon 1 of genes HSP90AA1 and HSP90AB1 in both alleles of the mutant clones, which provide shifts of the open reading frames of Hsp90α and Hsp90β. As a result, HT1080 mutant lines with a biallelic knockout of the HSP90AA1 gene encoding Hsp90α and a biallelic knockout of the HSP90AB1 gene encoding Hsp90β were obtained.
Next, we investigated how the absence of Hsp90α and Hsp90β in HT1080 cells affects their migration ability. Cell migration assessment was performed using the method of healing of a "wound" created on a cell monolayer. The "wound", was created using silicone 2-well culture inserts (Ibidi, USA), which ensured the formation of a gap (~500 μm) on a confluent monolayer of cells. Following "wound" formation on the cell monolayer, the "wound" was photographed at certain time intervals, and the rate of "wound" overgrowth by cells was estimated. In the experiments, cells in different physiological states (cell cultivation before the start of the experiment in the presence or absence of serum) were used, as well as various conditions during migration (different serum concentrations, serum-free medium). It was shown that the migration rate of the original HT1080 cells and all cell monoclones with Hsp90α or Hsp90β knockout was comparable regardless of the experimental conditions. It should be noted that all clones retained an ability for active proliferation, which did not differ from that of HT1080 cells.
Thus, on mutant lines with HSP90AA1 or HSP90AB1 knockouts, the absence of the Hsp90α or Hsp90β isoform was shown to exert nearly no effect on cell migration. The results obtained may indicate the absence of client proteins strictly specific to Hsp90α or Hsp90β among the proteins involved in the process of cell migration. On the other hand, only one of the Hsp90 isoforms was absent in the obtained monoclones. It is possible that one isoform compensates for the absence of another Hsp90 isoform and fully or partly fulfils its functions. Our results also call into question the generally accepted view concerning the critical role of extracellular Hsp90α in cellular locomotion. All HT1080 cell lines that do not express intracellular and, as a result, extracellular Hsp90α migrated in vitro at a rate comparable to that of the original HT1080 culture. Additional studies are needed to elucidate in more detail the role and interchangeability of Hsp90α and Hsp90β in various cellular processes, including cell migration.

### S4.257. The role of phosphorylation of cardiac myosin-binding protein-C in calcium regulation of actin-myosin interaction

Kopylova G.[2]*, Kochurova A.M.[2], Beldiia E.A.[1,2], Titova K.[1,2], Kruzhaev A.[1,2], Bershitsky S.Y.[2], Shchepkin S.V.[2]
[1]*UrFU, Yekaterinburg, Russia;*
[2]*Institute of Immunology and Physiology of the RAS, Yekaterinburg, Russia;*
* g_rodionova@mail.ru

Introduction
The heart contraction occurs due to the interaction of myosin of thick filaments with actin of thin filaments in sarcomere and is regulated by Ca2+. In the absence of calcium, tropomyosin (Tpm) closes myosin binding sites on actin ('blocked' state); when Ca2+ is bound by troponin C, Tpm on actin shifts aside, opening some of these sites

('closed' state); finally the myosin attachment further activates thin filaments ('open' state) [McKillop, Geeves, Biophys J., 1993]. The myosin-binding protein-C (cMyBP-C) takes part in the Ca2+ regulation of cardiac muscle contraction by shifting Tpm from its 'blocked' state to the 'closed' one [Mun et al., PNAS, 2014; Wang et al., Compr Physiol., 2018]. The N-terminal part of the cMyBP-C molecule contains phosphorylation sites Ser275, Ser284, and Ser304. Phosphorylation of cMyBP-C accelerates contraction, increasing the probability of myosin binding to actin and the rate of force development [Sadayappan, de Tombe, Biophys Rev, 2012; Lynch et al. J Mol Cell Cardiol., 2021]. We investigated the effect of the cMyBP-C phosphorylation on the calcium regulation of the actin-myosin interaction in the myocardium using an in vitro motility assay (IVMA).

Methods

Actin was extracted from rabbit skeletal muscles. Myosin was obtained from the left ventricle of the porcine heart. Human cardiac α-Tpm was expressed in E. coli. The human troponin (Tn) complex was provided by I.A. Katrukha (M.V. Lomonosov Moscow State University). Regulated thin filaments were reconstructed from F-actin, Tn and Tpm. N-terminal fragments of human cMyBP-C (C0-C2 fragments) with amino acid substitutions S275D, S284D, and S304D mimicking phosphorylation were expressed in E. coli.

We studied the effect of cMyBP-C phosphorylation on actin-myosin interaction using IVMA, which allows recording the movement of fluorescently labeled F-actin or thin filaments on the myosin surface in a flow cell [Kopylova et al., BBRC, 2017]. The filament velocity was analyzed using GMimPro software [Mashanov&Molloy, Biophys. J., 2007; Matyushenko et al., Biochemistry, 2017]. We fitted the Ca2+ dependence of the filament velocity by the Hill equation. Following parameters were determined: the maximum filament velocity (Vmax) at saturating calcium concentration; calcium sensitivity (pCa50), i.e. pCa at which the velocity is half-maximum, and the Hill cooperativity coefficient. We repeated each experiment three times. Data presented as mean ± SD, comparisons made with the Mann-Whitney U-test ($p<0.05$).

To study the effect of cMyBP-C fragments on crossbridge-crossbridge (Xb-Xb) cooperativity, we analyzed the dependence of the velocity of thin filaments on myosin concentration and determined its concentration (c50) at which the filament velocity is half-maximum.

Results

To study the effect of cMyBP-C phosphorylation on the actin-myosin interaction, we analyzed the dependence of the velocity of F-actin sliding on myosin in IVMA on the concentration of C0-C2 fragments. The fragment addition dose-dependently reduced the F-actin velocity. We identified the values of the C0-C2 fragment concentration, at which the F-actin velocity decreased twofold. These values were 288.4±35.9 nM, 161.8±48.9 nM, and 244.2±13.4 nM for C0-C2, S304D C0-C2, and S274D/S285D C0-C2, respectively.

We investigated the effects of the C0-C2 fragment phosphorylation on the calcium regulation of actin-myosin interaction by analyzing the calcium dependence of the thin filament velocity over myosin in the IVMA. The addition of 0.5 μM C0-C2 fragment reduced Vmax by 25% and increased its calcium sensitivity from 4.93±0.05 to 5.72±0.02 pCa. The phosphorylated forms of the C0-C2 fragment reduced Vmax of the filaments, its calcium sensitivity (to 5.34±0.03 with S304D C0-C2 and to 5.50±0.04 with S274D/S285D C0-C2) and the Hill cooperativity coefficient by 30% compared to the non-phosphorylated form. In addition, the phosphorylated C0-C2 fragment increased the velocity of thin filaments at low calcium concentrations.

Xb-Xb cooperativity is one of the mechanisms of the calcium regulation of actin-myosin interaction. Phosphorylation of the C0-C2 fragment affected Xb-Xb cooperativity. The c50 value without cMyBP-C fragments was 60.4±10.5 μg/ml of myosin; the addition of C0-C2 fragments at concentrations of 0.5 μM and 1 μM increased the Xb-Xb cooperativity, reducing this value. At both concentrations, the non-phosphorylated fragment decreased c50 by about 30 μg/ml, and the phosphorylated forms by 40 μg/ml.

Discussion

In the transgenic mice model, it was shown that at low calcium concentrations, phosphorylation of the N-terminal part of cMyBP accelerates the cross-bridge cycling; however, at high concentrations, phosphorylation slowed it down and prolonged the cross-bridge lifetime by strengthening the binding of cMyBP-C molecules to actin [Lynch et al. J Mol Cell Cardiol., 2021]. We found that the cMyBP-C phosphorylation enhances the actin-myosin interaction at low calcium concentrations and decelerates cross-bridge cycling at saturated calcium concentrations. The results of our experiments show that the mechanism of action of cMyBP-C phosphorylation on the actin-myosin interaction can differ at saturated and nonsaturated calcium concentrations.

The experiments were performed with the equipment of the Shared Research Center of Scientific Equipment of Institute of Immunology and Physiology and supported by the RSF grant no. 22-14-00174.

**S4.258. The role of sarcomeric cytoskeleton giant proteins in muscle plasticity of hibernators: facts and assumptions**

Vikhlyantsev I.M.[1]*, Zakharova N.M.[2]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
[2]*Institute of Cell Biophysics of RAS;*
* vikhlyantsev@gmail.com

The reportwill present data on the role of changes in the isoform composition, content, phosphorylation levels and gene expression of the giant proteins titin, nebulin and obscurin in adaptation of striated muscles of hibernators (long-tailed ground squirrel, edible dormouse, brown bear) to hibernation conditions. We will considerresultsof studies supporting the hypothesis about the role of hyperactivation of calcium-dependent calpain proteases in the regulation of giant protein contents during periods of interboutactivity. Obtained data on the role of the RNA-binding protein Rbm20 in the regulation of alternative titin splicingwill bediscussed. The results expand our understanding of plasticity – one of the most important properties of muscle tissue –which allows muscles and the organism as a whole to adapt to changing conditions of functioning.

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**S4.259. The role of sarcoplasmic Ca/ATPase SERCA channels in the regulation of signaling during 3 day functional unloading of rat muscles**

Zaripova K.A.[1]*, Belova S.P.[1], Shenkman B.S.[1], Nemirovskaya T.L.[1]
[1]*Institute of Biomedical Problems RAS;*
* katsu.no.himitsu@gmail.com

With prolonged hypokinesia, gravitational unloading, limb immobilization, as well as with a long-term absence of normal motor activity, skeletal muscles undergo atrophy as a result of an imbalance between protein synthesis and degradation. We assumed that ATP and "slow" Ca2+ stimulate the start of processes. In skeletal muscle, depolarizing stimuli induce a fast metabolic signal associated with contraction as well as a slow signal that regulates gene expression. The calcium accumulating in the cytoplasm of muscle tissues is actively pumped out to the sarcoplasmic reticulum using the SERCA. This is an active process because calcium is pumped into the sarcoplasmic reticulum against a concentration gradient, and calcium-dependent ATP hydrolysis is used for it. We hypothesized that during functional unloading, SERCA is inactivated, due to which calcium can accumulate in the cytoplasm and activate catabolic signaling pathways.

CDN1163 (a specific SERCA activator) was used to test the hypothesis of SERCA activity in the regulation of cellular signaling pathways and a decrease in muscle contractile characteristics with limited functional muscle activity. Hindlimb suspension was performed using a traction method of noninvasive tailcasting. With this method of unloading rats are free to move around the cage using forelimbs and have food and water ad libitum. For the experiment, 24 male Wistar rats were divided into 3 groups of 8 animals each: C - control; HS - three-day of hindlimb suspension with placebo; CDN - three-day hindlimb suspension with CDN1163 (50 mg/kg, intraperitoneally). Experiments were performed at the Institute of Biomedical Problems, RAS, Russia. The experiments were approved by the Committee on Bioethics of the Russian Academy of Sciences (protocol 584). The study was conducted in accordance with the internationally accepted regulations and rules of biomedical ethics.

After 3 days of unloading in HS group, a decrease in the weight of the soleus muscle compared with C was revealed, however, in CDN group, the decrease was less than in HS gr. ($p<0.05$). The ATP content in the HS group was 24% higher than in group C ($p<0.05$). SERCA activator in 3CDN group prevented these changes. With the accumulation of ATP during suspension, a tendency to a decrease in AMPK phosphorylation. At the same time, in CDN group, where ATP accumulation is prevented, there is a twofold increase in AMPK phosphorylation compared with HS group, indicating a correlation between AMPK activity and ATP accumulation. Since SERCA regulates the content of Ca2+ ions in the myoplasm, the content of markers of calcium-dependent signalling pathways was determined. In HS group was an increase in CaMKII phosphorylation by 204% and IP3R content by 41% compared with C group ($p<0.05$). The administration of CDN prevents an increase in CaMKII phosphorylation and IP3R content. It has been previously shown that IP3R-dependent delayed Ca2+ signals are involved in the activation of specific transcriptional programs of the phenotype of slow and fast muscle fibers.

We assessed the expression levels in atrophic process of ubiquitin and E3 ubiquitin ligase. The mRNA expression of ubiquitin and E3 ubiquitin ligase MuRF1, MAFbx and Cbl-b was significantly increased in HS group compared with control by 158, 37, 136 and 101%, respectively. CDN administration at 3 days of suspension prevented an increase in MurF1 mRNA expression (but not MAFbx) and significantly lowered increase in Cbl-b and ubiquitin expression ($p<0.05$). The expression of E3 ubiquitin ligases can be regulated by a variety of transcription factors, such as MYOG, FoxO3. The level of FoxO3 phosphorylation was slightly reduced in all suspended groups (HS and CDN) relative to control. MYOG expression was increased in HS group by 14% relative to control ($p<0.05$). In CDN group, the level of MYOG mRNA did not differ from the control group. Thus, a decrease in the expression of MuRF1 E3 ligase upon the administration of SERCA activator is associated with a decrease in transcriptional activity of myogenin.

Analysis of anabolic signaling markers showed that level of p-p70S6K phosphorylation did not change after suspension for 3 days, while phosphorylation of 4E-BP and P90RSK was equally reduced in both suspended groups – HS and CDN. However, phosphorylation of ribosomal protein S6 (Ser240/244) and (Ser235/236) was reduced only in HS group by 83 and 55%, respectively, compared with control ($p<0.05$), and the administration of CDN prevented this decrease. The level of eEF2 phosphorylation increases by 148% in HS group compared to control, which reduces the rate of elongation processes in unloaded muscles. Administration of the SERCA activator completely prevented the increase in eEF2 phosphorylation. GSK-3b can regulate both anabolic and proteolytic processes, as it is an endogenous inhibitor of protein synthesis and a promoter of protein degradation. We found a 26% reduction in GSK-3b phosphorylation in HS group compared with control ($p<0.05$), which was completely prevented by CDN1163 administration.

Conclusion: with a 3-day m. soleus functional unloading, the administration of the SERCA activator did not affect markers of mTORC1-dependent signalling, but prevented a decrease in phosphorylation of anabolic markers – GSK3b, eEF2, and S6 ribosomal protein, which together could improve the efficiency of translation. The transcription factors FoxO3 and MYOG are activated during functional unloading of m. soleus, but MYOG may be involved in the regulation of E3 expression by MurF1 and Cbl-b ligases upon SERCA activation. The SERCA CDN1163 activator influences the regulation of Ca-dependent signalling pathways during muscle unloading through changes in CaMKII phosphorylation and IP3R levels.

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### S4.260. Theoretical study of orientation, thermodynamic and relaxation properties of eukaryotic cells

Nikitiuk A.S.[1]*, Bayandin Yu.V.[1], Naimark O.B.[1]
[1]*Institute of Continuous Media Mechanics UrB RAS;*
* nas@icmm.ru

The mechanical properties of eukaryotic cells are important biophysical markers of oncological pathology. In particular, there is a large amount of direct evidence that tumor cells are softer and less viscous compared to normal cells. It has also been found that cell stiffness is directly related to the invasiveness of tumor disease. A number of other studies have shown that metastatic cancer cells that have been isolated from the tumor are less rigid and have a higher migration and invasive potential than primary tumor cells. In addition, recently there is more and more evidence that the actin skeleton of living cells, along with elasticity, may show signs of ductility, and the cytoskeleton of tumor cells is prone to fradgility.

Three key features of the mechanical behavior of eukaryotic cells can be distinguished. Depending on the experimental approach and the time scales studied, the cells exhibit both elastic and viscous properties. The viscoelastic behavior of a cell can be described by a power law with one or two exponents for a wide range of time or frequency scales. The elastic modulus of the cells is proportional to the magnitude of the preload, with the exception of a small residual stiffness at zero preload. Currently, there is no model reflecting the complete phenomenology of the mechanical behavior of eukaryotic cells.

For the theoretical study of the mechanics of single cells, the most common are structural-mechanical and rheological models with a power law, while they have serious drawbacks. To describe the power-law character of the viscoelastic behavior of a cell, a large number of parallel connected Maxwell elements in structural and mechanical models are necessary. In this regard, there is a large number of parameters that require experimental identification and verification. The most proven rheological model with a power law in terms of correspondence of experimental results in a wide range of time scales is a model with one or more fractal elements. These elements are described using fractional derivatives, the physical meaning of which from the point of view of the structure of the object of study remains unclear.

The main components affecting the mechanical properties of cancer cells are the actin skeleton, nucleus and extracellular matrix. When considering a single cell isolated from tissue, the actin skeleton can be represented as an active mechanical element, the nucleus as a passive mechanical element with viscoelastic properties, while the influence of the extracellular matrix can be neglected. The actin skeleton should be considered as an active element of the mechanical system due to two experimentally established facts. Firstly, with a certain external mechanical or biochemical effect, actin fibers tend to orient themselves in certain directions. Secondly, in the absence of any effects, depolymerization of the fibers of the cytoskeleton of the cell occurs.

Then the mechanical behavior of the cell should be considered similar to the characteristic deformation dependencies of polymer systems. As a consequence, it is possible to assume that the progression of a

non-cancerous cell into a cancerous one may be accompanied by a phase transition during a mechanical examination of the problem. The most proven approaches to the study of phase transitions are methods of statistical thermodynamics.
The aim of the work is to apply statistical-thermodynamic theory to the development of a theoretical description of the mechanical behavior of a eukaryotic cell, taking into account its structural properties. The orientation properties of the actin skeleton of eukaryotic cells are discussed and an order parameter describing them is introduced. The type of free energy of actin fibers of the cytoskeleton of the cell was obtained. On the basis of the first and second laws of thermodynamics, as well as the Onsager's principle for the linear case of the coupling of forces and flows, evolutionary equations for elastic and orientation elements of a mechanical system are obtained. It is proposed to use an orientation-viscoelastic body as a model of the representative volume of a eukaryotic cell and evolutionary equations are obtained. The relaxation spectrum for structural-mechanical models is also introduced. The results of modeling based on various existing cell models and the model proposed in this paper are compared, in addition, the possibility of describing the double power law of viscoelastic relaxation of living cells is analyzed.
The work was financially supported by the Ministry of Science and Higher Education of the Russian Federation (Government contract No. AAAA-A19-119013090021-5) and the Grant of the President of the Russian Federation for State support of young Russian scientists No. MK-44.2022.1.1 (Agreement No. 075-15-2022-362).

### S4.261. Transfer function of electromechanical coupling

Grishin S.N.[1]*, Khairullin A.E.[1], Gabdrakhmanov A.I.[1], Teplov A.Y.[1], Efimova D.V.[1], Ziganshin A.U.[1]
[1]*Kazan State Medical University;*
* sgrishin@inbox.ru

It is well known that, upon excitation of a motor neuron, an action potential (AP) of a muscle fiber can normally be recorded, and the fiber itself will contract. AP of muscle fibers is preceded by depolarizing potentials of the end plate. These arise in response to the entry of impulses along motor neurons and are called the end plate potentials (EPPs) and belong to the postsynaptic potentials. EPPs have maximum amplitude in the region of the end plate, decrease exponentially and disappear as they move away from it.
Classical experiments in Bernard Katz's laboratory showed that if a neuromuscular agent is affected by curare (or its synthetic analogue - d–tubocurarine), a blocker of postsynaptic H-cholinergic receptors, the EPP value becomes smaller. d-tubocurarine is used for muscle relaxation during surgeries under general anesthesia. It is known that d-tubocurarine is a competitive acetylcholine antagonist. That is, at a certain concentration, it completely displaces acetylcholine, and does not allow the latter to bind to nicotinic cholinergic receptors. Thus, when using H-cholinergic antagonist, it is impossible to trace the entire course of the correlation between the amplitude modulation of the evoked end plate currents and the muscle contraction force, since at a certain concentration of the agent, postsynaptic potentials simply disappear. In addition, the cholinergic receptor blockers not only reduce the amplitude of EPP, but also change their temporal characteristics, and that has a significant modulating effect on AP generation in the near-synaptic region of the muscle fiber.
A gradual decrease of the postsynaptic response amplitudes can be obtained using the modulators of the neurotransmitter composition. The search for effective (with extremely pronounced but reversible effect) presynaptic modulators of myoneural transmission has been ongoing throughout the history of synaptology. On the basis of comprehensive studies it become apparent that the purinergic endogenous synaptic agents - the co-mediators of acetylcholine - adenosine triphosphate (ATP) and its most stable metabolite - adenosine are among the most effective modulators of neuromuscular transmission.
Earlier we obtained data that these two purines in physiological concentrations have a pronounced depressant effect both on the amplitude of postsynaptic responses and on the contraction force of the frog sartorius muscle (tailor's muscle). Moreover, the enhancement reserve of the inhibitory effect on these parameters with an increase of these purines concentration is pronounced like no other endogenous modulator gives. We have shown, for example, that ATP is involved in the accumulation of muscle efforts released under stress and hyperthermia. Taking all this into account, in order to reveal the transmission correlations in the work of the motor unit, we aimed to record and compare changes in the parameters of the evoked responses of the end plate and contraction of the frog skeletal muscle under the influence of ATP and adenosine at concentrations differing by up to 5 orders of magnitude.
Standard electrophysiological experiments were performed on Rana radibunda frogs at room temperature in accordance with the European Convention for the Protection of Laboratory Vertebrate Animals with the permission of the Ethical Committee of Kazan State Medical University. Statistical analysis of the data was performed using the OriginPro 8 software package. The normality of the data distribution was verified by the Kolmogorov-Smirnov test. Experimental results were normalized relative to the control and presented as the mean ± standard deviation, in percentages. Regression analysis for the presence of a functional relationship was performed, as well as the Akaike information criterion test, to select the optimal regression equation. The orthogonal regression method was used to construct a non-linear curve of the relationship between the amplitude of the end plate currents and the contraction force. Statistical significance was tested with analysis of variance (ANOVA).
We found that when inhibiting by either adenosine or ATP the amplitudes of postsynaptic responses of down to 2/3 of the initial values, the decrease of contraction force was less pronounced - as the square root of the value of the reduced amplitude of the postsynaptic responses (with trivial normalization). With further depression induced by these inhibitors the effect on the amplitude of postsynaptic responses became equal in value to a decrease of the contraction force. The obtained ratios can be used to recalculate the effect on the muscle contraction force, when only the effect in the neuromuscular synapse of some modulator is known.

## S5. Biophysics of complex multicomponent systems. Math modeling. Bioinformatics

### S5.262. A new mathematical method for constructing a multiple alignment of highly divergent nucleotide and amino acid sequences

Korotkov E.V.[1]*
[1]*Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences" (Research Center of Biotechnology RAS;*
* bioinf@yandex.ru

The problem of multiple alignment is one of the central problems of bioinformatics. Much attention has been paid to the development of mathematical algorithms for constructing multiple alignments, and various mathematical methods have been developed. Dynamic programming, progressive alignment, iterative methods, as well as hidden Markov models and genetic algorithms are most commonly used to construct multiple alignments. However, all currently developed approaches, methods and algorithms will not allow building multiple alignment if there is no statistically significant pairwise alignment in the analyzed sequences. In this case, it is not possible to construct a statistically significant guide tree for progressive alignment. If the

sequences are very different, then it is also impossible to find statistically significant "seeds" or common "words". It turns out that it is currently extremely difficult or impossible to build a multiple alignment for very different sequences. By strongly differing sequences we mean sequences that have accumulated more than 2.5 random substitutions (x) per nucleotide relative to each other (x>2.5). It would be possible to find such an alignment by constructing a multiple alignment using N-dimensional dynamic programming for all analyzed sequences. But such an approach requires huge computer resources and is currently impossible to implement. It turns out that in modern methods developed for multiple alignment of amino acid or nucleotide sequences there is a certain gap. In the present work, we filled this gap and developed a mathematical method for generating multiple alignments for highly diverged sequences (MAHDS), which allows the construction of multiple alignments for such sequences where any pairwise alignment does not have sufficient statistical significance. We explored the possibilities of ClustalW, Clustal-omega, T-coffee, Kalign, Mafft, Muscle and AllAlign and some other programs to create multiple alignments of nucleotide and amino acid sequences depending on the degree of their evolutionary divergence (x). It was possible to show that these programs work well up to x<2.0. However, MAHDS makes it possible to build statistically significant alignments for the degree of evolutionary divergence x in the range from 2.5 to 4.4. This opens up new possibilities for studying the evolutionary divergence of both nucleotide and amino acid sequences.

The main idea of the MAHDS method is to find such an image of the multiple alignment of random sequences that would most accurately describe the multiple alignment of the analyzed sequences. In this case, we do not build a multiple alignment for the analyzed sequences by any method, but only optimize some images of multiple alignments. The optimization is to take images of different multiple alignments and adapt them to the available sequences. As an optimal multiple alignment for the analyzed sequences, it is convenient to take such a pattern (or PWM) that will have an extremum of the similarity function.

The mathematical method we developed was applied to align promoter sequences from the genomes of A. thaliana [Korotkov et al., 2021a], Oriza sativa [Korotkov et al., 2021b], Capsicum annuum [Rudenko, Korotkov, 2022]. Promoter sequences were taken from the EPD databank (https://epd.epfl.ch//index.php). This work shows that many regions of promoter sequences from -499 to +1 are highly conserved. Also, the +1 to +70 regions contribute greatly to creating a multiple promoter alignment. In total, it was possible to obtain from 5 to 16 classes of multiple promoter alignments for the studied genomes, which contain from 55 to 75% of known promoters. The generated multiple alignments were used to search for potential promoter sequences in the rice genome [Korotkov et al., 2021b]. In the genome of Oriza sativa, 145277 potential promoter sequences (PPS) were found. Of these, 18,563 are promoter sequences of known genes, 87,233 PPPs are part of transposons, and 37,390 PPPs are found in unannotated sequences. For the Capsicum annuum genome (genome size ~3 billion nucleotides), the number of PPPs is approximately 960 thousand, with the number of false positives less than 1%. For the human genome, we have found more than 1 million PPPs.

The developed method was also applied to align amino acid sequences from highly divergent protein families [Kostenko and Korotkov, 2022]. Using 21 protein families as an example, it was shown that MASHDS allows finding more statistically significant alignments than all previously developed methods. Any user can build a multiple alignment using the MAHDS method at http://victoria.biengi.ac.ru/mahds/auth.

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### S5.263. Active control of a robotized fluid in a convective loop with swimming micro-cyborges

Stupnikova A.V.[1]*, Bracun D.A.[1]
[1]*Perm National Research Polytechnic University;*
* stypnast2014@yandex.ru

In recent years, the attention of researchers has been attracted by the active fluid, which is understood as a medium with active elements capable of moving independently. A stricter definition defines an active fluid as a viscous suspension of particles, cells, macromolecules, or bacteria that can convert chemical energy into mechanical work by creating microscopic stresses in the fluid [1]. It has been shown that the efforts of many active elements can lead to such macroscopic phenomena in liquids as bio-convection or spontaneous transition to a superfluid state.

Some recent works demonstrated the possibility of creating not just an active medium with self-moving elements, but a new type of the medium, in which a swarm of micro-robots operates. For example, the technology for producing numerous inexpensive micro-cyborgs was presented in [2,3]. The cheapness of production is determined by the fact that the authors used a living bacterium as the biological basis of the swimming micro-cyborg. To externally control the bacterium, its body was covered with nanoparticles sensitive to the magnetic field. In addition, the bacterial genome was modified to produce a quorum sensing (QS) of the swarm. It means that bacteria can use diffusion signals to regulate gene expression depending on population density. A technique was developed to create micro-cyborgs possessing chemosensitivity, and the time of its activation was determined.

Thus, creating an environment saturated with millions of simple but effective devices capable of self-motion and communication is no longer fantasy. Fluid mechanics specialists are not yet familiar with these works, because micro-cyborg swimmers are discussed in the closed robotics community. Specialists in robotic control systems, who consider the liquid only as an environment for their robots, are also not fully aware of the perspectives. Thus, we are convinced that a new type of medium named by us as a “robotized fluid” needs to be investigated in detail. This paper studies the dynamics of bacterial self-organization in a thermal field in which QS is a behavioral adaptation and a collective response to environmental change. We assume that since micro-cyborgs are artificially altered bacteria, they can be programmed with the necessary list of properties. In this work, we assume that swimming micro-cyborgs have special heat-sensitive inserts. As they enter the thermal field, the swarm elements move along the temperature gradient. The swarm behavior is studied using the simplest convective system: a closed channel, the cross section of which is much smaller than the characteristic size of the loop [4,5]. We suppose that the convective loop is filled with incompressible fluid, inhomogeneously heated and placed under the gravity field. The motion equation averaged across the channel describes 1-D fluid flow, which makes the convective loop problem the simplest model for studying Rayleigh-Benard convection, as well as a model problem for considering a variety of hydrodynamic phenomena. An issue of controlling various modes of convective instability in the loop was considered in numerous papers [4,5]. The control of a continuous medium, which generally has an infinite number of degrees of freedom, is the most important direction of continuum mechanics.

In this paper, we consider a toroidal loop and assume that the fluid contains a swarm of swimming micro-cyborgs. The basic idea of control is that each micro-cyborg is heavier than a liquid and its movement in the medium locally changes the density of that medium. When moving a large swarm of swimmers, the effect can be significant. Two approaches are proposed to describe the properties of the system: hybrid and continuous-medium. In the first approach, we consider the micro-cyborgs as a complex system of locally interacting microscopic elements with individual dynamics based on Aristotelian mechanics (because of the strong dissipativity of the cyborg movement process). The hybridity of the approach is determined by the fact that the calculation of swarm behavior at each time step is synchronized with the numerical simulation of fluid motion. In the second approach, we developed a continuum model, in which a swarm of micro-cyborgs is treated as an effective liquid suspension with an active phase.
Numerical experiments show that a swarm of micro-swimmers can successfully maintain the stability of an inhomogeneously heated robotized fluid, even at high Rayleigh numbers, when convection already should occur. Since the velocity of micro-cyborg in the liquid is limited by the capabilities of the bacterium, the most important parameter of the convective system is its characteristic size. The critical size of the loop, at which rearrangements of the micro-swimmers swarm do not have enough time to make the necessary changes in the state of the robotized fluid, is determined.
The work was financially supported by the Ministry of Science and Higher Education of the Russian Federation (Project No. FSNM-2020-0026).
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### S5.264. Agent-based model of tumor growth with account of solid stress and nutrient supply

Kolobov A.V.[1]*, Kuznetsov M.B.[1]
[1] *P.N.Lebedev Physical Institute of RAS (LPI);*
* kolobov@lebedev.ru

In this talk an off-lattice agent-based model of tumor growth is presented, that describes tumor as a network of proliferating cells, which dynamics depends on the stress generated by intercellular bonds. A method of consideration of tumor cells and accounting for intercellular bonds is introduced, that ensures smooth dynamics of cell network and allows to maintain relative numerical cheapness but at the same time naturally reproduces the effects typical to more complex approaches, like the elongation of cells towards the low pressure regions and the tendency of maximizing the contact area between cells. The simulations of free tumor growth, restricted only by the residual stress generated within the tumor, demonstrate the influence of tissue hydraulic conductivity and strength of cell-cell interaction on tumor shape and growth rate. The simulations of compact tumor growth within the normal tissue show that strong interaction between tumor cells is a major factor significantly limiting tumor growth. Moreover, they detect ambiguous effects of the normal tissue size and the strength of interaction of normal cells on the tumor growth which natures change qualitatively depending on the value of tissue hydraulic conductivity. Simulations of tumor growth in normal tissue with account of nutrients, which also influence tumor cell behavior, yield different regimes of tumor growth, including growth without saturation during at least several years with formation of large necrotic cores in case of low tissue hydraulic conductivity and sufficiently high nutrient supply, which qualitatively correlates with the known clinical data.
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### S5.265. Analysis of genes assotiated with the development of reccurent urolithiasis

Faizullina E.A.[1]*, Sheveleva O.Y.[1], Molotkov T.P.[1], Orlov Y.L.[1], Alfimov A.E.[1]
[1] *I.M.Sechenov First Moscow State Medical University*
* faiz.eline2000@yandex.ru

This study aims to build a list of genes associated with the development of recurrent urolithiasis (kidney stone disease, KSD), analyze gene ontologies, and reconstruct the gene network to identify key genes associated with this disease. Bioinformatic approaches to the analysis of the structure of the gene network, previously presented in the works of students of Sechenov University for other diseases, were methodically used (Orlov et al., 2021; Dokhoyan et al., 2022).
The relevance of the study is due to the fact that urolithiasis occupies one of the leading places in the structure of urological diseases in terms of frequency of occurrence, frequency of calls for emergency medical care and hospitalizations (Apolikhin et al., 2014). According to official statistics, in 2012 the incidence rate of KSD per 100,000 population in the Russian Federation was about 550, while there was a significant increase in the number of cases compared to 2002 (Apolikhin et al., 2014). Recurrence of urolithiasis after various surgical interventions within 5 years may occur in half of patients (Tiselius, 2006) risk factors for stone formation. In the last decade, the main direction in the study of genetic risk factors for the development of KSD has been to identify the association of polymorphism of a particular gene with urolithiasis. The results of such studies are to establish the presence or absence of an association of gene polymorphism with urolithiasis. The study of such associations is of great importance for understanding the pathogenesis of the disease and choosing the tactics of managing patients with urolithiasis.
To compile a list of genes associated with the development of urolithiasis, the OMIM (https://omim.org/) and GeneCards (https://www.genecards.org/) databases were used. Further, a wide range of bioinformatic tools, such as Metascape (https://metascape.org/), GeneMANIA (https://genemania.org/), STRING-DB (https://string-db .org/). To analyze the categories of gene ontologies, DAVID resources (Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/) were used. The study of the structure of the gene network and comparison with previous studies on this topic made it possible to identify 45 candidate genes and several key genes, including CASR, SLC26A1, AGXT. The alleles of these genes are potentially risk factors for the development of various forms of KSD, including recurrent urolithiasis, and can be used in a diagnostic gene panel.
Analysis of the clusters of the constructed network showed that the greatest contribution to the manifestation of the disease is made by genes related to clusters: transport of small molecules, transport of proximal tubules, monoatomic cation transport, synthesis, secretion and action of parathyroid hormone, glyoxylic acid metabolism, processes of the renal system, absorption of minerals. A study was made of the relationship between the list of genes and the structure of the network of interactions (gene network) with the development of this

disease and other pathological conditions. First of all, these include: nephrolithiasis, nephrocalcinosis, kidney stones, hypercalciuria, polyuria, renal failure. A systematic computer search for marker genes and drug compounds reflects modern approaches to the analysis of pharmaceutical products (Koshechkin et al., 2022). The obtained results on the isolation of marker genes may be useful for further research on recurrent urolithiasis and for the development of new methods of treating this disease, as they provide information about genes that can be potential drug targets (Peerapen and Thongboonkerd, 2023).

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## S5.266. Analysis of palindromic sequences in SARS-CoV-2 genome

Kapunac S.K.[1], Beljanski M.B.[1], Mitić N.M.[1]*
[1]*University of Belgrade, Faculty of Mathematics, Belgrade, Serbia;*
* nenad.mitic@matf.bg.ac.rs

Recently, the RNA genome of SARS-CoV-2 was shown to be organised into structural and functional blocks of RNA information that are demarcated by short RNA breakpoint sequences that promote recombination at specific non-random locations within the viral genome consisting of short repetitive sequences, namely palindromes. Palindromic sequences are involved in the formation of RNA secondary structures. They can be locations recognised by RNA-binding proteins as well as places of RNA recombination [1].

We analyse SARS-COV -2 genomes with particular attention to mutations within palindromic sequences. A dataset of 423.425 complete isolate nucleotide sequences was extracted from https://www.ncbi.nlm.nih.gov/sars-cov-2 (database access on August 25, 2021. After the cleanup process, 347.962 isolates with 123.667 unique (related nucleotide sequences) with 226.624 corresponding unique protein (nucleotide) coding sequences and 141.926 unique protein (AA) sequences remain. The consistency of the two sequences was checked using the standard genetic code table (transl_table 1). Each sequence was annotated with a World Health Organisation (WHO) SARS-CoV-2 annotation.

Each nucleotide sequence was individually aligned to the reference sequence SARS-COV -2 (NC_045512.2) using the MAFFT alignment program [2]. The StatRepeats program [3] was used to determine all palindromes with a minimum length of 8. A total of 801.935.394 palindromes were determined. Among them, 785.854.841 repeats were identical with their pair in reference sequence NC_045512.2. Other (16.080.553) palindromes have some mutations related to reference sequence.

The analysis of the number of palindrome occurrences was performed in 5 time intervals of 4 months from 31.12.2019. to 25.08.2021. The average number of palindromes per isolate shows a constant increase, respectively by intervals: 1.92, 3.51, 9.31, 14.84, and 20.66.

We analyse mutations in all 12 types of ORFs present in the set of extracted sequences (ORF1a polyprotein, ORF1ab polyprotein, surface glycoprotein, ORF3a protein, envelope protein, membrane glycoprotein, ORF6 protein, ORF7a protein, ORF7b protein, ORF8 protein, nucleocapsid phosphoprotein, ORF10 protein). Among them, normalised on average protein length, after ORF1a and ORF1ab,the surface glycoprotein (S-protein) has the highest number of repeats, on average 4.65 palindromes with a length of >=8. The highest number of palindromes is located around positions 22.000 (left part) and 24.300 (right part), counting the positions with respect to the beginning of the isolates. For the total number of mutations, almost 78% resulted in amino-acid changes in the corresponding proteins.

In further research, we plan to perform a detailed analysis of mutations of palindromic sequences according to the SARS-CoV-2 WHO variant classification and also their influence on amino-acid changes and occurring RNA secondary structures or locations recognized by RNA binding proteins.

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## S5.267. Analysis of the Human Mortality Database survival curves using a multiphase numerical model

Alekseev A.A.[1,2]*
[1]*Lomonosov Moscow State University;*
[2]*Russian Gerontological Research and Clinical Center;*
* alekseev@physics.msu.ru

Introduction

In the study of aging, survival curves, which show the decrease in the number of animals remaining in the experiment for survival over time relative to the initial number of animals, are quite often considered. These curves are used both for the general assessment of mortality dynamics and for calculating the mean (MeanL) and maximum (MaxL) lifespan of animals. The values of MeanL and MaxL are used for comparative estimation of the effect of different environmental factors and genetic modifications in the search for geroprotectors (substances that slow down aging processes), as well as genes associated with basic aging processes or simply having a significant correlation with age (what is behind such a correlation is not always possible to judge reliably).

Methods of mathematical biology are also used to analyze such curves, in particular the theoretical models of Gohmertz and Weibull [2]. Although these models have a detailed mathematical basis, they do

not allow us to accurately describe the survival curve in some cases, including data from the Human Mortality Database [3] or experiments on model animals, where the curve is obviously biphasic - with low mortality at young age, and with non-smooth transition to the stage of accelerated growth of mortality, for Ames dwarf mice, where the effect of "postponing" of exponential phase of aging as the result of modification of growth hormone receptor GHR [1], or for experiments on survival with 8926 individuals of Drosophila melanogaster [4].
However, until recently, we are not aware of an explicit assumption of biphasic or multiphasic phase in the description of the lifespan curve in the literature, with one exception - in one chapter in [5], a biphasic model of aging for the lifespan curves of Drosophila melanogaster was considered.
Methods
We set out to describe the entire variety of mortality curves for of a number of animals of different groups (insects, reptiles, mammals) with one model with different sets of parameters for different animal species. As a first step, we took data (survival curves) from the database [3], with several geographically distant countries, and cohorts of different birth years.
The basis of our numerical model was a piecewise-adjusted dependence for the mortality rate (MR) on age, which has 5 phases, including a high mortality phase after birth, a decrease in mortality in adulthood, and an exponential increase in mortality (from a certain age) due to aging. The model has three parameters defining mortality at the initial point in time, at the minimum of mortality, and at the "plateau" at middle age. In addition, there are four parameters for the transition ages between phases, as well as a coefficient in the exponent exponent. Also added to the model is the coefficient of "stochastic" mortality, which is independent of age and unrelated to aging. In total, there are 9 parameters in the model.
In addition, the model explicitly takes into account the heterogeneity of the population by "initial health" and describes the decrease in the stress-resistance (SR) of the organism under the influence of random external factors, and the rate of SR decrease is proportional to the a priori set multiphase dependence of the MR on age.
In the course of calculations, the "health reserve" of each group of "virtual individuals" decreased, and after the decrease below a certain limit (which is also a parameter of the model), the fact of death was recorded for the "individual". Thus, the entire survival curve was calculated. In order to obtain an averaged curve, this algorithm was iteratively executed a certain number of times (its optimal knowledge was determined in the course of numerical "experiments").
Then, for each considered curve, the procedure of model parameter instantiation (fitting) was performed. Calculations were performed using the R programming language, the optimization problem was solved using the optim function, and confidence intervals for the parameters were estimated using the bootstrap procedure.
Results and Conclusions
Thus, we identified the model parameters for a set of human survival curves from the base [3], and, in general, showed the effectiveness of the multiphase approach in modeling of survival curves. We plan to use this approach for further analysis of the endurance curves for model animals, in order to work out a general approach to estimate model parameters for the effect of "delayed" aging [1], and justify the rationale for using MeanL and MaxL values in many experimental studies of aging, since MeanL and MaxL values reflect both the effects of aging itself and many specific features of the experiments, and the influence of environmental factors.
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**S5.268. Application of a reduced mathematical model of photosystem II reaction centers to determine the heterogeneity of its antenna under stress conditions**

Pluysnina T.Y.[1]*, Khruschev S.S.[1], Degtereva N.S.[1], Riznichenko G.Y.[1], Rubin A.B.[1]
[1]*Lomonosov Moscow State University;*
* plusn@yandex.ru

Under optimal conditions most of the PSII RCs form dimers and can attach light harvesting proteins, the so-called α-centers. Under unfavorable conditions, an increase of the portion of RCs with a reduced antenna, the β- and the γ-centers, is often observed. The ratio of PSII RCs with different sizes of light harvesting antenna depends on growth conditions and stress factors. The change in the ratio of different types of PSII RCs can serve as an indicator of the stress impact on the algae culture. The assessment of such changes can be useful for biotechnology and environmental monitoring. A common way to assess the ratio of reaction centers with different antenna size is to analyze chlorophyll fluorescence rise (FR) of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) treated samples [1]. To quantify such ratios multiexponential decomposition of the complemental area above the FR is commonly used. However, this approach has certain limitations [2, 3]. To overcome these limitations, we propose an approach based on mathematical modeling.
The constructed detailed mathematical model of the processes in the DCMU treated PSII contains 24 ordinary differential equations (ODEs) and includes redox changes of the oxygen evolving complex (OEC), photoexcitation and redox changes of the RC pigment P680, and redox changes of pheophytin and the primary quinone QA. The hierarchy of characteristic times of these processes makes it possible to reduce the model to a system of three ODEs. The solution of the reduced three-state model exactly reproduces the solution of the complete system in the range from microseconds to seconds. The combination of several such models made it possible to describe different types: α- β- and the γ-centers. The parameters of the reduced model were identified by fitting model solutions to the FR for DCMU- treated of Chlorella microalgae under nitrogen depletion and of Chlamydomonas microalgae under sulfur depletion.
Using the model, we tested the existing hypotheses about the nature of additional phases on the fluorescence rise, whether they reflect RC with a different antenna or some process. The model demonstrates that the presence or absence of an additional phase on the fluorescence rise measured for DCMU treated samples may depend on the balance of reaction rates on the donor and acceptor sides of the PSII. If the characteristic time of reactions on the donor side is longer than the characteristic time of reactions on the acceptor side, the initial part of the FR till ~100 μs demonstrates a slower rise compared to an exponential increase. If, for example due to stress, transitions in OEC slow down, and the characteristic time of the processes on the donor side becomes shorter than the characteristic time of the processes on the acceptor side, then an additional positive phase with characteristic times of 1–10 ms is observed.
An analysis of the experimental curves showed that under mineral starvation additional phases on the induction curve can indicate both the appearance of RCs with reduced antenna size and the slowing down of the S1–S2 transition in OEC. The model shows the fundamental possibility of the appearance of an additional phase on the FR obtained from samples treated with DCMU due to the processes in OEC. It is hypothesized that under normal conditions the phases on the curve reflect the presence of α- β- and the γ-centers, while under stress conditions,

one of the positive phases may reflect a slowdown in processes on the PSII donor side.

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### S5.269. Application of text complexity estimation methods to the analysis of genomic clusters of transcription factor binding sites

Dergilev A .I.[1,2]*, Orlova N.G.[3], Mitina A.V.[4], Orlov Y.L.[1,4]
[1]*Novosibirsk State University;*
[2]*Institute of Cytology and Genetics SB RAS;*
[3]*Financial University under the Government of the RF;*
[4]*I.M.Sechenov First Moscow State Medical University (Sechenov University;*
* arturd1993@yandex.ru

A software application is presented for studying the molecular mechanisms of formation of protein-DNA complexes using the example of assessing the informational complexity of genomic sequences containing transcription factor binding sites. The work is based on the application of modern mathematical and computer methods of the theory of information transmission and data compression (Orlov and Potapov, 2004), as well as the theory of data analysis and search for patterns to the study of genetic sequences (Vityaev et al., 2001; Orlov et al., 2002). Understanding biological processes requires the development of new software tools (Dergilev A.I. et al., 2021) to determine binding sites (sequence regions) from sequencing data, including in new model plant genomes, based on the processing of large data sets and the implementation of algorithms for estimating complexity, including the Lempel-Ziv algorithm and Shannon entropy estimates (Orlov and Potapov, 2004).

The convenience of using programs for the analysis of genetic texts on personal computers and the ability to process large amounts of data make them a necessary tool in the experimental work of molecular biologists. A huge amount of experimental data on DNA sequences, accumulated in specialized databases, makes it possible to obtain qualitatively new knowledge about the structure and evolution of genomes. Thus, a decrease in text complexity (including Shannon entropy and linguistic complexity estimates) was previously shown in DNA regions containing regions of single nucleotide polymorphisms (Safronova et al., 2015). Next, the tasks were to evaluate groups (clusters) of co-located transcription factor binding sites on DNA (Dergilev and Orlov, 2020). The change in the complexity of the DNA text on average (in a sliding window) for samples of nucleotide sequences containing clusters of transcription factor binding sites is shown.

A larger amount of data confirmed the difference in text complexity for the coding and regulatory parts of the genome, which include site clusters. In the presented work, the tasks are set to develop and apply new computer methods for statistical analysis of the complexity of genetic texts (Orlov et al., 2006), prediction of functional sites and regulatory regions in genomic DNA, search for repeats in genomes and analysis of their structure. Python scripts have been developed for fast encoding and decoding using the LZ77 method. A program for working with compact compression of texts of a large amount of information has been obtained, methods and scripts have been tested on experimental data.

A proprietary set of "Genomic Texts Complexity Analysis" tools has been developed using the latest version of the Python language in the PyCharm programming environment, using the Qt5 graphical module, which has the convenience and simplicity of an interface that allows you to conveniently work in one window with several tasks, providing the user with the ability to encode / decode texts, the calculation procedure complexity profile according to the modified Lempel-Ziv algorithm in a sliding window, visualization of the obtained complexity profiles. Thus, the software tool for analyzing the information content of DNA has been updated, and new estimates of the information content and entropy of the regulatory regions of genes have been made.

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### S5.270. Application of the rat brain arterial system model to estimate the oxygen and glucose distributions

Kopylova V.S.[1]*, Boronovskiy S.E.[1], Nartsissov Y.R.[1,2]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Moscow, Russia;*
[2]*Biomedical Research Group, BiDiPharma GmbH, Siek, Germany;*
* kopilova.veronika@yandex.ru

The permanent supply of oxygen to the brain is fundamental to the normal body functioning. A decrease in the brain oxygen concentration below the physiological level for even a short time can lead to catastrophic consequences. Along with oxygen glucose is another vital metabolite that keeps neurons functioning. It should be noted that when measuring in brain tissues in vivo the concentration of oxygen and other metabolites, researchers face a number of problems that make it difficult to obtain results. The brain arterial network is a complex system consisting of a huge number of vessels of various radii that provide blood flow for all areas of the brain. Therein the use of mathematical modeling methods both for assessing the metabolite flow in the complete arterial tree and for calculating the concentration of oxygen and other compounds in the entire brain volume requires serious computing capacity. In this work the assessment of the oxygen and

glucose distributions in tissues was carried out on the basis of a rat brain arterial system model via brain volume segmentation into local areas followed by calculating the concentration in each segment. Withal the concentration of metabolites near the corresponding vessels was calculated using the SSDF with subsequent validation by the finite element method. Using the constructed model of the rat brain arterial system with optimal values of the branching parameters distributions of oxygen and glucose concentration in the entire brain volume were obtained. The corresponding average concentrations for these compounds in turn are consistent with the experimental data. The main feature of the approach proposed in the work is the ability to quantify the distribution density of the concentration of metabolites when considering large anatomical structures of the brain as well as the entire organ as a whole. Accordingly, it allows one to obtain statistical characteristics as, for example, average values taking into account the architecture of the arterial network. At the same time, the described mechanism of brain volume segmentation the into local areas can significantly reduce the computational complexity of both the segmentation process and the concentration assessment in a single segment.

### S5.271. Assessment of connectivity between brain hempispheres in epikeptiform activity caused by administration of pentylentetraxole

Erhsova A.S.[2,1]*, Grishchenko1 A.A.[1,2], Suleymanova3 E.M.[3], Vinogradova L.V.[3], Sysoev I.V.[1,2]
[1]*Saratov Branch of Kotel'nikov Institute of Radioengineering and Electronics of Russian Academy of Sciences, Saratov, Russia;*
[2]*Saratov State University, Saratov, Russia;*
[3]*Institute of Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences, Moscow, Russia;*
* anshova2002@gmail.com

Peptilentetrazole is traditionally used to provoke epileptic seizures in healthy animals: rats, mice and guinea pigs. Such pharmacological models of epilepsy allow us to study the work of deep brain structures, including the thalamus and hippocampus, actively involved in the development of pathological activity [1], [2]. To study the connectivity between the hemispheres, various methods were used, such as calculating the values of the Kolmogorov-Smirnov and Mann-Whitney tests and the phase synchronization coefficient.

The data of 9 animals were used in the work: 6-7 month-old males of the Wistar line from the Stolbovaya nursery in the Moscow region. All experiments were conducted in accordance with the principles of the European Community for conducting experiments on animals and approved by the Committee on the Ethics of Animal Experiments. The electrical activity of the neocortex was recorded using electrodes [3] (stainless steel screws) implanted in symmetrical areas of the frontal cortex of both hemispheres. The average duration of the recordings was one hour, the number of secretions for each animal ranged from 42 to 477.

In order to use various connectivity calculation metrics, it was necessary to obtain an accurate markup. To do this, an algorithm was created based on the detection of a single discharge from single-channel data. The accuracy of the method was confirmed by the calculation of specificity and sensitivity [4]. The values obtained were high enough for two characteristics at the same time, which indicated that the method works quite accurately.

In this work, connectivity between the hemispheres of the brain was evaluated using two different methods. The first was based on applying and obtaining results using Kolmogorov-Smirnov and Mann-Whitney tests. The tests are based on a similar logic, which consists in determining the appropriate distribution in the sample [5]. It was hypothesized that the hemispheres of the brain are interconnected, thus the distributions of two samples of these hemispheres were compared. The value 0.05 was taken as the standard p-value. If the test result is lower than p, then the hypothesis is correct, and if it is higher, then it is not. The peculiarity was that the values obtained were calculated for all discharges in each rat. The second approach was the calculation of the phase synchronization coefficient, showing the quantitative characteristic of synchronization between the two leads of each discharge. To study phase synchronization, instantaneous phase signals were isolated from the marked signals using the Hilbert transform and their difference was calculated. Obtaining the values of the phase synchronization coefficient Ixy was carried out by finding the modulus of the arithmetic mean exponent raised to the power of an imaginary unit multiplied by the phase difference. Accordingly, the value of Ixy changed from 0 (full asynchrony) to 1 (full synchronicity). As a result, an unusual pattern was revealed: quite often, the coefficient values were high for the discharges allocated by the algorithm in two hemispheres, and for those discharges that appeared only in one hemisphere. The practical importance of detecting the synchronization phenomenon makes it necessary to obtain reliable estimates of the synchronization coefficients from the observed data. At the same time, in most cases it is necessary to deal with short signals. In this case, we have to take into account that it is likely to get a large value of the estimate for disconnected systems and mistakenly interpret it as a characteristic of the existing connection of systems.

In the course of the work, an algorithm for detecting discharges was written, and methods for calculating the values of the Kolmogorov-Smirnov and Mann-Whitney tests and the phase synchronization coefficient were implemented. Quantitative conditions were demonstrated under which large (close to one) values of the estimation of the synchronization phase were observed, which indicated high connectivity between discharges at the same time in different hemispheres of the brain.

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### S5.272. Assessment of linguistic complexity of genetic sequences of SARS-CoV-2 strains

Mitina A.V.[1]*, Orlov Y.L.[1]
[1]*I.M. Sechenov First Moscow State Medical University (Sechenov University);*
* alinamitina44@gmail.com

Motivation and purposes. The active spread of coronavirus infection requires the development of new models of variants of the connection of nucleotide sequences of viruses and their functional significance and possible pathogenicity. To date, several thousand strains of SARS-CoV-2 have been described, which are the causative agents of

COVID-19. Some variants are of particular interest, as they have high transmissivity and lethality rates. The existing variety of data obtained by sequencing makes it possible to analyze the genomic sequences of various strains of coronavirus using mathematical methods for assessing the structure of the genome, studying "hot spots" of mutations.
Methods. Sequences of SARS-CoV-2 strains obtained from Genbank were analyzed by evaluating the linguistic (combinatorial) complexity of the text (Orlov, Potapov, 2004). The value of combinatorial complexity is determined by the ratio of the number of words encountered to the number of possible words in a sequence of fixed length. This method allows you to set the level of saturation of the genetic text with repetitions. Evaluation of the flanking regions of nucleotide polymorphism points showed the presence of areas of low text complexity, which is associated with repetitions, increased probability of DNA breaks with subsequent repair errors. Sections of low text complexity are less evolutionarily conservative and are subject to mutations with a higher frequency.
Results. An algorithm for estimating the linguistic complexity of the genome for delta and omicron variants of SARS-CoV-2 is applied. The sites corresponding to the low and high levels of saturation of the genetic text have been identified. The complexity profile of the text is compared with the location of the open reading frames of the coronavirus. The data were compared with the variability of the genome according to the calculations of the Beijing Institute of Genomics (Beijing Institute of Genomics).
Conclusion. Infection with the SARS-CoV-2 virus can have a number of adverse consequences for the human body, ranging from mild malaise to death. The number of cases continues to grow in the world at the moment, and the number of deaths from COVID-19 is approaching the mark of 7 million people. The complexity for diagnosis and treatment is due to the continuous process of mutation, which leads to the emergence of new variants of the coronavirus. It is assumed that computer methods will allow assessing the pathogenicity of new strains based on their genetic sequence.

### S5.273. Automated sleep stage detection technique based on parallel computing technology

Zhuravlev M.[1]*, Ukolov R.V.[1], Runnova A.E.[1,2]
[1]*Saratov State University;*
[2]*SSMU;*
* zhuravlevmo@gmail.com

The state of sleep today is seen as an active neurophysiological process, accompanied by relatively cyclical changes in physiological and psychological activity. Accordingly, several studies have demonstrated the importance of sleep for various functions such as synaptic homeostasis [1], brain recovery from toxic disorders [2], memory consolidation [3], and emotion processing [4, 5].
Currently, a separate and very laborious task in the field of sleep research is the detection of various stages of sleep [6, 7] during the analysis of polysomnographic data. Currently, there are a significant number of methods and algorithms for automatic detection of sleep stages based on the analysis of various signals: electroencephalogram (EEG) [8, 9, 10], airflow [11], accelerometer signals [12], electrocardiogram (ECG) [13 , 14]. However, despite significant progress in the development of automatic algorithms for detecting sleep stages for medical purposes, these algorithms are almost never used due to the low level of accuracy, which is primarily associated with the high variability of polysomnographic records, as well as due to the significant analysis time with using existing methods.
This work is devoted to the development of an automated algorithm for detecting various stages of sleep based on the time-frequency analysis of biophysical signals recorded during night monitoring using GPU parallel computing technology. The adaptive algorithm developed in the framework of this work for automatic detection of various stages of sleep is based on the use of continuous wavelet transform methods [9, 10, 15] using parallel computing technology, the developed technique has shown its operability and a fairly high quality of recognition of sleep stages, on average, the difference between automated system for marking sleep stages and marking a sleep doctor accounted for 80-85%, which is not inferior to its predecessors in terms of the quality of stage detection, but a significant advantage of the developed technique is a significant reduction in the time required for marking sleep stages. It is worth noting that another undoubted advantage of the developed algorithm is the implementation of adaptive algorithms for identifying sleep stages for each patient based on a preliminary analysis of bioelectric signals, which in the future can increase the accuracy of labeling, with the introduction of additional tests before registering polysomnography.
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### S5.274. Bacterial genomic molecular clock

Sheinman M.[1]*
[1]*Sevastopol state university;*
* msheinman@mail.sevsu.ru

Studying the evolution of bacteria remains a challenge. In contrast to more complex organisms, genomic sequence divergence in bacteria is highly dependent on the frequency of horizontal gene transfer, which generates a mosaic structure of the bacterial genome: for a pair of bacteria, different homologous loci have different similarity. The same mosaic effect is generated by the fact that different loci are subject to selection of different strengths, so that they mutate at different effective rates.
Moreover, due to the relatively short generation times, neutral mutations accumulate very quickly and homology can only be detected at conserved loci with a relatively low effective mutation rate. The above properties of bacteria do not allow direct use of the molecular clock of whole genome sequences. Instead, a few slowly changing genes are used, sometimes it is possible to link the evolution of bacteria and their hosts, etc.
In this work, we have constructed a model for the evolution of the bacterial genome. The model takes into account different mutation rates of different loci and horizontal gene transfer. The predictions of the considered model very well describe the empirical data: how changes the length of the homologous region in two bacteria, the average similairy, and other genomic properties with divergence time. Using the model, we estimated the divergence times for bacteria from the Enterobacteriaceae family and built an ultrametric tree demonstrating the correctness and simplicity of the method.
The obtained results shed light on evolution of bacterial genomes and allow us to assess the divergence of bacterial species more robustly, based on their whole genome sequences.

**S5.275. Basic regularities of intercellular interactions through permeable junctions**

Aslanidi K.B.[1]*
[1] *Institute of Theoretical and Experimental Biophysics of RAS;*
* kbaslanidi@gmail.com

The paper analyses the functioning of various multicellular systems, including trichomes of blue-green algae, hyphae of mycelial fungi, as well as tissues of planaria and mammalian cell cultures. The similarity of electrical coupling parameters in taxonomically distant biological systems with a different structure of hydrophilic intercellular channels and different mechanisms of membrane electrogenesis was revealed.
Based on our own experimental results and the results of other researchers, we can conclude that energy transfer in the form of ion fluxes through permeable junction is a universal function of all permeable junction and is inherent to all cellular populations. The conversion of the energy of ATP hydrolysis into the energy of the electric potential on the plasma membrane has been evaluated. It is shown that the transfer of a unit of charge from the cytoplasm through the plasma membrane into the external environment or the transfer of a unit of charge through permeable junction is equivalent to 5.1 10-20J. If the fluxes of Na+ and K+ ions through the plasma membrane are 107 ions/s, an individual cell consumes about 10-13 J/s to maintain membrane potential.
A necessary and sufficient condition for energy transfer through permeable junction is the presence of electric current between cells. The power transferred from a donor cell to an acceptor cell is proportional to the electric current flowing through the permeable contacts. Energy flows through individual cells in a multicellular system can vary significantly. Experiments on cells differing in their sensitivity to ouabain showed that effective communication through highly permeable junctions reduces the observed difference. Moreover, energetic cooperation by means of electric currents through permeable junctions increases the energy flow through the system as a whole and creates conditions for the development of functional specialization of individual cells. Note that in multinucleated cells of micellar fungi, there are gradients of inorganic ion activity, which should lead to the specialization of individual nuclei. Verification of this assumption requires experimental confirmation.
It was shown that after formation of permeable contacts the values of membrane potentials of all depolarized cells shifted towards values of membrane potential of hyperpolarized cell. The changes in activity of Na+,K+-ATPase or ion fluxes through permeable contacts practically did not influence membrane potential values and ion composition of cytoplasm of hyperpolarized cell - energy donor, but led to changes in membrane potential values of depolarized cells, which, in its turn, changed activity of inorganic ions in cytoplasm of energy acceptor cells. This means that the hyperpolarized cell is an energy donor for depolarized neighbours, and the membrane potential of the hyperpolarized cell determaines the membrane potentials of all depolarized neighbors.
In the work, special attention is paid to the existence of functional compartments, within which the energy of the electrochemical potential gradient is created and used. On the one hand, an increase in the number of cells in the system creates conditions for narrower functional specialization of individual cells, increasing the efficiency of the system as a whole. On the other hand, increasing the distance between the edge cells inevitably leads to worsening the coordination of individual cell behaviour. This means that the size of the compartment is determined by the dynamic equilibrium between the efficiency of electrodiffusion interactions through permeable contacts and the processes of functional specialization. Functional compartments in cell systems bound by permeable contacts have been found in all classes of multicellular organisms. In our experiments, the size of the compartment in a variety of organisms and tissues did not exceed 1.0-1.5 mm. Thus, continuous bioelectric networks cannot store morphogenetic information on objects whose dimensions exceed 1.0 mm.
The paper shows that the membrane potential is created and maintained by ionic flows through the plasma membrane and through the permeable intercellular contacts. For its part, the membrane potential determines the ionic composition of cytoplasm and the content of inorganic ions in the cell cytoplasm determines the expression of genes responsible for proliferation, differentiation, or apoptosis. High values of [Na+]i and pH 8.2 induced the expression of genes responsible for proliferation and differentiation processes. Low [Na+]i values and slightly acidified cytoplasm caused expression of genes characteristic of an adult differentiated cell. High [Na+]i values and cytoplasm acidification caused the expression of genes responsible for the processes of programmed cell death.
It is evident from the above that the flow of energy coming into the living system from the external environment determines the flow of inorganic ions through the plasma membrane and further all other processes of vital activity.

**S5.276. BioGraph: Data Model for Linking and Querying Diverse Biological Metadata**

Veljković A.V.[1], Orlov Y.O.[2], Mitić N.M.[1]*
[1] *Faculty of Mathematics, University of Belgrade, Serbia;*
[2]*I.M.Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia;*
* nenad.mitic@matf.bg.ac.rs

Studying of the association of gene function, diseases, and regulatory gene network reconstruction demands data compatibility. Data from different databases follow distinct schemas and are accessible in heterogenic ways. Although the experiments differ, data may still be related to the same biological entities. Some entities may not be strictly biological, like geolocations of habitats or paper references, but they provide a broader context for other entities. The same entities from different datasets can share similar properties, which may

or may not be found within other datasets. Joint, simultaneous data fetching from multiple data sources is complicated for the end-user or, in many cases, unsupported and inefficient due to differences in data structures and ways of accessing the data. We propose BioGraph - a new model that enables connecting and retrieving information from the linked biological data that originated from diverse datasets. We have tested the model on metadata collected from 5 diverse public datasets and successfully constructed a knowledge graph containing more than 2,500,000 individual entity objects, interconnected with more than 4 million relations. The model enables the selection of complex patterns and retrieval of matched results that can be discovered only by joining the data from multiple sources.

Biological data is highly diverse. Data produced from protein crystallization experiments are very different from those from protein disorder experiments. However, both experiments may give information about the exact biological entities, in this case, the same proteins. As the proteins are sourced from genes, experiments related to their respective genes can also supply valuable information in a broader picture when linked with the protein data. However, a protein record from one database may not contain an exact property that connects it to its respective gene from the other database, but possibly requires a third database to establish that connection. Some databases, like MobiDB [1], contain a wide range of entity identifiers sourced from several databases, but the search is based only on exact property matching, without the ability to create complex queries using various metadata attributes. A practical example of a complex query over multiple databases would be selecting human tumor antigen genes associated with proteins with disorder content higher than a specific value. Such a powerful querying mechanism is not available using available data querying methods on individual databases but requires a certain level of data unification and linking.

Using a knowledge graph for interconnecting data from biological data sources is not a novel idea [2]. Knowledge graphs are the foundational structure for intelligent health care [3]. There are many active initiatives to join data from multiple datasets into a knowledge graph, but most nowadays available solutions focus on particular subdomains, like drug discovery and proteomics, rarely on the overall connection of general biological data from various domains. We present a new model which enables simultaneous querying of biological data properties from multiple datasets based on querying metadata available from the original databases. The model is not focused on copying the data from the original datasets but linking the metadata in a way that can be used for efficiently executing complex queries on linked data. The model allows adding properties to entities and relations and unifying metadata from diverse data formats. A tool and a Web interface that use the new data model were also developed. The tool and the corresponding packages can be deployed locally as a standalone system so that the queries can be executed offline. The predeployed BioGraph Web interface is currently available on http://andromeda.matf.bg.ac.rs:54321.

For verification of the proposed model and its implementation, we successfully collected and joined metadata from five diversely formatted datasets: DisProt [4], HGNC [5], Tantigen 2.0 [6], IEDB [7] and DisGeNET [8].

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**S5.277. Bioinformatic analysis of the distribution and some functions of self-complementary microRNAs in different species**

Kuzmichev S.A.[1]*

[1]*Moscow State University of Medicine and Dentistry,Research Institute ofCarcinogenesis;*
* kuzs19782005@mail.ru

The researches on interactions between small RNAs, whose main role is to regulate the expression of multiple genes, expands our knowledge of their functions. Various methods have shown the ability of a number of microRNAs (miRNAs) to form homo-duplexes [1,2], i.e. duplexes between self-complementary miRNAs, with the same nucleotide sequences. The formation of duplexes between miRNAs, which increase their resistance to nuclease degradation [2], can occur at minimum free energy (MFE) values ≤ –13 kcal/mol [2,3]. Non-canonical functions of miRNA homo-duplexes, i.e., outside the involvement of miRNA in the regulation of mRNA translation, has been suggested [2]. Species that differ in the size of genomes process different amounts of miRNA - from tens in some viruses to several thousand in mammals. The probability of duplex formation increases with a decrease in MFE [3], therefore, to interpret the differences [2] in the percentage (%) of miRNAs capable of forming homo-duplexes, it is important to analyze changes in their level with the increase of MFE. The aim of this work was to analyze the distribution of miRNAs capable of forming homo-duplexes with different MFEs in different organisms. The ability of miRNAs to participate in the regulation of processing based on the determination of MFE for different duplexes was evaluated. The miRNA and premicroRNA (pre-miRNA) sequences from the miRBase base, version 22.1, in different species were taken for analysis: in house mouse (M. musculus), in human (H. sapiens), and in 6 types of herpes viruses infecting H. sapiens - Epstein-Barr virus (EBV), herpes virus type 8 (HHV-8), cytomegalovirus (HCMV), herpes viruses 1 (HSV-1) and type 2 (HSV-2), herpes virus 6B (HHV-6B). Bioinformatic analysis for detecting miRNAs capable of forming homo-duplexes and determine their MFEs was carried out using the RNAup program as previously described [3]. It was determined of % of self-complementary miRNA with an increase in the lower limit of the homo-duplex formation range from ≤ -13 to ≤ -30 kcal/mol. The probability of hetero-duplex formation between miRNA and pre-miRNA was determined using RNAhybrid 2.2., (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/),the smallest MFE was comparable to ≤ -13 kcal/mol.Statistical analysis of the obtained data was carried out using the Statistica 10 program. Results and discussion:Correlations of MFE parameter calculation data (in kcal/mol) obtained for miRNA homoduplexes based on the Turner, Matthews free energy model method and constraint generation method [4] were high (correlation coefficient, R = 0.97-0.98).The distribution of miRNAs capable of forming homo-duplexes showed that for mice and human producing more than a thousand miRNAs, the percentage of these self-complementary miRNAs in the MFE range from ≤ -13 to ≤ -17 was less than in various types

of herpes viruses. The difference in % of self-complementary miRNAs between different types of herpes was maintained in this range MFE, which indicated a slight effect of errors in the determination of MFE [4] on the analysis results. The observed non-uniformity of the percentage of self-complementary miRNAs in changing MFE homo-duplexes suggests different involvement of these miRNAs in regulatory processes. Comparison of data from the ViRmiRNA database (http://crdd.osdd.net/servers/virmirna) revealed no differences in the number of mRNA targets for self - and non-self-complementary miRNAs ($p > 0.05$).Some miRNAs, like short oligonucleotides, can participate in the regulation of miRNA processing by binding to complementary sequences of pri-microRNA and pre-miRNA [2,5]. Our analysis showed that self-complementary miRNAs are more likely to produce hetero-duplexes.A large number of hybridization sites between self-complementary miRNAs and different pre-miRNAs compared to non-self-complementary miRNAs in different herpes species (differences are significant for Mann-Whitney U test: for EBV- $p < 0.05$, for HCMV - $p < 0.01$, for HSV-1 - $p < 0.001$, for HHV-8 - $p < 0.05$) may indicate a greater contribution of these miRNAs to processing regulation. The formation of homo-duplexes with MFE≤ -25 kcal/mol in mice and human for some miRNAs may contribute to the accumulation of these miRNAs in cells, significantly reducing the likelihood of their isolation in exosomes [3]. The reasons for the relative increase in % of self-complementary miRNAs with MFE ≤ -25 kcal/mol of their homo-duplexes in mice and humans can be identified in further studies in vitro, given the possibility of miRNA hybridization and with other types of non-coding RNAs.

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**S5.278. Bioinformatic analysis of data on the detection of proteomic analysis of blood serum proteins in patients with bipolar affective disorder and in healthy individuals using the PeptideShaker software**

Smirnova L.[1]*, Seregin A.[1], Dmitrieva E.[1], Ivanova S.[1]
[1]*Tomsk National Research Medical Center RAS;*
[2]*Mental Health Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences;*
* lpsmirnova2016@gmail.com

Affective disorders, especially bipolar affective disorder (BAD), have a negative impact on the professional and social aspects of the life of patients and lead to a significant decrease in the quality of life. BAD affects up to 3% of the world's population, being a mental illness with unclear etiology and pathogenesis. The main negative consequence of this disorder is a high risk of suicide (10-15%) in these patients, and the frequency of parasuicides reaches 25-50%. Therefore, early detection and proper treatment is of particular importance for bipolar disorder. Currently, there are no paraclinical methods for diagnosing mental disorders. To make a diagnosis, only anamnestic and clinical and psychopathological data are used, based on clinical assessments of the prevalence of certain psychogenic symptoms.

Recently, new approaches to the diagnosis of mental illness and the search for their pathogenetic markers, including those using proteomic analysis, have been actively developed. There are few publications on proteomic studies of patients with mental disorders, and are mainly represented by works on schizophrenia. A feature of proteomic analysis is the ability to use it to detect protein biomarkers associated with functional disorders involved in the pathophysiology of diseases, without the need to put forward a hypothesis and limit its search area.

It is assumed that the pathogenesis of BAD is associated with a violation of synaptic transmission in the neuronal system of the hypothalamus and other basal parts of the brain, as well as damage to the underlying energy pathways. Therefore, it is logical to assume the presence of biomarkers reflecting these pathogenetic changes. But so far, not a single biological marker of BAD has been identified in world practice. This is due, among other things, to the large labor costs in processing a large amount of mass spectrometric proteomic data. In this regard, we used the PeptideShaker software package to identify protein biomarkers. It is a search engine independent platform for interpreting proteomic identification results from multiple search engines and de novo engines, currently supporting X!Tandem, MS-GF+, MS Amanda, OMSSA, MyriMatch, Comet, Tide, Mascot, Andromeda, MetaMorpheus, Novor , DirectTag and mzIdentML. PeptideShaker combines results into a single identification set, annotates spectra, calculates consensus score, matches sequences and infers proteins, evaluates localization of post-translational modifications, performs statistical validation, quality control, and annotates results using multiple information sources such as Gene Ontology, UniProt annotations and Ensembl and protein structures. Thus, the purpose of this work was to conduct a comparative bioinformatics analysis of mass spectrometry data from the blood serum of patients with bipolar disorder and healthy individuals using the PeptideShaker software package. The paper analyzed the protein spectrum of the blood serum of 10 people with a diagnosis of bipolar disorder in comparison with a similar control group.

Peak lists derived from MS/MS spectra were identified using OMSSA version 2.1.9 and X!Tandem version X! Tandem Vengeance (2015.12.15.2). The search was performed using the SearchGUI version [4.1.14]. Protein identification was carried out using the unified version of the UniProtKB database [PMID 20013364]. Peptides and proteins were determined from the results of spectrum identification using PeptideShaker version 2.2.9. Peptide spectrum matches (PSM), peptides and proteins were tested with a false detection rate of 1.0% (FDR). Only proteins quantified with at least two peptides were counted. Unlabeled quantification based on emPAI intensity was used to assess differences between study groups. The emPAI intensities for proteins were taken log2 and normalized to ensure equal mean protein content in all samples. Statistical analysis was performed using a two-tailed unpaired Student's t-test (FDR 0.05 and $S0 = 2$).

As a result of the analysis, more than 1300 proteins were identified in each study group. Further, taking into account the analysis of statistically significant differences in the parameters of normalized mean intensities (emPAI) of the peptides of the studied groups, 10 proteins were isolated.

The minimum p value ($p=0.0003$) was found for the Transforming growth factor-beta-induced protein ig-h3, which plays a role in cell adhesion and is a structural component of the extracellular matrix. The following 5 proteins Disabled homolog 2-interacting protein, coiled-coil domain-containing protein 80, B-cell CLL/lymphoma 9 protein, coatomer subunit gamma-1, ras GTPase-activating-like protein IQGAP1 ($p=0.001$) were also different structural components of the extracellular matrix, regulators of a wide range of different signaling pathways, primarily inflammatory, as well as regulating the immune response, embryonic development, transcription and cell differentiation, and apoptosis. In addition, ras GTPase-activating-like protein IQGAP1 is essentially a neurospecific protein and promotes neurite outgrowth. Proteins such as ectonucleoside triphosphate diphosphohydrolasea adhesion G protein-coupled receptor B1 and 14-3-3 protein zeta/delta ($p=0.002$-$0.005$) are mainly responsible for phosphorylation and dephosphorylation, hydrolyze ATP and other nucleoside diphosphates, regulate apoptosis and ubiquitination; in neurons regulate the formation of dendritic spines.

Thus, the use of the PeptideShaker software package helped to select several of the most promising protein candidate markers of BAD for further quantitative research.

### S5.279. Bioinformatics and Chemoinformatics in Drug Repositioning. Lessons from the COVID-19 Pandemic

Poroikov V.V.[1]*
[1]*Institute of Biomedical Chemistry;*
* vvp1951@yandex.ru

The pandemic of a new coronavirus infection has a significant impact on all aspects of human activity. Over the past three years, scientific knowledge about SARS-CoV-2 and the progression of the infectious process of COVID-19 has expanded significantly. The virus genomes have been deciphered, some functions of viral proteins and certain mechanisms of their interaction with human cells have been established, clinical data on the pathogenesis and symptoms in various groups of patients have been accumulated. A strategy has been developed for the widespread use of diagnostic test systems and vaccination of the population, which made it possible to personalize and increase the effectiveness of clinical approaches to the treatment of the disease. Compliance with sanitary and hygienic recommendations and vaccination limit the spread of the virus in the population, however, the emergence of new mutant strains of SARS-CoV-2 with increased virulence reduces the effectiveness of preventive measures. The lack of knowledge about the pathobiology of the SARS-CoV-2 virus and the mechanisms of development of the pathological process requires further basic and exploratory biomedical research, identification of the main cellular and molecular targets for tissue and organ damage, and the search for new treatments and prevention of coronavirus infection.
Drug repositioning – identifying new indications for approved pharmaceuticals – is the only possible immediate response to the COVID-19 pandemic and future biogenic threats. The availability of information on the pharmacological and toxicological characteristics of a known drug provides the conditions for its rapid use in a new nosology. The search for new pharmacological effects of known drugs is carried out in silico and in vitro. Computer estimates are obtained by modeling the interaction of the analyzed compounds with molecular targets, identifying analogs based on structural similarity, analyzing structure-activity relationships, and establishing associations using network pharmacology methods. The selection of potentially active compounds is carried out by virtual screening in silico, followed by experimental validation of computer predictions in vitro. The determination of anticoronavirus activity in vitro is carried out using biochemical and cellular model systems. The correlation between the results of studies obtained in silico, in biochemical and cellular test systems in vitro, and in experimental animals in vivo is low, which is explained by the lack of standardization of the test systems and reference drugs that can be used for the appropriate validation.
In order to effectively use the received in 2020-2023 information, we are developing the Anti-COVID-19 portal (https://way2drug.com/anticovidinfo/) aimed to drug repositioning for SARS-CoV-2/COVID-19 therapy. The portal provides information on the mechanisms of the development of the pathological process in COVID-19, the impact of this infection on biological processes in the body, pharmacological targets for therapeutic intervention, anticoronavirus agents used to treat the disease, as well as studies on drugs repositioning for the treatment of COVID-19.
To evaluate in silico characteristics of repositioned drugs and new pharmacological substances, web services have been implemented that provide a search for structural analogues of active compounds among 4000 drugs approved for medical use, predict anticoronavirus effects, side effects and toxicity for molecules planned for synthesis, etc.
An analysis of the available experimental data on in vitro testing of the anticoronavirus activity of known drugs allowed to establish priorities for their further research. Three teams from the Chumakov Center, Zelinsky Institute and IBMC, independently conducted molecular modeling of the interaction of fifteen drugs (disulfiram, omeprazole, silibinin, saquinavir, montelukast, imatinib, atazanavir, dasatinib, ciprofloxacin, glycyrrhizic acid, dihydroquercetin, narlaprevir, teicoplanin, bedaquiline, doxazosin) with the main SARS-CoV-2 protease 3CLpro. The Chumakov Center confirmed by the experiment anticoronavirus activity for narlaprevir (IC50=2.75 μM, EC50=64 μM, CC50=106 μM). Imatinib inhibited virus replication in cell culture with EC50 = 40.0 μM, but was practically inactive against the main protease 3CLpro.
In March 2020, an international project for the virtual screening of potential anti-SARS-CoV-2 compounds "JEDI Billion Molecules Against COVID-19 Grand Challenge" was announced (https://www.jedi.foundation/covid19challenge). The project participants were requested to conduct in silico screening of potential anti-coronavirus compounds among at least one billion structures available for synthesis and testing, in relation to three or more molecular targets, by three independent computer methods, and submit a list of ten thousand hits for synthesis and biological testing. 130 teams from different countries, which took part in the project, offered 639,024 hits for synthesis and testing; 820 compounds synthesized and tested; 28 "actives" found (success rate 3.19%). We performed in silico screening among 1.08 billion structures on 4 targets (3CLpro, PLpro, RdRp, TMPRSS2); ten thousand hits were chosen. We were among the 20 teams whose proposals were selected for experimental verification; 36 molecules synthesized; the activity of one molecule (PLpro inhibition) was confirmed in the experiment.
Opportunities and limitations of drug repositioning in the context of the COVID-19 pandemic and ways to reduce the risks of new biogenic threats in the future will be discussed.
The study is performed in the framework of the Program for Basic Research in the Russian Federation for a long-term period (2021-2030) (No. 122030100170-6).

### S5.280. Bioinformatics, Next-Generation Neuroscience, and Artificial Intelligence

Osypov A.A.[1,2]*
[1]*Institute of Higher Nervous Activity and Neurophysiology of RAS;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS;*
* aosypov@gmail.com

The interconnection between bioinformatics, next-generation neuroscience, and artificial intelligence is presented.
Bioinformatics uses methods of traditional artificial intelligence for data analysis and, in turn, supplies data and ontologies for AI tools such as medical decision support systems.
We introduce the concept: "next-generation neuroscience" - a stage of development of neurobiology, which is characterized by the use of methods of automatic acquisition and analysis of massive data, the use of non-invasive high-resolution methods, close integration with bioinformatics and developments in the field of traditional AI. Next-generation neuroscience uses bioinformatics to acquire and analyze massive genomic data, and adapts its approaches and methods to analyze big data of its own specific types. Examples of studies that use said data and methods are given, and contemporary systems for depositing integrated massive data are described.
Next-generation neuroscience supplies data for studying structural and functional organization and fundamental mechanisms of brain functioning as a carrier of natural intelligence in order to create its functional model, capable of performing basic cognitive tasks of strong artificial intelligence.

We introduce the concept of "medium neuromorphic artificial intelligence" as an intermediate between highly specialized reactive weak AI and universal proactive strong AI. Medium neuromorphic artificial intelligence is characterized by universality, reactivity and limited neuromorphism. Universality allows for the necessary complex cognitive functions. Reactivity removes the ethical problems of mankind security and non-increasing suffering of thinking beings, as well as the task of creating an emotional-motivational unit, a system of attention regulation, etc. Limited neuromorphism is caused by our incomplete knowledge of the structure of the brain as the substrate of cognitive activity, the lack of the need for life support systems, the emotional-motivational block, the attention regulation system, etc. Also the limitation of neuromorphism is caused by the need to solve some problems of input/output, processing and storage of information, which are not available for living systems, but are desirable for the functioning of artificial intelligence as a tool of human activity.
In turn, the creation of an average artificial intelligence will allow to organize the accumulated scientific knowledge on an unprecedented level and fundamentally improve the efficiency of scientific work.

### S5.281. Cardioprotective effect of NO donor in a model of heart failure

Karimova R.G.[1]*
[1]*Kazan (Volga region) Federal University;*
* Rufiya77@yandex.ru

Many cellular signaling pathways are involved in the development of heart failure, including nitric oxide (NO). The reduced bioavailability of NO in chronic heart failure is the basis for the use of nitric oxide (II) donors in order to compensate physiological processes in this pathology.
The effect of NO donor on electrocardiographic parameters was studied in rats with a phenylephrine model of chronic heart failure. A compound containing the furoxan ring, 4-chloro-6,7-furoxanobenzofurazan, was chosen as the NO donor. Previously, we have proven its NO-donor activity. The results of electrocardiography showed that the phenylephrine model of chronic heart failure is characterized by changes in the electrocardiogram of rats. There was a decrease in the duration of the P wave, an increase in the duration of the QRS complex, the RR and QT intervals. In addition, oblique ascending depression of the ST segment was registered in 15% of rats. The heart rate of rats with the model of chronic heart failure was increased, which was restored by 36.8% ($p < 0.05$) with the administration of 4-chloro-6,7-furoxanobenzofurazan.
In 46% of rats in the model of chronic heart failure, tachyarrhythmia was detected, in 9% of rats - atrial fibrillation. The noted arrhythmias fully recovered after the administration of an NO donor. The duration and amplitude of the P wave decreased in rats with the model of chronic heart failure by 1.4 times ($p < 0.05$) and 2.3 times ($p < 0.05$), respectively. Administration of 4-chloro-6,7-furoxanobenzofurazan load increased the duration of the P wave to the previous level, while its amplitude remained unchanged. Normally, rats do not have a Q wave, so instead of the duration of the P-Q interval, the duration of the P-R interval is measured. In rats with the model of chronic heart failure, the duration of the PR interval is increased by 1.66 times ($p < 0.05$). With the introduction of 4-chloro-6,7-furoxanobenzofurazan, the P-R interval decreased to the level of intact animals. The Q wave appeared in 55% of rats with chronic heart failure. After the introduction of an NO donor, the presence of Q waves and their amplitude in rat model of chronic heart failure was preserved.
The administration of the NO donor led to the restoration of the duration of the (Q)RS complex by 23% ($p < 0.05$).
Thus, the method of electrocardiography established an increase in heart rate, narrowing of the P wave, a decrease in the amplitude of the P, R, and T waves, and an elongation of the (Q)RS and (Q)RT complex in rat model of chronic heart failure up to the parameters of intact animals.
The cardioprotective effect of the NO donor was also confirmed by biochemical studies: the restoration of serum lactate dehydrogenase activity, as well as the level of sodium and chlorine.

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### S5.282. Comparative analysis of the effect of heavy metals on the photosynthetic apparatus of Chlorella vulgaris cells

Chervitsov R.N.[1]*, Plyusnina T.Yu.[1], Khruschev S.S.[1], Todorenko D.A.[1]
[1]*MSU, Faculty of Biology;*
* roman123qwe123@gmail.com

One of relevant environmental issues is the determining the presence of various toxicants in the aquatic environment, such as heavy metal ions. The presence of these ions causes disruption of photosynthesis processes and damage to various structures in algae cells, which can induce mass death of phytoplankton and disruption of the balance of aquatic ecosystems. One of the methods to determine the presence of heavy metals in the medium is based on the measurement of chlorophyll a fluorescence induction curves that characterize the state of the photosynthetic apparatus of cells of photosynthetic organisms and alter their shape when exposed to various stress factors. The parameters of the JIP test characterizing the state of individual elements of the photosynthetic apparatus can be calculated from the induction curves. When processing large arrays of such data, it is advisable to use machine learning methods, in particular, the "random forest" classification algorithm.
In this work, the green algae Chlorella vulgaris is used as a test organism. The algae cells were incubated for 60 hours, and the fluorescence induction curve was measured once an hour. Toxicants (CdSO4 or K2Cr2O7 at a concentration of 20 or 50 μM) were added at the 17th hour of incubation. From the obtained induction curves, 12 parameters of the JIP test were calculated using the PyPhotoSyn program [1], which the data array analyzed in this work consists of.
The first stage is devoted to consideration of dynamics of JIP-test parameters alteration for the samples with toxicants and without them. It is shown that the impact of cadmium ions in low concentration (20 μM) is almost unnoticeable, because the dynamics of most of parameters for these samples is similar to the control. The impact of chromium ions is noticeable for both concentrations. Fv/Fm, which characterizes the efficiency of photosystem II work, remain about 0.7 for the control data during incubation. PI, which characterizes the overall condition of photosynthetic apparatus, is significantly increased and later decreased. The toxicants inhibit the photosynthetic reaction centers, which is expressed in a decrease of values of these parameters. What is more, this decrease for cadmium samples is quick at the early hours and slow later, unlike chromium samples, for which this decrease is relatively slow. The equality of values of these parameters for cadmium and chromium is detected after 20 hours of toxicant exposure, when it is significantly expressed. ABS/RC characterizes the area of antenna complex per reaction center, the increase of this parameter is usually induced by inhibition of reaction centers. Thus, the inhibition of reaction centers in samples with toxicants induces the increase of ABS/RC and the dynamics of alteration rate of parameters, which is detected earlier, is also noticeable for this parameter. The alteration of Sm, which characterizes the pool of oxidized quinones, are usually induced by alterations in photosynthetic apparatus outside the photosystem II. It is shown that for samples with chromium Sm is decreased and later remains the same, unlike samples with cadmium, for which Sm is decreased and later significantly increased, which can be triggered by chloroplast breath, which is usually observed during

stress. Therefore, the cadmium and chromium ions induce the damage in photosynthetic apparatus. However, the influences of these metals are different.
The next stage is devoted to use of classifiers. The data which is obtained during the period from 17 to 60 hours of incubation is used to build the random forest classifiers. The JIP-test parameters are the features for the classification. The accuracy of classifier for detection of presence of toxicant is 95% (94% for control samples and 96 % for samples with toxicants). The accuracy of classifier for detection of type of toxicant (cadmium or chromium) is 93%, the best accuracy is for control samples (96%) and samples with chromium (95%), the accuracy for samples with cadmium is lower (87%). The most significant JIP-test parameter for classification is Fv/Fm (the quantum yield of primary photochemistry, which characterizes the efficiency of photosystem II work).
To sum it up, the impact of cadmium and chromium ions on algae cells induce the inhibition of reaction centers of photosystem II, which is expressed in a decrease in Fv/Fm and in an increase in ABS/RC. The toxic effect of cadmium is expressed earlier than the effect of chromium. The classifiers for this data could detect the presence and type of toxicant with a high accuracy. These methods for data analysis have prospects of application for estimation of the state of natural habitats.
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### S5.283. Comparative study of the structure and taxonomy on the example of 5 S and 16 S RNA of bacteria

Ovchinnikova I.[1]*
[1]*Siberian Federal University ;*
* july.l4o6@mail.ru

The most important subject of research for molecular biologists and bioinformatics is the relationship between the structure of biological macromolecules and the carrier taxonomy. 16 S RNA sequences are a classic subject of study, but other sequences are of no less interest. In the framework of this study, we studied the relationship between the two structures using the 5 S RNA and 16 S RNA sequences of bacteria as an example.
The aim of this work is to reveal the similarities and differences between the two structures determined by the 16S RNA and 5S RNA sequences. Previously [1], it was shown that the triplet composition of genetic systems correlates very well with the taxonomic position of carriers of the corresponding genes. However, the species composition of genetic databases (in our case, SILVA databases) is often quite displaced. Therefore, database indexing was carried out: deletion of some of the records located in overrepresented groups.
Let's proceed to the description of the work itself and the results. Comparison of the structure and taxonomy of the indexed databases of bacterial 5S and 16S RNA genes was carried out using the method of elastic maps [3]. Clustering of bacterial 5S RNA genes according to their frequency dictionaries of triplets was carried out using the freely distributed VidaExpert software.
The result of this work is the establishment of a link between the clustering of 5 S and 16 S RNA genes. Thus, the possible outcomes are completely different clustering patterns observed on these two sets of genes, on the contrary, their noticeable similarity. Another important question is the answer to the question: is it true that the triplet composition of these two groups of genes makes it possible to distinguish between the genes of 16 S RNA and 5 S RNA of bacteria.

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### S5.284. Computational modeling of Stokes flows in complex channels

Markovskiy A.[1]*, Gamayunova D.Y.[1]
[1]*Kuban State University;*
* mrkvsk@yandex.ru

The classical Stokes problem of constructing a viscous slow flow inside a bounded region requires setting the velocity on the entire boundary. But, in cases where the speed is unknown on part of the border, it is necessary to determine it artificially. However, it is possible to construct flows using only the boundary data of the desired current function under the natural condition of minimum RMS vorticity. In such a new formulation, the current function of the desired flow is represented by a logarithmic potential with an unknown density. Belonging of the desired density to the space of harmonic functions ensures the uniqueness of the solution of the problem and corresponds to the extreme condition. The desired harmonic density is proposed to be determined by the method of basic potentials, which is based on the proven completeness of the systems of shifts of the fundamental solution of the Laplace equation.
The paper proposes a simple algorithm for constructing Stokes flows based only on boundary data. The proposed approach makes it possible to easily build flows in channels of complex geometry, which can be used in modeling blood flow in a branched vascular system.
The paper presents the results of computational experiments for different fields.

### S5.285. Computer analysis of the structure of the gene network of glioblastoma and brain tumors

Turkina V.A.[1]*, Mayorova A.A.[1], Dergilev A.I.[2], Krasilnikova A.A.[1], Lanskikh D.V.[3], Kumeiko V.V.[3], Orlov Y.L.[1,2,3]
[1]*I.M.Sechenov First Moscow State Medical University (Sechenov University);*
[2]*Novosibirsk State University;*
[3] *Far Eastern Federal University, Vladivostok, Russia;*
* y.orlov@sechenov.ru

A computer model of the glioblastoma gene network was considered in order to study for associations with other brain tumors (Gubanova et al., 2021). The relevance of the problem is due to the fact that glioblastoma multiforme is the most common brain cancer and the second most common brain tumor (Louis et al, 2007). Glioblastoma cells can develop from normal cells as well as from existing low grade (5%) astrocytoma cells. The cause of most cases of glioblastoma is unknown (Gulaia et al., 2022). Non-standard risk factors are genetic disorders such as neurofibromatosis and Li-Fraumeni syndrome, as well as previous radiation therapy. The incidence of glioblastoma has been reported to be associated with SV40 viruses (Vilchez et al, 2003), HHV-6 and cytomegalovirus.
The task was to collect a list of genes associated with the development of glioblastoma, analyze the categories of gene ontologies for such a list, and reconstruct the gene network. The databases of clinical trials are considered and the most significant drugs for the treatment of glioblastoma and their "targets" are selected. The Internet resources OMIM (https://www.omim.org/), MalaCards.org and modeling tools

were used using previously published approaches (Orlov et al., 2021). The list contained 551 gene names: ENO1, MTOR, PTEN, KRAS, etc. The DAVID resource (https://david.ncifcrf.gov/summary.jsp) was used to analyze the categories of gene ontologies.

It was shown that the most significant categories for glioblastoma genes according to DAVID are enzyme binding and negative regulation of gene expression. In general, most genes fall into categories that regulate tumor formation, including apoptosis, enzyme and protein kinase binding, negative regulation of cell proliferation and genes, nervous system-specific terms such as regulation of neuronal apoptosis, and regulation of beta-amyloid precursor catabolism. Gene ontology analysis resources (DAVID, PANTHER, GOST) distinguish angiogenesis, regulation of signaling and apoptosis, binding of enzymes and proteins as the main gene ontologies of glioblastoma.

The online gene network reconstruction tools GeneMANIA, STRING-DB, Metascape (https://metascape.org/) were used. STRING-DB statistics show that the network has a non-randomly large number of connections (with significance <1.0e-16), the average degree of network node (protein) connectivity is 9.61, and the clustering coefficient is 0.468. In the center of the network are genes with the greatest influence on other genes and, most likely, the most associated with the occurrence and development of glioblastoma: PTEN, PIK3CA, MTOR, KRAS, VEGF, etc. An extended analysis of protein-protein interactions was performed using the Metascape tool.

In the structure of the glioblastoma network, seven clusters of protein-protein interactions were identified, in total about 50 genes are involved - 16% of the total number, which cannot be called a large number, but, nevertheless, it cannot be denied that the proteins of these genes are completely isolated from each other and do not interact. The common pathways found by Metascape correspond to the main ways of initiating carcinogenesis - resistance to inhibition of the epidermal growth factor receptor (EGFR tyrosine kinase inhibitor resistance) and regulation of the PI3K-Akt signaling pathway, also responsible for cell proliferation. DNA repair complexes - MSH2-MSH6-PMS2-MLH1 and PCNA-MutS-alpha-MutL-alpha-DNA (with improper DNA repair, the risk of developing oncology increases), nuclear complexes (LINC complex) and chromatin regulation (Chromatin modifying enzymes , Chromatin organization), glycolysis (NADH regeneration, canonical glycolysis, glucose catabolic process to pyruvate) (tumor cells are subject to the Warburg effect - the tendency of most cancer cells to produce energy mainly through very active glycolysis followed by the formation of lactic acid, and not through slow glycolysis and pyruvate oxidation in mitochondria using oxygen, as in most normal cells).

Drug data from MalaCards and clinicaltrials.gov show that the classic drugs Temozolomide and Bevacizunab are the most used. These drugs were found using the Drugbank online resource (https://go.drugbank.com) and summarized in the table describing the mechanism of action. The study of gene ontologies confirms that the most successful pharmacological "targets" would be the inhibition of angiogenesis, the regulation of apoptosis and protein/enzymatic pathways, signaling receptors. The construction of gene networks has shown that some genes associated with glioblastoma are sufficiently interconnected, while others are “drop-out”, which means that they cannot be influenced by other “targets”.

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## S5.286. Construction of multiple alignments of amino acid sequences by means of the MAHDS method

Kostenko D.O.[1,2]*, Korotkov E.V.[1,2]
[1]*National Research Nuclear University MEPhI;*
[2]*The Federal Research Centre “Fundamentals of Biotechnology” RAS;*
* dk0stenko@yandex.ru

Multiple sequences alignments construction is an important task of bioinformatics which allows one to find evolutionary and functional relationships in the chains of nucleic acids and proteins of living organisms. However, this problem is NP-complete. This means that the time it takes to compute its deterministic solution depends exponentially on the size of the input (in particular, the number of sequences). For this reason, all methods used in practice for constructing multiple alignments use a variety of heuristics that allow solving this problem in an acceptable time, but with a loss of accuracy.

Previously, we developed the MAHDS method, which allows building multiple alignments in a time that depends linearly on the number of sequences and quadratically on their average length. This method has shown that it is able to build statistically significant DNA sequence alignments even with x=4.4 substitutions per symbol. At the same time, for other methods compared with MAHDS (T-Coffee, Muscle, ClustalW, MAFFT, Kalign), this value was x<2.5.

We have adapted MAHDS for amino acid sequences alignment [1]. MAHDS was compared with the multiple alignment methods presented on the EMBL-EBI resource, for which a Web API is available. These include T-Coffee, MUSCLE, PRANK, Clustal Omega, Kalign, MAFFT. To compare MAHDS with other methods, the following test datasets were used: the BAliBASE database of reference protein family alignments, artificial sequences with certain properties, and real protein families with a low percentage of identity (<20%) taken from the Pfam and HOMSTRAD databases. The CS and Z criteria were used to evaluate the quality of the alignments. CS reflects the measure of similarity of two alignments (reference and estimated), and Z is the statistical significance of the alignment.

In the course of testing on BAliBASE, the average values of CS and Z were calculated for the alignments of all protein families presented in this database. The purpose of the testing was not only to compare MAHDS with other methods, but also to select the most biologically appropriate parameters of the MAHDS method (which also affect the Z score). For the selection of parameters, we maximized CS, trying to prevent significant drops in Z. We used fixed parameters that were selected at this step further in the research. In general MAHDS showed superiority in Z, but lagging behind in CS, in comparison with other methods in the context of BAliBASE.

Before conducting further tests a threshold of statistical significance was determined according to the 3-sigma rule. It shows Z at which the alignment can be considered non-random. Alignments of sets of random sequences were estimated for this. The Zt threshold turned out to be equal to 10.

For testing on artificial sequences, sets of descendants were generated from random ancestral sequences by adding random insertions, deletions, and substitutions (the ancestral sequence was not included in the final

set). 81 different sets of artificial sequences were generated. The length of the ancestral sequence was 600 characters. The number of descendant sequences is 100. Sets were created with properties from the following range. Number of insertions (and the same number of deletions): [2, 5, 10]. Insertion length (and the same for deletions): [1,5,20]. The number of substitutions per character: [0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7], respectively, the distance x between descendant sequences (in substitutions per character) in the generated sets of sequences was [0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4]. It was shown that MAHDS, in cases of a small number and length of insertions and deletions, is able to build statistically significant alignments even when distance between aligned sequences is equal to x=4.8 substitutions per symbol. MUSCLE showed the best results among other methods. Under the same conditions, MUSCLE is able to build statistically significant alignments at x=2.4 substitutions per symbol, which is significantly worse than the MAHDS score.
In the context of testing MSA methods on real protein sequences, we compared MAHDS with the T-Coffee and MUSCLE methods, because these 2 methods showed good results in previous tests. We constructed alignments using three methods for 21 protein families and scored the resulting Z alignments. For 16 out of 21 families, MAHDS constructed the most statistically significant alignments (for the other 5 families, either alignments by all methods turned out to be significant, or vice versa, they were insignificant for all methods). At the same time, for 4 families (PF00915, PF10846, PF10895, and PF13944), only MAHDS was able to construct a statistically significant alignment.
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### S5.287. Database of Potential Promoter Sequences in Eukaryotic Genomes

Rudenko V.M.[1]*, Korotkov E.V.[1]
[1]*Research Center of Biotechnology RAS;*
* v.m.rudenko@gmail.com

Promoter regions serve to initiate the transcription process. They are located upstream of the coding regions of DNA sequences. Determining their location is extremely important for solving the problem of genome annotation.
Promoters have a complex composition in eukaryotic genomes. They contain specific binding sites for RNA polymerase, as well as transcription regulation sites, including the initiator (Inr), TATA-box, DPE, and some others. At the same time, the composition of sites and their position is inconstant. The situation is complicated by the fact that genes can have not one, but several promoters. The presence of alternative promoters causes transcription of different mRNAs from one gene, depending on the tissues and developmental stages of the organism.
These difficulties lead to the fact that today most bioinformatic methods for searching for promoters can reveal only a small part of the promoter sequences. A priori knowledge of the promoter structure is also required. Known promoters, mostly for model organisms, have been determined experimentally and are stored in the EPD database. Using these data, it is possible to train a neural network or build a Markov model and achieve a high sensitivity of the method in recognizing promoters similar to those presented in the training set [1]. However, it becomes difficult to recognize promoter sequences if they contain a large number of mutations compared to the sequences of the training set, or if it is necessary to determine the promoters of species that are absent in the EPD.
In our work we used MAHDS, a highly divergent sequence multiple alignment method, to search for promoters. MAHDS consists of 4 steps. At the first step, classes are determined in the known set of promoters of a particular biological species. If the experimentally confirmed promoters are unknown than sequences of length 600 bp, which are located at -500..+100 bp from the transcription start site are taken as a training set. In the second step, a multiple alignment of the sequences included in each class is built; a class profile is generated based on this alignment. There are some modifications of the method when creating a profile, the frequencies of nucleotides or dinucleotides in different positions of the promoter are taken into account. Further, for all possible fragments of length 650 bp of chromosomes, local alignment with the profile of each class of promoters is determined. The length of the promoter was determined by us as 600 bp, an additional 50 bp was provided for the case of multiple insertions. If the alignment weight is statistically significant, it is considered that a potential promoter sequence (PPS) has been found. The last step is to remove overlapping PPS in order to eliminate redundancy in the results. By using the term PPS, we emphasize the fact that certain promoter sequences are found using bioinformatics methods but have not been verified experimentally.
As can be seen, MAHDS can be used to search for PPS in any genome. MAHDS does not require any a priori information about the structure of the promoters, since it independently determines these structures such as profiles.
MAHDS has been applied to search for PPS in the genomes of various eukaryotes: Oriza sativa, Capsicum annuum, Lactuca sativa, and Homo sapiens. All detected PPS were placed in the database installed on the server of the Bioengineering Center of the Federal Research Center for Biotechnology of the RAS [2]. The link: http://victoria.biengi.ac.ru/cgi-bin/dbPPS/index.cgi. It is assumed that the database will be fill in with PPS of other genomes. For each PPS, an identifier, species name, chromosome number, DNA strand (direct or reverse), PPS length, left and right positions in the chromosome, left and right positions in the profile, the profile (or class matrix), and the value of statistical significance are stored. The filtering function by these parameters has been implemented. After specifying the parameters or their possible ranges, the results are presented as a paginated list. It is also possible to view more detailed information for each PPS, that opens by clicking on the ">>" button. In the opened window, there is a hyperlink to the original chromosome sequence and the alignment between PPS and profile. Also here you can see the multiple alignment used to calculate the PPS profile.
The number of PPS identified by the MAHDS method significantly exceeds the number of genes in these genomes. For example, for Capsicum annuum genome, the number of PPS is 825136, while there are only 31600 annotated genes, i.e. there is a difference of the indicated values by 26 times. A similar picture is observed for other genomes. There may be several reasons for this discrepancy. First, we think that there are currently unannotated genes thats promoters have not been identified yet. Secondly, some promoters may be alternative. Also, part of PPS intersects with mobile genetic elements, that are common in plant genomes.
We believe that the PPS database may be helpful for studying the genetic regulation of the transcription process. Also, the presented data can be used in experimental studies, on the study of alternative transcription pathways and in the field of genetic engineering.
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### S5.288. Development of a patient-specific ablation correction technique

Berezhnoy A.C.[1,2,3]*, Slotvitsky M.[1,2,3], Sergeeva T.[1,3], Tsvelaya V.[1,2,3], Kalinin A.[1,3], Agladze K.[1]
[1]*Moscow Institute of Physics and Technology;*
[2]*Moscow Regional Research Clinical Institute named after M. F. Vladimirsky;*
[3]*Almetyevsk State Oil Institute;*
* berezhnoi.ak@phystech.edu

Cardiovascular diseases are a group of diseases of the heart and blood vessels, which includes coronary heart disease, heart failure and other pathologies. This group of diseases is one of the most common causes of death among the population of developed countries. One of the most common diseases of the cardiovascular system is a persistent form of atrial fibrillation (atrial fibrillation)- cardiac arrhythmias, which can lead to the development of heart failure and sudden cardiac death.
The main method of treating this disease is surgical ablation - the creation of foci of fibrosis that prevent the development of arrhythmia (spiral wave rotation). Unfortunately, more than half of the patients return for repeated ablation, there are cases of up to 10 operations.
This paper describes the creation of a system to help a doctor with surgical ablations, which allows to increase the effectiveness of the procedure. The system is based on the consideration of histological analysis of atrial tissue morphology and MRI/CT.
The key ideas of this work are the influence of cellular morphology on the dynamics of waves in the tissue, as well as the role of fibrosis in the occurrence and development of spiral reentry waves (fibrosis acts as a substrate for their occurrence).
At the final stage of development, the system will create an individual model of cardiac tissue for the patient, with which the surgeon will be able to test various ablation protocols to reduce the number of relapses. An important feature of the system in comparison with existing analogues is the consideration of different types of fibrosis (both infarct scars and diffuse of varying degrees).

**S5.289. Development of an algorithm for analyzing images obtained by FLIM time-resolved microscopy**

Shchechkin I.[1,2]*, Rodimova S.[1,2], Bobrov N.[1,3], Mozherov A.[1,2], Kuznetsova D.[1,2]
[1]*Institute of Experimental Oncology and Biomedical Technologies Privolzhky Research Medical University;*
[2]*Lobachevsky State University of Nizhny Novgorod;*
[3]*Volga District Medical Center of the Federal Medical and Biological Agency;*
* iliahasa1992@gmail.com

Keywords: time-resolved microscopy, FLIM, machine learning, neural networks
Introduction. Multiphoton time-resolved microscopy FLIM is a research method based on the analysis of the fluorescence lifetime of endogenous fluorophores, in particular nicotinamide adenine dinucleotide (NAD(P)H). To describe the decay of NAD(P)H fluorescence, a two-exponential model is usually used, where t1 and t2 correspond to the lifetimes of the free and bound forms of NAD(P)H, respectively. The relative amplitudes a1 and a2 in this case are the contributions of the lifetimes of the free and bound forms of the coenzyme, respectively. A three-exponential model is also used, which additionally includes the phosphorylated form of NADPH, which corresponds to the parameters t3 and a3. It is known that the NAD(P)H fluorescence decay curve can serve as an indicator of metabolic changes. Thus, an increase in the proportion of free NAD(P)H (a1) correlates with an increase in anaerobic glycolysis, and an increase in the contribution of the bound form (a2) may be associated with a transition to oxidative phosphorylation. An increase in the contribution of the phosphorylated form (a3), in turn, corresponds to the activation of the synthetic function of the tissue. This method is widely used to study the cellular metabolism of biological tissues. However, to date, there is no established approach to the analysis of data obtained by the FLIM method. Existing automated methods of computational analysis using machine learning are able to extract more data from the received images compared to traditional analysis methods. One of the new approaches is to apply machine learning methods to images obtained using FLIM multiphoton microscopy in order to objectify the results obtained and obtain more information about the structural and functional state of biological tissues.
Purpose of the work: to develop an algorithm based on machine learning methods that can automatically determine the characteristics of fluorescence (t1, t2, t3, a1, a2, a3) of liver tissue based on FLIM images.
Materials and methods. For FLIM imaging, a liver regeneration model was chosen. In order to start the regenerative process, surgical resection of the liver was performed on 18 Wistar rats with the removal of different volumes (30 and 70% of the mass) of the liver. On day 7 after resection, the liver was harvested and ex vivo imaging of the specimens was performed by FLIM. In general, the pool of images included: control (liver samples before resection) in the amount of 80 images; liver samples at 30% resection on the 7th day after the start of regeneration in the amount of 11 images; liver samples at 70% resection on day 7 in the amount of 17 images. Cell boundaries were marked on the images in the ImageJ program (Fiji) for subsequent training of the neural network. After that, the images were augmented to increase the amount of training material.
Results. Using the obtained pool of FLIM images, a neural network based on Unet++ was trained with a multicomponent loss function consisting of BCA, Focal and Dice functions. The neural network was trained to determine the boundaries of cells, the accuracy of predictions was 80%. The predicted results were used for Instance segmentation, followed by the calculation of the kinetics of the obtained ROIs corresponding to the cell cytoplasm, based on the restored functions according to the manuals provided for SPCImage.
Conclusion. Thus, an algorithm was developed that can determine cell boundaries with high accuracy, isolate them in a FLIM image, and calculate the corresponding fluorescence parameters: component lifetimes (t1, t2, t3) and their contributions (a1, a2, a3).
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**S5.290. Development of approaches to model DNA sliding in the nucleosome by all-atom MD**

Kniazeva A.S.[1]*, Armeev G.A.[1], Shaytan A.K.[1]
[1]*Lomonosov Moscow State University ;*
* kniazeva.anastasiia.2015@post.bio.msu.ru

DNA in cells exists in a complex with proteins called chromatin. At a bottom level of organization, chromatin is a sequence of nucleosomes - protein disks around which DNA forms two incomplete turns. Nucleosomes, like chromatin in general, are steady-dynamic structures. Depending on the DNA sequence, different equilibrium states of DNA supercoiling are observed in the nucleosome (145, 146 or 147 base pairs are engaged). DNA in nucleosomes can partially unwrap, form twist defects (loops) and slide relative to the protein core of the nucleosome. DNA sliding inside the nucleosome is the currently accepted model of nucleosomes repositioning in chromatin; nucleosome mobility on DNA has been shown experimentally [1,2]. The ATP-dependent sliding of DNA in the nucleosome by remodelers has been studied by the method of cryo-electron microcopy [3]. In these works, the structures of nucleosomes at different stages of the remodeling cycle have been obtained using non-hydrolysable ATP analogues. This allowed to show the connection between DNA rearrangements and the protein core of the nucleosome (represented as the octamer of histone proteins). There have also been works on computational modelling of the dynamics of DNA sliding in nucleosomes in a coarse representation [4]. However, this representation does not provide a dynamic model of the process on an atomistic scale and does not allow us to study the coupling of dynamic modes within the nucleosome. DNA sliding refers to the micro/millisecond dynamics of nucleosomes, and is not currently available for direct modelling using classical molecular dynamics (MD). Previously, our work [5] has seen a DNA sliding by one nucleotide pair close to the nucleosome exit (at SSHL 5) - only in one of a series of microsecond trajectories. The

challenge now is to model DNA sliding in the MD using heuristics and amplified techniques.
We used three approaches in this work. Firstly, we calculated the classical MD of nucleosomes that already had large DNA twist defects in the inner site of the nucleosome (SHL 2). Such a state was discovered for the nucleosome in complex with the Snf2 remodeler (PDB ID 5Z3L). The expected relaxation of the twist defect towards the nearest exit of the nucleosome was observed. This movement was accompanied by a rearrangement of the DNA-protein contacts; the key histone contacts shifted by one DNA base pair and moved to the opposite DNA strand. Thus, the sliding in this experiment proceeded according to the accepted model of the corkscrew mechanism. Subsequent calculations have been performed using enhanced MD methods using external potentials on crystal structures of nucleosomes without DNA twist defects (PDB ID 3LZ0). In particular, a DNA twist defect in the SHL 5 nucleosome site has been force moved by applying a harmonic potential on the collective variable describing the torsion. In the another approach, the nucleosome dyad region was 'swayed' using the metadynamics. Both cases show shifts of DNA strands within the nucleosome. An interesting observation was that the nucleosomes showed stability to such an external disturbance, and observed small shifts did not cause DNA unwrapping. Note that the DNA unwrapping occurs spontaneously on the microsecond timescale and is shown in previous MD experiments. Further work involves both the further development of methods for setting collective variables describing torsion states of DNA and the development of modelling protocols, as the classical approach poorly reproduces dynamic transitions between different forms of DNA, stabilizing the B-form.
Thus, it is not yet possible to simulate the full DNA sliding cycle in the nucleosome, but dynamic models of local DNA sliding in the nucleosome have been obtained. This work was supported by grant no. 18-74-10006 from the Russian Science Foundation. The work was carried out using the equipment of the Lomonosov Moscow State University's Super High Performance Computing Centre.
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### S5.291. Distributed agent model of photosynthetic electron transport

Khruschev S.S.[1]*, Plyusnina T.Yu.[1], Riznichenko G.Yu.[1], Rubin A.B.[1]
[1]*Lomonosov Moscow State University;*
* styx@biophys.msu.ru

An agent-based mathematical model reproducing the dynamics of redox transformations of the components of the photosynthetic electron transport chain with explicit simulation of mobile electron carriers diffusion in the complex interior of the intrachloroplast space is considered. The geometry of granal and stromal thylakoids is set analytically on the basis of modern ideas about the spatial organization of thylakoid membranes. The model considers a grana, consisting of several (from 2 to several dozen) cylindrical thylakoids, and stromal lamellae connected with it. It is assumed that the grana, which consists of stacked cylindrical thylakoids, "pierces" the stromal lamellae, and spiral structures are formed in the contact area. The dimensions and shape of the compartments are specified as numerical parameters of the model. The model is discrete in time and space. The space inside the chloroplast is divided into rhombic dodecahedral cells with a volume of 2 cubic nm, toroidal (periodic) boundary conditions are used.
The model considers redox reactions occurring with the participation of agents - transmembrane protein complexes (photosystems 1 and 2 and the cytochrome b6f complex) and mobile electron carriers - plastoquinone, plastocyanin and ferredoxin. One cell of the model is used to represent the plastoquinone molecule; protein molecules occupy several adjacent cells, their shape is set according to X-ray diffraction analysis or cryoelectron microscopy, and the rotation angle is determined randomly when the model is run. All agents in the model are considered as mobile; for transmembrane protein complexes, lateral diffusion in the membrane plane is available; for mobile carriers, three-dimensional diffusion within the corresponding compartment (the lipid layer of the membrane for plastoquinone, the thylakoid lumen for plastocyanin, and the stroma for ferredoxin) is available. In one step of the model (2 us), the agent can move one cell in any direction; for each type of agents, the probability of displacement in one step of the model is set. For mobile carriers, the displacement probability was chosen in such a way that the diffusion coefficients observed in the model corresponded to the experimentally measured ones.
Each of the agents can be in one of several redox states. The way the transitions between the states of agents occur is determined by the program specified for each type of agents, so one can easily change the level of detail of the processes occurring in each of the agents, regardless of other agents. One or several cells near the surface of the transmembrane complexes are set as active sites, the hit of mobile electron carriers into which leads to the launch of a program that analyzes this interaction. A maximally simplified system was considered, in which all processes of electron transport between transmembrane complexes are modeled as irreversible diffusion-controlled reactions. If, at the current step of the model, a molecule of an agent, a reaction partner, is in the interaction site, and the redox states of two agents allow the exchange of electrons between them, then a corresponding change in the states of these agents occurs. In addition, spontaneous reactions can occur in complexes, which correspond to light-induced charge separation in photosystems and electron transfer between the carriers included in the complex.
With the help of the developed model, the influence of the geometric parameters of the grana on the transient redox processes occurring under the illumination of dark-adapted leaves was studied. Despite its simplicity, such a model made it possible to reproduce the main features of the curves observed in experiments using a small number of adjustable parameters.
The model is implemented as a program in the Python programming language. This work was supported by RFBR grant 20-04-00465 and RSCF grant 22-11-00009.

### S5.292. Dynamic models of divergent evolution initial stages

Frisman E.Ya.[1]*, Kulakov M.P.[1], Zhdanova O.L.[2]
[1]*Institute for Complex Analysis of Regional Problems FEB RAS, Birobidzhan, Russia;*
[2]*Institute of Automation and Control Processes FEB RAS, Vladivostok, Russia;*
* frisman@mail.ru

Within the framework of the classical theory of population genetics, the preservation of balanced polymorphism in a stationary environment

is possible under the overdominance. Although it is extremely difficult to detect this type of selection, polymorphism is quite common in nature, which supports the unrelenting research interest in identifying and studying the mechanisms that prevent the loss of genetic diversity. Polymorphism may be the first step necessary for the primary divergent evolution of populations, i.e. the emergence of differences in the subpopulations genetic structures of an initially homogeneous population system. At the same time, stable primary genetic divergence in itself is an additional factor for maintaining polymorphism in the population system. The most interesting and even somewhat paradoxical in terms of population genetics is the possibility of establishing stable differences in a selection-homogeneous range, i.e. under conditions of the same selection action in all subpopulations. The only type of selection that can lead to such divergence is disruptive selection. In the simplest case of single-locus selection, it is reduced fitness of heterozygotes.

In the first part of this report, based on a mathematical model, we investigate the bifurcation mechanisms that lead to the emergence of primary genetic divergence i.e. stable genetic differences between two adjacent populations coupled by migration of individuals. The following simple situation is considered. The populations are panmictic with Mendelian rules of inheritance. The fitness of individuals is strictly determined by a single diallelic locus. We use a dynamic model that describes the change in the concentration of one of the alleles in each subpopulation and the ratio (weight) of first population to the total size. We showed that the genetic divergence in such a model population is reached only with reduced fitness of heterozygotes and is the result of the subcritical flip bifurcation (period doubling) of the unstable equilibrium state corresponding to polymorphic populations. In free populations with unlimited growth in numbers, solutions corresponding to the genetic divergence are always unstable and are just a part of the transient process when the trajectory moves to one of the monomorphic states. Genetic divergence is stable only for populations that maintain the ratio of their numbers at a constant level. For example, this is possible for populations with limited or fully synchronous dynamics in adjacent sites. In this case, the divergence is preceded by a saddle-node bifurcation and the dynamic becomes quad-stable, i.e. depending on the initial conditions, the populations are homogeneous in their genetic structure, or there are significant differences between populations. Thus, the appearance of genetic divergence is impossible without a significant ecological limitation of population growth in addition to genetic factors.

To show the possibility of genetic divergence in a homogeneous areal with more evidence, one can only experiment with laboratory populations without the influence of habitat heterogeneity as much as possible. A team led by Yu. P. Altukhov carried out such large-scale experiments with box populations of Drosophila melanogaster. In particular, the experiments produce pronounced primary divergence of the genetic structures of the subpopulation at the α-GDH locus with almost identical environment characteristics in each of the 30 boxes. In the second part of our report, using mathematical modeling, we study the role of disruptive selection (namely, the presence of reduced fitness of heterozygotes for α-GDH) in maintaining the primary genetic divergence observed in the system. The alternative hypothesis is the genetic drift that fixed the difference in the genetic structures of subpopulations. We analyzed mathematical models of the allele frequencies' dynamics in a large panmictic population and a system of 30 local migration coupled ones. Then we compared the model dynamics of allele frequencies under the disruptive selection and without it with data obtained in the experimental work of Yu. P. Altukhov and co-authors. The simulation results show that with disruptive selection, in the system of populations connected by stochastic migrations, primary genetic divergence occurs with a sufficiently high probability and turns out to be structurally stable. The simulation results comparison with those of the experiment allows us to conclude that in the considered experimental population system, disruptive selection at the α-GDH locus facilitated the primary genetic divergence.

Looking at the ongoing processes somewhat broader, one can see the following generalization. Since the hybrid individuals created the initial population nucleus of the experimental system, one can assume that two oppositely directed processes took place simultaneously during development of this system. The first was in formation of gene blocks that provided increased fitness for heterozygous forms. Natural selection here maintains the polymorphism of the alleles of these genes. During the second process, segregation of heterozygous forms and identification of genes with increased fitness of homozygotes occurred. It results in disruptive dynamics of allele frequencies. Each of these processes also involved alleles of adaptively neutral genes. Whatever the reason, the main of which, apparently, is linkage, these neutral alleles turned out to be genetic markers of the corresponding adaptive processes. Thus, the esterase-6 locus became a marker for the processes maintaining allele polymorphism, and the α-GDH locus turned out to be a marker of disruptive selection that occurs in the system and promotes primary genetic divergence.

### S5.293. Dynamics of DNA-Dps crystals under dehydration conditions

Tereshkina K.B.[1]*, Tereshkin E.V.[1], Loiko N.G.[2], Kovalenko V.V.[1], Krupyanskii Y.F.[1]
[1]*N.N. Semenov Federal Research Center for Chemical Physics Russian Academy of Sciences;*
[2]*Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences;*
* quebra-mola@yandex.ru

The growth of a bacterial colony undergoes multiple types of stress and adjusts its life cycle to the existing environmental conditions. The survival of bacteria under adverse conditions is largely determined by the ability of the bacterium to keep intact its genetic material, mainly nucleoid DNA. When unfavorable conditions occur, the growth of the colony slows down, it enters the stationary phase of growth, in which special DNA-stabilizing proteins Dps (DNA-binding protein from starved cells) begin to be produced [1-2]. Dps are homododecamers with a cavity inside. The point symmetry group is 23. Due to the ability to bind iron ions, inactivate them and accumulate in the intraprotein cavity, the Dps protein family belongs to the ferritin superfamily. The termini of all subunits are extremely labile and bind DNA. Crystallization of the Dps protein results in the formation of intracellular DNA-Dps crystals. Dps homologues have been identified in many bacteria and archaea. However, the molecular mechanisms that allow these proteins to protect DNA have not yet been studied.

In this work, the processes of formation and evolution of DNA-Dps co-crystals of the bacterium Escherichia coli (PDB ID: 6GCM) are considered by molecular dynamics methods. The dynamics of Dps-DNA nanocrystals during drying, accompanied by a decrease in the percentage of water and an increase in the ionic strength of the solution, was studied. The initial concentrations of ions corresponded to the cytoplasm of the E. coli cell. As a control, the following systems were studied: 1) Dps-DNA crystals in solution with counterions, 2) free DNA in solutions. The calculations were carried out using the coarse-grained representation of molecules in Gromacs. The crystal contained 15 Dps and DNA of 165 bp (the yhis gene, presumably having a high adsorption capacity for the Dps protein [3]). Simulations were carried out in periodic box at a constant number of particles, temperature and. The integration step was 10 fs, the trajectory length was 0.1 μs for all systems, except for strongly dehydrated ones. The trajectory length of such systems is reduced to 0.03 μs due to the faster plateauing of the studied parameters. The temperature of 300 K was maintained using a Langevin thermostat (t=1 ps). The pressure was maintained using a Parrinello-Raman barostat (t=4 ps, p=1 bar, isothermal compressibility of water was 3.0*10^4 bar^-1). The cutoff radii for the Coulomb and

van der Waals interactions were taken to be 1.2 nm. The dielectric constant of the medium was equal to 15 to provide clear shielding. The number of water molecules varied from 100% for non-dehydrated system. The first stage of dehydration corresponded to a decrease in the number of water molecules up to 75%, the second stage up to 50%, the third stage up to 25%, the fourth stage up to 10%. The fifth stage of dehydration was characterized by the presence of exclusively bound water. The spatial and energy characteristics of the systems were obtained. Principal component analysis made it possible to evaluate the differences in the dynamics of Dps and DNA during dehydration. It was found that at normal (100%) and close to normal (75%) amounts of water molecules, the nanocrystals retain their structure and are almost identical. The DNA inside the crystal adjusted to the shape of the channels. The terminal regions of DNA lying outside the channels, as well as free DNA in solution, did not undergo significant conformational changes. With a decrease in the amount of water in the system by 50%, dehydration effects began to appear. The diameter of Dps molecules remained unchanged, and the approach and interaction of Dps molecules that did not contact each other in native crystals took place. At this stage of dehydration, crystal defects began to appear. Defects could both eliminate themselves and grow as the calculation proceeds. The DNA inside the crystal turned out to be stabilized, adjusting to the internal environment of the channels. However, kinks began to appear in the regions of the DNA molecules lying outside the crystal. With a decrease in the amount of water in the system to 25% or less, the crystal core formed by internal Dps molecules continued to densify. At this stage, densification occurred both due to the approach of Dps molecules and a change in the diameter (compression) of the dodecamers themselves. Detachment of some surface Dps molecules was observed. The compression of Dps molecules arose due to the release of water molecules that were in the cavity of the dodecamer. This effect manifested itself at both high and low ion concentrations and was apparently due to a local increase in the ion concentration on the surface of the dodecamers. The DNA inside the crystal did not undergo significant changes. Free DNA regions were compressed or adsorbed on the surface of the nanocrystal. A further decrease in the amount of water only slightly affected the shape of Dps molecules and crystals with DNA inside them, but caused significant conformational changes in free DNA regions. There were kinks and bends that could lead to degradation of the DNA structure. It was shown that, during dehydration, Dps molecules have a critical effect on the preservation of DNA and are able to preserve the native DNA structure in the internal channels of the crystal.

The work was performed under a grant from the Russian Science Foundation (no. 23-24-00250). The calculations were carried out on the MVS-10P high-performance computer system at the Interdepartmental Supercomputer Center of the Russian Academy of Sciences (MSC RAS).

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### S5.294. Dynamics of connectivity between the hemispheres of the rat brain induced by spreading depression

Lachinova D.A.[1,2]*, Sysoev I.V.[1,2], Vinogradova L.V.[1]
[1]*Institute of Higher Nervous Activity and Neurophysiology of RAS;*
[2]*Saratov State University;*
* lachinova-dasha@yandex.ru

Spreading depression (SD) is a response of nervous tissue in the form of short-term reversible self-propagating intense cellular depolarization [1]. Acute brain damage (traumatic brain injury, stroke) and epileptic seizures can provoke waves of spreading depression. It is believed that a local increase in the concentration of potassium ions in the extracellular environment, leading to depolarization of neurons, is necessary for the occurrence of SD. Extracellularly, the SD wave is registered as a high-amplitude negative shift of the constant potential, which is the most reliable indicator of its development in the nervous tissue. The amplitude of the SD wave reflects the number of neurons involved in depolarization, and its duration reflects the rate of cell membrane repolarization. In focal occurrence, SD propagates relatively slowly (2-6 mm/min) through the brain gray matter. Low propagation velocity and long duration (0.5-1 min) of the SD wave indicate that the main contribution to its development is made by extrasynaptic processes.

To determine changes in the connectivity between the two hemispheres in the cortico-limbic system (neocortex) of the rat brain, electrophysiological experiments were performed on adult male Wistar rats. Two weeks before the experiment, the rats were implanted with electrodes for electrocorticogram (ECoG) registration and guiding cannulas for focal microdamage of the nervous tissue. Electrical activity of the frontal cortex in a wide frequency band was recorded using a high input impedance DC amplifier and an ADC (E14-440, L-Card, Russia) under the conditions of a chronic experiment.

We had at our disposal ECoG recordings obtained under conditions of free behavior in eight awake rats. Several recordings, 14 in total, were obtained in a number of animals. The appearance of SD in the recording areas was determined by characteristic slow shifts of the extracellular potential. We analyzed 600-second epochs of ECoG recordings of the frontal cortex of both hemispheres before and after unilateral damage to the amygdala and induction of a single SD wave. The recordings were rectified by the moving average method, low-frequency trends were removed, bandpass filtering (50 Hz) was applied, and then artifacts caused by the signal exceeding the dynamic range of the ADC were corrected. The segments were divided into 20-second non-overlapping consecutive intervals. For each interval, a measure of connectivity, a function of mutual information, was calculated according to the method proposed in [2].

Analysis of interhemispheric functional connectivity in the signal as a whole using the mutual information function (a nonlinear, frequency unresolved measure of non-directional interaction) showed a significant change in interhemispheric connectivity associated with SD. It turned out that simultaneously with a decrease in the power of cortical oscillations, the unilateral SD wave caused a significant (2-5 times) decrease in functional connectivity between the hemispheres (a decrease in functional similarity of signals). The minimum of mutual information was reached in about 100 s after the beginning of the decrease. It took another 100-200 s to restore connectivity to the background level. This work was supported by the Russian Science Foundation (grant No. 22-15-00327).

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### S5.295. Experimental and theoretical study of the quorum effect in batch culture of Photobacterium phosphoreum 1889

Sarangova A.B.[1]*, Bartsev S.I.[2]
[1]*Siberian Federal University;*
[2]*IBP SB RAS, FRC KSC SB RAS;*
* sarangova.antonina@yandex.ru

One of the objectives of modern microbiology is to study the mechanisms of triggering the quorum effect, which was discovered in the study of bioluminescence [1] in bacterial populations. Then the topic has received more development in the field of medical microbiology. Attention to this effect is explained by the fact that the detected quorum effect regulates the physiological activity of microbes, namely, it affects symbiosis, virulence, conjugation, antibiotic synthesis, motility, spore formation, biofilm formation, and bioluminescence. The purpose of the work is to describe theoretically the launch of the luciferase operon in the batch culture of Ph.phosphoreum 1889 and to assess the role of the metabolic influence on its launch.
The obtained experimental regularities are consistent with the known data [1, 2]. The growth dynamics of the culture biomass is well described by a model that takes into account substrate inhibition. To describe the dynamics of the amount of luciferase, two equations were used that describe the actual average amount of luciferase in a culture cell and the concentration of the autoinducer in the medium. It is shown that for an acceptable description of the luminescence dynamics it is necessary to accept that the activation of the luciferase operon in bacteria occurs at a certain stage of cell development and the sensitivity of cell receptors to the concentration of the autoinducer varies significantly.
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**S5.296. Features of the behavior of magnetically marked biological cells at phase transitions in a ferrite-garnet film**

Siryuk Ju.A.[1], Bezus A.V.[1]*, Kapshukov R.A.[1], Kononenko V.V.[2], Bondarev I.S.[1]
[1]*Donetsk National University;*
[2]*Galkin Donetsk Institute for Physics and Engineering;*
* a.bezus@donnu.ru

Introduction. Domain structures of magnetics have been the subject of intensive experimental and theoretical research for many years, caused by the interests of both applied and fundamental science. Interest in the study of ferrite-garnet films is explained by the fact that the degree of manifestation of various properties in such films is much higher than in the bulk single-crystals of the same property.
In recent years, many works have been published related to the controlled transfer of paramagnetic colloidal particles over the surface of a ferrite-garnet film [1]. The motion of particles both on the surface of stripe domains and on the surface of a lattice of cylindrical magnetic domains (CMD) is being studied. The motion of paramagnetic particles over the surface of the pattern of domain structures (DS) occurs under the influence of external magnetic fields. But these fields also affect the DS, causing phase transitions (PT) in it. This influence must be taken into account in the controlled transport of magnetic particles over the film surface.
The aim of work is to study the peculiarities of behavior of paramagnetic colloidal particles on the surface of a ferrite-garnet film during PT induced by an external magnetic field in domain structures.
Such studies are relevant. As time shows, these results can be demand in different scopes: in microelectronics - recording and storage of information; in medicine - directed transportation of biological particles, i.e. local treatment; in chemistry - sorting particles according to their size. In addition, when creating microdevices, these scopes of applicability can be combined.
Main part. The investigations were carried out on a ferrite-garnet film with a developed surface <111> grown by liquid-phase epitaxy method on a gadolinium gallium substrate, a composition of rare-earth sublattice YSmLuCa. Film thickness h=6.8μm. The film at a room temperature has the saturation magnetization 0.258mT and quality factor Q>5.
Yeast cells (Saccharomyces cerevisiae) magneto-marked with iron oxide ions are used as magnetic particles [2]. The test solution is prepared as follows: 10 microliters of the magneto-marked cell suspension is placed in an Eppendorf tube. Then 5 microliters of 10% sodium dodecyl sulfate and 1 milliliter of distilled water are added to it. The contents of the tube are thoroughly mixed. Five microliters of the prepared suspension is applied to the surface of the ferrite-garnet film cleaned with alcohol.
Transportation of magneto-marked cells occurs under the action of a magnetic field with the following parameters: induction B=54mT, frequency $\nu$=2Hz, the shape of the control signal is sawtooth. The formation of external magnetic fields is carried out using the magnetic system. The visual pattern of the DS is observed due to the Faraday Effect on a polarizing microscope MKD-R and recorded with a digital camera. The action of a pulsed magnetic field creates a lattice of CMD in the ferrite-garnet film.
In this work, the localized movement of magneto-marked cells around CMD has been studied. Such movement is created by the control magnetic circular field of induction B=22mT in the frequency range (1-10)Hz. The most pronounced effects of localized cell movement around the CMD are observed in the film saturation magnetization range (0.1–0.22)4πMS. It has been noted that the dynamics of localized cell movement depends on the magnitude of the field induction normal to the film surface (displacement field). Cell movement is observed with an increase in the displacement field within (2.97-5.69)mT. This is explained by the fact that the CMD lattice is preserved in such range of the displacement field. If the displacement field is antiparallel to the magnetization inside the CMD, then at an induction value of B≥5.69mT, a first-order PT occurs in the CMD lattice due to the disappearance of the central domain of hexagonal packing into a new lattice with a smaller CMD diameter and a large period.
If the displacement field is coincides in direction with the magnetization inside the CMD, then at an induction value of B≥2.97mT, a second-order PT into a honeycomb structure occurs in the lattice. In this case, the number of domains is conserved and CMDs acquire the shape of a hexagon. Magneto-marked cells line up along domain boundaries and visualize the domain structure.
With a subsequent increase in the induction of the field coinciding with the direction of magnetization inside the CMD, a first-order PT occurs in the honeycomb structure into a cluster structure. The magneto-marked cells visualize the domain boundaries of this structure as well. As the field induction increases, changes occur in the cluster structure. The sizes of some domains decrease, and the length of domain boundaries also decreases. In this case, the paramagnetic cells move on the surface of domain under the influence of the magnetic field gradient from the pattern of domains.
Conclusion. The action of low value of the induction of external magnetic field (compared to the value of saturation magnetization of the ferrite-garnet film) occur a phase transitions in the domain structure. Domain boundary generates a strong local magnetic field gradient, which affects on peculiarities of the behavior of magneto-marked biological cells. Thus, the cells are localized near the domain boundaries and visualize the magnetic pattern obtained during phase transitions in the ferrite-garnet film.
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### S5.297. Fractional derivatives for pressure pulse wave simulations

Gamilov T.M.[1,2,3]*, Rogov A.V.[1,2], Kirichenko Ya.Yu.[1]
[1]*Sechenov University, Moscow, Russia;*
[2]*Moscow Institute of Physics and Technology, Dolgoprudny, Russia;*
[3]*Sirius University, Sochi, Russia;*
* gamilov.tm@mipt.ru

Recently, mathematical models with fractional derivatives were used to describe various phenomena in physics, mechanics, economics, and the natural sciences. Fractional differential equations provide more adequate and accurate description of many processes than classical equations with integer derivatives. They are successfully used in mathematical biology, hydrogeology in modeling heat and mass transfer processes in highly non-homogeneous media, problems of elasticity, epidemiology, etc.

One of such applications is the use of fractional derivative models to describe hemodynamic processes. Fractional derivatives allow hemodynamic specialists to simulate complex flows with visco-elasticity, anisotropic viscosity, memory effects, etc., and to create more realistic pulse wave forms. The latter task has become especially relevant in recent years with the development of computer technology and increasing interest in inverse problems.

In this paper, we propose to use a model of one-dimensional hemodynamics to describe the blood flow in the aorta and coronary arteries. Each vessel is a one-dimensional elastic tube with a viscous incompressible liquid. Bifurcation points require calclation of the redistribution of blood flows. It is calculated with the help of law of conservation of mass and continuity of total pressure. The constructed network of vessels requires imposing boundary conditions at the inlet (near the heart) and at the outlets (microcirculation areas). At the inlet, the flow is imposed. This flow corresponds to the cardiac output of the average person. The pressure drop through the hydrodynamic resistances is set at the outlets. Fractional derivatives are used to modify the boundary conditions at the outlet of the aorta and describe the distensibility of the microcirculatory bed. Various values of the Caputo fractional derivative index are considered. The value of the index affects the shape of the pressure pulse wave in the aorta and, as a consequence, in the coronary arteries. The shape of the pulse wave can influence the indices used to assess the hemodynamic significance of coronary artery stenoses, for example, the iFR (wave free ratio). iFR is calculated as the ratio of pressures after and before stenosis during the relaxation of the ventricles of the heart. iFR values are used in diagnostics to make a decision about surgical intervention.

Fractional derivatives can be used to personalize the blood flow model by selecting a Caputo index and reproducing the pulse wave shape of the patient. Such a personification of the model is important in the accurate assessment of various hemodynamic indices that affect the choice of a patient's treatment strategy.

### S5.298. Generation of a statistically significant predictors system for the application of machine learning in predicting the secondary structure of proteins

Milchevsky Y.V.[1]*, Milchevskaya V.Y.[1], Tevonyan L.L.[1], Arutyunyan A.F.[1], Kravatsky Y.V.[1,2]
[1]*Engelhardt Institute of Molecular Biology of RAS;*
[2]*Center for Precision Genome Editing and Genetic Technologies for Biomedicine, EIMB RAS;*
* milch@eimb.ru

In recent years, the improvement of protein structure prediction accuracy has been closely related to the application and refinement of machine learning methods. Encoding the amino acid sequence is the initial stage of structure prediction, and therefore plays a fundamental role in the success of these methods.

Significant progress has been observed in protein structure and function prediction methods based on sequence over the past few years. Substantial advancements have been made in tasks such as predicting secondary and local protein structure, protein contacts, protein-binding sites, and more. The use of machine learning methods, particularly deep learning methods, has been essential in achieving progress in these tasks.

Data preparation for predictive models generation is one of the most challenging tasks, both methodologically and algorithmically. Generating input data for machine learning tasks for predicting protein structures and functions is not a standard procedure and is usually implemented in the context of a specific task.

Thus, the choice of the initial set of predictors is crucial for building a model that describes the relationship between sequence and protein local structure. It is necessary to create and implement an algorithm that allows reducing the number of features, simplifying the model and excluding any redundant information, while preserving the statistical significance of the predictor system formed.

It should be noted that application of machine learning methods doesn't permit to assess the contribution and significance of individual predictors. Therefore, during model debugging, changing the input set of predictors usually performs by rundown or by researcher's personal choice. Our approach to generation of the initial set of predictors consists of using a set of statistically significant features previously obtained by other methods, which allow this statistical significance to be quantitatively evaluated [1]. We applied predictors from our previous work on predicting local structure [2]. In this work, for each of the 16 protein blocks (PBs) [3], we identified significant predictors based on both the physicochemical properties of amino acid residues and the statistical characteristics of structural elements [1]. The prediction for each element of the sequence is a set of distances calculated by the RMSD metric to each of the 16 protein blocks (PB). Thus, creating a predictive model involved 16 separate tasks, each of which identified a set of statistically significant predictors from a large initial set of predictors. Each predictor formalized assumptions about the physical factors that determine local structure. The selection of the most significant predictors for each PB was performed using stepwise regression analysis [1]. All significant predictors were combined into a set for subsequent use in deep learning methods. Many predictors were found to be significant for predicting multiple, and even all 16 PBs. The final set consists of 243 predictors, selected from 852 input predictors. Among the selected predictors are those based on the properties of amino acids (AAindex [4] database) as well as predictors reflecting statistical characteristics of the occurrence of structural elements.

The obtained predictor system can be expanded or reduced in a predictable way (i.e. by adding or excluding certain physicochemical and/or structural parameters of protein chains). The generation of predictor systems is described in detail in our works [1] and [2]. Programs that implement stepwise regression and discriminant analyses are freely available on Github: https://github.com/Milchevskiy/protein-encoding-projects.

The work was supported by RFBR grant 22-24-01088.

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**S5.299. Glutamate-cysteine ligase modeling on the basis of stochastic methods**

Boronovskiy S.E.[1]*, Kopylova V.S.[1], Nartsissov Y.R.[1,2]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Moscow, Russia;*
[2]*Biomedical Research Group, BiDiPharma GmbH, Siek, Germany;*
* scihazard@yandex.ru

Glutathione is one of the main intracellular antioxidants that play an important role in cell functioning. In mammalian cells it is synthesized in the cytosol via two successive ATP dependent reactions catalyzed by glutamate-cysteine ligase (GCL) and glutathione synthetase (GS). The study of GCL is of greatest interest, since the activity of this enzyme, along with the cysteine concentration, are limiting factors of the glutathione biosynthesis. Also, with an increase in the glutathione intracellular concentration, the rate of its synthesis is significantly reduced due to GCL feedback inhibition. Based on the results of various experimental studies of the glutamate-cysteine ligase kinetics several assumptions about the order of substrate binding during the reaction have been made. They include both primary ATP binding with random binding of glutamate and cysteine, and completely random binding of substrates. In this work we use a stochastic algorithm based on continuous-time Markov chains to resolve the uncertainty in the choice of a possible enzyme mechanism. Based on the obtained results it can be concluded that only the mechanism involving the primary ATP binding allows obtaining a value for the reaction rate that is consistent with the experimentally measured activity of human erythrocyte glutamate-cysteine ligase at physiological levels of substrates. Moreover, modeling of glutathione biosynthesis under the assumption of ATP saturation does not correspond to physiological conditions. Herewith the concentration of ATP molecules can have the greatest effect on the glutamate-cysteine ligase activity under conditions that lead to disruption of the biochemical pathways of ATP production.

**S5.300. Hidden symmetries of proteinaceous viral shells**

Rochal S.B.[1]*, Konevtsova O.V.[1], Golushko I.Yu.[1], Podgornik R.[2]
[1]*Southern Federal University;*
[2]*University of Chinese Academy of Sciences;*
* rochal_s@yahoo.fr

Viruses occupy an intermediate area between living and non-living matter and at certain stages of their life, cycle they require a host cell to reproduce, using its material and machinery to replicate viral genome and produce proteins of viral shell (capsid). Self-assembly of new viruses is a multi-stage, many-hour process with some steps controlled exclusively by physical and chemical laws. For many viruses, the final stage of assembly involves structural transitions that convert a noninfectious precursor particle into an infectious agent. Only after the completion of all stages of this process, called maturation, the virion becomes able to infect host cells. Therefore, understanding principles of self-assembly and structural transformations of capsids during maturation is very important for the development of antiviral strategies.

Proteins encoded by a relatively simple viral genome cannot be too complex and are characterized by a minimum number of conformational states and types of bonds with their nearest neighbors. However, an infinite number of crystallographically equivalent positions exists only in ideal periodic structures. In any closed discrete shell, this number is limited and for identical asymmetric proteins, in the case of icosahedral capsids, it is equal to 60, which is the order of the capsid symmetry group I. Therefore, even environments of structurally equivalent proteins located in different crystallographic positions cannot be strictly the same. Therefore, while it is geometrically impossible for proteins to occupy equivalent crystallographic positions, proteins manage to locate in quasi-equivalent positions with approximately the same local environments. This principle of quasi-equivalence was introduced by Caspar and Klug (CK) [1] and allowed them to describe structures of icosahedral capsids consisting of pentamers and hexamers within their well-known viral shell model. Due to the quasi-equivalence principle, most capsids have the specific static hidden (approximate) symmetry and are characterized by a local periodic order.

In the CK model, this local periodic order is formed by the centers of pentamers and hexamers. As we have shown in articles [2-5], in abnormal capsids (violating the CK model), the centers of mass of individual proteins or other symmetrical structural units, such as trimers, can also form the locally periodic order. Using the theory of icosahedral spherical lattices (SLs), which are smooth mappings of an appropriate periodic hexagonal order onto a sphere via icosahedron net, we discuss structural organization of anormal viral shells After mapping the hexagonal order onto the sphere, the nodes that do not coincide with symmetry axes of the icosahedron lose their local symmetry, which allows asymmetric structural units, or structural units with 'inappropriate' symmetry, to be placed in these positions on the sphere. Examples of small and giant virus shells arranged in this way are discussed.

Along with the static hidden symmetry, viral shells can also have dynamic hidden symmetry. Note that capsids lack exact translational symmetry. Therefore, the analysis of normal modes of the shells is much more difficult. Following the standard procedure for the determination of normal modes, one can calculate and diagonalize the Hessian matrix of 3N dimensions, where N is the total number of the rigid structural units (SUs) in the capsid. However, the selection of elastically coupled rigid SUs is not an unambiguous approach, since capsid proteins consist of domains, which have a complex internal structure. An alternative methodology is to implement the continuum approach. As we show, traces of condensation of certain phonon modes can be seen in the structures of icosahedral viral shells and these modes approximately corresponds to the phonon modes of a thin elastic spherical shell. The fact that the modes under consideration must preserve the icosahedral symmetry of the capsid imposes strict symmetry restrictions on their explicit form. Such modes, like ordinary spherical harmonics, can be classified according to their wave numbers l. Interestingly, the widespread icosahedral and relatively rare dodecahedral capsid faceting is associated with the l=6 mode. We also discuss the collective shear displacements of proteins that occur during the maturation of viral proteinaceous capsids and are similar to the shear modes of a spherical shell.

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**S5.301. How changes in the electrical charges of proteins contribute to their repacking in the capsids of Zika and Dengue viruses during their maturation**

Golushko I.Yu.[1]*, Konevtsova O.V.[1], Podgornik R.[2], Rochal S.B.[1]
[1]*Southern Federal University;*
[2]*University of Chinese Academy of Sciences;*
* vaniagolushko@yandex.ru

Electrostatic forces play a crucial role in many biological processes at the molecular level, being a part of both short-range and long-range interactions. Except for an isoelectric point corresponding to the solution of a certain acidity, proteins always carry a nonzero electric charge, which can reach several tens of elementary charges per molecule. One of the key features of the electrostatics of proteins compared to the classical electrostatics of colloids is the presence of ionizable amino acid residues. Because of this, the presence of mobile ions in the surrounding solution not only screens protein charges, but also directly affects their magnitude. Variation in the pH of the solution leads to the dissociation of ionizable residues, changing both the total protein charge and its distribution in space. Corresponding changes in electrostatic interactions can affect the secondary, ternary, and quaternary structures of the protein.

In this work, we study the role of electrostatic forces in the large-scale reconstruction of the shells of Dengue and Zika viruses during their maturation. These viruses, belonging to the same genus, have a complex three-layer structure of the capsid. Its inner protein core is surrounded by a lipid membrane, which is in turn covered by an outer shell consisting of elongated structural proteins. Dengue and Zika are initially formed in the endoplasmic reticulum at neutral pH. To become able to infect cells, they must enter an acidic environment, where their porous shell with a developed morphology undergoes large-scale conformational changes, repacking proteins into a denser and smoother structure. We analyze the structures of Dengue and Zika viruses at different stages of their life cycle within the framework of the developed semi-quantitative electrostatic model [1,2]. Given the considerable size of the system (~10^7 atoms), we consider the viral envelope as a set of point charges corresponding to ionizable residues. Using the Henderson-Hasselbalch isotherm, we determine the partial charges of the amino acids in the proteins for different pH values and then calculate the corresponding electrostatic interaction energies between the proteins within the Debye-Hückel approximation.

We show that as pH decreases, the electrostatic energy of the initial state becomes greater than that of the final mature state, which promotes the repackaging of the proteins of the outer shell. Several mechanisms may contribute to the activation of the transition. Firstly, it is a change in the interactions between the outer shell proteins caused both by a change in the total charge and its redistribution between parts of the molecule. For example, in Dengue virus, the positive charge of pr domains located at the ends of the structural proteins increases with decreasing pH. Before the conformational changes, the pr domains are combined in triplets, but after the reconstruction, they are almost evenly distributed across the virus surface, which significantly reduces the repulsive electrostatic forces between them. Second, there is an increased attraction between the negatively charged lipid membrane of the virus and the structural proteins of the outer shell, which acquire a large positive charge in the acidic environment, making the denser, mature structure energetically more favorable. Finally, interaction with the inner nucleus can also contribute to the rearrangement of the outer envelope.

Studying and modelling of the unique maturation mechanism of Dengue and Zika viruses have several important practical applications. First, it is the development of new drugs aimed at its disruption. On the other hand, the obtained results could be used in the development of new crystalline porous composite nanobiomaterials, consisting of lipid membranes and associated protein complexes, and capable, like Dengue and Zika viruses, of structural rearrangement induced by the pH of the surrounding environment. Such materials can be used for targeted drug delivery and their sustained release.

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**S5.302. How does AlphaFold identifies 3D protein structure: Is it a physics-based prediction or sequence similarity-based recognition using huge databases?**

Finkelstein A.V.[1,2,3]*
[1]*Institute of Protein Research of the Russian Academy of Sciences;*
[2]*Biotechnology Department of the Lomonosov Moscow State University;*
[3]*Biology Department of the Lomonosov Moscow State University;*
* afinkel@vega.protres.ru

The great success of AlphaFold-kind programs [1-3] in protein structure identification from the sequence poses the questions: (i) What is the main reason for this success? (ii) What exactly do AlphaFolds do: the physics-based prediction of the spatial structure of a protein from its amino acid sequence or recognition of this structure from similarity of the target amino acid sequence to some parts of sequences with already known spatial structures? The answers given in this talk are: the main reason for the success of AlphaFold-kind programs is (i) the usage of huge databases which already cover virtually all protein superfamilies existing in Nature; (ii) using these databases, multiple sequence alignments, and coevolutionary information like correlations in triplets of amino acid residues of the contacting chain regions, AlphaFold-kind programs recognize a structure by similarity of the examined amino acid sequence (or its parts) to related sequence(s) with already known 3D structures [4]. I emphasize that this does not diminish the merit and utility of AlphaFold; it only explains the basis of its success.

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**S5.303. How to increase the cultivation efficiency of the diatom alga Skeletonema costatum?**

Suglobov A.S.[1], Kuznetsov A.V.[2,3]*
[1]*Center for Additional Education "Small Academy of Sciences", Sevastopol, 299055;*
[2]*A.O. Kovalevsky Institute of Biology of Southern Seas, Russian Academy of Sciences, Sevastopol, 299011;*
[3]*Sevastopol State University, Sevastopol, 299053;*
* andrei_kouznetsov@hotmail.com

Introduction

Many bacteria use cell-to-cell communication to arrange population density-dependent changes in behavior, called quorum sensing. This phenomenon is based on the secretion into the environment and perception of signaling molecules, the concentration of which varies depending on the number of surrounding cells [Popat et al., 2015; Abisado et al., 2018]. Quorum sensing has been found in marine bacteria [Zhao et al., 2019], yeast [Rosselló and Bouza, 2013], sponges [Padder et al., 2018], and other single-cell species, particularly microalgae [Zhang et al., 2021].

Diatom algae (Diatomeae) are photosynthetic organisms characterized by the silica "shell," called a frustule, whose structure is unique to each species, making them attractive to nanotechnology [Rabiee et al., 2021]. Diatoms create up to a quarter of all organic matter on the planet as the most important component of marine plankton [Belyakova, 2006].

The cosmopolitan diatom Skeletonema costatum [Shevchenko et al., 2022], which inhabits the Black Sea and whose biology and cultivation conditions are well studied [Kumar et al., 2014], is of interest. The genome of S. costatum has been sequenced, is 51.13 Mb in length with a GC content of 45.1%, the ecology and physiology are described, which gives an understanding of the biology of these organisms and can clarify a number of applied questions. For example, how to increase the yield of biomass when culturing microalgae under artificial conditions?

Bioinformatics analysis

Two death-specific proteins are known in Thalassiosira pseudonana, such as DSP1 and DSP2 (death-specific protein, DSP), which allowed us to find orthologs in S. costatum by applying the pBLASTp procedure to the nr database [Sayers et al., 2021]. We found protein sequences AAY27742.1 and ABU86410.1 in S. costatum, using both matrices ABU86411.1 and ABU86412.1 from T. pseudonana. Further analysis of the AAY27742.1 and ABU86410.1 proteins was performed on the Protomenal server [NOVEL, 2004], which allows identification of functional domains. It turned out that both proteins contain a regulatory EF-hand domain at the C-terminus of the polypeptide chain, but differ in the actuator domains.

Mathematical modeling

Let the activity of proteins involved in death processes in S. costatum prevents total extinction during intensive growth, and possibly limits the growth characteristics of cultures of these cells under laboratory conditions. We performed simulations in the NetLogo environment [Wilensky, 1998, 1999; Liu, 2001]. Initially, we applied exponential and logistic growth models, which are included in the list of standard NetLogo models that use system dynamics, where the idea of growth limitation from outside, such as under the influence of unfavorable environmental factors or as a result of nutrient depletion, is exploited. Nevertheless, as our data on the presence of DSP genes in the S. costatum genome show, internal control offers the possibility of preventive regulation, which may have arisen during evolution as a mechanism ensuring the reliable survival of the population under rapidly changing environmental conditions. Consequently, it is more logical to represent each cell as an autonomous agent configured for uninterrupted reproduction, and to program death in case the quorum threshold is reached or to perform a simulation without reproduction boundary conditions, which corresponds to the absence of DSP protein activity in the cells. The simulation began by generating an initial random sparse population of quorum-sensing agents. Reproduction of the agents was accompanied by the release of signaling molecules, when their maximum local density was reached, partial death of the agents occurred, and then everything was repeated from the beginning. The system is stable over a long time interval.

Conclusion

Thus, we found 2 death-specific proteins in the diatom alga S. costatum by bioinformatic analysis. Agent-based simulations demonstrated sustained oscillatory growth regulated by quorum sensing, as well as unrestrained exponential growth when internal regulation was disabled. Molecular biological evidence suggests that the quorum system carries out host cell elimination in real bacteria and microalgae by means of DSP proteins [Thamatrakoln et al., 2013; Hao et al., 2021], 2 of which (AAY27742.1, ABU86410.1) we found encoded in the genome of the diatom alga S. costatum. Our finding is in agreement with the studies on S. costatum [Chung et al., 2005, 2008] on the autoregulation of the abundance of these organisms under stress. Turning off the activity of these genes theoretically can lead to uncontrolled growth of cultures in the absence of nutritional limits, which is a most interesting finding for large-scale cultivation in biotechnology. This can be realized by RNA interference [Han, 2018] at the transcriptional level or, more, by CRISPR/Cas9 targeting [Ma et al., 2014] of the relevant genes at the genome level, else by using low-molecular-weight DSP protein inhibitors to prevent autocatalytic cell lysis.

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**S5.304. Identification of serotonergic neurons in Hymenolepis diminuta and Schmidtea mediterranea by means of confocal laser scanning microscopy (CLSM)**

Kreshchenko N.D.[1]*, Kuznetsov G.V.[1], Mitkovskii D.E.[2], Mochalova N.V.[3], Terenina N.B.[3], Movsesyan S.O.[3]
[1]*Institute of Cell Biophysics Russian Academy of Sciences;*
[2]*State budgetary professional educational Institution of Moscow region "Serpukhovskiy college", Moscow region, Russia;*
[3]*A.N. Severtsov Institute of Ecology and Evolution of RAS, Center of Parasitology (Moscow, Russia);*
* nkreshch@rambler.ru

The study is dedicated to the investigation of serotonergic components in the nervous system of free-living planarians and parasitic flatworms (Platyhelminthes). Schmidtea mediterranea planarians are a biological model object that is used to study the molecular mechanisms of regenerative processes and the stem cells functioning. The cestode Hymenolepis diminuta is a dangerous intestinal parasite of a human and animals. Planarian S. mediterranea of about 1 mm in size (7 specimens) were used, as well as cestode larvae (the cysticircoids) of H. diminuta, 149–170 μm long and up to 99–106 μm wide. The specimens were flat fixed with 4% paraformaldehyde and then placed in a 15% sucrose solution. Serial frozen sections were used to identify serotonin in planarians. For the study of H. diminuta the whole-mount preparations (4 individuals) were used. Samples were stained by an indirect immunocytochemical method with primary antibodies to serotonin (Immunostar) and secondary fluorescently labeled immunoglobulines (Sigma), and analyzed using a Leica DM6000 B fluorescent microscope, as well as a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Germany), Sector of Optical Microscopy and Spectrophotometry of the Center for Collective Use (ICB PSC RAS, Pushchino). The measurements were carried out on micrographs using the AxioVision Rel 4.8.1.0 software. The size of the neurons was measured. Quantitative evaluation of serotonin neurons is carried out for the first time.

A confocal laser scanning microscopy of preparations stained with antibodies to serotonin showed that the nervous system in H. diminuta is represented by the head ganglia located at the head end between four sucker, and by two pairs of the nerve cords connected by serotonin-immunopositive (-IP) nerve commissures. Localization of serotonin immunoreactivity was observed in the bodies of nerve cells and nerve fibers in the central nervous system of H. diminuta larvae. Serotonin-IP nerve elements were present in the lateral (or cerebral) ganglia, in the connective commissure, in the proboscis ganglia, as well as in the longitudinal nerve cords and in the transverse nerve commissures. A

pair of serotonergic nerves is found to run from the lateral ganglia to the proboscis ganglia to join them. Several serotonin-IP neurons have been identified in each of the two lateral ganglia. In total, from 14 to 18 serotonin neurons could be observed at the head end of the body. Serotonin-IP nerve fibers are present in the nerve plexuses within each sucker. Positive staining for serotonin is observed in the neurites that comprise the longitudinal nerve cord, two of which are the most pronounced with a distance of about 50–64 μm between them. Serotonergic neurons (6–8) were found along the nerve cords, which are connected by several thin serotonin-IP nerve commissures. The average size of serotonin neurons in H. diminuta was: in length of 4.80±0.28 μm, in width 3.53±0.34 μm (n=54, hereinafter – the mean±standard deviation, n – number of measurements). The average size of serotonin neurons in the anterior 1/3 third (head end) of the larval body was: in length 4.91±0.68, in width 3.67±0.70 μm (n=19).

In S. mediterranea planarians, an abundance of serotonin-IP neurons and nerve fibers was found in the central and peripheral parts of the nervous system. The nervous system is represented by the head nerve ganglion, which has the shape of an arc, and a pair of ventral nerve cords extending along the planarian body. The number of serotonin neurons in the head ganglion of S. mediterranea observed on one tissue section was estimated as 25.7±3.1. The width of the head ganglion was 109.2±15.3 μm. The inner part of the head ganglion consists of thin nerve fibers stained with antibodies to serotonin (the so-called "neuropil"), while the bodies of serotonin neurons in S. mediterranea are located on the periphery of the ganglion. Mainly bipolar and multipolar, rarely unipolar serotonin-IP neurons were observed in the head ganglion. The average length of a neuron in the head ganglion of planarian is 13.39±3.59 μm, and its width is 9.34±2.42 μm (n=120). Numerous serotonin-IP nerve fibers and neuron cell bodies were found in the peripheral parts of the nervous system, namely, in the submuscular nerve network. These were mainly multipolar neurons, less often bipolar, the shape of the bodies of the neurons is oval, elongated, or irregular. Sizes of the peripheral serotonin neurons of planarian S. mediterrenea were: in length 10.19±2.55 μm, in width 7.51±1.78 μm (n=87).

Thus, a confocal laser scanning microscopy, together with the immunocytochemical staining of tissues with specific fluorescently labeled antibodies, made it possible to identify and characterize the serotoninergic neurons in the nervous system of representatives of parasitic and free-living flatworms. The study made it possible to determine the total number as well as the localization and morphological characteristics of serotonin neurons in the central nervous system of planarian S. mediterranea and cestoda H. diminuta.

Morphometric data on the serotoninergic neurons were obtained on a large number of measurements and are presented for the first time. The results point to the widespread distribution of the neurotransmitter serotonin in flatworms. It was found that the serotonergic neurons of the brain ganglion of planarians are larger than those located in the peripheral parts of the nervous system. The number and size of planarian neurons significantly exceeds the size of neurons in the larvae of parasitic cestodes. It is necessary to conduct studies of the nervous system using a larger number of species for subsequent comparative analysis of the morphometric characteristics of serotonin-IP components of the nervous system in free-living and parasitic worms. The information may be useful to reveal the evolutionary patterns of the centralization of the nervous system in Bilateria.

### S5.305. Impact of copy number variations (CNVs) on specification and strain diversity of Leishmania

Novozhilova T.S.[1]*, Chistyakov D.S.[2,3], Akhmadishina L.V.[2], Lukashev A.N.[2], Gerasimov E.S.[1], Yurchenko V.[4]
[1]*Faculty of Biology, M.V. Lomonosov Moscow State University;*
[2]*Martsinovsky Institute of Medical Parasitology, Sechenov University;*
[3]*Faculty of Bioengineering and Bioinformatics, M.V. Lomonosov Moscow State University;*
[4]*Life Science Research Centre, Faculty of Science, University of Ostrava;*
* nota-ru@yandex.ru

The genus Leishmania is a group of species that cause neglected tropical disease called leishmaniasis in humans and can infect different animal hosts. There are more than 20 Leishmania species each associated with different clinical manifestations, drug resistance and different hosts [1]. Remarkably some of Leishmania species can even share the animal host [2]. Nowadays various approaches have been applied to study genomic, proteomic, and metabolic factors that establish the relationship between Leishmania species and the mechanisms underlying host-specificity and pathogenicity. Despite this research, much remains unclear about the diversity, evolution and genetics of the Leishmania species complex.

In our work we focused on the system of two closely related Leishmania species that naturally share the same animal host to shed light on genetic factors, which may drive speciation of these important parasites. In Central Asia, great gerbils (Rhombomys opimus) serve as the main animal reservoirs for several Leishmania species: L. major, L. gerbili and L. turanica. While Leishmania major is pathogenic for human, it's neighbor L. turanica is gerbil-restricted [3]. There are two types of gerbil populations: those, co-infected with L. major and L. turanica (will be further referred as 'sympatric'), and those infected only with L. turanica ('allopatric'). We have sequenced 5 genomes of 'sympatric' and 6 genomes of 'allopatric' strains of L. turanica and performed extensive comparative genomics analysis of these data. We analyzed genome coverage variations in order to detect CNVs (copy number variations), because it was previously shown that CNVs may play important role for Trypanosomatids [4]. Specifically we applied GIP method [5] and cn.MOPs method [6].

We analyzed gene and chromosome copy number variation and revealed that in all strains, the chromosome 31 has a ploidy of 4, which is typical for other Leishmania spp. In individual strains, we detected either full chromosome amplifications, or increased coverage in large regions of the chromosome. At average 244 genes had significantly increased coverage indicating that gene duplication events shaped the diversity between strains. We did not observe specific patterns of chromosome or gene copy number amplifications in 'sympatric' or 'allopatric' strains. To conclude, these results confirm that CNVs are the fundamental drivers that shape Leishmania diversity, but there seems to be no prominent genomic differentiation between so-called 'sympatric' and 'allopatric' strains.

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### S5.306. Impact of turbulence effects in aortic valve hemodynamics simulation in normal and pathological states

Pil N.E.[1]*, Kuchumov A.G.[1]
[1]*Perm National Research Polytechnic University;*
* nikitapil32@gmail.com

Aortic stenosis is one of the most common aortic valve disorders, over 15% of people elder than 70 years are affected. Aortic stenosis results in a narrowing of the valve orifice and/or deformation of the valve cusps. Severe aortic stenosis is a leading cause of disease and mortality in the elderly. More than 275 000 aortic valve replacement surgeries are performed worldwide each year and this is predicted to increase to 850 000 per year by 2050.

Biomechanical modelling of hemodynamics in the aortic valve is essential for long-term prediction of surgical outcomes. To date, full model has not been proposed due to many unresolved issues. One of them is to consider the effects of turbulence on hemodynamic processes. Turbulent flow increases the risk of hemolysis and platelet destruction occurs at high values of shear Reynolds stresses.

In this work we analyzed the use of two approaches to model turbulent processes: using the method of large eddies and based on turbulent viscosity models. The axisymmetric problem was solved on an idealized three-dimensional geometry constructed on the basis of ultrasound images and literature review. The problem was solved within the FSI approach using the COMSOL Multiphysics software package. Blood flow is modelled as an incompressible Newtonian fluid with constant density and viscosity.

The Holzapfel-Hasser-Ogden anisotropic hyperelasticity model is used to simulate the mechanical behavior of aortic valve leaflets in normal conditions. The pathological state of aortic valve cusps is described by a linear elastic model.

The mathematical formulation includes Navier-Stokes equation with incompressibility condition, equations to describe turbulence patterns. The equation of motion for solids is also written. The system is closed by initial and boundary conditions as well as fluid-solid coupling conditions. A velocity profile is given at the inlet to the computational domain. To determine the pressure at the outlet of the computational domain, a two-element windkessel model is used, where the velocity profile is taken as an input.

The results described the changes in the main haemodynamic indices: velocity, pressure, near wall tangential stresses and the index of tangential stress fluctuations. Comparison of the results for kinetic and turbulent kinetic energy values between the two turbulence models and the normal and abnormal states is also carried out.

### S5.307. Influence of phytoplankton photosynthetic cell structures dynamics on biomass production

Abakumov A.I.[1]*, Pak S.Y.[1]
[1]*Institute of Automation and Control Processes, FEB RAS;*
* abakumov@dvo.ru

Two models have been proposed to study the effect of photosynthetic cell structures on biomass production. Both of them are based on the generally accepted Droop model [1] for the intracellular content of mineral matter in phytoplankton. Description of the photosynthetic processes in phytoplankton includes in the model structure. The concept of chlorophyll quota is used. It is the proportion of photosynthetic substances in plant cells. In addition to the chlorophyll quota, the photosynthetic activity of phytoplankton is determined by external conditions, primarily by the level of photosynthetically active radiation (PAR). The model is based on separating the dependence of phytoplankton reproduction on external conditions according to the stages of photosynthesis. The light stage is largely determined by the PAR, and the dark stage is limited by the nutrient resource under the controlling influence of the temperature of the aquatic environment. In order to develop the model, the storage of energy in the light stage of photosynthesis is described in detail. Energy is stored in the form of energy-intensive substances in macroergic molecules (macroergs). The most common cell macroerg is adenosine triphosphate (ATP). The proportion of ATP in phytoplankton varies depending on the light regime and on the energy amount stored in the dark stage. The model includes the Droop kinetics and equations for the dynamics of the chlorophyll quota and the ATP pool. The conditions for the existence and stability of equilibrium solutions are compared for the same values of parameters common to both models. The greatest influence on the dynamic modes of the minimum value of the cell quota has been established. The proportion of biomass associated with the light period of photosynthesis is also significant. For the first model that is the biomass produced during daylight hours. And in terms of the second model, it is the biomass formed due to the energy of ATP stored in the light phase. The influence of the structure of dynamic models on the daily and annual dynamics of phytoplankton was revealed. Scenarios of behavior of models under various lighting conditions, including constant and periodically changing lighting, have been studied.

When stable equilibria do not exist both models have complex dynamics. This dynamics obeys the attractor of the Droop model. It can have periodic, quasi-periodic and quasi-stochastic properties in a constant environment.

The conditions for changing illumination were modeled under other constant conditions. The model behavior of the chlorophyll quota and the proportion of ATP in the biomass is in full agreement with the results of published laboratory experiments [2]. In particular, the introduction of the equation of ATP dynamics into the model system makes it possible to avoid the direct influence of the chlorophyll quota on the intracellular content of nutrients. Thus, a time lag is formed. The system has time to accumulate the energy necessary to start the enzymatic reaction. Important to note that chlorophyll begins to be consumed only when a sufficient amount of energy is accumulated. In the absence of illumination, phytoplankton cells use only ATP as an energy source. The reaction of organic matter synthesis proceeds more slowly. Therefore, chlorophyll is also consumed slowly. Consequently, its highest concentration in phytoplankton cells is noted in absolute darkness. On the contrary, the ATP concentration drops to a minimum, since the consumption of energy-intensive substances increases in the dark due to the lack of other energy sources [3].

Modeling of the annual cycle takes into account seasonal changes in the habitat and demonstrates adequate dynamics in both models. It is shown that such changes lead to spring and autumn bursts of biomass productivity. This is consistent with known in situ observations [4]. In general, the models demonstrate the ability to more accurately describe the processes of bioproduction in comparison with the ones developed by us earlier. Given the availability of data from remote observations and the results of laboratory experiments, the presented models can be used to assess the bioproductivity of aquatic ecosystems.

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**S5.308. Influence of the substitutions of Ca atoms on Sr, Mg, Mn, Fe atoms in the Hydroxyapatite structure and electric field changes on its physical properties important for biomedicine**

Bystrov V.S.[1]*, Paramonova E.V.[1], Bystrova A.V.[1], Avakyan L.A.[2], Makarova S.V.[3], Isaev D.D.[3], Bulina N.V.[3]
[1]*Institute of Mathematical Problems of Biology RAS - the Branch of Keldysh Institute of Applied Mathematics of Russian Academy of Sciences ;*
[2]*Physics Department, Southern Federal University, Rostov-on-Don, 344090 Russia;*
[3]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630128 Russia;*
* vsbys@mail.ru

Hydroxyapatite (HAP) is a widely used biomaterial in various medical applications, due to its natural biocompatibility with bone tissues of the human body, as the main mineral component (up to 70%) of human bones and teeth [1]. Bone is a complex multicomponent system that includes collagen fibers surrounded by hydroxyapatite crystals, which form plates (~450 Å by 250 Å in size) and various bone tissue cells: osteoblasts are involved in the creation and mineralization of bones, osteocytes maintain the structure, and osteoclasts provide resorption bone tissue. In a healthy body, the processes of bone regeneration or remodeling usually constantly occur - sequential resorption of bone tissue with the formation of a new strong bone matrix. The process of bone tissue renewal occurs in such a way that 4-6% of the entire skeletal mass of the body is replaced per year. In bone injuries, fractures, extraction of teeth or the introduction of bone implants, remodeling processes are triggered by inflammation, which activates the actions of osteoclasts, which stimulates the splitting of the affected bone tissue, and accelerates the rate of resorption of new tissue due to the work of osteoblasts. In order to accelerate this process, the growth of new bone tissue and the survival of the implant, doctors use special osteogenic biomaterials. Among them, HAP is the main component here, which is used, among other things, for coating both titanium and other implants. However, to improve the biocompatibility properties of synthetic HAP, it must be modified. One way is to introduce additions and substitutions of atoms in the HAP structure. This changes the properties of HAP and thus affects the activity of osteo-cells.
Previous studies, modeling, and calculations of the structure and properties of HAP showed that the properties of real HAP samples are determined by the presence of structural defects in HAP (vacancies, interstitials, and substitutions of atoms in the HAP structure) [1–3]. In this work, models are considered, calculations are made, and the physical properties of HAP with substitutions of Ca atoms for other atoms (Sr, Mg, Mn, Fe) [1–3] are studied and analyzed, as well as changes in the properties of HAP under the influence of an electric field and the piezoelectric effect [4, 5]. Modeling and calculations of HAP properties were carried out by methods of density functional theory (DFT) in different approximations and in combination with quantum semiempirical PM3, PM7, PM6-D3H4 methods. An important feature of the substitution of Ca atoms in HAP for other atoms is the change in the entire energy band structure of HAP and, as a result, the change in the electronic work function. This leads to a change in the surface potential of HAP, which affects the adhesion and growth of the number of osteocells on its surface, i.e., the growth of bone tissue on the surface of an implant coated with such a HAP. This makes it possible to regulate the biocompatibility of HAP. It is essential here that substitutions of different types lead to different effects. For example, Mg/Ca and Sr/Ca substitutions cause opposite changes in the band gap Eg and, accordingly, in the work function: Sr/Ca substitutions increase Eg, while Mg/Ca substitutions decrease Eg [1]. The Mg and Sr atoms are in the same group with Ca in the periodic table, and their substitutions do not cause strong deformations of the HAP lattice. At the same time, substitutions of the Mn/Ca and Fe/Ca types (atoms from different groups) cause more significant rearrangements in the unit cells of the HAP lattice. As a result, in addition to a shift in the band gap Eg, additional energy levels Ei appear inside it, which serve as electron donors/acceptors and change the optical properties of HAP [1]. Another effect that affects the energy level shift of the HAP band structure and changes the band gap Eg is the electric field E. Model calculations performed by different methods on pure HAP have shown that the shifts of the energy levels Ev, Ec, and Eg under the influence of an electric field turn out to be comparable in magnitude with shifts arising from the substitution of atoms in HAP, and also change the surface potential of HAP. It is important that in this case, the piezoelectric effect also has an effect — due to the compression deformations that occur in the bones during walking, and the piezoelectric effect of HAP nanocrystals [4, 5] (which are part of the bone tissue), an electric field is generated on their surface, the magnitude of which is sufficient to create such a shift in Eg, a change in the work function and the surface potential of HAP, leading to the activation of the work of bone cells and the growth of bone tissues. Thus, by this way the process of bone tissue regeneration takes place. The modeling of the processes of the influence of the electric field and the substitution of HAP atoms, carried out in this work, and the calculations performed by various DFT and semiempirical methods show that the obtained values of the shifts in energy levels, the band gap Eg, and the change in the HAP surface potential are comparable with the experimental data. The results obtained describe the physical mechanism of the influence of the electric field, the piezoelectric effect and substitutions of atoms in HAP on the processes of bone tissue regeneration. This is of practical importance both for biomedicine and for the technology of synthesis of new nanomaterials for medical and other purposes based on HAP. This work was supported by the RSF grant no. 21-12-00251.
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**S5.309. Informatics based on nanobioelectronics**

Lakhno V.[1]*
[1]*Institute of Mathematical Problems of Biology RAS;*
* lak@impb.ru

Bioinformatics is the area that develops methods and software tools for understanding of biological data, which includes sequence analysis, gene and protein expression, analysis of cellular organization, structural bioinformatics, data centers etc. A new and more general direction is to consider bioinformatics as informatics on the bases of nanobioelectronics and biocomputer technologies.
DNA molecular is an important example of data storage and biocomputing. Performing millions of operations simultaneously DNA – biocomputer allows the performance rate to increase exponentially. The limitation problem is that each stage of paralleled operations requires time measured hours or days. To overcome this problem can nanobioelectronics [1]-[3].
The central problem of nanobioelectronics is the realization of effective charge transfer in biomacromolecules. The most promising molecule for this goal is DNA. Computer simulation of charge transfer can make up natural experiment in such complex object as DNA. Such processes of charge transport as Bloch oscillations, soliton evolution, polaron dynamics, breather creation and breather inspired charge transfer are modeled. The supercomputer simulation of charge dynamics at finite

temperatures is presented. Different molecular devices based on DNA are considered. These make the basis for solution of informatics problems on biomolecular technologies.

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### S5.310. Investigation of biomolecular solvation by classical site density functional theory

Chuev G.N.[1]*, Kruchinin S.E.[2], Fedotova M.V.[2]
[1]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
[2]*G.A. Krestov Institute of Solution Chemistry of RAS, Ivanovo, Russia;*
* genchuev@rambler.ru

Biomolecular solvation in solutions plays one of the important roles in the running of many vital processes. In a living organism, biomolecules are involved in a large number of intermolecular interactions such as the interactions with water (hydration), with each other (association, self-aggregation), with ions present in the media (association), or with ligands (complex formation). Their biological activity depends essentially on the specifics of the manifestation of these interactions. However, in many cases a determination of the parameters of hydration, association, or complex formation of molecules by the experimental methods presents a significant problem, not only technical but also the financial one. Simulations are also extremely costly for the solvated biomolecules.

In this contribution, the challenges in non-empirical study of the solvation effects are discussed, including the calculations of biomolecule hydration free energy and protein–ligand binding free energy. To overcome the problems of accuracy and speed of such calculations, it is proposed to use the classical site density functional theory with some new approaches. The first technique is aimed at the correct analysis of the three-dimensional hydration structure using a new straightforward procedure of accurately determining the thickness of the hydration shell and the hydration numbers of biomolecules. The second approach is realized by calculating the parameters of complex formation of biomolecules in a limited region of their hydration shell to increase the speed of calculations. The third methodology is based on new parameterization of the solvation (hydration) free energy functional to improve the accuracy of calculations. The application of these approaches to in silico study of biomolecular hydration are considered on the examples of proteins bovine pancreatic trypsin inhibitor (BPTI) and tyrosine phosphatase 1B (PTP1B) as well as a set of drug compounds for which protein 1B (PTP1B) is a target. The results are also presented for a complex involving the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein and the human cellular membrane receptor angiotensin-converting enzyme 2 (hACE2).

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### S5.311. MD modeling of interaction of Lys2Leu peptide dendrimer with bioactive peptides

Mikhtaniuk S.E.[1], Shcherban-Fikimoshkin V.A.[1], Shavykin O.V.[1], Neelov I.M.[1]*
[1]*ITMO University;*
* i.neelov@mail.ru

Dendrimers are nanoscale molecules that consist of a central core, branched repetitive blocks, and end groups. High-generation dendrimers are toxic for biomedical applications because of the large number of charged end groups. This problem can be overcome by lower-generation peptide dendrimers and their modification with amino acid inserts between all branching points [1-3]. In the present work, we studied the possibility of using the lysine dendrimer Lys2Leu with leucine amino acid dipeptide spacers as drug carriers for the delivery of 16 biologically active tetrapeptides, such as Ala-Glu-Asp-Gly (AEDG), Ala-Gly-Ala-Gly (AGAG), Ala-Leu-Leu-Gly (ALLG). Molecular dynamics simulations of the interaction between the dendrimer and peptides were performed in an aqueous solution with explicit consideration of counterions at temperature T=310 K and pressure P=1 atm using the Gromacs 4.5.6 software package. It was found that negatively charged AEDG peptides (8 of 16 peptides) form the most stable and compact complexes with dendrimer Lys-2Leu. The shapes of the distributions of the radii of inertia for the systems with AEDG and ALLG peptides look similar; the distribution for the first peptide is shifted toward lower values of the radii of inertia. The AEDG peptides form approximately twice as many hydrogen bonds with the Lys2Leu dendrimer compared to the ALLG peptide. The most hydrophobic peptide ALLG forms a complex with 6 of 16 peptides, whereas the peptide AGAG forms a complex with only 2 of 16. The internal structure of the formed complexes was also studied, and the radial atom group density distribution functions were obtained. The radial distribution of the density of Lys2Leu dendrimer atoms in the system with ALLG peptides has characteristic minima corresponding to the penetration of peptides into the inner space of the dendrimer due to hydrophobic interactions. Thus, the greatest contribution to the formation of complexes is made by the electrostatic interaction between the positively charged end groups of the dendrimer and the negatively charged groups of the peptides, but hydrophobic interactions are also sufficient for the transfer of hydrophobic molecules by the dendrimer with hydrophobic inserts.

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### S5.312. Mathematical Theory of Reliability and Aging: A Little Bit of History and State of Art

Koltover V.K.[1]*
[1]*Federal Research Center of Problems of Chemical Physics and Medical Chemistry, RAS, Chernogolovka, Moscow Region, Russian Federation;*
* koltover@icp.ac.ru

The foundations of the mathematical theory of reliability were laid in 1950s due to the needs of aeronautic machinery and problems of communication, management, etc. In engineering, reliability is defined as ability of a device to perform its function for a given time under given specific conditions [1]. Biological systems perform their functions in presence of a great number of random factors which disturb all functional strata, starting from the molecular level of organization ("nanobioconstructs") to ecosystems. Therefore, similarly to technical devices, biological constructs are not perfectly reliable in operation. Namely, normal operation acts alternate with random malfunctions, i.e. - failures. The field of systems biology, in dealing with the problem of reliability, incorporates systematization and classification of failures in biological systems of different levels of complexity, investigations of quantitative characteristics and mechanisms of failures, possible ways to evaluate biomolecular failures in functional breaks, renewal processes, and elaborations of methods for testing reliability and predicting failures in biological systems [2, 3]. The special Committee on Reliability of Biological Systems, to deal with the problems of reliability of biological systems, was organized at the Scientific Council on Biological Physics of the USSR Academy of Sciences in 1978, and many prominent biophysicists were the members of this Committee. The regular conferences on reliability of biological systems, starting from the first one in 1975, have given the strong impetus to research in this direction. A quarter of a century after, it has spurred similar biological studies under the style of "robustness [4]. The problem of bioreliability has direct bond to the problem of aging. The systems reliability approach, that was developed in our papers, is based on the several general postulates [5-8]. First, all biological constructs are designed in keeping with the genetic program in order to perform the preset functions. Second, all constructs operate with the limited reliability, namely, for each and every biological device normal operation acts alternate with accidental malfunctions (recurrent failures). Third, the main line of assuring the high systems reliability is the preventive maintenance, i.e., unreliable elements should be timely replaced for novel ones ahead the phase of their wear-out begins. Forth, there are a finite number of critical elements of highest hierarchic level which perform the supervisory functions over the preventive maintenance (metabolic turnover). And, five, the supervisors also operate with the limited genetically preset reliability. On this basis, the universal features of aging, the exponential growth of mortality rate with time (Gompertz law of mortality) and the correlation of longevity with the species-specific resting metabolism (Rubner law), are naturally explained. The stochastic malfunctions of the mitochondrial electron transport nanoreactors, that produce oxygen anion-radicals (superoxide radicals) as by-products of oxidative phosphorylation, are of first importance. This radical, as the reducing agent, affects the NAD(P)H/NAD(P)+ ratio, thereby impacting the epigenetic sirtuin regulators of metabolic repair and renewal processes. As the consequence, the oxidative-stress products and other metabolic slags accumulate with the resulting impetus to autophagic or apoptotic cell death and age-associated clinical disorders. Basing on the theory of reliability, one can estimate that longevity of human brain could reach 250 years, should the enzyme antioxidant defense against the oxygen free radicals be perfect. Thus, aging occurs as the inevitable consequence of the genetically preset deficiency in reliability of the biomolecular constructs while the free-radical redox-timer, located presumably in the special cells of hypothalamus, serves as the effective stochastic mechanism of realization of the genetic program. Furthermore, the systems reliability approach serves as the heuristic methodology for anti-aging medicine, in patt, for searching the real mechanisms of the antioxidant pharmacology [9]. In vivo, the so-called antioxidants, natural and synthetic ones, work not so much as the inhibitors of free radical reactions but they prevent the generation of reactive oxygen species, via neuro-endocrine system and the organism's microbiome, thereby providing the prophylactic maintenance against the oxidation processes.

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**S5.313. Mathematical approaches to estimating the duration of the QT interval of the electrocardiogram**

Iskakov N.G.[1]*, Chershintseva N.N.[1], Nazarenko A.S.[1], Zverev A.A.[1]
[1]*The Volga Region University of Sports and Tourism;*
* Nikitaiskakov1992@mail.ru

Currently, ECG is one of the most accessible and used methods for analyzing the work of the heart. The interpretation of each ECG includes the measurement and assessment of the QT interval, a change in the duration of which may be associated with the risk of ventricular arrhythmias and sudden cardiac death. The evaluation of the ECG requires the accuracy of different amplitude-temporal characteristics of the teeth. The amplitude of the Q and T waves is necessary to determine the time of their end to assess the QT[3]. The QT interval depends on many factors, among which heart rate plays the most important role[2,4]. Many cases of QT prolongation have been described; both associated with genetic diseases, and associated with taking medications, electrolyte disorders or cardiovascular pathology, collectively called "Long QT Syndrome". Short QT syndrome was described in 2000. I. Gussac associated it with an increased risk of sudden death due to the development of ventricular fibrillation. To determine the indicator of the electrical systole of the ventricles of the heart, different formulas for its calculation are used, and these indicators must meet the requirements of the physiology of heart contractions, in conditions of changing heart rate[1]. In recent decades, several formulas have been used to estimate the temporal characteristics of individual teeth. One of the first formulas for correcting the QT depending on the heart rate was proposed by Bazett and to this day, it remains the main formula for determining the corrected QT interval, both in scientific research and in clinical practice. Most electrocardiographs in automated analysis use the Bazett formula, which uses an exponential method to determine QT, where QT=QT/RR1/2. However, Bazett's formula is not entirely correct. There was a tendency to overcorrect in tachycardia and under correct in bradycardia. Fridericia proposed to calculate the corrected interval as the ratio of the duration of the QT interval to the cube root of the previous RR interval, denoted by QT=QT/(RR)1/3. This formula gives more reliable results at high or low heart rate. Formulas using the linear correction method can reduce the errors of the exponential method. The most famous of them is the Framingham

formula QTc(S)=QT+0.154×(1000–RR)[3]. This formula can be used in atrial fibrillation. The Hodges formula QTc=QT+1.75(HR-60) is a linear correction. The Matsunaga QTc=log600×QT/logRR formula and VandeWater et al QTc=QT-0.087×[RR-1000] differ in the calculation of the RR interval unit[4]. The aim of this study was to conduct a comparative analysis of the use of various formulas for calculating the corrected QT interval in different body positions.

Materials and methods.ECG registration was performed at the Volga Region UFKSiT using the PowerLab device (ADInstruments) (n=15, 8-10 years old). The subjects performed the Romberg stabilographic test (test with open and closed eyes) with parallel ECG recording. The electrodes were placed on the chest in the Holter type. Processing was performed using the built-in ECG analysis module in the LabChartPro software. The duration of the QT interval was estimated, corrected according to the formulas of Bazett, Fridericia, Framingham, Hodges, Matsunaga, Mitchell et al, Van de Water et al. The Bazett formula was taken as control values. The statistical significance of the effect was determined using paired Student's t-test and ANOVA($p<0.05$).

Results and discussion. Despite the relevance of measuring the QT interval, when calculating its duration on the same ECG, different specialists often come to different results. The problem is that there is no well-established standard for exactly where to define the start and end of the QT interval. In experiments, the subjects were in horizontal and vertical positions, and in the vertical position, registration was carried out with open and closed eyes. Analysis of ECG parameters (in the supine position) showed the maximum spread of only temporal ECG values. The smallest value of QT interval in a horizontal position was when calculating the Mitchell, which was 70%, relative to the Bazett. With a change in position, there is a tendency to increase the values of the QT interval both with open and closed eyes. When calculating in a horizontal position, the Bazett always showed the longest QTc interval. When the position was changed, the opposite reaction was observed from the side of the heart, which may be associated with an additional load on the heart. The duration of the QT interval is longer with closed eyes, which may indicate the relationship of the visual analyzer to the temporal characteristics of the ECG. When analyzing the amplitude characteristics of the QT interval with different formulas and with different positions of the subjects, the amplitude of all the main teeth does not change. Thus, when analyzing the temporal characteristics of the ECG, it is necessary to use various formulas that take into account, first of all, the initial heart rate, the position of the body and the presence of different types of arrhythmias.

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### S5.314. Mathematical modeling in problems of personalized optimizing the placement of vascular bypasses

Kuianova Iu.O.[1], Dubovoy A.V.[1,2], Bervitsky A.V.[1,2], Shutov A.V.[1], Tegiltsev I.I.[1], Chupakhin A.P.[1], Parshin D.V.[1]*
[1]*LIH SB RAS;*
[2]*Federal Neurosurgical Center (Novosibirsk);*
* danilo.skiman@gmail.com

Shunting is widely used in the treatment of cardiovascular pathologies. The surgeon faces a number of non-trivial tasks: the need for a bypass as such, the optimal angle of its installation, the optimal shape of the arteriotomy, and others. From the point of view of hydrodynamics and mechanics, this corresponds to a number of specific problems, the solution of which was proposed in the series [1,2,3]. As part of the task of determining the optimal angle of installation of the vascular anastomosis during a neurosurgical treatment, the hydrodynamics of the tee was studied. Four possible mounting angles were considered: $\pi/6$, $\pi/4$, $\pi/3$, and $\pi/2$, corresponding to the most commonly used real configurations. The problem was solved numerically using ANSYS CFX software. As an optimality criterion, the condition for the minimum value of the kinetic energy dissipation is used. It is shown that the anastomosis angle $\pi/3$ is optimal, the angle $\pi/4$ is the least favorable. An electrical circuit for the recirculation of large cerebral vessels was also constructed based on the configurations of the circle of Willis in real patients, the optimal parameters of which were determined numerically using swarm intelligence methods.The value of pressure after shunting compared with the pressure before surgery was chosen as the objective optimization function. This method was first used in solving the problem of the formation of cerebral vascular anastomoses. It is shown that the obtained results are in good agreement with the data of real operations. In addition, various options for connecting the recipient vessel were considered from the point of view of the optimality of the stress concentrator zones arising during the articulation with the donor vessel.

Based on the results of the study, a computer program was developed and registered, which allows, according to the data of a real patient, using the electrical analogy of the circulatory system, to suggest the optimal location for the installation of a vascular bypass. The results obtained in the course of the cycle of works significantly expand the fundamental knowledge of vascular bifurcations, as well as provide answers to practical questions of clinical surgeons.

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### S5.315. Mathematical modeling of quantum yields of chemiluminescence activated by coumarins c-314, c-334 and c-525 under the action of cytochrome C complex with cardiolipin

Levchenko I.L.[1]*, Vladimirov G.V.[2], Volodyaev I.V.[3]
[1]*Moscow State University, Faculty of Physics, Moscow, Russia, 119234, Kolmogorova, 1 , b2;;*
[2] *I.M. Sechenov First Moscow State Medical University, Institute of Regenerative Medicine, Moscow, Russia, 119048, Trubetskaya, 8;;*
[3]*Moscow State University, Faculty of Biology, Moscow, Russia, 119991 Moscow, Leninskie gory, 1, b12;;*
* irnlevchenko@yandex.ru

Comparing the mathematical modeling of the quantum outputs of the physical activators C-314, C-334 and C-525, which intercept the excitation of triplet-excited ketones and have values of quantum yields of

chemiluminescence 3-4 orders of magnitude higher than the excited ketones themselves, we obtain chemiluminescence activated by coumarins C-314, C-334 and C-525, which shows the intensity values ~1500 times, ~1600 times and ~2000 times higher than spontaneous chemiluminescence of lipids, while it does not differ from it in terms of kinetic curve parameters and has velocity constants of the same order. The accuracy of the comparison of mathematical modeling of quantum outputs is determined by the presence of cardiolipin for pH stabilization, the quenching of Fe2+ and the presence of physical activators C-314, C-334 and C-525. Among the factors that distort the value of mathematical modeling of quantum yields, insufficient addition of hydrogen peroxide, excessive amounts of nitrogen (II), methanol, protein denaturation, as well as a change in the conformation of CytC in the CytC-CL complex are highlighted.

In search of optimal excitation conditions, the systems of lipoperoxidase and quasi-lipoxygenase reactions activated by physical activators - coumarins C-314, C-334 and C-525 were analyzed.

In our work, based on the analysis of the parameters of cytochrome C with cardiolipin, physical activators C-314, C-334 and C-525, as well as horseradish and luminol peroxidase, comparisons of studies of the sensitizing ability of coumarins C-314, C-334 and C-525 as physical activators were carried out in order to compare the magnitude of quantum yields C-314*,C-334* and C-525*.

The cytochrome C complex with cardiolipin differs from the native cytochrome C in the following properties:(1)has the fluorescence of tyrosine and tryptophan residues; (2) loses absorption in the Cope band(405-410 nm) reflecting the existence of the Fe(heme)***S(Met80) bond;(3)has peroxidase activity and catalyzes the formation of lipid radicals in the membrane;(4)C-525 is a "classic" physical activator of chemiluminescence, as well as C-314 and C-334, is actively oxidized by the CytC-CL complex, while the rate of this oxidation is limited only by the concentration of cytochrome C itself, which is also destroyed as part of the CytC-CL complex under the action of hydrogen peroxide.

### S5.316. Mathematical modeling of the growth of epithelial tissue in the pores of a solid scaffold

Krasnyakov I.[1]*, Bratsun D.[1]
[1]*Perm National Research Polytechnic University;*
* krasnyakov_ivan@mail.ru

The main problem of tissue engineering is that any tissue is a multicellular formation. Thus, a researcher who sets himself the task of growing an artificial tissue from a single cell faces a serious challenge: how to organize cooperation between thousands of new cells in such a way that they would form correctly structured cellular ensembles, which could then be included in already working organism without unpleasant consequences.

The main tool with which tissue engineering attempts to assemble puzzles from individual cells is the scaffold. It is understood as either a porous or fibrous matrix of an arbitrary structure, which is seeded with stem cells of the future tissue. It is important to note that the solid body with which the cell comes into contact is not the natural habitat of the cells. Cells evolved either in a liquid multicomponent solution or as part of a multicellular organism. Thus, the processes near the tissue-solid body surface require a individual study. All the details of the interaction between cells and solid surfaces are still unknown. In the case of a porous scaffold, experimental observations show that cells prefer to lay flat on the walls of microchannels. Perhaps they perceive the walls of the pores as a basement membrane, which plays an important role in the structuring of epithelial tissues in the organism. Cell division leads to the filling of pores, while the cells grow both along the channel and towards its center creating a multilayer structure. In the case of a fibrous scaffold, cells attach to the fibers of the matrix and tissue growth occurs in a less orderly manner, as immediate contact between cells is weakened. However, experiments show that cells can change strategy and attach across the channel in a porous scaffold, and vice versa, cover the surface of cord in a fibrous scaffold.

It is important to note that the scaffold allows you to control the growth of the tissue so that the cells differentiate in accordance with the requirements of the organ for which the tissue is grown. The matrix is made of a material that decomposes over time, leaving behind a tissue of structured cells. Hydrodynamic phenomena play an important role in the process of seeding and growing cells. Cell culture is called static or dynamic, depending on whether the nutrient saturating the scaffold is at rest or moving. It is argued that the filtration of fluid through the pores of the scaffold has a mechanical effect on the upper layer of cells, which stimulates them to accelerate division. Thus, the tissue–liquid interfacial surface plays a fundamental role in cell growth. It has been observed that tissue preferentially grows transversely to the flow of nutrient, which places mechanical stress on superficial cells. In the case of a porous scaffold, the width of the pores, the shape of the channels, and the total porosity of the material play an important role.

In this paper, we present a mathematical model for the growth of epithelial tissue in the pores of a solid scaffold. Because epithelial tissues are paving the surfaces of organs, mucous membranes, and its cells are attached to the basement membrane, then the problem can be reduced to a two-dimensional formulation. The elementary unit of a living mother is cell, is represented in the model as a polygon with a dynamically changing number of vertices. The whole system is calibrated so that the most probable cell shape is a hexagon, but the appearance of cells with a different number of vertices is not excluded. The hexagonal cell is the most energetically favorable in the system and is quite close to a circle in shape.

To calculate the dynamics of the system, we set the potential energy equation, in which the main control parameters are the coefficients of elasticity, which determine the deformation properties of the medium. The first term in the equation describes the contractility of the cell perimeter, which can be interpreted as resistance to their excessive stretching, and the second term characterizes the tendency of cells to maintain their average area and resist the action of tension and compression forces from the environment. Each cell-polygon changes its position in space by moving its nodes (polygon vertices). The resultant of the mechanical forces applied to a node is the potential energy gradient along the radius vector of the node. Displacement of nodes leads to deformation of the cell. We use Aristotle's mechanics to describe the equation for cell movement velocity. Firstly, the cell tissue is a highly dissipative medium where movement occurs without the effect of inertia, and secondly, this approach can significantly reduce the amount of calculations. So in the equation of motion, forces directly determine the speed of an object. For epithelial cells, the model assumes that the probability of cell division depends on the number of its nodes. An important property of the epithelium is cell intercalation. It is necessary to facilitate the pressure exerted on the cell in the tissue, to relieve local stresses, and also to reduce the potential energy of the entire tissue in the event of its structural restructuring. This mechanism is triggered whenever the jumper between adjacent cells becomes less than a critical value. The system also contains a reaction-diffusion equation that describes the exchange of chemical signals between cells across their overall boundary. By introducing such an equation into the model, we can set the chemomechanical interaction of the elements of the entire system.

The developed mathematical model contributes to the development of mathematical modeling methods in static and dynamic cell tissue cultures growing in scaffolds.

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## S5.317. Mathematical modeling of the main rhythm generator of limbic seizures in the hippocampus

Kornilov M.V.[1,2]*, Sysoev I.V.[1,2]
[1]*Saratov Branch of Kotel'nikov Institute of Radio Engineering and Electronics of Russian Academy of Sciences;*
[2]*Saratov State University;*
* kornilovmv@gmail.com

The question of the origin and evolution of the main oscillation frequency of brain neurons in various normal and pathological conditions is an urgent task of modern science. For the study and modeling of epilepsy, the mathematical description of synchronization is very important [1], since epilepsy is characterized by highly synchronous activity patterns [2]. In this paper, we propose the concept that the main frequency is not the result of the activity of a single cell, but is formed due to the collective dynamics in the ring of model neurons associated with delay.

To confirm this hypothesis, a test system was used, consisting of a ring of Hodgkin-Huxley neurons [3] (this model, which describes the propagation of action potentials in neurons, is considered one of the reference ones), unidirectionaly connected with a delay. In this case, the parameters of neurons were considered the same and were chosen in accordance with [4] so that they demonstrated the dynamics of cortical-pyramidal neurons. The parameters of the neurons have changed in such a way that no oscillations occur in the rest mode. The dynamic mode was achieved with the help of a starting neuron, which acted on one of the ring neurons for 30 ms. After that, an oscillations was excited in the system, the main frequency of which was estimated. The number of neurons in the ring varied from 10 to 100; the delay time in the coupling was from 0.2 ms to 1 ms. The coupling coefficients between neurons were considered as equal and 4 values were considered (30, 40, 50, 60).

As a result of a numerical experiment, it was shown that the proposed scheme can generate oscillations with frequencies close to those observed experimentally. By changing the number of neurons in the ring, the delay time in the coupling, and the coupling coefficient, one can control the main frequency of the signal in neurons. At the same time, the effect of increasing the number of neurons in the ring has a greater effect on the signal frequency. And increasing the value of the coupling coefficient allows to get higher frequencies. Additionally, an empirical formula was proposed for estimating the period of oscillations depending on the number of neurons and the delay time in connection. On its basis, the dependence was reconstructed, which showed sufficient accuracy in comparison with the data of a numerical experiment.

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## S5.318. Mechanical models of DNA

Bezhenar M.V.[1]*
[1]*Federal State Budgetary Educational Institution of Higher Education «Kuban State University»;*
* mia1610@yandex.ru

Experimental studies of the DNA dynamics and open state generation are limited by the spatial resolution of the available biophysical tools [1]. Mathematical modeling is therefore a main method to study the open states of DNA [2].

L.V. Yakushevich proposed a theoretical model of DNA, considering the open state as a result of base pair rotation [3]. This model considers hydrogen bonds between base pairs and stacking interactions between neighbor base pairs are accounted for in the model. The disadvantage of this model is that the fluctuations of the bases in the extreme pairs are not taken into account.

L.V. Yakushevich Yakushevich obtained an analytical solution of the model with the averaged equation coefficients, this solution does not take into account the helical nature of the DNA structure and the interaction of angular displacements of nitrogenous bases. In addition, a kink-type solution can only be obtained in the case of homogeneous synthetic DNA. Several simplifications (averaging the characteristics of a DNA strand) were shown to impair the significance of the solution [4].

The above models are angular and predict soliton movement along the DNA molecule. The Peyrard- Bishop (PB) model [5, 6], and its two expanded versions provide well-known examples of translational models. The two versions are the helicoid Peyrard–Bishop model and the Peyrard–Bishop–Dauxois model. A main requirement to the translational approach is that a nonlinear potential is used to describe the complementary H bonds. The Peyrard–Bishop model assumes that a DNA molecule consists of two polynucleotide strands and imitates the strands by two chains of disks, which are connected together via longitudinal and transverse springs. Longitudinal interactions are strong covalent bonds, which are modeled using harmonic potentials. Transversal interactions between nucleotides of different strands are week hydrogen interactions, and require an anharmonic potential.

The method of self-stressed, or tensegrity, structures is no less interesting. The method is used to describe the behavior of complex biological systems. A respective model of the cell explains how mechanical cell behavior arises as a result of physical interactions between various systems of molecular threads that form the cytoskeleton [7, 8].

Each model has its advantages and disadvantages. Thus, the Yakushevich angular model allows modeling hydrogen bonds between bases. Based on calculations carried out using the angular model, we found that the isotopic ^2H/^1H composition of the medium has a significant effect on the probability of occurrence of open states [9, 10]. Deuterium present in a nucleotide chain was shown to decrease or to increase the probability for an open state to arise, depending on the energy of hydrogen bond breakage. [11]. External periodic factors with frequencies ranging from 10^(-11) to 10^(-8) s^(–1) were shown to affect the dynamics of the DNA molecule. In response to an external periodic force, oscillatory movements may arise in the DNA molecule with a frequency differing from that of the external factor.

Numerical solutions were obtained with the mechanical mathematical model of the DNA molecule for the interferon α17 gene and a Drosophila gene fragment. The results showed that the rate of changes in angular oscillations of nitrogenous bases decreases with the increasing viscosity of the medium, leading to base stabilization. Oppositely, a decrease in medium viscosity increases the angular deflection velocity of nitrogenous bases and increases angular deformations of DNA strands. Thus, a decrease in medium viscosity causes DNA instability, which increases with time [12].

A torsion moment applied to the DNA molecule causes rotational movements of nitrogenous bases and thus leads to the formation of open states, which most often arise at the ends of the DNA molecule and in regions where A–T base pairs predominate [13]. The probability for an open state to form when a torsion moment is applied to a certain gene region depends on the A-T content of the region, the region size, and the time during which the torsion moment acts on the region.

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### S5.319. Mechanisms of aggregate sizes regulation in a discrete Dictyostelium discoideum aggregation model

Kruchinin I.[1]*, Yakovenko L.[1]
[1]*Lomonosov MSU, Faculty of Physics;*
* iv.kruchinin@physics.msu.ru

Dictyostelium discoideum ( DD ) is a species of soil-dwelling amoeba belonging to the phylum Amoebozoa, infraphylum Mycetozoa. The life cycle of this organism, commonly referred to as a slime mold, consists of unicellular and multicellular stages: when food is scarce, myxamoebae stop feeding, move to each other, collide and stick together, forming intermediate clusters, which then aggregate to form a so-called slug, which includes up to 〖10〗^5 cells. This process is controlled by interacting intracellular and extracellular signaling systems.

We propose a model of the initial stages of myxamoeba aggregation based on moving finite automata. The model includes four interconnected grids, one of which, that describes the movement of myxamoebae, consists of movable finite automata and has four layers. The other three grids describe the release and diffusion of three main signal components forming the chemotactic field that determines the behavior of myxamoebae [1]. This model is modified by taking into account the dependence of myxamoebae movement on the intracellular Ca^(2+) concentration. Movements of amoebae can be either directed along the concentration gradient of cyclic adenosine monophosphate, or random walk. Also, the direction of movement of amoebas is affected by the concentration of Ca^(2+) in the immediate environment.

The calculation results show that when the aggregate reaches a certain size its growth stops and the number of cells in it fluctuates around the average value. Such behavior suggests that aggregation is determined by two competing mechanisms: one promotes the growth of the aggregate while the other promotes detachment of cells or small clusters from it [2].

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### S5.320. Model of action potential generation and propagation, taking into account the dependence of the membrane capacitance on voltage

Hernandez-Caceres J.[3], Dzhimak S.S.[1,2], Drobotenko M.I.[2]*, Nechipurenko Y.D.[4,5]
[1]*Southern Scientific Centre of the RAS;*
[2]*Kuban State University;*
[3]*Cuban Center for Neurosciences;*
[4]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences;*
[5]*Sevastopol State University;*
* mdrobotenko@mail.ru

During excitation, the axon membrane changes its thickness, and this mechanical change accompanies the propagation of the action potential. This phenomenon is not considered in the classical model of Hodgkin-Huxley (H.-H.) [1], and a number of authors represent nerve excitation as a mechanical soliton moving along the axon bilayer [2].

Remaining within the conceptual framework of the H.-H. model [3], we considered the possibility that changes in membrane thickness are a consequence of mechanical pressure caused by the action of the membrane potential on the membrane. Given the known values of the Young's modulus of the axonal membrane, this possibility seems realistic. We have modified the X.-X equations by making the membrane capacitance a function of the membrane potential. A wide range of possible parameters was investigated, as well as the possibility of a linear and quadratic dependence of the capacitance respect to the membrane potential. We obtained the following results:

1. For a wide range of dependence of membrane capacitance on membrane potential, the generation and propagation of action potentials were observed.
2. The dependence of the capacitance on the membrane potential led to a change in the amplitude and speed of the pulse propagation.
3. The shape of the action potential remained practically unchanged.

With this model, we showed that including plausible assumptions it is possible to obtain a propagating action potential that is accompanied by a mechanical deformation wave, with no need to postulate the existence of a mechanical soliton.

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### S5.321. Modeling of Spreading Cortical Depolarization Caused by Epipial Potassium Application

Terenina M.S.[1]*, Khazipov R.N.[1], Zakharov A.V.[1,2]
[1]*Institute of Fundamental Medicine and Biology;*
[2]*Kazan State Medical University;*
* mari_terenina@inbox.ru

The aim of this work is to model the local field potential (LFP) corresponding to the spreading depolarization (SD) induced by the epipial application of potassium chloride [1]. For this purpose, we used the NEURON Python system and an extracellular module [2], which allow us to reproduce the features of the functioning of single neurons and calculate changes in the macroscopic parameters of the simulated space (ion concentrations, potentials, etc.).
To reproduce the effect of the epipial application of potassium chloride, the following parameters and model settings were used: the size of the space is 1600x1000x1000 μm with the Neumann boundary conditions, all parameters corresponding to the operation of the neuron are included. The density of neurons is 90,000 per mm3. An epipial application was imitated by setting a potassium bolus at one of the space boundaries. LFP was calculated from the total transmembrane currents of potassium and sodium based on the biophysical model implemented in NEURON. The action potentials of neurons located at a distance of no more than 200 micrometers from each electrode were taken into account [3].
The dynamics of the local potential was compared with the results of in vivo recording of the extracellular potential in the rat cerebral cortex during epipial application of potassium chloride. The general dynamics of the characteristic parameter, the LFP value, corresponds to experimental observations. The average value of the SD amplitude is ~ -22 mV, which agrees with the experiment. The speed of vertical propagation of the SD of these LFP is 60 mm/min, which also corresponds to the experimental data [1]
Further modeling of the neuron network is planned with the addition of an external source of current stimulation in the frequency range of epileptic patterns.
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### S5.322. Modeling of cyclic processes based on experimental data in the form of time series by solving piecewise linear difference equations with constant coefficients

Smirnov V.Ju.[2]*, Kuznetsova A.V.[1]
[1]*N.M. Emanuel Institute of Biochemical Physics (IBCP);*
[2]*Azforus, Ltd;*
* azforus@yandex.ru

The paper proposes modeling of cyclic processes of the real macrocosm by a system of linear difference equations with constant coefficients. Such a model can be transferred from any initial state to a given final state in a given number of steps. The conditions for the existence of a cyclic solution on a plane or in a space of any dimension are obtained. For a cyclic process, the systems of equations switch when the integral curves reach the boundaries on the phase plane (space). The convergence rate of such cyclic solutions is analyzed.
The model in the form of autoregressions is associated with experimental data – time series and approximates them by the criterion of minimizing the standard deviation. The model allows us to solve the problems of achieving a given value of the indicator (biological, pharmacological, etc.) by a given moment. For modeling biological systems, a large number of methods are described in Riznichenko G.Yu. [2]. These studies serve, however, for a qualitative description of systems and are not intended to link models with any experimental data.
We consider models of dynamic, in particular cyclic processes, in the form of two (or more) systems of linear difference equations with constant coefficients, the integral curves of the systems are connected in continuity. Switching from one system of equations to another occurs when the integral curves reach threshold values – boundaries on the phase plane. The models we propose use experimental data presented by time series, the approximation of the data is carried out by functions-solutions of systems of linear difference equations with constant coefficients, and the criterion of minimizing the standard deviation is fulfilled.
Such a choice of approximating functions seems to be the most appropriate to the physical essence of many real dynamic processes of the macrocosm, since even relaxation-type processes have a description of fronts in the form of rapidly decaying exponentials. For the processes of accumulation or expenditure of some interrelated resources, in which the rate of accumulation (expenditure) is proportional to the available mass of resources, such a description follows from the very concepts of mass and accumulation rate. This is the nature of the processes of growth of populations of organisms in conditions of sufficient food supply and the absence of enemies [3], the excretion of drugs from the body [4], as well as such physiological processes as the reaction of the pupil to a light pulse [5] and the “sodium-potassium pump” on cell membranes [6].
The use of separate links (equations) in our model appeals to the “bottleneck” control principle, the essence of which is that the amount of those resources that have accumulated in excess does not affect the flow of the process corresponding to the “bottleneck" in any way. Examples of such systems are, in particular: biological enzymatic processes [3], [4], the work of the heart (the stage of the QRS complex and the stage of changes in ion concentrations): the electrical activity of the myocardium at the time interval corresponding to the QRS complex does not depend on the processes of changes in the concentrations of potassium or calcium ions and, on the contrary, upon completion The processes of changes in ion concentrations of the QRS complex do not depend on an almost constant state (called isolation) of the electrical activity of myocardial cells.
We present models in the form of solutions of linear difference equations with constant coefficients, assuming that such a model is applied to processes for which it is physically logical due to the nature of the material under study. In addition, the proposed models may be of interest only in cases where experimentally obtained time series are available as the material under study. The proposed description of dynamic, in particular cyclic, processes by the model in the form of piecewise linear difference equations is presented in the form of two (or more) systems of linear difference equations with constant coefficients; the integral curves of the systems are connected in continuity. Switching from one system of equations to another occurs when the integral curves reach threshold values – boundaries on the phase plane. The increase in the dimension of the phase space does not cause the appearance of fundamental difficulties both from the side of the cycle existence condition and from the side of the experimental data approximation algorithm [1]. This model can be useful for describing many biological, chemical phenomena that occur cyclically or once in time. As a result of applying this approach, experimental data turn out to be approximated curves that are solutions of linear difference equations with constant coefficients (sets of real or complex-conjugate exponentials, as well as alternating curves).

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### S5.323. Modeling of effect of 4-hexylresorcinol, an analogue of the anabiosis autoinducer of bacteria, on the biopolymers of the Escherichia coli cell

Tereshkin E.V.[1]*, Tereshkina K.B.[1], Krupyanskii Y.F.[1]
[1]*N.N. Semenov Federal Research Center for Chemical Physics Russian Academy of Sciences;*
* ramm@mail.ru

To obtain survival, bacteria use small molecules that change development parameters: the formation of biofilms, the transition to the initial state under the influence of stress factors, etc. The transition of pathogens to various states is accompanied by the acquisition of resistance to appearance, which is a serious problem in various states of the national economy and medicine . Some of them relate to metabolites that are produced in the cytoplasm by the stationary phase of growth of a bacterial colony - autoinducers of anabiosis, derivatives of alkylresorcinols. The regulation of bacterial infection transmission depends on their influence [1]. The most widely used chemical analog of suspended animation inducers is 4-hexylresorcinol (4HR). It has been shown that the effect depends on growth phases of bacterial colony. The stress sustainability of cells increases, it is observed in the presence or mummified state [2-3]. Dose-dependent effects are detected and in proteins [4] and DNA [5-6] in vitro.
In this work, the concentration dependence and molecular mechanisms of the interaction of 4HR with Escherichia coli cell biopolymers were studied by molecular dynamics methods. The effect of 4HR on the outer and inner membranes, peptidoglycan, membrane protein porin, cytoplasmic DNA-stabilizing protein Dps and DNA was studied. Simulations was carried out with Gromacs in all-atom force field AMBER99-PARMBSC1. A temperature was maintained by a Langevin thermostat with a friction coefficient of 0.5 ps^-1. Constant pressure was maintained by Parrinello-Raman barostat with a time constant of 2 ps. For DNA, Dps, and peptidoglycan systems, the pressure is 1 atm. maintained isotropically. When calculating model membranes, the pressure was maintained in a semi-isotropic way. The interaction parameters of bound atoms and short-range interactions were calculated for each time step. Electrostatic interactions at long distances were calculated by the Particle Mesh Ewald method (PME). The cutoff radii for all types of interaction were taken to be 1.5 nm. The list of neighbors was maintained using a Verlet cutoff scheme and updated every 10 fs. Fast degrees of freedom were limited using the LINCS algorithm. The integration step was 2 fs (for peptidoglycan, 1 fs), the trajectory length was 0.1 μs. Before finding the dynamic characteristics of DNA and proteins, a principal component analysis was performed. To obtain data on the free energy of 4HR migration through the bilayer, the approaches of controlled molecular dynamics and umbrella sampling were used [2]. To determine the thermodynamic characteristics of DNA binding to Dps, we used the method of searching for the linear interaction energy (LIE) with previously selected parameters [3].
Simulations showed that the effect of 4HR is not limited to any particular cell compartment, but is characterized as a complex effect of this compound on the biopolymers of the bacterial cell membrane and cytoplasm. When migrating across membranes, 4HR preferentially migrates through the bilayer rather than porin channels. It interacts both with polysaccharide residues of the outer membrane and with membrane proteins and membrane lipids. The thickness of the 4HR layer at the outer membrane varies depending on the concentration. Penetration of 4HR into the bilayer, at the porin–lipid interface, is determined by its hydrophobic interaction with the transmembrane regions of the porin protein. Upon passage through the outer membrane, 4GH is partially absorbed by peptidoglycan, where carbohydrate residues are the initial binding sites. During the growth of such a complex, a change in the shape and size of peptidoglycan occurs due to the fact that 4HR molecules pass into the region of peptides, causing compression and stretching. When interacting with DNA, 4HR molecules have the ability to insert themselves into both the major and minor grooves to form an O–H…O(–)–P bond (the hydroxyl group of resorcinol and the phosphate group of DNA). The fluctuation of Dps atoms is significantly reduced by the addition of DNA. The ability of 4HR molecules to adsorb on the Dps surface either increases or decreases the mobility of the N-termini, and vibrations are significantly excited on the outer surface of DNA, which is clearly noticeable at medium 4HR concentrations. These vibrations have the ability to accelerate DNA binding. The method of searching for the linear interaction energy was used to obtain the free energy of DNA binding to Dps, which varies from -49 kJ/mol (in the absence of 4HR), slightly increasing at low concentrations, but remaining negative. up to -150 kJ/mol (at high concentrations). Thus, the effect of the dynamic behavior of the considered components of the bacterial cell depends on the ability of 4HR to convert it, while being directly determined by its concentration and is in good agreement with experimental data, where at low and medium concentrations of the autoregulator, the stress resistance of bacteria increases, and at high concentrations of 4HR, stable, but unviable forms.
The computations were carried out on MVS-10P at Joint Supercomputer Center of the Russian Academy of Sciences (JSCC RAS). This work was supported within frameworks of the state task for FRC CP RAS FFZE-2022-0011 (#122040400089-6)
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### S5.324. Modeling of self-organization of hybrid molecules consisting of a lysine dendron with several hydrophobic tails

Mikhtaniuk S.E.[1], Fatullaev E.I.[1], Shavykin O.V.[1], Neelov I.M.[1]*
[1]*ITMO University;*
* i.neelov@mail.ru

Amphiphilic molecules in aqueous solutions self-organize, which is a well-known phenomenon seen in all living things. The design and chemical composition of the hydrophobic and hydrophilic components may be changed to produce a wide range of aggregates with various sizes and morphologies. Excellent prospects for drug and gene carriers are dendromicelles, which are generated by hybrid molecules, including hydrophilic dendrons and hydrophobic linear units [1-4]. Dendromicelles must have good biocompatibility and minimal toxicity for use in biological applications. Peptides and, in particular, lysine dendrons [5-7] fulfill this criterion. The numerical self-consistent field approach was used in this study to examine spherical micelles made of linear dendritic amphiphilic block copolymers with a number of linear hydrophobic tails.
First, the boundaries between different micelle morphologies were determined using the dependencies of the large thermodynamic potential and chemical potential on the aggregation number. The next stage was to choose the most likely micelle shape that matched the surfactant molecule's chemical potential at its lowest value. Spherical morphology

predominates at short tail length values; as tail length increases, a shift to cylindrical morphology occurs; lamellar morphology only predominates at long tail length values. Additionally, the internal structure of the spherical micelles and how it relates to the hybrid molecules' structure were investigated. It was discovered that dividing a single hydrophobic tail into multiple causes the number of aggregations and the core radius to decrease. Finally, we looked at the electrostatic properties of micelles generated by hybrid molecules with charged lysine heads and hydrophobic tails. With more micelle generations, the maxima of the electrostatic potential radial distribution curves move closer to the nucleus, showing a reduction in the number of aggregations. The relative effective charge rises, and the zeta potential falls as the number of tails for a given number of hydrophobic segments in the surfactant increases. Two sets of dendrons and their associated charges make up the micelle's corona: one is located close to the micelle's core, while the other is close to its surface. Due to the intermediate region's wide breadth, molecules with biological activity can enter there. The latter finding may be relevant if the investigated spherical dendromicells are used as nanocontainers for medication delivery.

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### S5.325. Modeling the accumulation of charged photo-sensitizers in tumor cells with variable electric fields gradients on internal membranes

Askarova K.A.[1], Morozova G.M.[1]*
[1]*Russian Peoples Frendship University;*
* gimorozova@mail.ru

The photodynamic therapy (PDT) effectiveness for oncology patients is significantly determined by the photo-sensitizer (PS) accumulation in tumor cells (TC).The latter depends both on the physicochemical properties of PS molecules (charge, hydrophobicity) and on the metabolic cells state in the tumor associated with their mitotic cycle and oxygenation level [1-3]. Based on the biophysical approach, TC heterogeneity in different tumor layers can be characterized by the sum of three electrical transmembrane potentials (TMP) in cell. This sum includes negative plasmatic and mitochondrial TMPs as well as the energy-dependent positive nuclear TMP [3-6]. According to Nernst's theory, the kinetics penetration of amphiphilic charged molecules into cell and their accumulation in its membrane compartments depends on the total TMP value, which vary in different TC [3,5]. In particular, it is important to consider this fact by using some chlorine PS [1]. Previously, stationary solutions were obtained for the distribution of anionic PS in cellular compartments at various cell energy states within the framework of a kinetic model with constant TMPs [5]. The purpose of this work is the modeling the accumulation kinetics of charged PSs in TC differing in energy metabolism at variable TMPs.

The kinetic model including a set of nonlinear differential equations describing the accumulation of amphiphilic charged PS molecules in cell membrane system is suggested. This system (environment-cell) consists of four parallel-sequential compartments separated by permeable membranes with initially different TMPs on the plasmatic and internal mitochondrial and nuclear membranes. The experimental growth curves of TC fluorescence intensity in HT29 culture incubated with chlorine E6 and with its derivative - ether E6 (DME) were used for modeling according to [1]. The transfer rate constants (RC) of DME and E6 initial values into TC were determined from the linear part slopes of these curves. In our model these constants depend on the mitochondria and nucleus TMPs, which change exponentially with given modulating parameters $\alpha$ and $\beta$, respectively. Moreover, the plasma TMP is assumed to be constant during the experiment time. Based on this model, a computational experiment using a special computer program was performed. The changes of theoretical curves in the PS-chlorin concentrations in the cytoplasm, mitochondria and nucleus were obtained. These turned out to be correct in the first 10 minutes from the computational experiment start for the selected modulation parameters. It follows from the results analysis that cationic DME can accumulate more efficiently in the tumor zone with active mitochondria, whereas, in contrast, anionic E6 accumulation rate in TC will be higher under hypoxic conditions.
We conclude that the accumulation kinetics of charged PS in the TC (as well as some cytostatics), mainly depends on the relationship between RC through inner cell membranes, these constants being functions of TMPs.The dynamic relationship changes between TMPs in mitochondria and nucleus can affect the ion homeostasis fluctuations in nuclei and, consequently, the cells mitotic activity in different tumor zones. Comparative studies and data analysis allow us to hypothesize the existence of an invariant of three TMPs sum for normal cells of different histological types. The changes of this invariant can be serve a fundamental criterion of cells transition from normal to certain pathological phases.

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### S5.326. Molecular modeling of gold nanoparticle interaction with P2Y12 protein

Kapitunova A.[1]*, Kundalevich A.A.[1], Zyubin A.U.[1]
[1]*Immanuel Kant Baltic Federal University, Rec «fundamental and Applied Photonics. Nanophotonics», 14 A, Nevskogo str.;*
* AIKapitunova@mail.ru

P2Y receptors (P2YRs), a family of G protein-coupled purinergic receptors (GPCRs), are activated by extracellular nucleotides. There is a total of eight different functional P2YRs expressed in humans, which are classified into P2Y1-like receptors and P2Y12-like receptors. Their ligands are typically charged molecules with relatively low in vivo bioavailability and stability, limiting understanding of this receptor family. P2Y12R regulates platelet activation and thrombus formation. Thrombus caused by abnormal activation of platelets is the pathological basis for the formation of many diseases. Under various physiological and pathological conditions, platelets can be activated and undergo processes such as adhesion, deformation, aggregation, release of particles and synthesis of thromboxane A2, which ultimately leads to physiological hemostasis or pathological thrombosis.

In the field of cardiovascular diseases, active attempts are being made to use the method of Raman Spectroscopy in biomedical studies of platelets. Raman Spectroscopy is a non-invasive and highly sensitive analytical method that allows you to get "fingerprints" of molecules. It can be used as a tool to analyze unknown samples or target molecules in a mixture of components. Surface enhanced Raman Spectroscopy (SERS) is based on the amplification of electromagnetic fields around metal nanoparticles, which greatly increases the Raman scattering signals of a sample surrounded by nanoparticles. There are reports on the use of gold nanoparticles of different structures to obtain SERS spectra of plasma, blood and platelets, in particular, from healthy patients and those taking anti-thrombotic drugs. However, it is not known how nanoparticles affect platelets and its receptors, as well as what kind of interaction between them occurs.

Here, for the first time, we performed molecular modeling of the P2Y12 protein with a golden surface. The results of this study can provide insight into the mechanisms of interaction of nanoparticles with biological objects, as well as become the basis for further research and modeling of the interaction of platelets with nanoparticles.

Since the known structure obtained by X-ray diffraction has artifacts and empty spaces in the crystal lattice, we completed the completion of the protein using homologous modeling. The human P2Y12 amino acid sequence (PDB: 4NTJ) was used as a query sequence to search for homologue models with known structures from the Protein Data Bank (PDB) using NCBI-BLAST. A similar human P2Y12 protein (PDB: 4PZX) was chosen for homologous modeling. The 3D human model P2Y12 was created using the MODELER program (version 10.2).

The protein and gold structures were used as initial coordinates for the simulation. A cubic box with a size of 160x160x200A3 was built, including TIP3P water molecules with the addition of chlorine ions to balance the charge, protein and the gold surface Au(111). The protein was placed at a distance of about 60 Å from the surface. All simulations were performed using the GROMACS 2021.5 package. In all simulations, CHARMM27 was chosen to model the peptide, and a modified TIP3P potential was used to represent water. Peptide–gold interactions at each of the various gold water interfaces have been described by GolP-CHARMM. Bond lengths were limited by h-bonds. Surface gold atoms and bulk gold atoms were frozen during all simulations, but gold dipole charges remained free. Classical MD simulation was carried out at a constant volume and temperature (T = 300 K). Periodic boundary conditions, the PME algorithm, and an integration time step of 2 fs were used. The total electrostatic energies of the systems (1-4 van der Waals energy, 1-4 electrostatic energy) were calculated from unit cell parameters to understand protein structural rearrangements.

To evaluate the behavior of the protein in the presence of a gold nanoparticle, simulations were performed for 5000 ps. At the beginning of the simulation, the protein moves away from the nanoparticle at a distance of about 4 nm, after 1500 ps it approaches to about 3.5 nm, and after 3500 ps it again moves away to 4.5 nm.

Evaluation of the evolution of the radius of rotation in time during the entire simulation time showed a decrease in the value by tenths of units from 3.4 to 3.15 nm, which indicates a dense packing of the protein. Basically, the unfolding and conformational change is associated with a flexible intracellular region of the protein.

Next, both the Lennard-Jones potential and the Coulomb electrostatic potential were calculated. The Lennard-Jones potential describes the potential energy of interaction between two non-bonding atoms or molecules depending on the distance of their separation. This is useful for accounting for Pauli repulsion and hydrophobic/van der Waals attractions. On the other hand, the Coulomb potential can describe electrostatic interactions between atomic (partial) charges. The total energy of the system slightly decreased during the simulation from -5278469 to -5280000 kJ/mol. The Lennard-Jones potential increased throughout the simulation from 880250 to 883488 kJ/mol. The value of the electrostatic potential increased by 2200 ps of simulation to a level of 65000 kJ/mol, then decreased to 64500 kJ/mol for 1500 ps, and after 5000 ps it returned to the maximum level of about 64875 kJ/mol.

The results of our modeling indicate that there is no aggregation of the P2Y12 protein on the surface of the nanoparticle, and the protein conformation does not change. These data can form the basis for further modeling of P2Y12 with nanoparticles of other shapes, as well as for modeling the effect of nanoparticles on the area of the platelet membrane with this receptor.

### S5.327. Morphological features and mechanism of structural transformation in the flavivirus shell during maturation

Konevtsova O.V.[2]*, Golushko I.Yu.[2], Podgornik R.[1], Rochal S.B.[2]
[1]*University of Chinese Academy of Sciences;*
[2]*Southern Federal University;*
* khelgla@yandex.ru

Flavivirus is the most common genus of the Flaviviridae family including over 50 species, which are transmitted predominantly through bites of infectious mosquitoes and ticks to both animals and humans. Flavivirus causes various pathologies ranging from asymptomatic to life-threatening ones, including encephalitis and hemorrhagic fever. Although millions of people are infected with flavivirus every year, currently there are no approved antiviral drugs for the treatment of flavivirus infections, and known vaccines are effective only against certain serotypes. Understanding the principles of structural organization and the mechanisms driving morphological transformations in virus shells (capsids) during their maturation can be pivotal for the development of new antiviral strategies.

All flaviviruses exhibit similar icosahedral proteinaceous capsid structures. The inner protein shell of the capsid is formed from capsid protein C. The capsid is surrounded by a lipid membrane, which is then covered by an outer protein shell, consisting of 180 complex proteins called E heterodimers. The interposed membrane thus mediates the interactions between the two protein layers. The outer shell assembles on the endoplasmic reticulum membrane from 60 pre-assembled symmetrical trimers consisting of three identical heterodimers. While being transported to the cell surface through the trans-Golgi network, the trimeric outer shell reconstructs to smooth densely packed structures formed by 90 dimers. The inner capsid consists of 60 C protein dimers. Despite its relative simplicity, the structure of the capsid was obtained only in 2020. Using recent flavivirus structural data we reveal the hidden features of protein order in a complex flavivirus shell, which selfassembles simultaneously on two opposite sides of an interposed lipid membrane [1]. As elucidated in this work, the arrangement of proteins within the icosahedron faces of the immature flavivirus surface is based on the trihexagonal lattice, while the radial projections of the mass centers calculated for the proteins of both inner and outer immature shells form a common icosahedral triangular spherical lattice (geodesic polyhedron) (5,0). Thus, despite the interposed lipid membrane separating the proteinaceous layers, their structural organization is consistent with the

close packing principle of layered structures: the positions of surface proteins (outer side) reside between those of capsid proteins (inner side), which makes the whole system more homogeneous and possibly also more stable. Within the proposed structural model, we furthermore rationalize the structural organization of misassembled non-infectious subviral particles that have no inner capsid.

During the maturation, the self-assembled outer shell goes through a transition from a trimer into a dimer protein state, so that the protein locations coincide with the spherical lattice (3,2). Unfortunately, there are no detailed structural data on the flavivirus inner shells in the mature state. However, since the capsid is protected by the lipid membrane, it seems reasonable to assume that upon transition, the capsid structure remains unchanged. In that case the mass centers of capsid C dimers should still correspond to the spherical lattice (5,0), whereas in the dimeric outer shell, the protein mass centers belong to the spherical lattice (3,2). As the above spherical lattices do not have common nodes, commensurability and matching between the layers decrease after the trimer-to-dimer reconstruction.

By establishing a correspondence between the heterodimer positions in the trimeric and dimeric states, it is possible to completely determine the still unclear structural mechanism of the transition that occurs during the maturation of flavivirus. For this aim, we superimpose the centers of mass of heterodimers before and after the structure transformation and assume that the displacements of the centers of mass of proteins are small. Such an assumption leads to a well-defined mechanism of the trimeric to dimeric rearrangement [2].

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### S5.328. Mutational analysis of the arginine transporter structure

Koltovaya N.A.[1]*, Dushanov E.B.[1]
[1]*Joint Institute for nuclear research;*
[2]*Joint Institute for Nuclear Research;*
* koltovaya@jinr.ru

The superfamily of APC (amino acid-polyamine-orhanocation) transporters found in all living organisms and in humans consists of numerous families of solute carriers (SLC). The transport of amino acids across the cell membrane is carried out using secondary active transport and the glutathione transport system. Secondary active transport is the transfer of substrates using a concentration gradient of sodium or protons between the inner and outer sides of the cell membrane. Canonical APC transporters include the sodium-independent SLC7 glutamate/cysteine antiporter that exchanges extracellular cysteine for intracellular glutamate [1, 2]. Cationic amino acid transporters (CATs) are also members of the SLC7 family. They supply arginine for the synthesis of nitric oxide, which makes a major contribution to asthma pathogenesis [3]. In cancer, the APC L-type amino acid transporter I provides cells with the amino acids necessary for tumor cell growth, deregulates tumor cells [4], and mediates the uptake of melphalin, a chemotherapy drug [5]. In the central nervous system, the SLC12 transporters play an essential role in setting the concentration of Cl- and γ-amino butyric acid (GABA)- and glycine-mediated neurotransmission [6]. Other APC transporters are involved in a variety of biochemical processes in the nervous system, including the packing of inhibitory neurotransmitters in synaptic vesicles [7] and sodium- or proton-dependent symport of glutamine, a critical step in the recycling of glutamate and GABA [8]. To gain a deeper understanding of APC transporters in general and to understand the similarities and differences between proton-linked and sodium-linked transporters, we studied the atomic structure and mechanism of the eukaryotic proton-dependent yeast APC transporter.

The incorporation of amino acids into yeast cells is mediated by ~16 plasma membrane permiases, most of which belong to the APC superfamily of transporters. Arginine incorporation is carried out mainly by three permyases: Gap1, Can1, and Alp1, which work both as a transporter and as a receptor. The main amino acid permiase Gap1 transports all amino acids; Alp1 transports only arginine; Can1 transports arginine and, less efficiently, lysine. Can1 catalyzes H+/arginine symport, and arginine transport requires a proton driving force [9].

Yeast arginine permiase Can1 is a homodimer and consists of 590 a.a. The crystal structure of eukaryotic yeast permiases is missing from the PDB database, but there are atomic structures of three bacterial proteins of the APC family: antiporters arginine/agmatine (AdiC) and glutamate/γ-aminobutyric acid (GadC) and a broad specificity proton/amino acid symporter (ApcT). The proteins are similar in structure and consist of 12 alpha-helical transmembrane segments (TMs) flanking the hydrophilic tail directed into the cytoplasm. TMs form two "5+5" rings that span the substrate binding site. TM11 and TM12 carry out binding of monomers. Such stacking is typical of several families of transporters.

Previously [10, 11], for modeling, we used the crystal structure of the AdiC monomer (PDB: 3L1L and 3OB6) [12, 13] in an open conformation in an aqueous environment with arginine as a substrate. In this work, we used a high-resolution (1.7 Å) crystal structure of the AdiC homodimer (PDB: 7O82) embedded in a membrane hydrated with a 17 Å thick water layer as a basis for modeling Can1 [14]. This system was assembled using the CHARMM-GUI package [15]. Next, the system was balanced and simulated at a temperature of 310 K using Gromacs 2022.4 package [16]. When modeling the system, a Berendsen thermostat was used; the pressure was maintained at 1 bar using a Parrinello-Rahman barostat. Periodic boundary conditions were used in all three dimensions. The equations of motion were integrated using the leap-frog algorithm with a time step of 1 fs. The system was balanced for approximately 10 ns. The simulation was run for approximately 0.5 μs. The results obtained were analyzed using the built-in utilities of the Gromacs package. As a result of modeling, the structure of the eukaryotic proton transporter was obtained, which made it possible to clarify the interaction of the Can1 permease with the membrane and water molecules. We analyzed 1712 mutations that disrupt the transport of the arginine analogue canavanine into the cell and lead to resistance to this fungicide. Among them, 322 single missense mutations inactivating the enzyme were selected. They were localized in 158 a.a. Analysis of their localization makes it possible to clarify the mechanisms of functioning of amino acid transport.

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### S5.329. Network dynamic model of thalamo-cortical brain activity during sleep, wakefulness and absence seizures

Sysoeva M.V.[1]*, Dolinina A.Yu.[1,2], Sysoev I.V.[2]
[1]*Yuri Gagarin State Technical University of Saratov;*
[2]*Saratov State University;*
* bobrichek@mail.ru

In this work the problem of mathematical modeling of three types of experimentally observed brain activity is considered; in particular epileptiform activity (spike-wave discharges), passive wakefulness (regular alpha rhythm), slow-wave sleep (chaotic multifrequency activity). The simplified mathematical model of thalamocortical brain network in form of ensemble of FitzHughNagumo oscillators is studied This model is able to demonstrate all three desired behaviors with unchanged parameters of individual network elements and unchanged network architecture, but with minimal changes in the coupling strength between these elements. These processes are analyzed, clustered and compared to experimental local field potentials of the brain.

The main approach to modeling proposed in this article is to generate morphologically [1] and physiologically [2] sound rules of connectivity matrices, using which models are constructed in the form of ensembles of model neurons. During generation, the couplings can appear following the preliminary states set of rules, for example, couplings between thalamocortical neurons are forbidden. Two approaches are possible to setting the allowed couplings. One is that the probabilities of couplings are given (empirically selected) and then matrices with random connections are generated [3, 4]. The other approach is to impose additional restrictions, for example, on the total number of couplings or couplings within a single structure, the prohibition of unconnected or one-way connected neurons, since they do not participate in the dynamics of the network [5, 6]. Thus, the total number of possible options is significantly reduced and a more complete analysis becomes possible: iterating over a large but foreseeable number of connectivity matrices from a certain class instead of randomly generating them. In this paper, the first approach will be used.

To simulate all three studied brain states, matrices with the same connection architecture were generated, only the strength of the connection for some pairs of structures differed. Many connectivity matrices were generated and only those that simultaneously satisfied the following conditions were selected. 1) To simulate an epileptic peak-wave discharge, allowed connections were established kSWD = 0.18, including from an external input neuron, then external exposure was applied and those matrices were selected that were in a non-oscillatory mode before the external stimulus, and after the end of exposure showed residual fluctuations. 2) Only these selected matrices were used to simulate passive wakefulness, but all connections were increased by 10% compared to kSWD, while no external stimulus was provided, the network itself simply began to fluctuate due to the greater strength of the connection. 3) To simulate slow sleep, the same matrices were taken again to be selected for peak wave discharges, but now the strength of the connection from reticular cells to thalamocortical cells was increased by 10%, and the connections of reticular cells to each other were reduced by 10% compared to similar interactions for peak wave discharges; the network fluctuated without an external incentive. Ten different 28-element matrices and ten different 280-element matrices were found. They differed in the communication architecture due to the random generation of the probability of occurrence of these connections. Thus, the structural stability of the observed modes to small variations in the matrix of connections was tested, which is important, since connections in the brain are continuously rebuilt during life, and small rearrangements do not qualitatively affect behavior. One set of SWD, PW and SWS states was obtained from each matrix.

It is shown that in models with the same connectivity matrix, it is possible to realize various types of vibrational activity observed experimentally and corresponding to epilepsy, passive wakefulness and sleep. At the same time, one mode corresponds to the dynamics on the attractor, the other – long transients. It is shown that there is a class (set) such connectivity matrices, while the time series obtained from models using them are qualitatively similar, but differ in details as the brain activity of different animals from the same genetic line.

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### S5.330. New mega dataset combined with deep neural network makes a progress in predicting impact of mutation on protein stability

S5.330. New mega dataset combined with deep neural network makes a progress in predicting impact of mutation on protein stability

Pak M.A.[1]*, Dovidchenko N.V.[2,3], Sharma S.M.[1], Ivankov D.N.[1]
[1]*Skolkovo Institute of Science and Technology;*
[2]*Institute of Protein Research RAS;*
[3]*Atlas Biomed Group-Knomx LLC;*
* Marina.Pak@skoltech.ru

Prediction of protein stability change (ΔΔG) upon mutation is one of the most important unsolved problems of structural bioinformatics. The recent success of AlphaFold in predicting 3D protein structures at near-to-experimental accuracy showed the perspectives of deep learning techniques for solving biological problems. The vast amount of known protein sequences (Uniprot Consortium, 2012) and known crystallographic structures played a crucial role in AlphaFold's success. Field of ΔΔG prediction always suffered from the lack of data: by the middle of 2022, only ~14k experimental records were collected, which may be too low to learn the ΔΔG prediction by a deep neural network.

Recently, Tsuboyama et al. published the experimentally measured ΔΔG values for 851,552 mutations, with 376,918 being high-quality single mutations [1]. The dataset is much larger than any dataset used before and has no bias towards 'truncating' mutations to smaller amino acids, especially to alanine. Thus, it provides a unique opportunity to develop an unbiased state-of-the-art ΔΔG predictor using one of the powerful deep learning models.

Here we present ABYSSAL (Mega dataset and Deep neural network with attention-like mechanism), the first predictor of protein stability change due to a single mutation trained on such a large amount of data. ABYSSAL takes advantage of the state-of-the-art deep neural network model ESM2 [2]. ABYSSAL predicts experimental ΔΔG values with the Pearson correlation coefficient (PCC) of 0.85, which amounts to near-to-experimental quality [1]. We have shown that a training dataset should contain around ~100,000 data points is enough to take full advantage of the current state-of-the-art deep neural network models like ESM2 [2].

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**S5.331. New methods of analysis of stochastic nonlinear dynamics of living systems**

Ryashko L.[1]*
[1]*Ural Federal University;*
* lev.ryashko@urfu.ru

The transition from traditional methods of statistical processing of time series to the construction and analysis of adequate mathematical models is a distinctive feature of modern research in the life sciences. Actual problems of the analysis of complex modes of behavior in biological systems are solved with the help of the modern mathematical theory of bifurcations. Random perturbations, which are inevitably present in such systems, can significantly change behavior scenarios and generate phenomena whose causes are not explained within the framework of the original deterministic models. Here, we can note such phenomena as noise-induced transitions between qualitatively different regimes, stochastic bifurcations, noise-induced transitions from order to chaos and excitability, stochastic resonance, phantom attractors. The construction of adequate stochastic models and the development of constructive methods for their analysis is an urgent problem in modern mathematical biophysics.
The widely used method of direct numerical simulation of solutions to the corresponding stochastic dynamic models is very costly and only allows one to state certain phenomena without giving an answer to the question of the underlying mechanisms. In a series of recent works by the authors, an analytical approach is developed that uses the stochastic sensitivity technique and the method of confidence regions. This approach focuses on a constructive parametric analysis of the impact of random disturbances on the dynamic regimes of nonlinear systems with continuous and discrete time. The mathematical technique of stochastic sensitivity, developed for regular attractors (equilibrium, periodic, and quasi-periodic) and for chaotic ones, makes it possible to approximate the dispersion of random states in the form of confidence regions (ellipsoids and bands) [1,2].
In the analysis of noise-induced transitions between attractors, the geometry of attraction basins and separatrices detaching them plays an important role. In this case, an estimate of the critical values of the intensity of the noise that causes transitions can be obtained from an analysis of the mutual arrangement of the confidence regions and these separatrices.
This approach has been successfully applied to the analysis of the mechanisms of noise-induced phenomena in biophysical models belonging to different hierarchical levels and having different physical nature: in the processes of intra- and intercellular exchanges [3], neuronal dynamics [4], cardiac activity [5], population dynamics [6] and metapopulations [7], infection spreading processes [8], immunotumor interactions [9].
The universality of the developed methods of probabilistic analysis makes it possible to use them in the actively developed modern field of nonlinear stochastic dynamics of complex biophysical processes.
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**S5.332. Nonlinear properties of spike-wave discharge signals**

Dolinina A.Yu.[1,2,3]*, Sysoeva M.V.[1,2], Sysoev I.V.[1,3]
[1]*Saratov Branch of Kotel'nikov Institute of Radioengineering and Electronics of RAS;*
[2]*Yuri Gagarin State Technical University of Saratov;*
[3]*Saratov State University;*
* dolinina13nastya@yandex.ru

Diseases of the nervous system are nowadays among the quite common ailments for people. Epilepsy, as one of the types of such a disorder, is generally characterized by the occurrence of high-amplitude electrical discharges in the brain, synchronous in several brain structures and resulting from the simultaneous excitation of numerous neurons. A fairly common method, electroencephalography (EEG), is used to record such discharges. In the case of animal genetic models (rat WAG/Rij and GAERS lines) or pharmacological models (kainate and pilocarpine models), intracranial measurements of local brain potentials are also used.
In the human electroencephalogram, the basic frequency of epileptic activity may be the same, whereas in animal models it is different. For example, in humans the peak-wave discharges have a frequency of about 3 Hz, whereas in rats it is mostly 6-8 Hz. However, the frequency-time structure of spontaneous spike-wave discharges in humans and animals is similar (scalable).
In some cases, the onset of the discharge is characterized by a sharp increase in the signal frequency followed by a rapid decrease to a certain value. This dynamic can be characteristic of both absence seizures and limbic ones. Such changes in the discharge structure are usually only considered within a range corresponding to the fundamental (first) harmonic, for example, in the range of 7-12 Hz in WAG/Rij rats.
In this research, we performed a time-frequency analysis of the dynamics of epileptic discharges in two ranges corresponding to the first and second harmonics of the signal. The data under study were time series of brain local potentials of rats.
Fourier transform in a moving time window was used for the analysis. A Hann window of length $\Delta T=1$ s was chosen as the window, which

corresponded to an acceptable compromise between the frequency resolution Δf. In our case, the main frequency lay in the range [7Δf; 12Δf], and the second harmonic in the range [14Δf; 24Δf]. The time window was shifted along the entire studied interval with the minimum possible time step equal to one sampling. The frequency and time characteristics obtained after transformation were used to construct skeleton — structures representing the dependence of the seizure basic frequency on it's duration. Such structures allow characterizing the dynamics of seizure frequency changes, as well as visual estimation of it's frequency structure in the specified range. The raw skeletons had a significant number of rapid short-term jumps between frequencies, therefore, in addition to the signal processing described above, skeletons were filtered (smoothened) in the time domain with a cut-off time of 0.1 s. The skeletons of two harmonics were combined. For visual estimation of the similarity of frequency dynamics, the values of frequencies corresponding to the second harmonic were divided by two (we will call such frequencies adjusted).

As a result, for the vast majority of the analyzed discharges, the time-frequency structure of the second harmonic repeated the structure of the first harmonic with an error of no more than 1 Hz. Outside the discharge, the skeletons of the two harmonics looked, conversely, quite independent. The moments of time when the harmonics did not differ by more than the specified frequency threshold (1 Hz in the values given) could correspond to the moments of seizure time. To test the proposed method 200 s signal intervals of local potentials and including both discharges and background dynamics were used.

According to the obtained graphs in the places of marked discharges the method marks the coincidence of frequencies with a solid line without gaps, and in the gaps between the discharges the marks are arranged episodically, with varying lengths of the gaps between them. This means that the frequency matches found there are of random or short-term nature. In order to remove them, the filtering of time series corresponding to the matching of two harmonics was performed. For this purpose, the difference between the next and the previous time value was calculated, and if it was more than 0.4 seconds, the next time moment was not memorized. Thus, a series consisting of a certain number of values was formed. If the length of such series was less than 350 points, then it was not considered and was excluded from the whole chain.

After removing the short-term matches of the reduced frequencies of the two harmonics, only those series of moments of time remained, when these frequencies changed synchronously, which was characteristic of most of the studied intervals.

Evaluation of the dynamics of the frequency evolution of the two harmonics showed that the main frequencies in these bands are tightly coupled during the discharge, i.e., they have synchronization. At the same time, during the background dynamics, the synchronism of the two harmonics was absent. This demonstrates that the spike-wave discharge is a nonlinear process.

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### S5.333. Numerical modeling of shear-induced platelet activation in hemodialysis catheters

Salikhova T.[1,2]*, Pushin D.M.[1], Guria G.Th.[1,2]
[1]*National Medical Research Center for Hematology, Moscow, Russia;*
[2]*Moscow Institute of Physics and Technology, Dolgoprudny, Russia;*
* salikhova.ty@gmail.com

Platelets are sensitive to blood shear conditions via the interaction with specific macromolecular sensors known as von Willebrand factor [Löf et al., 2017]. VWF macromolecules being bounded to platelet surface keep a globular conformation under low shear stress. However, high shear stress can induce a VWF globule-stretch transition. The stretched VWF macromolecules are capable of interacting with multiple platelet receptors. The multivalent binding may trigger a platelet activation, resulting in the development of life-threatening thrombotic complications (heart attack, stroke). It is of great interest to develop an approach for the estimation of shear-induced platelet activation risk in high shear flow.

The platelet activation condition includes both magnitude and duration of shear stress. Cumulative shear stress is an indicator accounting for both factors [Bluestein et al., 1997]. The indicator is defined as an integral of shear stress along the part of platelet trajectory passing through a high shear zone. If cumulative shear stress exceeds a definite threshold value, shear induced platelet activation will take place.

This work is aimed at the modeling of shear induced platelet activation in catheters used for hemodialysis. The latter procedure requires two catheters. The first catheter is used to draw blood from a vein into the dialysis machine and the second one allows cleaned blood to return to the same vein. The shear stress inside both catheters is high enough to induce platelet activation and accompanied by downstream thrombus formation. Platelet activation modeling was conducted using recently established dependence of threshold cumulative shear stress value on VWF size [Pushin et al., 2020]. As a measure of shear induced platelet activation intensity the cycle-averaged proportion of activated platelets in convective flux at the vein outlet was used [Pushin et al., 2021]. The influence of flow rate through catheters and VWF size on risk of shear induced platelet activation arising in several commonly used catheter configurations was investigated.

The conducted analysis allows us to rank the catheter configurations according to presumable risk of platelet activation. Practical recommendations on lowering the risk of thrombus formation during hemodialysis were formulated.

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### S5.334. Numerical study of the strain affecting the propagation of the excitation-contraction waves in the myocardium sample with coupled electromechanical model of the cardiac muscle

Syomin F.A.[1]*, Galushka V.A.[1], Tsaturyan A.K.[1]
[1]*Institute of Mechanics, Lomonosov Moscow State University, Moscow, Russia;*
* f.syomin@imec.msu.ru

Currently, various scientific groups are actively developing electromechanical models of the tissue of the cardiac muscle, myocardium, aiming for further application of such models in personalized numerical simulation of the heart and its chambers. In particular, the phenomena of re-entry waves are widely studied, which include the formation of detaching and twisting excitation waves in the myocardium of the heart wall. The models presented in the scientific literature rarely describe the mechanics of the cardiac muscle in detail, which is important for reproduction of mechano-electrical feedback observed in experiments and clinical practice: a decrease in the myocardial conduction velocity

upon its stretch. Although there are a number of models that take into account the effect of myocardial strain on the dynamics of the waves of electrical excitation within myocardial wall, such feedback is often based on the strain dependence of the conductivity of some ion channels of the cell membrane and/or the direct effect of the tissue geometry on its conductivity due to changes in the length and area of the conductor. At the same time, experimental data show that the contribution of these effects is insignificant compared to the effects caused by a change in the capacitance of the cell membrane during cardiac muscle stretch [1].

The report presents the results of an application of the electromechanical model of the myocardium, developed by the authors earlier [2], to the problem of electrical excitation and mechanical contraction of a myocardial sample with a non-excitable and non-conductive region. The model combines a block that describes the electrophysiology of the myocardium by a simple phenomenological Aliev-Panfilov model [3] with a detailed model of the mechanics of myocardial contraction and activation [4]. The equations specifying the electromechanical coupling, including the processes of calcium-induced calcium release, were taken from the model [5] with some modifications and simplifications, which allowed the complete coupled model to reproduce important dependences of the contraction force and relaxation rate of the cardiac muscle on the its stimulation frequency with relatively small computational requirements of the model. The model also describes the mechano-electrical feedback in the form of a dependence of the capacity of the cardiomyocyte membrane on strain. At the same time, we took into account that the change in the capacitance of the membrane and, accordingly, the conduction velocity of the excitation waves lags behind the strain. The existence of two different components of myocardial conductivity was also taken into account: the conductivity of the cell cytoplasm, which changes with myocardial strain, and the strain-independent conductivity of the cell membrane.

The simulation results showed that under certain conditions, such as an increased stimulation frequency (2 Hz) of the sample and an increased tissue excitation threshold, excitation-contraction waves detach from the boundaries of non-excitable regions and twist around them. In the case when the mechano-electrical feedback was turned off during these numerical experiments, no wave detachment was observed. These results, in our opinion, demonstrate the importance of consideration for the effect of strain on the myocardium electrophysiology in the simulations of its excitation and contraction, which, in its turn, shows that a correct description of the stress-strain state of the myocardium by electromechanical models is required, even if they are used only to study the dynamics of excitation waves. The results also show the possible influence of mechano-electrical feedback on the occurrence of arrhythmias.

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### S5.335. On the dimensionless model of heterogeneous DNA

Krasnobaeva L.A.[1,2]*, Yakushevich L.V.[3]
[1]*Tomsk State University;*
[2]*Siberian State Medical University;*
[3]*Institute of Cell Biophysics, Russian Academy of Sciences;*
* kla1983@mail.ru

Mathematical models that simulate the internal mobility DNA molecule contain many dynamic parameters, such as moments of inertia of nitrous bases, distances between base pairs, distances from the centers of mass of bases to sugar-phosphate chains, rigidity of the sugar-phosphate backbone, interactions between bases within pairs. Estimates of the parameter values are often difficult, and it is very problematic to guarantee their accuracy.

In mathematics, instead of a system of differential equations with many parameters, their dimensionless analogs often used. It is believed that the using of the dimensionless analogs permits to reduce the number of parameters, to make it easier the analysis of equations and finding of their solutions. Moreover, dimensionless equations are of interest due to the fact that they are not "tied" to a specific object and can be used more widely. So, it can describe can describe the nonlinear dynamics not only of the DNA molecule, but also of mechanical, electronic, and other nonlinear systems.

In this paper, we considered dimensionless equations that model the motion of nonlinear conformational perturbations of kinks, in heterogeneous DNA. It turned out that the nondimensionalization procedure actually leads to a decrease in the number of model parameters. Besides this, the dimensionless model permits to justify the validity of applying the perturbation theory and the McLaughlin-Scott method based on it, which greatly facilitates finding solutions and understanding the transcription bubbles behavior.

The dimensionless one-soliton solutions (kinks) and the McLaughlin-Scott equation are obtained, the velocity of transcription bubbles is calculated and their motion trajectories is ploted.

### S5.336. On the estimation of the emergence of cognitive ability during biological evolution

Antonets V.A.[1,2]*
[1]*Institute of Applied Physics RAS;*
[2]*Lobachevsky University;*
* antonetsva@gmail.com

In the last century, the presence of cognitive ability, i.e. the ability to perceive the surrounding world to a certain extent and act accordingly, has been reliably established for many animal species by ethologists. Of course, this ability was realized in different forms and used different methods of obtaining and using information about the surrounding world and oneself among different researched biological species.

Due to the diversity of mechanisms of cognitive ability and evolutionary niches occupied, it is not easy to establish reasonable criteria for comparing this ability among different species, including humans.

Paleontology is a scientific field that reconstructs the time of origin and living conditions of various species based on discovered evidence. This leads to the natural question of determining the time when multicellular animals first developed the ability to perceive.

The analysis in this work is based on the well-known fact that the size of single-celled organisms has been and still is 10-100 micrometers. The existence of single-celled organisms implies that this size allows for the use of diffusion to deliver oxygen from the surrounding environment into the cell for breathing, which is sufficient for the plastic and energy needs of single-celled organisms to exist and reproduce.

With the appearance of multicellular organisms, the biological mechanism of respiration remained diffusion-based while the mechanism of

nutrition became diffusion-based. The cells integrated within the organism cannot consume each other.
However, the energy and plastic supply of each cell in the macroscopic organism due to direct diffusion absorption of substances from the environment through its outer shell is not possible because it is a very slow process.
Multicellular animals have proven to be viable by transitioning to a different type of nutrition. Not being autotrophs, they can only be consumers, consuming comparable in size fragments of other multicellulars - plants and animals, including the consumption of prey in its entirety. However, this requires that the consumer and its victim be able to approach each other. Therefore, multicellular animals must possess macroscopic mobility that far exceeds the size of their body.
However, if a navigation system is not integrated into the localization apparatus, the movements of the multicellular organism cannot be ordered. The trajectory of such motivated but unordered movement looks like a tangled curve with self-intersections. Therefore, the consumer and food can only approach each other by chance. In primitive cases, for example, when a small multicellular organism consumes unicellular ones, the probability of approach appears to be sufficient for survival.
Thus, directed (not chaotic) movement is a sign of the presence of a navigation mechanism in multicellular animals. This mechanism, in turn, assumes the presence of sensors of one or another physical nature that allow one to detect a remote goal (food or threat) in the surrounding world. It also assumes the ability of the animal to use this information to control its own movements, i.e. to direct them towards the target. All together, this is the independent element of cognitive ability, i.e. the ability to perceive the surrounding world and use the information received to carry out necessary actions for survival. Any fact of directed animal movement confirms the presence of cognitive ability in it.
Thus, the emergence of cognitive ability in multicellular animals can be estimated in two ways: a) by dating the first paleontological traces of directed movement, and b) by comparing the evolutionary age of the last common ancestor of capable and incapable relatives capable of ordered movement.
It can definitely be stated that cognitive ability (intelligence) emerged no later than 520 million years ago. This corresponds to the age of Diania cactiformis, which was known to be capable of directed movement (doi:10.1038/nature09704).
The obtained conclusion is somewhat disappointing in its simplicity. However, such simple proofs of important scientific positions that change the world view have been encountered before. For example, the demonstration of the heliocentric organization of our planetary system, based on Galileo's discovery of the phases of Venus using the simplest telescope, also looks simple.
The proposed approach is intentionally oversimplified. It is not intended to consider all the details and exceptions. But it allows formulating new tasks for cognitive and paleontological sciences, directing their attention to the study of the evolution of cognitive ability up to the emergence of thinking as an element of cognitive ability.
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### S5.337. Optimization of nanosensitizers size for antitumor radiotherapy using mathematical modeling

Kolobov A.V.[1]*, Kuznetsov M.B.[1]
[1]*P.N.Lebedev Physical Institute of RAS (LPI) ;*
* kolobov@lebedev.ru

One of the promising technologies for increasing the efficacy of antitumor radiotherapy is the use of non-radioactive nanosensitizers that emit secondary radiation when activated by a primary beam [1]. A rational approach in practice is the use of nanoparticles, each of which represents a small volume of the active radiosensitizing substance, covered with a layer of polymers, several nanometers in width, with built-in antigens specific to the considered tumor cells. This approach facilitates the accumulation of the active substance within the tumor and prevents its accumulation in the normal tissues, which should lead to the increase of the treatment efficacy while minimizing side effects associated with normal tissues irradiation. The most important factor that determines the success of using nanosensitizers in this case is the efficiency of the delivery of active substance to the tumor, which essentially depends on the size of the nanoparticles in use. Upon its increase, the volume fraction of the active substance in the nanoparticles grows, but their penetration through the pores in the walls of capillaries into the tumor becomes more difficult [2], their movement through the tissue becomes more complicated due to the decrease of their diffusion coefficient, and the rate of their clearance from the body via filtration by the liver increases.
This talk presents a mathematical model of tumor growth and its radiotherapy with the use of nanosensitizers, specific to the tumor cells, which are administered intravenously. The results of mathematical modeling suggest that the optimal size of nanoparticles for maximum tumor radiosensitization after a single intravenous injection of a fixed total volume of particles depends on the degree of permeability of the capillaries formed in result of tumor angiogenesis. Under physiologically justified pore spectra of such capillaries, with the width of the polymer layer of nanoparticles of 7 nm and physiologically justified values of other parameters of the mathematical model, the optimal radius of nanoparticles lies within the range of 13-17 nm, where the upper value is achieved when considering a quite extreme spectrum of tumor capillary pores, in which pores with any radii up to 100 nm are possible.
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### S5.338. Peculiarities of electron-phonon interaction in photosynthetic pigments revealed in optical response modeling

Chesalin D.D.[1]*, Pishchalnikov R.Y.[1]
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences;*
* genoa-and-pittsburgh@mail.ru

Over the past 20-30 years, the development of computer technology and artificial intelligence has made it possible to take a fresh look at the problem of theoretical description of the optical properties of pigments in photosynthetic antenna complexes. Carotenoids are the object of this study. These are molecules whose chemical structure contains two main elements: a polyene chain and rings. Despite the apparent simplicity, the absorption spectra of carotenoids are nontrivial, and the calculation of the optical response has certain difficulties. Carotenoids are characterized by four vibrational frequencies of chemical bonds: $\nu_1$ and $\nu_2$ – vibrations of double and single carbon bonds, $\nu_3$ – vibrations of the methyl group and $\nu_4$ – vibrations of hydrogen. For correct modeling of the optical response of carotenoids by semiclassical methods that describe a set of effective parameters of the system, it is necessary to compare experimental data (for example, absorption spectra) with

the corresponding theory. Such calculations are based on an approach known as the multimode Brownian oscillator (MMBO) model, in which an infinite set of vibronic states interacting with an electronic transition is replaced by a finite set of effective vibronic modes. In calculations, in addition to these four general modes, we used two overtones 〖2ν〗_1 and 〖2ν〗_2 and the sum mode ν_1+ν_2. Each mode is represented by set {ω_j,S_j,γ_j }, where ω_j is the electronic transition frequency; S_j is the Huang-Rhys factor characterizing the electron-phonon interaction; γ_j is the damping factor. While ω_j and γ_j could be found experimentally, S_j is an effective model parameter that cannot be found by measurements. As the number of free parameters increases, the fitting of experimental data becomes impossible, therefore, to solve this problem, it is necessary to use optimization methods, for example, the differential evolution algorithm [1]. This is a global optimization method based on the idea of natural selection and is a more refined and advanced version of genetic algorithms. Its advantage is that the target function can be non-linear and non-differentiable. In this work, the absorption spectra of carotenoids in various organic solvents were modeled using MMBO. There were 12 free parameters: Ω_eg is the energy of the electronic transition between the ground and excited states of the system, 〖FWHM〗_Ω is the full width at half maximum of the absorption spectrum, {ω_low,S_low,γ_low } are the parameters of the lowest vibronic mode, and 7 Huang-Rhys factors [2,3]. The performed calculations have shown that when modeling almost identical spectra, differential evolution makes it possible to determine with high accuracy the effect of polar and nonpolar solvents on a number of microparameters. 9 of 12 parameters were determined with high accuracy, only for the parameters of the lowest vibronic mode the values of the standard deviation were significant. Thus, the use of the differential evolution algorithm makes it possible to create correct models of the optical response of biological pigments and use them to model data in optical spectroscopy.

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### S5.339. Percolation model of a branching network of active vessels

Antonets V.A.[1,2]*, Antonets M.A.[3]
[1]*Institute of Applied Physics RAS;*
[2]*Lobachevsky University;*
[3]*JSC Ma-tek;*
* antonetsva@gmail.com

The proposed work is dedicated to the theoretical analysis of the mechanism of spatial distribution and regulation of blood flow in tissues, i.e., the mechanism of tissue perfusion with blood.

At the beginning of the last 20th century, Krough introduced the concept that the control of blood flow into the capillaries of muscles occurs through the opening/closing of the lumen of arteries possessing a muscular wall. Later, this concept was extended to other organs. It is precisely this concept that we have used as the main one for building a percolation model of blood flow regulation.

We will start from the fact that blood enters the tissue capillaries through a branching system of vessels starting from the aorta. At some level of branching, it enters arterioles with an active smooth muscle wall, whose contraction changes the diameter of the vessel. At the finest branches of this tree - the terminal arterioles - are capillary cells containing several parallel capillaries. At the same time, these cells belong to the finest branches of the venous tree - venules. In this way, the large circle of blood circulation can be seen as two merged "crowns" of a tree - the arterial and venous.

In the model, we will only consider its arterial part and identify the arterial tree with a dichotomously branching graph. The triad of vessels consisting of a parent and two descendants will be considered the main element of the tree-like network. Thus, each vessel A is a part of two triads - one as a parent, the other as a descendant.

The state of a vessel A at a discrete moment in time t will be described by a logical variable X(A,t), which has a value of "true" at a discrete moment in time t if the vessel is open and "false" if the vessel is closed. We will introduce the natural conditions of flow continuity in the network: if vessel A is open, then its predecessor A0 is also open. Otherwise, there is nowhere for the flow in vessel A to come from.

Active vasomotions will be described by random logical variables. Positive activity m(A,t) means opening of vessel A at time (t+1), i.e. the truth of X(A,t+1). Negative activity n(A,t) means closing of vessel A at time (t+1). The variables m(A,t) and n(A,t) describe the interaction of vessels with each other, but we will limit this assumption by assuming that each vessel A interacts only with its predecessor A0 and two descendants A1 and A2. Then the behavior of the network of active vessels is described by the following recurrent logical equations:

$$X(A,t+1) = [X(A,t) * (1 - n(A,t)] + [m(A,t)* (1 - X(A,t) * X(A0,t)] + [ X(A1,t + X(A2,t)] \quad (1)$$

If X(A,t) changes only due to its own activity and the interaction with A0, A1 and A2 is reduced to compliance with the continuity condition, then the stationary solution of the system of equations (1) is a fully open network or a set of size-finite sub-trees, i.e. the absence of a through flow. It follows that for regulation of blood flow, a more complex interaction between vessels is required.

Stationary states with more complex interactions will be considered by introducing conditional probabilities that with an open vessel A, both of its descendants are open with probability P11, descendant A1 is open with probability Π10, descendant A2 is open with probability Π01, and both descendants are closed with probability Π22. The sum of probabilities $\Pi11 + \Pi12 + \Pi21 + \Pi22 = 1$.

Let's consider the sequence of random numbers Nk, each of which represents the number of open vessels in the k-th generation of branching network. It forms a branching process. The analysis of its generating function shows that if $\Pi22/\Pi11 > 1$, then the flow through the network is impossible. If $\Pi22/\Pi11 < 1$, then the probability P of flow through the network, i.e. the formation of infinite clusters, is equal to $P = 1 - \Pi22/\Pi11 = (\Pi11 - \Pi22) / \Pi11$. Since the average number M of nearest open descendants of an open vessel is equal to $M = 1 * \Pi12 + 1 * \Pi21 + 2 * P11$, then $P = (M - 1) / \Pi11$. That is, flow occurs when $M > 1$, when the average number of open descendants of a vessel is greater than 1. A mixture of finite and infinite clusters is formed. This means that tissue perfusion can occur with a partially open vessel network. This corresponds to the well-known fact that the volume of circulating blood is less than the total volume of the network vessels.

If it is assumed that each vessel in the network exhibits its activity independently of the others and is open with probability Π, then the probability of flow $P= (2\Pi-1)/\Pi*\Pi$. The flow occurs when $\Pi > 1/2$. This corresponds to the conclusions of percolation theory for tree-like graphs. In these, the flow threshold is reached when $\Pi = 1/K$, where K is the branching multiplicity of the graph.

The dependence of the probability of flow P on the excess of Π over the threshold is very strong. An excess of Π over the threshold by 10% increases the probability of flow P from 0 to 0.56. This is what signifies the efficiency of blood flow regulation through changes in its structure. In particular, this may explain the fact that an increase in the minute blood flow volume by 3-5 times with an increase in systolic pressure by only 30-40%.

Additional regulation of blood flow can be provided by collaterals, which could connect terminal clusters. In them, the average number of open vessels is equal to H = 1 / (Π22 - Π11), and the average number of those without open descendants, Γ = Π22 / (Π22 - Π11). It can be considered as a quantitative measure of the boundary of the terminal cluster or as a branching multiplicity of the original vessel. Then, with a concentration of collaterals C = (Π22 - Π11) / Π22, the threshold of flow in the system of these clusters is reached.

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### S5.340. Phylogenetic analysis and classification of short H2A histone variants

Singh-Palchevskaia L.[1]*, Shaytan A.K.[1]
[1]*Lomonosov Moscow State University;*
* l.singh@intbio.org

Histone proteins are a key factor in epigenetic mechanisms. They are responsible for the compaction and functioning of chromatin by influencing its packing density. There are five types of eukaryotic histones: core H2A, H2B, H3, H4, and linker histone H1 (H5 in birds). The main part of histone proteins in chromatin are canonical histones expressed during replication and are responsible for DNA packaging. However, during the eukaryotic cell cycle, there are histone variants that are integrated into the nucleosome and change its stability in order to regulate the work of certain parts of the genome.

For a long time, it was believed that histone proteins evolve slowly and have practically no differences, with the exception of tails, which have a huge number of modifications. However, today it is known that even evolutionarily related organisms can have a wide range of histone variants with different functional features. For example, macroH2A and H2A.B variants are responsible for the regulation of gene expression, while H2A.X and H2A.Z respond to DNA damage [1]. It should be noted that some variants separated from the canonical histone proteins much earlier than others. Thus, phylogenetic analysis [2] demonstrates that H2A.Z separated from other H2A before eukaryotic diversification, while macroH2A appeared much later. There are histone variants found only in one taxonomic group or one cell type. For example, the histone variant H2A.W is specific for plants [3], while OO H1.8 is special for mammalian oocytes. Despite the existence of so many proves that demonstrate the phylogenetic, functional and species diversity of histone proteins, there is still no comprehensive understanding and systematized knowledge about the various variants and their significance. Short H2A is a class consisting of several histone variants of the H2A family in placental mammals, expressed mainly during the development of male germ cells of mammals to the almost complete replacement of histones by protamines in the nuclei of spermatozoa [4]. In order to conduct a phylogenetic analysis and identify their features, we collected their amino acid sequences, including those for the recently discovered H2A.Q variant [4], and some sequences of other variants from the H2A family. Based on the multiple alignments that were obtained using MUSCLE, we built a phylogenetic tree using PhyML algorithms [5], which are based on maximum likelihood methods. Based on it, we were able to conclude that short H2A arose the most recent in evolutionary history to date. The closest ortholog is the histone variant H2A.R. It is important to note that separate clades are distinguished within each subfamily (H2A.B, H2A.P, H2A.Q, H2A.L). Moreover, the most pronounced ones are within the H2A.Q subfamily: at least 4 clades can be distinguished.

We also clustered the short H2A amino acid sequences using the UPGMA hierarchical clustering algorithm and analyzed the pairwise identity matrix. The results showed that the median identity among all short H2A sequences was less than 36%, while within each individual subfamily it was not less than 43%, but not more than 59%. Also, the resulting clusters fit well into the concept of evolutionary analysis. We see that each cluster contains all the sequences of one or more clades observed on the phylogenetic tree. This fact means that evolutionarily formed clades may have important functional differences.

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### S5.341. Processing of heart cell morphology data to create a mathematical model of atrial tissue

Sergeeva T.).[1]*
[1]*MIPT;*
* sergeeva4247@gmail.com

Cardiovascular diseases are a group of diseases of the heart and blood vessels, which includes coronary heart disease, heart failure, heart defects and other pathologies. This group of diseases is one of the most common causes of death in developed countries [1]. One of the most common diseases of the cardiovascular system is a persistent form of atrial fibrillation (AF) - a violation of the heart rhythm that can lead to the development of heart failure and sudden cardiac death [2].

A common method of treating atrial fibrillation is surgical ablation – the creation of non-conducting topology zones in the atrial tissue that can prevent the occurrence and development of spiral reentry waves. The effectiveness of this procedure is extremely low: more than half of the patients return for repeated surgery [3], there are cases of up to 10 repeated operations. This paper describes the creation of a system of assistance to a doctor during surgical ablations, which allows to increase the effectiveness of the procedure.

The system is based on the consideration of histological analysis of atrial tissue morphology. The key ideas for this work are the influence of cellular morphology on the wave dynamics in the tissue, as well

as the role of fibrosis in the occurrence and development of spiral reentry waves (fibrosis acts as a substrate for the occurrence of spiral waves) [4].
Based on the results of the study, a mathematical model of the patient's atrial tissue is created, using which it is possible to determine the optimal ablation protocols.
The mathematical model of cell morphology is based on the Potts cell model [5]. This model characterizes the morphology of cardiac tissue with consideration of two types of cells – cardiomyocytes and fibroblasts. The electromechanical function of the heart is performed by excitable cells – cardiomyocytes, which are able to generate an action potential and mechanical contraction. Non–excitable cells are fibroblasts, their mutual arrangement with excitable cells can significantly affect the propagation of the wave. The interaction between cardiomyocytes, fibroblasts and extracellular proteins leads to the formation of a complex tissue texture. This cellular structure of interaction is the basis of the Potts model.
The creation of a patient-specific computer model takes place in several stages. Initially, the electrophysiological data of single cells are removed from the biopsy of patients by the patch-clamp method [6], then an inhomogeneous cardiac tissue is generated based on a mathematical model of cell morphology.
During the development of the model, the following tasks are performed:
• preparation, immunohistochemistry and confocal microscopy of sections of atrial tissue of patients (pathanatomic material) with and without fibrosis. Staining of thin sections prepared on cryotome is carried out with dyes DAPI, f-actin, a-actinin to evaluate the quantitative and structural analysis of cell morphology;
• development of a model of morphology of atrial single cardiomyocyte and fibroblast in 2D according to patient sections, taking into account observed and variable (iterative) morphology parameters (cell area, transverse cell dimensions, number of protrusions in the membrane, energy of cell interaction, etc.);
• development and adaptation of atrial electrophysiology model according to electrophysiology of patients' cell material (obtained using the patch-clamp method and optical mapping);
• development of a model of human atrial tissue reproducing the excitation and predicting the probability of a spiral wave in 2D tissue based on the obtained data of cell morphology and their electrophysiology.
As a result, a surgical decision support system will be created in the form of software that performs the following functions:
• determination of fibrous areas and the nature of fibrosis according to MRI and CT scans of patients;
• analysis and modeling of identified areas from the point of view of the structure of atrial tissue;
• presentation of the analysis result in the form of a visualizing map of the atria with possible arrhythmia foci and predicted locations for ablation surgery.
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## S5.342. Protein-protein interaction study by molecular computer modeling

Kovalenko I.B.[1]*, Fedorov V.A.[1], Khruschev S.S.[1], Riznichenko G. Yu.[1], Rubin A.B.[1]
[1]*Lomonosov Moscow State University;*
* ikovalenko78@gmail.com

In most biological systems, protein molecules play a key role. Using the methods of X-ray diffraction analysis and nuclear magnetic resonance, the spatial structures of many different proteins and their complexes have been studied. However, it still remains unclear in detail to what extent electrostatic and hydrophobic interactions affect the rate of formation of protein complexes at various stages of molecular approach. The formation of a protein complex is a multi-stage process that requires taking into account many factors, such as long-range electrostatic interactions between protein surfaces, geometric and chemical complementarity of binding regions, molecular mobility in the protein-protein interface, and hydrophobic interactions. We have developed an original approach that allows, thanks to the combined use of Brownian and molecular dynamics methods, to predict the structure of the formed complex and the molecular mechanisms that led to its formation. In this approach, the Brownian dynamics method is used to model the formation of a collision complex by two proteins, taking into account diffusion processes and electrostatic interactions, and molecular dynamics is used to model the transformation of a preliminary complex into a final one, taking into account the mobility of atoms, conformational changes, and solvent molecules.
This approach allowed us to reveal the role of electrostatic and hydrophobic interactions in the formation of the complex of plastocyanin and cytochrome f proteins in cyanobacteria, green algae and higher plants, and to show that their role in complex formation changes along with evolutionary changes in protein sequences.
The work was supported by the grant of the Russian Science Foundation #21-74-20035.

## S5.343. RNA polymerase II promoters: similarities and differences between core promoter regions for mRNAs and non-coding RNAs

Savina E.A.[2], Anashkina A.A.[1,2], Tumanyan V.G.[1], Il'icheva I.A.[1]*
[1]*V.A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia;*
[2]*I.M. Sechenov First Moscow State Medical University of the Russian Ministry of Health (Sechenov University), Moscow, Russia;*
* imb_irina@rambler.ru

RNA polymerase II (POL II) is responsible for the transcription of messenger RNAs (mRNA) and a number of long non-coding RNAs (lncRNA), including antisense RNA (asRNA). Currently known mRNA exons encoding proteins account for less than 3% of all transcripts. The remaining 97% of the transcripts present in cells do not encode proteins, being precursors of non-coding RNAs. A significant part of them are POL II transcripts. The apparatus used POL II is the same for mRNA and lncRNA transcription. The transcription initiation step occurs at core promoters that are about 100 bp in length and whose sequences are extremely diverse. These regulatory fragments may differ from each other in motifs of short elements, the presence

and combination of which determine the level of gene expression. However, the general pattern of transcription initiation is always the same. Its beginning is the recognition by the TATA-binding protein (TBP) of the octanucleotide fragment of the core promoter located at a distance of ~-28 bp from the transcription start position.
In this work, we compared the textual and structural characteristics of H. sapiens core promoters that affect the processes of mRNA and lncRNA transcription initiation. We used representative samples of nucleotide sequences aligned at transcription start site (TSS) from EPD New database (http://epd.vital-it.ch). Sets contain 29597 promoters for mRNA, and 2339 promoters for lncRNA. Profiles of different textual and structural characteristics were constructed on 80 bp fragments, in positions (-50 - +30). We analyzed the frequencies of occurrence of nucleotides in every position, logo representation of the nucleotide sequences, and changes of different parameters characterizing the local 3D structure of the DNA double helix as well as local changes of conformational dynamics. Textual and structural characteristics of mRNA promoters have been described earlier [1]. All characteristics of lncRNA promoters will be described in detail in a forthcoming paper. Changes of the indexes, characterizing the structure of DNA and its dynamics on the profiles of lncRNA promoters turned out to be completely similar to their changes on the profiles of mRNA promoters. Namely, the architecture of the core promoter also includes two singular regions -- a hexanucleotide surrounding the transcription site start (TSS), where the equilibrium parameters of the double helix dinucleotides change sharply with each step, and an octanucleotide (TATA box) disposed at ~ -28 n.p., which is characterized by minor groove widening and a decrease in the intensity of conformational dynamics. However, the logo representations of the nucleotide sequences of mRNA and lncRNA core promoters reveal significant differences. These differences concern the entropy component of the nucleotide texts. The exception is the TATA box, where both types of promoters are characterized by an extremely high entropy component. Another singular region of the promoters, the hexanucleotide surrounding the TSS, in the lncRNA promoters has a lower entropy component than in the mRNA promoters. In the other parts of the text, on the contrary, the mRNA promoters have a lower entropy component. Perhaps this is why short motifs in mRNA promoters can play the role of gene expression level regulators. They can interact with proteins of the POLII transcription apparatus by the mechanism of direct recognition, which seems unlikely to us for lncRNA promoters.
The higher percentage of PyPu in the TSS position in lncRNA core promoters points, that the open complex formation should be easier (on average) than in mRNA core promoters. And this may provide them with a sufficiently high level of expression.
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### S5.344. Radioengineering model of connected neurons with synapses

Egorov N.M.[1,2]*, Ponomarenko V.I.[3,2], Sysoev I.V.[3,2], Sysoeva M.V.[1,2]
[1]*Yuri Gagarin State Technical University of Saratov;*
[2]*Saratov Branch of Kotel'nikov Institute of Radioengineering and Electronics of Russian Academy of Sciences;*
[3]*Saratov State University;*
* egorovnm@sstu.ru

Novadays, the device, structure and principles of the brain are one of the most relevant areas of modern research in the field of biological sciences and biomedicine. The most complete understanding of the mechanisms of the brain will allow us to accelerate the development of new things that depend on our knowledge about the structure of the brain, for example, the creation of neural interfaces or finding new effective ways to combat brain pathologies. The study of pathology will be discussed further, and specifically, the modeling of epilepsy. Epilepsy is one of the most common diseases, which for the most part is the result of pathological structural and functional connections between the cortex and deep brain structures.
Traditionally, the study of processes in biosystems is divided into several stages, one of which is the creation of models. The aim of this work is to implement a radio engineering model of network-connected neurons with synapses to simulate the electromagnetic activity of the brain during the pathological state of an absence epilepsy attack. With the help of radio engineering simulation, one can check the roughness of model representations, such as sensitivity to noise or the achievability of model behavior in a real experiment.
To reproduce the pathological condition in the form of an electromagnetic signal, a radio installation based on analog components was soldered to implement a model of the thalamo-cortical network of the brain. As one node of this network, a popular simple model of an excitable cell, the FitzHugh-Nagumo oscillator, was chosen. Due to the fact that the model has a low order of nonlinearity and dimensionless quantities, it was relatively easy to implement. The architecture of connections between neurons is characterized by the model proposed earlier in [1]. Cells are divided into pyramidal, reticular, thalamocortical and interneurons. All neurons are made similar to each other, the difference between cell types is in which cells they can act on and with what strength of connection. To implement the inhibitory function of the synaptic connection, the linear connection in the model was replaced by a sigmoid function of half hyperbolic tangent with a shift [2]. This type of connection reproduces the mechanism of signal inhibition between cells through inhibitory connections and, at the same time, continues to have an excitatory effect on neurons connected by an excitatory connection. According to modern concepts of the initiation of an epileptic effect on an external trigger, a model neuron similar to the others was also introduced into the network, with the difference that it is tuned to a self-oscillatory mode, that is, it represents an external effect from a trigeminal effect.
Thus, in this work, a radio engineering model of the thalamo-cortical network of the rat brain in the pathological state of an absence epilepsy attack was implemented. Neurons are interconnected according to certain rules of the hierarchical model by means of a sigmoid function that mimics the properties of synapses. The network consists of 14 oscillators, including the trigeminal nerve. Temporal realizations were obtained that have characteristic similarities with the real data of epileptic discharges in rats.
The work was supported by the Russian Science Foundation grant No. 21-72-00015.
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### S5.345. Reconstruction of gene circuits associated with type 2 diabetes mellitus using online bioinformatics tools

Zakharov D.V.[1]*, Orlov Y.L.[1]
[1]*I.M. Sechenov First Moscow State Medical University;*
* exsterol@gmail.com

The epidemic of diabetes mellitus (DM) is a challenge to the global health system. According to the World Health Organization (WHO), in 1980 there were about 150 million people on the planet suffering from diabetes, and in 2014 - about 421 million. Unfortunately, no positive dynamics of incidence over the past decades has been observed, and today we can already say that diabetes mellitus is one of the most common and serious diseases. Type 2 diabetes mellitus is a chronic non-infectious, endocrine disease, which is manifested by profound disorders of lipid, protein and carbohydrate metabolism associated with an absolute or relative deficiency of a hormone produced by the pancreas. In patients with this disease, the pancreas produces a sufficient amount of insulin, a hormone that regulates carbohydrate metabolism in the body. However, due to a violation of metabolic reactions in response to the action of insulin, a deficiency of this hormone occurs. The task was to collect a list of genes associated with diabetes, reconstruct the gene network and develop a computer model of the disease

The genetic predisposition to the development of type II diabetes mellitus is provided by a set of genes that control the sensitivity of peripheral tissues to insulin and are thus responsible for the state of insulin resistance, which is primary, leading to the need for increased secretion of insulin by pancreatic islet b-cells and contributing to the depletion of their functional activity.

In recent years, a lot of molecular data have been collected that reveal the pathogenetic mechanisms of the development of DM and its complications. More than 100 genes associated with the risk of developing this disease and products that affect insulin secretion, adipogenesis, and insulin resistance have been described, but for most genes, point molecular mechanisms of participation in the pathogenesis of T2DM have not been finally established.

Analysis of molecular genetic networks makes it possible to identify molecular interactions that are important for the development of DM and its complications. With the help of online bioinformatics tools, a list of genes associated with diabetes was compiled and a gene network of macromolecular interactions was reconstructed.

For this, databases such as OMIM (https://omim.org/) and GeneCards (https://www.genecards.org/) were used. When analyzing the interactions of the products of these genes, such gene network reconstruction resources as STRING-DB (https://string-db.org/), Metascape (https://metascape.org/), GeneMANIA (https://genemania .org/). To analyze the categories of gene ontologies, the DAVID resources (Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/summary.jsp) were used. The intellectual analysis of data and texts of scientific publications opens up new possibilities for processing this information.

### S5.346. Review of chronic disease markers and mechanisms stud molecular based on high-throughput sequencing

Orlov Y.L.[1,3], Chen W.L.[2], Volkov I.A.[1], Cai G.[4], Zhao X.[5], Li H.[5]*
[1]*I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia;*
[2]*Shanghai University of Traditional Chinese Medicine, Shanghai, China;*
[3]*Novosibirsk State University, Russia;*
[4]*University of South Carolina, Columbia, SC, United States;*
[5]*Shanghai Jiao Tong University, Shanghai, China;*
* kaikaixinxin@sjtu.edu.cn

We prepared special journal issue “High-throughput sequencing-based investigation of chronic disease markers and mechanisms” at Frontiers in genetics journal discussing the genomics studies on cancer and chronic diseases (Orlov et al., 2022). With the recent development of sequencing technology and the rapid reduction of sequencing costs, high-throughput sequencing (including second and third-generation sequencing) is revolutionizing basic life science research and clinical research. High-throughput sequencing often produces millions of sequencing reads at a time, and the alignment or assembly of these reads allows determination of various mutations at the genomic level, accurate gene expression quantification at the transcriptomic level, and identification of histone or DNA modification at the epigenomic level. The resulting accumulation of enormous multi-omics information has opened up a new era of finding effective disease markers and studying their roles in disease occurrence and development (Anashkina et al., 2021).

Using high-throughput sequencing, various markers of chronic diseases have been developed at all omics levels, which have been used for diagnosis and classification of diseases, prediction of treatment effects, and prevention of diseases. The chronic diseases include cancer, heart disease, diabetes, arthritis. The quickly and massively acquired multi-omics data, together with newly developed algorithms, provide excellent opportunities for the identification of more reliable biomarkers. This Research Topic aimed at 1) developing new chronic disease markers at four levels (i.e., genome, epigenome, transcriptome, and translatome) with the help of high-throughput sequencing, and 2) delineating potential marker-related mechanisms for chronic disease occurrence or development. More specifically, the Research Topic contains contributions including:

Identification of novel biomarkers and prediction signatures for chronic disease detection or prognosis prediction using high-throughput sequencing;

Analysis the possible pathological causes of markers as well as the potential roles they play in disease initiation and development;

Applications of new high-throughput sequencing techniques facilitating the development of more effective biomarkers of chronic disease;

New algorithms or tools for in silico identification of effective chronic disease markers based on high-throughput sequencing data.

Thus, we have organized this Research Topic to collect the papers focused on the frontiers of chronic disease markers. This Topic complements recent Research Topics “Bioinformatics of Genome Regulation” (https://www.frontiersin.org/research-topics/17947/bioinformatics-of-genome-regulation-volume-ii), and “Association between Individuals’ Genomic Ancestry and Variation in Disease Susceptibility” in Frontiers in Genetics (Das et al., 2022). The later journal issue collected papers focused on genetic background and ancestry rather than on molecular mechanisms of the human diseases. Due to importance of this topic, the paper collections are extended now for 2023 as “Volume II” (see https://www.frontiersin.org/research-topics/53085/high-throughput-sequencing-based-investigation-of-chronic-disease-markers-and-mechanisms---volume-ii )

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### S5.347. SARS-Cov-2 pandemic as a "predator-prey" system: biophysical, social and heliophysical factors by local epidemics

Ragulskaya M.V.[1]*
[1]*Institute of terrestrial magnetism and radio wave propagation of RAS;*
* ra_mary@mail.ru

The features of the coronavirus pandemic in various countries are considered within the framework of the “predator-prey” system

under the external modulating influence of solar activity. It is discussed that under the conditions of the global minimum of solar activity, the genetic composition of the population turned out to be the main factor in the difference by of local SARS-Cov-2 epidemics dynamics. Countries with the highest relative mortality from coronavirus have a dominant population with haplogroup R1b. The incidence ratio in haplogroups R1b : R1a : N in the first and second waves of coronavirus (before the start of universal vaccination in December 2020) was approximately 7:2:1, and practically did not depend on the severity of quarantine measures and the level of medical care for the population. The coincidence of the dynamics of morbidity and mortality in countries with a similar genetic composition is shown, regardless of the introduction or absence of a lockdown on their territory. The emergence of self-oscillatory waves in small countries with a hard lockdown in the first wave of the pandemic was revealed. It is led to a significant increase in the number of victims in subsequent waves. Universal vaccination lowered the level of relative mortality by 5-8 times for countries with haplogroup R1b. However, for countries with haplogroup N and R1a + N, by mid-2022, relative mortality increased by 2-4 times compared to 2020. Unfortunately, the effect of vaccination in countries with haplogroup R1b also decreased by mid-2022. Perhaps this is due to the increase in solar activity during the development of the 25th cycle. The influence of these multidirectional processes on the coronavirus pandemic during the maximum of the 25th cycle of solar activity requires further study.

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### S5.348. Solving the problem of calculating strain indicators in echocardiography using deep learning neural networks

Alekhina A.E.[1,2], Dorrer M.D.[1]*, Sadovskiy M.G.[3,4,5], Sakovich V.V.[5], Demichev I.A.[5]
[1]*Reshetnev Siberian State University of Science and Technology;*
[2]*Siberian Federal University;*
[3]*Institute of Computational Modeling SB RAS;*
[4]*Siberian Research and Clinical Center of FMBA of Russia;*
[5]*V.F. Voino-Yasenetsky Krasnoyarsk State Medical University;*
* mdorrer@mail.ru

The paper sets the task of calculating the parameters of deformation of the heart muscle according to echocardiogram data under interference conditions, for example, in the study of children.
Indicators of deformation (strain value) of the heart muscle were used by us to determine the presence and severity of dysfunction of the chambers of the heart in atrial septal defect – a congenital heart defect characterized by the presence of communication between the right and left atria.
The problem was solved by analyzing the video stream obtained from the installation of EchoCG using a set of deep learning neural network architectures designed for image segmentation. The study was conducted for neural network architecture U-net. The initial data for training were prepared on the basis of images of real video recordings of echocardiograms that had been preprocessed with median filters, by subtracting Gaussian filters, as well as by binarization of the image using the Father filter. As a result of processing the video stream, it was possible to solve the problem of segmentation of the walls of the heart muscle and binding of key points in the condition of interference in the removal of an echocardiogram on child patients unable to remain motionless during the study. The dynamics of the distance between the key points on the heart muscle in the sequence of EchoCG frames allows you to calculate strain indicators.
The obtained indicators provide the cardiologist with important information for determining the dysfunction of the chambers of the heart (especially the right atrium, the most compromised chamber of the heart in the studied cases) with a defect of the atrial septum. Accurate diagnosis of the degree of myocardial dysfunction in this anomaly will allow more accurately determining the indications and timing of surgical correction.

### S5.349. Some thermodynamic features of self-complementary microRNA(miRNA) processing

Kuzmichev S.A.[1,2]*
[1]*Moscow State University of Medicine and Dentistry,Research Institute ofCarcinogenesis;*
[2]*Research Institute of Carcinogenesis, N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia;*
* kuzs19782005@mail.ru

The prediction of possible targets of epigenetic regulation for miRNAs with different nucleotide sequences is based not only on the presence of nucleotide motifs complementary to them, but also on the analysis of intermolecular hybridization parameters [1], including minimum free energy (MFE). The peculiarities of processing self-complementary miRNAs capable of forming homo-duplexes, i.e. duplexes between miRNAs having the same nucleotide sequences, have not been sufficiently studied, taken into account that the content of different miRNAs can vary widely in various cells [2]. To fill this gap, we investigated some thermodynamic processing parameters. Considering that the stability of the structure of miRNA duplexes and premicroRNAs (pre-miRNAs) can affect the processing rate of miRNA molecules by Dicer RNase III [3], a comparative analysis of the MFE folding parameter was carried out between pre-miRNAs from which miRNAs having different MFE of their homo-duplexes are processed. The miRNA and pre-miRNA sequences were taken for analysis from the miRBase base, version 22.1, in species that differ in the amount of miRNA produced: in human herpes virus type 1 (HSV-1) - 27 miRNA, in Drosophila fly ananass (D. ananassae) - 74 miRNA, and in house mouse (M. musculus) - 1479 miRNA. Bioinformatic analysis for detecting miRNAs capable of forming homo-duplexes and determining their MFE was performed using the RNAup program, as described earlier [2]. RNAfold program was used to calculate the MFE of pre-miRNA folding (http://rna.tbi.univie.ac.at).Statistical analysis of the obtained data was carried out using the Statistica 10 program. Results and discussion: Correlations of MFE parameter calculation data (in kcal/mol) obtained for pre-miRNA and miRNA homoduplexes based on the Turner, Matthews free energy model method and constraint generation method [1] were high (correlation coefficient, R = 0.97-0.98). At the same time, our analysis showed that with an increase (absolute value) of MFE homo-duplexes of self-complementary miRNAs from MFE ≥14-16 kcal/mol in 3 species (HSV-1, D. ananassae, M. musculus) from different taxon, which differ significantly, including in the number of

genes and miRNA, significantly increases the MFE of the folding of their pre-miRNA (for HSV-1 – R =0,85 , for M. musculus – R=0,6 , for D. ananassae – R=0,58). For the house mouse, the analysis also showed a difference in the distribution of genes encoding sequences of self-complementary miRNAs between different chromosomes - with the largest number on chromosomes 2, 7 and 12 - 9%, 11% and 10%, respectively, and the smallest (of the order of 1%) on 3.6 and 13 chromosomes. Correlations between MFE homo-duplexes of miRNA and MFE folding of their pre-miRNAs on different chromosomes in mice were also significant. With a decrease in MFE of pre-miRNAs in them significantly increase the content of nucleotides G and C, which reflect high R (for HSV-1 – R =0,93 , for M. musculus – R=0,8 , for D. ananassae – R=0,82). This is consistent with the previously established relationship between an increase in the content of G and C in pre-miRNA, and a decrease in their MFE, which allows the formation of a more stable pre-miRNA structure [4]. An increase in G and C was found in self-complementary miRNAs with greater absolute value MFE homoduplex, but here the R were smaller (from 0.28 for M. musculus to 0.52 D. ananassae), which indicates the effect of other nucleotide pairs (A, U) on the MFE of homo-duplexes. Previously, the possibility of inhibiting the processing of some pre-miRNAs by hybridization with them complementary miRNAs has been shown [5]. The increase in the number of G and C in pre-miRNAs from which self-complementary miRNAs are processed, as they increase absolute value their MFE, suggests the possibility of forming more high-energy secondary structures (hetero-duplexes) of pre-miRNAs and complementary miRNAs. The latter indicates the presence of a mechanism for regulating the processing of self-complementary miRNAs by negative feedback loop. Different R's between MFE homo-duplexes of miRNA and MFE folding pre-miRNA in different species indicate that the effect of the nucleotide structure of their pre-miRNA on the self-regulation of miRNA processing may differ. The found tendency shows the possibility of a molecular mechanism in different species that allows increasing the efficiency of controlling pre-miRNA processing for self-complementary miRNAs capable of forming more stable homo-duplexes.

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## S5.350. Spatial pattern of the distribution of GC-content of the fragments of mitochondrial, chloroplast and bacterial genomes

Senashova M.Yu.[1]*, Sadovsky M.G.[1]
[1] *Institute of Computational Modeling SB RAS, Krasnoyarsk, Russia*
* msen@icm.krasn.ru

Introduction

The study of the features and peculiarities of the structure of nucleotide sequences is one of the most important tasks of biology at present. Revealing the relationship between structural components and their corresponding functions is a common problem in molecular and systems biology, and despite the large quantity of publications and research in this direction, it is still far from being completed.

The genome GC- composition, both in general and in individual regions, is widey used in studies of the genome structure and functions. Many works appeared in this issue, both in the case of genomes of chloroplasts [1–2] and mitochondria [3–4], and bacteria [5–6]. In this work, we consider how the selected fragment GC-composition values of these genomes are distributed in the genome spatial structure, obtained on the basis of fragment frequency dictionaries.

Materials and methods.

We consider a genetic sequence of length L, consisting of symbols of the alphabet {A,C,G,T}. This sequence is divided into subsequences of length d. For each of the subsequences, frequency dictionaries W of thickness 3 are compiled. The frequency dictionary of thickness 3 is a list of all triplets w=v1v2v3 of consecutive nucleotides, indicating the frequencies of these triples. Frequency fw is the ratio of the copies number nw of a given word to the total number of all triplets N, where N is the sum of all nw: fw=nw/N.

The dictionary W specifies the correspondence of the genome into a 64-dimensional metric space. To build triplet frequency dictionaries, the sequence of each genome was scanned by window of length d with a step t. For each window position i, a section of the genetic sequence was determined that coincided with the reading frame, for which the frequency dictionary Wi was calculated corresponding to the i-th point in 64-dimensional space.

The data contained in the open access in the EMBL-bank database were used for the study. The data visualization obtained was carried out by the VidaExpert program, which projects points of a multidimensional space into the space of the first three principal components. The form of the obtained projections in this space was analyzed in the course of the work.

Spatial distribution of the GC-content values of the chloroplasts, bacteria and mitochondria genome fragments.

We examined 570 chloroplast, 280 bacterial and 488 mitochondrial genomes from the EMBL database. The available GC-content values interval was divided into 7 subintervals of equal length for all genomes. The interval with minimum values corresponds to purple, and the interval with maximum values corresponds to red. It was found that the distribution of the GC-content of chloroplast genome fragments according to the same type spatial structure. GC-content of fragments is distributed along a gradient along the genome spatial structure symmetry axis.

A centrally symmetrical distribution of values is typical for GC-poor bacterial genomes (the value of GC-content is less than 50%). The minimum values are predominantly located in the center of the structure and the maximum values are at the edges for a centrally symmetrical distribution. A gradient distribution of values is observed for GC-rich genomes.

The mitochondrial genomes of land plants, liverworts, unicellular algae, mosses, and higher fungi have a pronounced gradient distribution of GC-content values. Multicellular algae, lichens and lower fungi also have a gradient distribution, but it is not so pronounced. The mitochondrial genomes of insects, arachnids, and crustaceans are characterized by a centrally symmetric distribution of GC-content values. There are distributions of GC-content values in the form of an implicit gradient distribution and a centrally symmetric one in the genomes of sponges, mollusks, flatworms, annelids, and roundworms. It was not possible to reveal any typical picture of the distribution of the GC-content of fragments within classes and between classes for vertebrates. It was found that all identified genome fragment GC-content distributions are stable with respect to different window lengths .

Conclusions.

The results described above show that there is an order in the distribution of different genome fragments GC-content values. Moreover, this ordering has a typical pattern for individual genome groups. Mitochondrial genomes have the greatest diversity in the types of fragment GC-content distributions.

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### S5.351. Statistical estimates of clustering of transcription factor binding sites in plant genomes

Dergilev A.I.[1,2]*, Ivanisenko V.A.[2], Orlov Y.L.[2,3], Chen M.[4]
[1]*Novosibirsk State University;*
[2]*Institute of Cytology and Genetics SB RAS;*
[3]*Agrarian and Technological Institute, Peoples' Friendship University of Russia, Moscow, Russia;*
[4] *Zhejiang University, Hangzhou, China;*
* arturd1993@yandex.ru

The development of high-throughput genomic sequencing combined with chromatin immunoprecipitation technologies makes it possible to study the binding sites of protein transcription factors (TF BS) at the genome scale. An increase in the volume of data on experimentally determined binding sites poses qualitatively new tasks for the analysis of gene expression regulation, prediction of target genes for transcription factors, and reconstruction of regulatory gene networks, including plant genomes analysis.
The co-location of binding sites for two or more different factors in the promoter region of a gene may define an element of the network of regulatory genes: two protein transcription factors can bind to the same promoter region of the gene, which corresponds to the interaction. The TF protein can bind to the promoter of its own gene, forming a regulatory circuit. The network of regulatory genes can be reconstructed from a set of binding site locations that form clusters in the genome using several different TFs. Such a regulatory network may persist between species.
The distribution of the number of bonds in the network of protein-protein interactions follows certain statistical patterns. The distribution of the number of nodes in such a network for transcription factors also has an exponentially decreasing character. New technologies for genome -wide determination of binding sites for protein transcription factors (ChIP - seq) make it possible to study the distribution of nodes in more detail for various model objects (human genome, mammalian and plant genomes). A hypothesis is put forward about the general nature of the distribution of clusters of binding sites in the genome according to the number of different transcription factors, which is determined by the structure of the regulatory gene network. A method for searching for regulatory regions based on statistics on the distribution of binding sites in plant genomes has been proposed; computer tools for such analysis and visualization have been presented (Dergilev et al., 2021).
The existence of non-random clusters of binding sites in all studied plant genomes has been shown, clusters in the Arabidopsis genome have been considered in detail. thaliana . It has been shown that the LFY factor, which is responsible for the growth of flowers in plants, is usually found in the genome of Arabidopsis . thaliana is the most common among all others, while the SRS group factors are the least expressed . In the Physcomitrella genome patens factor LFY is in the first place, and factor G2, which is involved in the process of the cell cycle and affects cell differentiation, is less common than others. It is also interesting to note that the LFY factor tends to be the most common among all the others, but weakly correlates with other factors. In general, it can be noted that evolutionarily older factors GATA and MYB are present in site clusters in all studied plant species.
For the plant genomes under consideration, analysis of an expanded set of transcription factor binding sites confirmed a broad co-clustering of binding sites for transcription factors of the GATA family, that is, transcription factors characterized by the ability to bind to the GATA DNA sequence.
Contrary to expectations, the LFY factor weakly correlates with other factors from the selected set. However, during the clustering process, LFY is actively associated with other factors.
In general, the proposed statistical estimates make it possible to identify nonrandom clusters of TF binding sites in plant genomes. The distribution of site clusters by size shows the general patterns of the formation of site clusters in eukaryotic genomes. Such regions of the plant genome need to be further investigated by experimental methods to identify cooperative interactions, determine the functional role of the detected clusters, including in response to stress ( Doroshkov et al., 2019).
In the general case, the problem of joint regulation has not been sufficiently studied. For plant genomes, such studies are presented in separate databases. To model gene networks - complexes of interacting macromolecules in a plant cell - a network approach will be used, tools STRING-DB, KEGG Pathways (Orlov et al., 2021).
The developed computer approaches can be applied to a wide range of tasks for assessing clusters of functional elements in the genome. These may include areas of low text complexity, tandem repetitions, and CpG islands (Babenko et al., 2018). Analysis of functional clusters makes it possible to statistically describe enhancers and annotate genomes. The integration of experimental genomic information, big data in general is an important problem of molecular biophysics that requires the integration of existing software tools and solutions.
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### S5.352. Stochastic modeling of protein fields with non-Markovian dynamics

Buzmakov M.D.[1]*, Bratsun D.A.[1]
[1]*Federal Autonomous Educational Institution of Higher Education "Perm National Research Polytechnic University";*
* maxim.buzmakov97@gmail.com

Protein fields, which are synthesized because of gene expression and are involved in gene regulation, are the most important part of the functionality of living systems. As is known, a deterministic description of such fields based on differential equations should be supplemented

by the stochastic approach to understand the ongoing processes in all details. The typical number of protein molecules involved in the regulation is relatively small. It leads to strong field fluctuations, which are caused by the noise of chemical reactions, as well as intercellular differences [2]. The standard tool for numerical analysis of protein concentration fluctuations is the Gillespie algorithm [5].

In this paper, we consider a specific class of protein fields whose dynamic behavior cannot be reduced to Markov processes. In recent years, it has become clear that gene expression includes of multi-stage reactions during which ensembles of complex organic compounds are successively formed. Thus, these processes are distributed over space, extended over time and depend on the entire prehistory. It should be noted that non-Markovian behavior can be a natural consequence of the processes mentioned above, or it can be artificially introduced into gene regulation through genetic engineering methods.

If the non-Markovian process is a fixed delay, we cannot neglect this effect if the delay time is of the same order of magnitude or greater compared to other characteristic times of dynamic processes. As a model system, we consider a repressilator [4], which is a typical product of synthetic biology. The repressilator plasmid includes the lacI, λcI, and tetR genes, which are naturally occurring but do not occur in such a combination in nature. The promoter of each gene controls the cistron following it through negative feedback, suppressing the expression of its neighbor, which leads to the excitation of oscillations. We have made several assumptions in the repressilator model, from which we highlight the following ones [3]. We supposed that gene regulation is carried out only through the dimeric form of the protein. In addition, the processes of transcription and translation proceed with some delay. In the numerical simulations, we used a version of the Gillespie algorithm generalized for the case of non-Markov processes [2]. The difference from the classical version is a stack for reactions that should occur after a predetermined delay time.

We considered two main repressilator configurations. In the first case, the protein production rates of all genes involved in the dynamics are different (asymmetric gene circuit). It was found that the difference between the transition times to the new regime in both the deterministic and stochastic descriptions is small here. In the second case, we considered a symmetrical gene circuit, in which reaction rates and expression delay times are the same. When studying the behavior of a repressilator, we show that a stochastic description provides new information about the behavior of a system, which does not reduce to deterministic dynamics even when averaged over sufficient realizations. We show that in the subcritical range of parameters, where deterministic analysis predicts the absolute stability of the system, quasi-regular oscillations may be excited because of the nonlinear interaction of noise and delay. We have discovered within the framework of the deterministic description that there is a long-lived transient regime, which is represented in the phase space by a slow manifold. This mode reflects the process of long-term synchronization of protein pulsations in the work of the repressilator genes. In this work, we show that the transition to the cooperative mode of gene operation occurs a two order of magnitude faster, when the effect of the intrinsic noise is considered. We calculate the probability distribution of the moment when the phase trajectory leaves the slow manifold and have determined the most probable time for such a transition.

Spatial modeling of protein fields was performed within the framework of a hybrid model of two-dimensional epithelial tissue, including a deterministic description of the diffusion process and stochastic of gene regulation [1]. It has been found that the combined action of delay, noise, and spatial signaling between cells can lead to the formation of patterns even when the deterministic description predicts an absolutely steady state.

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## S5.353. Structural-dynamic Models and IR Spectra of Chromenopyrydine Carbonytril and Pyrido[1,2-a]pyrimidine Systems

Peretokina (Ivlieva) I.V.[1]*, Babkov L.M.[1], Metscheryakova A.A.[1], Sorokin V.V.[1]
[1]*Saratov State University named after N.G. Chernyshevsky, Saratov, Russia;*
* Irine09@yandex.ru

The systems containing chromenopyridine fragment has antimicrobial, antitumor, antibacterial, anti-inflammatory and other types of biological activity. Pyrido[1,2-a]pyrimidine systems have a wide variety of pharmacological activity. The aim of our work was to study the structure and vibrational IR spectra of previously unknown chromeneopyridinecarbonitrile systems: 5-amino-2,4-diimino-3-(pyridin-2-yl)-2,3,4,10b-tetrahydro-1H-chromeno [3 , 4-c] pyridine-1-carbonitrile (I) and 4-amino-2,5-diimino-3- (pyridin-2-yl) -2,3,5,10b-tetrahydro-1-chromeno [3, 4-c] pyridine-1-carbonitrile (II) and pyrido[1,2-a]pyrimidine systems such as isomeric 4-amino-2-aryl-6H-pyrido[1,2-a]pyrimidine-3-carbonitriles(III, IV) and its open types such as 2-(aryl(pyridine-2-ilamino)methyl)malononitriles(V, VI) [1-3]. The systems (I) and (II) obtained during the synthesis at the base of 2-2-(2-amino-3-cyano-4H-chromene)malonitrile and 2-aminopyridine. 2-2-(2-amino-3-cyano-4H-chromene)malonitrile and 2-aminopyridine were taken at equimolar ratio of reagents and were boiled at isopropile alcohol. The systems were realized as a mixture of tautomers in the ratio 49.56 : 50.44%. [1]. Pyrido[1,2-a]pyrimidine systems (III, IV) anf their open types (V, VI) three-component reaction of 2-aminopyridine, malonitrile and aromatic aldehydes in ethanol [2, 3]. IR spectrum of the samples of the systems has been measured at room temperature ar 400-3700 cm-1 area on a Fourier spectrophotometer Shimadzu IRAFFINITY-1. The samples were pressed at KBr pellets. The structure-dynamic models of the systems (I) -(VI), fig.1, were obtained using the DFT method by the B3LYP/6-31g(d) functional. The simulation was obtained by the GAUSSIAN'03 program package [4]. According to the results of simulation of molecular structures tautomers (I) and (II) differ from each other. The angle that determine the orientation of the R4 ring relative to the remaining part of the molecule is different for different tautomers. Differences between the length of the bonds are less than 0.1 A.

During the interpretation of the measured spectra the bands that are most characteristic of a particular system were found out. Bands with high intensity at the areas 2295-2210cm-1 and 1580-1640 cm-1, assigned the the oscillations of -C≡N, =NH, -NH2 groups, are characteristic bands for the systems (I) and (II). Band 3483 cm-1 corresponds to the stretching vibration q (NH) of the NH2 group of system (I), band 3439 cm-1 corresponds to a vibration of q (NH) of the NH2 group of system (II). Bands with frequencies 2222 and 1543 cm-1 correspondes to the vibrations QR4(C≡N) and βR3(CCH), QR3(CC) systems (III) and (IV), bands with frequencies 959 and 754 cm-1 corresponds to the vibrations of R1 and R3 rings. Bands with frequencies 2208 and 1605 cm-1 correspondes to the vibrations QR4(C≡N), QR2R4(CC), QR3(NO), QR2(CC) and βR2(CCH), βR2 R(CCH) of the systems V and VI, bands with frequencies 1348 and 856 cm-1 corresponds to the vibrations of the R1 ring.

Measured IR spectra of the chromeneopyridinecarbonitrile and pyrido[1,2-a]pyrimidine systems were interpreted and their components I-VI are inentfiedi.

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### S5.354. Study of the structure-activity ratio of a number of chemical compounds with anti-tuberculosis activity

Bogdanova L.G.[1,2]*, Kezin V.A.[2], Anashkina A.A.[1,2]
[1]*I.M. Sechenov First Moscow State Medical University;*
[2]*V.A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences;*
* liliia.bgdnv@gmail.com

The infectious disease tuberculosis continues to be one of the major health problems worldwide. The emergence of new multidrug-resistant (MDR-TB) strains creates a need for modern drugs with improved properties and increased efficacy. One of the creation strategies is the search for new chemotherapeutic agents. In this regard, an urgent task is to search for new chemical compounds - potential drugs by analyzing the patterns of structures in chemical compounds that inhibit the growth and development of M. Tuberculosis strains.

The aim of this work was to analyze existing chemotherapeutic agents based on 5'-norcarbocyclic nucleoside analogs with antituberculous activity and to search for their properties responsible for the activity, as well as to predict new chemical compounds with improved properties.

To study patterns in the structure of chemical compounds with already known activity, the MOE program [1] was used. To describe the model of such compounds, 18 descriptors were chosen based on their statistical significance, in particular: the value of the potential energy, the total SCF energy (kcal/mol), the ionization potential (kcal/mol), etc. Next, using the model, a search was made for compounds with a predicted activity comparable to that of existing anti-tuberculosis drugs.

High anti-tuberculosis activity of seven chemical compounds was predicted, the best of which is Nc1c(C2=C/C(=C\C)/N=C2)cncn1. It is planned to synthesize the predicted compounds and experimentally test their activity against M. tuberculosis bacteria.

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### S5.355. Study of the structure-activity ratio of a number of chemical compounds with antiviral activity

Dokhoyan A.Yu.[1,2]*, Kezin V.A.[2], Anashkina A.A.[1,2]
[1]*I.M. Sechenov First Moscow State Medical University;*
[2]*Engelhardt Institute of Molecular Biology of Russian Academy of Sciences;*
* adnastasyia@gmail.com

In connection with the emergence of new drug-resistant strains of viruses, newly emerging infections, the high prevalence of coinfections and the increased susceptibility of the population to viral diseases, the creation of modern effective antiviral drugs with improved properties has become relevant. Previously, new compounds based on 5'-norcarbocyclic nucleoside analogues were synthesized to inhibit viral targets [1]. The aim of this work was to analyse the structures of chemical compounds with antiviral activity from [1] and to predict new compounds with antiviral activity.

For a comprehensive analysis of the structure-activity relationship (QSAR) of a number of chemical compounds-analogues of nucleosides with known data on antiviral activity, the MOE program was used [2]. 3D structures of chemical compounds with known antiviral activity are modelled in the MOE program and combined into a database.

For all compounds, more than 400 different physicochemical properties (descriptors) were calculated, of which 18 were included in the model describing the activity of the compounds. Among the selected descriptors were, for example, the sum of the areas of the van der Waals surface of hydrophobic atoms and the molecular weight of the compound. The model describes the activities of compounds with high accuracy. Using the libraries of nucleoside compounds and combinatorial libraries, based on the model, a search was made for compounds with potentially higher antiviral activity. Found 131 new chemical compounds with predicted antiviral properties. The highest predicted antiviral activity was found for the compound O=C1[N+@H](CCCCO)C=C2C(=N1)NC(c1ccc(CCCCC)cc1)=C2.

Also, with the help of MOE, 2D structures of the presented chemical compounds were built, a database was created, including the structures of the compounds and the activity obtained experimentally. Using the GUSAR software [3], this database was analysed: a search was made for structural elements responsible for activity. A systematic analysis of the "Structure-activity" ratio in the program made it possible to identify certain patterns in the structure of molecules and identify promising structures with the greatest contribution to the effectiveness of the action.

The results of the work performed can serve as a contribution to the development of a methodology for the chemical structural synthesis of potentially socially demanded drugs based on the use of the relevant properties of isolated chemical compounds. Subsequent synthesis and experimental verification of the predicted compounds are planned.

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### S5.356. The EAATs implication in excitotoxicity formation during elevated neurotransmitter level in glutamate convectional reaction-diffusion simulation

Zagubnaya O.A.[1,2]*, Nartsissov Y.R.[1,2]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Moscow, Russia;*
[2]*Biomedical Research Group, BiDiPharma GmbH, Siek, Germany;*
* oz_brg@icmph.org

Mathematical diffusion modelling of any biologically relevant molecule in nervous tissue is an extremely complicated challenge. Glutamate diffusion in brain parenchyma in vivo is constrained by many aspects. Physicochemical properties of environment and neurotransmitter

molecule itself and numerous implemented in glutamatergic neurotransmission proteins, i.e. receptors and transporters, are amid these aspects. It is proven matter, that disrupted glutamatergic neurotransmission play a pivotal role in neurodegeneration. Glutamate-induced excitotoxicity is mainly linked to an impaired ability of astrocytes to reuptake and respond to elevated glutamate level. This impairment is considered a common hallmark in many neurodegenerative diseases, traumatic brain injury and neuronal apoptosis.

The simulation of such diffusion is usually held within spherically defined synaptic space. The single-synaptic nervous tissue phantom domain with more biologically detailed geometry was reconstructed for more sophisticated results. This domain consists of pre- and postsynaptic neuron endings, synaptic cleft, surrounding astrocytic processes and a space restricted by neuronal and astrocytic plasma membranes. The free space among astrocytic part of the cleft in current work is suitable for numerical estimation of both a laminar interstitial flow and a convectional diffusion of the mediator into the interstitial space of the tissue. Glutamate convectional reaction-diffusion simulation was performed inside the cleft and onward interstitial space according to finite-element method using COMSOL Multiphysics. The inside part of synaptic cleft and free space among astrocytic part of the phantom was employed as simulation area. And different number and allocation of glutamate transporters, namely Excitatory amino acid transporters (EAATs), is included in the digital reconstruction of the nervous tissue phantom in an explicit form. The sufficient amount of EAATs is a general way to avoid excitiotoxic conditions and maintain inter-synaptic crosstalk. Now it was shown, that EAATs precise spatial allocation density with respect to synaptic cleft is also imperative in the setting toxic condition formation during elevated glutamate level.

### S5.357. The effect of the recrystallization procedure on the physicochemical characteristics of glycine and succinic acid: experiment and quantum chemical modeling

Zhulidin P.A.[1]*, Filin P.D.[1], Zakharov A.A.[1], Plastun I.L.[1], Yakovlev R.Y.[2]
[1]*Yuri Gagarin State Technical University of Saratov;*
[2]*LIMITED LIABILITY COMPANY "SCIENTIFIC CENTRE RTA";*
* zhulidin@mail.ru

In recent years, much attention has been paid to the search for means to increase the bioavailability and solubility of drugs to increase their therapeutic effect. The company "SCIENTIFIC CENTER OF THE MOUTH" LLC, which develops new polymorphic modifications of medicinal substances and their co-crystals, recently proposed new schemes for the modification of glycine and succinic acid based on the technology of cryochemical recrystallization of organic substances.

As noted in [1], as a result of recrystallization, solubility, fluidity, wettability, melting point, dissolution rate change and, as a consequence, the bioavailability of modified dosage forms increases in comparison with the main ones.

Succinic acid is a part of plant and animal tissues. It is widely used in medicine [2, 3], in particular, in the treatment of cardiac, neurological and endocrine diseases, as well as in toxicology. Glycine, as a drug, is used in neurological practice to eliminate increased muscle tone, improve metabolism and prevent the death of brain cells after a stroke [4]. Also, the study [5] proved that glycine, when taken orally in sufficiently large dosages (3-9 g), improves the quality of sleep and does not have serious side effects.

The technology of obtaining modified forms of glycine and succinic acid, developed by the SCIENTIFIC CENTER OF THE MOUTH LLC, is a technology of cryochemical recrystallization of organic substances and consists of several defining stages: 1) dissolution of the substance to form a true solution; 2) obtaining molecules in high-energy conformations, which is achieved by exposing the solution to temperature, laser or ultraviolet radiation; 3) cooling / freezing of the solution; 4) freeze drying.

As shown by experimental studies conducted at the SCIENTIFIC CENTER OF THE MOUTH LLC, after modification, there is a significant change in the morphology of glycine and succinic acid crystals. Morphological characteristics were studied using a TESCAN MIRA3 scanning electron microscope (Czech Republic) with a resolution of up to 5 microns (3.0 kV). A significant increase in the specific surface area of substances was found: for glycine from 0.08 m2/g before modification to 6.3 m2/g after modification; for succinic acid from 1.28 m2/g before modification to 4.54 m2/g after modification. During the experiment, photographs were obtained confirming that the nanoparticles of substances underwent restructuring during cryochemical recrystallization. Infrared radiation spectra were recorded on the Spectrum Two IR Fourier spectrometer in the range of 600 - 4000 cm-1 with a resolution of 2 cm-1 at a temperature of 20 °C.

To confirm the experimental data, numerical modeling of the structure and IR spectrum of molecular complexes of the studied substances was performed both separately and with the sequential addition of water molecules to monomers and polymers of glycine and succinic acid. Modeling of the structure and calculation of the spectra of molecules and their complexes were carried out on the basis of density functional theory (DFT) methods using the B3LYP functional and the basic set 6-31++G (3D2F,3P2D) [6]. All molecular modeling procedures, including optimization of molecular structures and calculation of IR spectra, were carried out on the basis of the Gaussian software package. Based on calculations of the structure and IR spectra of the monomer, dimer and tetramer of glycine, a qualitative assessment of its complexation with water was carried out. The analysis showed that with an increase in the number of water and glycine molecules, a large number of hydrogen bonds are formed, the frequency of which coincides with the main frequencies of the water spectrum. At the same time, there is a good agreement between the calculated and experimentally measured spectrum of modified glycine.

A similar study of the IR spectra and structure of succinic acid showed that modified succinic acid contains not only chain associates, but also succinic acid monomers, which indicates the possibility of breaking chains of bonds as a result of the recrystallization procedure. From the comparison of the calculated and experimental IR spectra, the presence of water and its effect on the physicochemical properties of modified succinic acid are clearly noticeable.

It can be concluded that during recrystallization of glycine and succinic acid, despite lyophilic drying, single water molecules remain. this leads to changes in the physicochemical properties of modified substances, expressed in an ultra-high rate of dissolution and to a change in the IR spectrum of the substances under consideration.

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### S5.358. The influence of a biological object spatial polymorphism on metabolites gradients during the modeling of convectional reaction-diffusion

Nartsissov Y.R.[1,2]*
[1]*Institute of cytochemistry and molecular pharmacology;*
[2]*Biomedical Research Group, BiDiPharma GmbH;*
* yarosl@biotic.dol.ru

The essential property of any biological object is a spatial distribution of considered system variables. Concentrations of metabolites involved in biochemical intracellular processes are among the most important characteristics. To describe spatial-time distributions of chemical compounds boundary problems for a parabolic type of partial differential equations (PDE) are usually used. In a common case a convectional term should be also included. It describes the movement of a solvent in some parts of a biological object [1]. In the present study convectional reaction-diffusion of metabolites is modelled using a finite element method (FEM) in COMSOL Multiphysics software. In some cases the formed obstacles yield the changes in a system reply on external influence. It was shown that the appearance of glucose concentration gradients essentially depends on geometrical parameters of neurovascular unit structural elements [2,3], meanwhile the gradient of oxygen is not effected by such a geometric polymorphism. Moreover, a symmetry of vessels geometry bifurcation determines the changes in nervous parenchyma supply under decreased blood flow in a daughter branch. These effects of an object geometry is observed under a subcellular level as well. In particular, a cluster location of glycine receptors on a post- synaptic membrane of a neuron causes a temporal alteration of a local chloride ions concentration. These changes occasion modification of the receptors reply on mediators' release from pre-synaptic vesicles. Similarly, a cluster position of mitochondria in a neuron's body leads to essential fluctuations in an ATP content. Moreover, it expounds the difference in some cells response to chemical compounds affecting oxidative phosphorylation. Thus, a set of mathematical models made for different biological system levels reveals an indispensable role of geometrical features of diffusion space structure during a metabolic gradient forming together with changes of physical-chemical properties of the medium.

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### S5.359. The physical model of the nuclear membrane permeability mechanism

Minasbekyan M.L.[1]*, Badalyan H.G.[1]
[1]*Yerevan State University;*
* minlia@ysu.am

Nuclear cytoplasmic transport is mediated by a large number of receptors that recognize specific signals on proteins and RNA and transport these substrates through nuclear pore complexes. It is known that transport receptors pass through complexes of nuclear pores by the mechanism of facilitated diffusion. The small protein selective filter is a network of unfolded hydrophobic nucleoporin polypeptide chains lining the central channel of the NPC. The main mechanism is Run-dependent transport. Through this mediator, the rapid and targeted transport of thousands of proteins and RNA to and from the nucleus is accomplished.

In this article, we discuss the experimental material we have accumulated on the change in the charge on the surface and inside the nucleus, the main contribution to which is made by anionic phospholipids, and on its basis, we propose a new physical model of the mechanism of RNA transport. The total surface charge created by phospholipids in the nuclear membrane can play an important role in the stability and functioning of the nuclear pore complex, including interaction with positively charged signals of nuclear localization mediating protein import. This model takes into account the contribution of electrostatic fields to the rate of facilitated transport and the change in pore size for facilitated GTP-dependent release of RNA from the nucleus.

### S5.360. The potential of differential scanning calorimetry to study protein-ligand binding

Sedov I.A.[1,2]*, Khaibrakhmanova D.R.[1]
[1]*Kazan Federal University;*
[2]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS;*
* igor_sedov@inbox.ru

Differential Scanning Calorimetry (DSC) is a powerful analytical technique used in biophysics to study the stability and denaturation of proteins. DSC relies on measuring the amount of heat evolved or absorbed upon a physical or chemical transition in a sample as its temperature is raised or lowered. It is commonly used for characterization of the thermal properties of individual proteins at different conditions, design of thermostable proteins, or characterization of monoclonal antibodies and vaccine antigens.

DSC can also able to study the binding interactions between proteins and ligands, which has been done much less commonly and usually in a qualitative manner. Ligand binding to the native protein results in an increase of its denaturation temperature, however, there is no simple relationship between the melting point shift and the thermodynamic binding constant. We developed a program for the numerical simulation of the DSC curve in protein-ligand systems with different binding stoichiometry. The calculations are made for a reversible denaturation model in an assumption that at any temperature during the scan, the folding-unfolding and binding-unbinding equilibria are established. This is true for the slow scanning rates.

Using known thermodynamic parameters of denaturation and binding of several drug substances for bovine serum albumin, we predicted the calorimetric curves for various ligand:protein ratios. Then the experimental thermograms for the same systems were recorded using a capillary DSC instrument. A good reproducibility of the denaturation peak maximum temperatures and the peak areas was observed for the ligand excess below 5:1. Higher ligand concentrations lead to the stronger than predicted temperature shifts which can be explained by the binding to the additional binding sites with weaker affinity.

The inverse problem of determining the binding constants from the DSC data was then solved for a number of drug ligands. We fitted the experimental temperature of the peak maximum and the peak area at different ligand concentrations and determined the first and second sequential protein-ligand binding constants for which the model gives the best agreement. These values were then independently confirmed by the isothermal titration calorimetry measurements.

The results indicate that DSC may help researchers to quantify the interaction strength between proteins and small molecules in order to better understand the mechanism of interaction and reveal the structure-affinity relationships. The insight into the differences in binding affinity among different ligands can be used to design and screen prospective drug candidates in de novo drug design. A significant advantage of the DSC method is its high reproducibility.
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**S5.361. The study of various diets using a mathematical model of carbohydrate-lipid metabolism in adipocytes**

Chistyakova I.A.[1]*, Fursova P.V.[1], Khrushchev S.S.[1], Plyusnina T.Y.[1]
[1]*Lomonosov Moscow State University;*
* chistyakyu@gmail.com

Impaired metabolism of fats and carbohydrates can lead to the development of various pathological conditions and diseases, such as atherosclerosis, type 2 diabetes and obesity. The number of people in the world who are overweight or obese has been growing over the past decades, so research on ways to combat excess body weight is quite active. The most commonly used approach to correct weight and improve metabolism is the selection of dietary nutrition. However, the empirical selection of diets may not always be correct, and sometimes fundamentally erroneous due to the characteristics of the metabolism of a particular person. Mathematical models can describe the processes of transformation of various substances and therefore are a very important tool for the study of metabolism. There are many different models of metabolism, including those applied to the metabolism of lipids and carbohydrates [1], [2], [3]. Such models can be of varying degrees of detail, depending on the goals of modeling, they can include one or more organs and tissues, many metabolites. However, there is a set of metabolites that are found in most models of carbohydrate-lipid metabolism, which include glucose, triglycerides, fatty acids, and insulin as an effector.
In this work, a mathematical model of the carbohydrate-lipid metabolism of the adipocyte was built. The model is a system of thirteen differential equations written according to Michaelis-Menten. The main variables of the model are the concentrations of blood plasma metabolites - glucose, insulin, triglycerides and fatty acids, adipocyte metabolites - glyceraldehyde-3-phosphate, fatty acids and fat droplet triglycerides, as well as fats, proteins and carbohydrates, measured in kilocalories, absorbed in the process of nutrition. In the model, insulin acts as an effector: it activates reactions aimed at the formation of lipid droplet triglycerides and inhibits the reactions of their breakdown. The model was verified on three types of data [2], [4] and showed a high level of agreement with experiments. Next, the behavior of the model was studied with a change in the number of meals, as well as with a different ratio of fats and carbohydrates in food. Two, three and five meals a day were considered, for each diet the effects of three types of diets were investigated: low-carbohydrate-high-fat, low-fat-high-carbohydrate, and the diet described in the article, according to which the verification of the model was carried out. It has been shown that with a high fat content in the diet, two-, three- and five meals a day leads to a decrease in the concentration of triglycerides in the fat drop. With normal and carbohydrate meals, two meals a day leads to a decrease, and five meals a day to an increase in the concentration of triglycerides, while three meals a day has a slight effect on their concentration.
It has been hypothesized that the differences in triglyceride dynamics in the fat droplet at different numbers of meals are due to the unequal insulin response. The production of insulin in response to an increase in glucose and the entry of glucose into the cell are interdependent processes that activate each other. Despite the same amount of carbohydrates absorbed per day with different frequency of nutrition, the amount of insulin formed per day varies. This is due to the non-linear dependence of the insulin response to an increase in blood glucose concentration. This dependence is a function with saturation, that is, when the glucose level rises above a certain concentration, the insulin response ceases to change. Thus, with two and three meals a day, although the amplitudes of the peaks of glucose concentration after meals differ greatly, the amplitudes of the peaks of insulin concentration practically do not differ, but in one case there are two peaks, and in the other three, due to which an unequal insulin response is observed.
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**S5.362. Three types of neuron models for modeling epileptiform activity**

Sysoev I.V.[1,2]*, Kapustnikov A.A.[1,2], Sysoeva M.V.[1,3]
[1]*Saratov Branch of Kotel'nikov Institute of Radioengineering and Electronics of RAS;*
[2]*Saratov State University;*
[3]*Yuri Gagarin State Technical University of Saratov;*
* dr.ilya.sysoev@yandex.ru

Epilepsy is a network phenomenon caused primarily by pathological changes in the organization of neural networks in the brain, often in very small subnetworks [1]. Absence epilepsy is a mild form. The seizures are accompanied by a short-term loss of consciousness and are expressed on the electroencephalogram as spike-wave discharges. Since to understand its mechanisms it is necessary to measure the activity of deep brain structures, the main results were obtained on genetic rats models [2]. Since absences end abruptly and no specific termination mechanism has been found [3], one of the main ways to model them is to represent spike-wave discharges as long transient processes [4]. Since the effects of generation of spike-wave discharges are considered primarily as network effects, the simplest of the known ones, most often the FitzHugh-Nagumo model, were taken as models for individual neurons and their groups. However, if the effects observed in [4] are indeed network effects, they should be repeated when using different equations for a single network element.
In this paper, we consider small network models of the epileptic focus, consisting of 14 neurons. Three popular models are used for nodes: Hodgkin-Huxley, Morris-Lecar and FitzHugh-Nagumo models [5]. A total of 88 models were considered, differing in the details of the architecture of connections built on the basis of the original model [6]. Both linear relationships and more physiologically substantiated ones, through the hyperbolic tangent, were considered. For each model for each type of node, a bifunctional analysis was carried out in terms of the connection parameter. For a significant number of models, it was possible to detect a saddle-node bifurcation of the limit cycle, at which the birth of a limit cycle of finite size occurs, coexisting with the equilibrium state. At the threshold of the birth of such a cycle, there were long quasi-regular transient processes. They can be caused

by a short-term external driving, as shown in [4,6], for example, from the trigeminal nerve. Such transient processes are considered by us as models of spike-wave discharges.
For each model, an analysis was made of the dependence of the transient process duration on the phase of driving. We selected the models which demonstrated the longest transient processes. It turned out that there are several connectivity matrices, when using which the generation of long transient processes occurs for all three neuron models used. In this case, the duration of the discharges ranges from several tens to several hundreds of oscillations, which is in good agreement with the average discharge durations described in the literature [2]. This proves that the mechanism for generating spike-wave discharges proposed in [6] is not only common for a number of models from the same class, but also relatively insensitive to which equations are used to describe an individual network node.
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### S5.363. Tissue-engineered constructions in biophysics, neurology and medicine

Reutov V.P.[1,2]*, Davydova L.A.[3], Sorokina E.G.[4]
[1]*Institute of Higher Nervous Acnivity and Neurophysiology of RAS;*
[2]*Institute of Higher Nervous Acnivity and Neurophysiology of RAS;*
[3]*Belarusian State Medical University;*
[4]*National Medical Research Center for Children's Health of the Ministry of Health of the Russian Federation ;*
* valentinreutov@mail.ru

The goal of tissue engineering is the construction and cultivation of living functional tissues or organs outside the human body, for subsequent transplantation to a patient in order to replace or stimulate the regeneration of damaged organ or tissue. Since the 1960s, scientific research has been carried out in this field in the laboratory of morphology of the Institute of Physiology of the National Academy of Sciences of the Republic of Belarus under the guidance of an outstanding Belarusian scientist - embriologist and neuromorphologist academician, doctor of medical sciences, professor and laureate of the USSR State Prize - D.M. Golub. Thus, the methods of gangliopexy - transpantation of autonomic ganglia on a neurovascular pedicle in order to create new centers of local neurohumoral regulation of neurogenically affected internal organs, organopexy with suturing of the small intestine to the wall of the denervated bladder and truncopexy, when the distal end of the cut nerve is implanted into the organ, have become revolutionary methods of rehabilitation of patients of different ages. The autors and developers of these methods were Belarusian scientists. There are materials in the literature pointing to the important role of the vascular factor in organ transplantation. Most researchers consider the degeneration of neurons in the transplanted ganglion as a violation of its blood supply and the occurrence of a state of hypoxia/ischemia. The authors analyze the possible role of reactive forms of nitrogen (NO/NO2) and oxygen (O2) in the mechanisms of neuronal damage in transplanted ganglion in the event of disruption of nitric oxide and superoxide anion radicals cycles under hypoxic/ischemia conditions. One of the ways to reduce the degree of damage to the neurons of the transplanted ganglion could be to improve the degree of oxygen deliveryto problem areas and reduce the nitrite reductase activity of heme-containing proteins, which contribute to the reduction of nitrite ions to NO. Food products with low content of nitrates/nitrites can contribute to the decrease in the nitrite reductase activity of heme-containing proteins, as well as shifts of the pH towards more alkaline values of the pH, at which R-conformers of Hb complexes with oxygen are stabilized. In this regards mineral hydrocarbamate water (Borjomi, Narzan, Essentuki №4 and №17) can be considered as an additional favorable factor in the recovery of patients after surgery using gangliopexy methods.

### S5.364. Topological features of proliferative and non-proliferative epithelial monolayers

Roshal D.S.[1]*, Azzag K.[2], Fedorenko K.K.[1], Rochal S.B.[1], Baghdiguian S.[3]
[1]*Southern Federal University;*
[2]*University of Minnesota;*
[3]*Université de Montpellier;*
* rochal.d@yandex.ru

In the early stages of embryonic development in mammals, plane epithelial monolayers can form structures of complex geometry. For example, the primitive endoderm transforms into the yolk sac, which performs many functions in the embryo. A change in shape also occurs during the formation of epithelial tubes, which may become part of gland, lungs, kidney and other organs of animals and humans. The buckling observed during the formation of intestinal villi is another example of the flat structure transformation.
This study is devoted to the topological aspects of changes in the shape and curvature of the epithelium. The main objectives of the work: to study the global and local topological characteristics of flat and spherical proliferative monolayers of monkey kidney cells (COS), as well as to compare the results with the characteristics of the non-proliferative epithelium of different species of ascidians and various model (theoretical) cell packings.
In this work more than 50 images of flat and spherical monolayers of COS cells obtained using a laser confocal microscope were analyzed. A program, that reveals the centers of cell nuclei in the images and draws a Delaunay triangulation on them, has been written. We calculate the topological charges of cells (q=6-i, where i is the number of cell neighbors), and construct cell distributions by the number of neighbors (CDNN). It is found that the percentage of cells with 6 neighbors is 41% and 36% in spherical and flat monolayers, respectively. This result is in good agreement with experimental data for other proliferative epithelia [1]. The difference in the number of cells with 6 neighbors in flat and spherical monolayers can be associated with different conditions for their growth: flat monolayers were seeded in ordinary Petri dishes,

and spherical ones were seeded in hydrophobic ones. It is shown that the main difference in the topology of flat and spherical monolayers is the presence of asymmetry in CDNN. In particular, the curvature of the sphere leads to the fact that the proportion of cells with five neighbors is greater in the spherical epithelium, and the proportion of cells with seven neighbors is smaller in the plane one.

Also, a new method of pair correlation functions has been developed for a deeper analysis of the monolayer topology. It compares the probabilities to be the neighbors of a cell with number of neighbors i with a cell with number of neighbors j in random theoretical packing and in a real monolayer. Detailed theoretical calculations will be presented in the report. It has been shown that both in spherical and flat COS cell monolayers, there is a tendency for cells with topological charges of different sign to be located near each other. Similar tendencies in abiotic structures, for example, in spherical colloidal crystals [2], lead to the formation of linear topological defects (scars and pleats), i.e., chains of particles with 5 and 7 neighbors. In the proliferative epithelium, it is impossible to distinguish individual topological defects, since their topological defectiveness (the number of cells with a nonzero topological charge) is much higher.

A similar topology analysis is also performed for 140 samples of nonproliferative epithelial monolayers with spherical geometry covering the larvae of 8 different ascidian species [3]. CDNN, as well as pairwise correlation functions, are calculated. It has been shown that the ascidian epithelium is less topologically defective than the proliferative epithelium. In monolayers of ascidians, a noticeable tendency to agglomeration of 6-valent cells was revealed, which is not observed in monolayers of COS cells. We attribute this fact to the presence of gap junctions, that carry out the exchange of substances between different cells, in the epithelium of ascidians, but not in monolayers of COS cells. Also, in the ascidians, there is a more pronounced tendency of cells with a topological charge of different sign to be located near each other.

The morphological features found in monolayers of COS cells and ascidian epithelium were also compared with the features of model structures, which are a random packing of cells on a sphere, and less random packings with a smaller spread of cells in their sizes. It is shown that the more highly ordered the model packing of cells, the greater the correlation between cells with 5 and 7 neighbors. The tendency to agglomerate of cells with 6 neighbors, observed in the epithelium of ascidians, is completely absent in all model structures.

Thus, in this work, the features of the topology of flat and spherical monolayers of COS cells, as well as the spherical epithelium of ascidian eggs, were considered. We propose that in normal epithelium, specific structural features, including specific topological defects and certain pairwise correlations, generate a subtle relationship between order and disorder that determines the elasticity of epithelial tissue throughout morphogenetic processes. The topological methods developed in this work can also be useful for creating computer algorithms for finding differences in topology between images of cancerous and healthy tissues [4].

The study was supported by the Russian Science Foundation grant No. 22-72-00128, https://rscf.ru/project/22-72-00128/.

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**S5.365. Variability of the bacterial rpsA gene in the polyfunctional family of ribosomal proteins S1 with structural repeats**

Galzitskaya O.V.[1]*, Machulin A.V.[2], Deryusheva E.I.[3], Kravchenko S.V. S.V.[4], Surin A.A.[5], Glyakina A.V.[1], Grishin S.Y.[1]

[1]*Institute of protein research, Pushchino, Russia;*

[2]*Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research, Pushchino, Russia;*

[3]*Institute for Biological Instrumentation, Federal Research Center "Pushchino Scientific Center for Biological Research, Pushchino, Russia;*

[4]*Institute of Environmental and Agricultural Biology (X-BIO), Tyumen State University, Tyumen, Russia;*

[5]*MIREA, Russian technological university, Moscow, Russia;*

* ogalzit@vega.protres.ru

Multiple copying of the same structural domain along the protein chain have been found in many biologically important proteins, such as, for example, integrin α, ankyrins, interleukin-1s, a number of trypsin inhibitors, and also in many protein toxins. The copying of domains is considered to be a "successful evolutionary strategy". Such proteins have a regular conserved secondary structure, while forming three-dimensional structures of various sizes and expanding the surface area available for binding. In this case, the limitations of interactions with ligands associated with the no conservatism of the primary structure of the protein are removed precisely by multiple copying of structural repeats. It is assumed, that protein domain repeats arise due to tandem gene duplications, but the mechanisms of this process have not yet been fully studied.

The polyfunctional ribosomal protein S1 encoded by the rpsA gene is part of the 30S subunit of the ribosome and plays an important role in the initiation of mRNA translation and elongation. The family of the ribosomal protein S1 accounts for approximately 20% of all bacterial proteins containing the S1 domain. A distinctive feature of this family is the multiple copying of structural domains in prokaryotes.

To study the variability of the bacterial rpsA gene, we examined 1324 sequences of this gene in the family of the ribosomal protein S1, with a different number of S1 structural domains (http://oka.protres.ru:4200). The rpsA gene encoding full-length S1 proteins containing from one to six S1 domains are 42%, 38%, 56%, 56%, 44%, and 58% identical to each other, respectively. At the same time, the analysis of consensus sequences showed that the regions of the rpsA gene encoding individual S1 domains do not have a strictly repeating structure between groups containing a different number of S1 domains. The part of the rpsA gene encoding the central domains in multidomain S1 proteins is more conserved than the terminal domains. This finding correlates with the fact that the duplication is found predominantly in the central region of the protein chain between other repeats. The third domain of six domain containing proteins has the most conserved consensus gene sequence compared to the others. This is in line with our assumption that this domain should be considered as the most conservative. The consensus sequence of the rpsA gene for all separate domains contains some conserved parts (nTTCGTnGAn, nTnGnn, nGAnnTn) corresponding to three (out of five) conserved residues on the surface of the S1 domain, which forms the RNA binding site in the bacterial, archeal, and eukaryotic protein containing the S1 domain. Thus, the tertiary structure of separate S1 domains (conservative OB-fold) plays a key role for the functioning of the family.

Analysis of the sequences before the start codons of the rpsA genes showed that the translation initiation region of the rpsA gene can be folded into the structure proposed for E. coli. We have found such rpsA gene translation initiation regions having structural similarities and some features of conserved sequences in representatives of the phyla Terenicutes, Bacteriodites, Cyanobacteria, Firmicutes, and Actinobacteria. All sequences contain a degenerate Shine-Dalgarno sequence (GAAG) in hairpin III with the start codon AUG. In addition,

the hairpins are separated by extended areas rich in A/U (ss1 and ss2). Found phylogenetic similarity suggests that the proposed fold of the rpsA gene translation initiation region in E. coli has a functional value and, most likely, is important for the translational control of rpsA gene expression in other phyla of bacteria, and not only in gamma proteobacteria.

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# S6. Biophotonics. Photobiology. Photosynthesis. Bioluminescence. Photoreception. Optogenetics

### S6.366. Comparison of PAM-fluorometry and hyperspectral imaging methods in presymptomatic detection of viral infection in Nicotiana benthamiana plants

Grishina A.I.[1]*, Zhavoronkova A.S.[1], Grinberg M.A.[1], Khlopkov A.D.[1], Ageeva M.N.[1], Brilkina A.A.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* 79159532707@yandex.ru

Phytopathogens are one of the significant causes of yield loss. Despite the fact that currently in agriculture there are mainly plant varieties that have a fairly high level of spread to infection with pathogens and their harmful effects, a decrease in crop yields by 10–20% is regularly observed in everything due to plant diseases, which poses a serious threat to global food security. multiple detected detections at once, detection of detection foci when symptoms are detected, and timely detection of diseases with pathogens.

The aim of this work is to compare the methods of PAM fluorimetry and hyperspectral imaging in the early detection of viral diseases in plants.

A stock study of four-week-old Nicotiana benthamiana plants that observed the spread of PVX virus highly cross-linked to a protein. Infection of plants is carried out with the help of agorobacterium infiltration of the fourth true leaf. PAM fluorometry, hyperspectral imaging, and RGB photography have been blamed as methods for detecting accusations. Measurements were made every day at the same time for 10 days using the IMAGING-PAM M-Series MINI and Specim IQ hyperspectral sensor.

In a non-inoculated (10 leaves) tobacco leaf, infection-induced chlorophyll fluorescence parameters (quantum yield of photosystem II (YII) and non-photochemical fluorescence quenching (NPQ)), as well as reflection spectra of affected and infected parts of the leaf, were recorded. After 12 minutes of plant adaptation in the infected area of the non-inoculated leaf, there is a significant difference in stationary YII and NPQ values compared to those in the healthy area of the same leaf. In contrast to stationary indicators, the dynamics of YII and NPQ, due to the inclusion of actinic light in infected and infected leaf areas, significantly increased in nature from the second days after infection with the virus in a non-inoculated leaf. The frequency of the reflection spectra of diseased and infected plants is also important.

Results have been obtained that may lead to the detection of infection virus in tobacco leaves. In particular, a clear distinction between healthy and infected areas based on the YII trait within 40–60 s after actinic light is turned on and available 20–40 s based on NPQ.

The analysis of the spectra showed that there are differences between the infected and healthy leaf area. infections are found in several regions of the virus. It seems to be optimal to use the normalized reflective index to eliminate the exception in the absolute value of the signal frequency. Comparison of the detection results showed that PAM is a more sensitive method and with the help of its properties, a viral infection can be already 1-2 days after the arrival of the virus in the leaf.

The work was carried out during the implementation of the project of the NTsMU "Photonics Center" with the Russian financial support of the Ministry of Science and Higher Education of the Federation, contract No. 075-15-2020-927.

### S6.367. A novel method for the determination of the optical spectral density of pigments; modelling of the absorption of carotenoids and porphyrinic dyes

Kurkov V.A.[1,2]*, Chesalin D.D.[1], Razjivin A.P.[3], Pishchalnikov R.Y.[1]
[1]*A.M. Prokhorov Institute of General Physics of the Russian Academy of Sciences;*
[2]*Moscow Institute of Physics and Technology ;*
[3]*Lomonosov Moscow State University, Belozersky Research Institute of Physico-Chemical Biology;*
* v.k27@yandex.ru

The use of semiclassical quantum theories of the interaction of electromagnetic radiation with matter to model the optical properties of photosynthetic monomeric pigments has shown [1,2] that the use of optimization algorithms allows not only calculating with a high degree of accuracy the absorption line profile of the electronic transition of the molecule, but also assessing the degree of statistical significance of the found parameters of the system under study. Taking into account the results of previous studies, we have proposed a method for the analysis of electron-phonon interactions for any organic molecule in solvents that absorbs in the visible range. The key computational object is the notion of generalized spectral density - the characteristic function of electron transition of a molecule with the help of which the correlation functions of dipole moment of electron transition are calculated. In our previous works to fit the experimental data we used as free parameters only Huang-Rhys factors - effective parameters of electron-phonon interaction, while the vibronic mode frequencies were taken from Raman scattering experiments. The new method is able to calculate absorption spectra and, at the same time, estimate the spectral density parameters just by the form of the electron transition spectrum. Moreover, the application of an evolutionary optimization algorithm makes it possible to estimate the statistical significance of the found quantum model parameters. Thus, the method is an effective tool for simulating the optical properties of organic molecules and allows the creation of quantum models of monomeric pigments and the use of the data obtained to calculate systems of interacting molecules and crystals. This study presents the results of processing of absorption spectra of carotenoids, porphyrin dyes and analysis of the obtained data.

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### S6.368. Analysis of reflectance spectra of healthy and infected leaves of Nicotiana Benthamiana

Zhavoronkova A.S.[1]*, Grishina A.I.[1], Grinberg M.A.[1], Ageeva M.N.[1], Brilkina A.A.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* ann.zhavoronkova38.95@gmail.com

Plant diseases caused by pathogens such as viruses, bacteria and fungi cause significant yield losses. For example, for world production of wheat and rice they are 21% and 30% respectively. Globalization, expansion of world trade, and population mobility are breaking down geographic barriers that have kept plant communities and their pathogens isolated, resulting in an increase in alien pests and their rapid spread. Spectrum analysis is necessary for the most effective presymptomatic detection of pathogens in plants, which in turn is important for reducing the spread of disease and facilitating plant protection, as well as for food security due to global population growth. The idea is not to treat diseases after symptoms appear, but to continuously monitor plant health and to eliminate the pathogen right at the point of onset of infection.

The purpose of this work is to analyze the reflectance spectra of healthy and infected leaves.

Studies were performed on four-week-old Nicotiana benthamiana plants in which the PVX virus, which has the fluorescent protein GFP cross-linked to the coat protein, was observed to spread. Infection of the plants was performed by agrobacterial infiltration of the fourth true leaf. Hyperspectral imaging and RGB photographs were used as methods of infection detection. Measurements were taken every day at the same time for 10 days using a Specim IQ hyperspectral sensor.

The whole plant was photographed with a hyperspectral camera under constant artificial light, then the reflection spectra of tenths of uninoculated tobacco leaves in healthy and infected parts as well as tenths of leaves of uninoculated plants were obtained. Differences in the spectra between healthy and infected regions were recorded in different parts of the spectrum, namely, at 530-550 nm and 700-750 nm. The differences increase every day.

The results obtained allow us to choose optimal and efficient reflectance indices for the detection of viral infection, which in turn makes it possible to select light filters for much more financially advantageous multispectral sensors.

Possible reasons for changes in the reflectance spectrum of leaf tissue during infection are discussed.

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### S6.369. Analytical system based on mKate2-Kv1.3 channel and Atto488-Hongotoxin for the study of peptide blockers

Orlov N.A.[1,2]*, Ignatova A.A.[2], Kryukova E.V.[2], Yakimov S.A.[2], Kirpichnikov M.P.[1,2], Nekrasova O.V.[2], Feofanov A.V.[1,2]
[1]*Lomonosov Moscow State University;*
[2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS;*
* n.orlov858@yandex.ru

The voltage-gated potassium channel Kv1.3 plays a key role in vital cellular processes: it provides the conduction of potassium ions through the membrane, participates in the propagation of action potential in muscle cells and neurons, it is associated with cell proliferation and migration. Kv1.3 is involved in the pathogenesis of some oncological [1], autoimmune [2], and neuroinflammatory diseases [3].

Pore blockers of this channel - organic compounds and peptide toxins, are considered as promising therapeutic agents for the treatment of pathologies associated with overexpression or increased activity of the channel [4, 5], and also serve as tools for studying its structure and functioning [6]. The search and study of new blockers require an effective system for assessing their ability to bind to the channels.

Expanding the variety of methods for studying the affinity of pore blockers, we report on the development of an analytical system for measuring the affinity of blockers to Kv1.3 expressed on the membrane of mammalian cells [7]. The components of the system are the peptide Hongotoxin 1 conjugated with the fluorophore Atto488 (A-HgTx), and the human Kv1.3 channel fused with the red fluorescent protein mKate2. Confocal microscopy is used for measurements, and a special image processing protocol has been developed to evaluate the results.

The cellular distribution of Kv1.3 channels fused with mKate2 at the N- or C-terminus (K-Kv1.3 and Kv1.3-K, respectively) during their transient expression in HEK293 cells was studied using fluorescent markers of cellular organelles. It was found that the position of mKate2 in the structure of the merged protein affects the localization of the channel in cells. The representation of the channel on the membrane is much higher in the case of K-Kv1.3, while for Kv1.3-K, the predominant accumulation of the channel in the cytoplasm is observed. To test the possibility of the previously described additional amplification of the membrane localization of Kv1.3 by removing its N-terminal fragment (which does not change the properties of the channel conductivity and the affinity of pore blockers [8, 9]), we created a plasmid encoding a truncated Kv1.3 fused at the N-terminus with mKate2 (K-ΔKv1.3). It is shown that K-ΔKv1.3 is characterized by increased expression in the membrane, and the overall distribution in the cell is similar to the distribution of K-Kv1.3.

Electrophysiology data have shown that K-Kv1.3 expressed in cells is a functionally active voltage-gated channel that is blocked by specific Kv1.3-channel blockers.

All three investigated variants of the Kv1.3 channel bind A-HgTx on the membrane of living cells. This binding is observed at nanomolar concentrations of A-HgTx and does not lead to noticeable changes in the membrane distribution of Kv1.3 channels. Washing cells with fresh medium leads to rapid dissociation of A-HgTx from the cell membrane. An excess of unlabeled HgTx1 displaces bound A-HgTx from the cell membrane. No binding of A-HgTx to the membrane of intact HEK293 cells was observed. Thus, the binding of A-HgTx is specific and reversible. It was found that the binding of A-HgTx to Kv1.3 is concentration-dependent and saturable, and the dissociation constant of the complexes amounts to 0.48±0.08 nM.

Competitive binding experiments have shown that A-HgTx is displaced from complexes with K-ΔKv1.3 on the membrane of living cells by various known peptide pore blockers of the Kv1.3 channel, including recombinant HgTx1, Charybdotoxin (ChTx), Agitoxin 2 (AgTx2), and Kaliotoxin 1 (KTx1), which were originally found in the poisons of various scorpions. A-HgTx is also displaced by the nonspecific pore blocker tetraethylammonium (TEA), which binds to both the inner side of the pore and the outer vestibule of the Kv channels. Data from competitive binding experiments were used to calculate the apparent dissociation constants of complexes, which equaled to 0,2±0,1; 4±2; 7±4 1,1±0,3; (2±1)×106 nM for peptides HgTx1, ChTx, AgTx2, KTx1, and a non-specific blocker TEA, respectively.

The results of our experiments demonstrate that with due attention to the factors affecting the interaction of blockers with the channel, the developed analytical approach based on confocal fluorescence microscopy of A-HgTx complexes with K-ΔKv1.3 channels on the membrane of living cells allows screening of Kv1.3 blockers that bind to the extracellular vestibule of K+-conducting pores, and analyze their affinity.

The presented approach complements the arsenal of techniques in the field of ion channel research and can potentially help in expanding the library of peptide blockers of the Kv1.3 channel, which are promising candidates for the development of therapeutic agents based on them. We believe, that by varying the fluorescent ligand and the type of channel expressed, the developed approach can be used to study interactions between different groups of peptide blockers and ion channels at the cellular level.

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### S6.370. Application of silicon nanoparticles for nanothermometry at the nanoscale

Gerasimova E.N.[1]*, Timin A.S.[1], Zyuzin M.V.[1]
[1]*ITMO University;*
* elena.gerasimova@metalab.ifmo.ru

In the Russian Federation, according to the Ministry of Health, about 10,000 new cases of melanoma are diagnosed every year. Modern methods of therapy for this disease are based either on surgery or on chemotherapy. However, these methods are associated with numerous side effects and can be ineffective. An alternative to these methods of treatment can be photothermal therapy.[1] The idea of this approach includes the nanoparticles accumulation in the tumor zone and their subsequent irradiation by laser radiation for heating. However, excessive temperature changes strongly affect cellular functions such as cell division, gene expression, growth factor activity, and metabolism.[2] Thus, real-time temperature monitoring during external heating is necessary to predict cell behavior, and to prevent possible side effects during therapy. [3, 4]

One of the most used methods of temperature monitoring is Raman stokes spectral shifts, for example, from silicon nanoparticles. They can convert laser radiation into thermal energy. [5,6] Also, such dielectric nanoparticles possess optically induced magnetic and electric Mie resonances, which allows them to amplify the Raman scattering signal, absorb light and stimulate optical heating. All the advantages of silicon nanoparticles make these carriers perspective for oncotherapy without side effects due to temperature control.

First, the diameter of silicon particles for the most effective heating was theoretically calculated, and it was equal to 180 nm. Then silicon particles were synthesized by laser ablation in a liquid. A silicon wafer was used as a substrate, and the ablation was carried out in water. To filter silicon nanoparticles by size, the technology of density gradient centrifugation was used. The next step was to test the optical properties of synthesized silicon nanoparticles. Experimentally measured dark-field scattering spectra showed the resonant nature of nanoparticles.

Further, in vitro experiments were also conducted to evaluate the cell viability and uptake of nanoparticles by the B16-F10 cell line. The cell viability was analyzed using the Alamar Blue test. As a result, it was shown that silicon nanoparticles have almost no toxic effect on cells and cell viability exceeded 90% even with the maximum number of particles (500 mcg/ml, 20 mcl). In turn, to study the uptake of silicon nanoparticles by cells, the carriers were modified with a Cy 5 dye conjugated with bovine serum albumin. The cell membrane was labeled with the Fluorescein dye. The uptake of silicon nanoparticles was studied using confocal laser microscopy. It has been demonstrated that silicon nanoparticles have been successfully uptaken by cells.

The heating of silicon nanoparticles outside and inside cells was studied using Raman stokes spectral shifts. For this, silicon nanoparticles were irradiated with a He Ne laser with a wavelength of 632.8 nm. The power density of the laser radiation was approximately I0 = 2 MW/m2. Raman stokes spectral shift for measurement outside the cells at maximum power was equal to 4 cm-1, which corresponds to a heating of about 120 degrees. However, the shift inside cells was less, about 2 cm-1, which corresponds to a heating of 70 degrees. Such a change in temperature is sufficient for therapy because the process of apoptosis occurs at about 42 degrees Celsius.

Then the biodistribution of the silicon nanoparticles was tested in vivo. The particles were modified with Cy 5 dye and injected locally into the tumor of a laboratory mouse. Then, after 1, 3 and 7 days, the mice were sacrificed, then main organs and the tumor were taken from the body. The biodistribution experiments showed that the particles were localized only in the tumor and did not migrate to other organs.

Thus, silicon nanoparticles can efficiently convert laser radiation into heat. They can be used to avoid overheating of the surrounding cells and tissues due to real time temperature monitoring. In the future, it is planned to perform photothermal therapy and temperature measurement in real time simultaneously on a living laboratory mouse.

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### S6.371. Bioanalytical systems based on firefly luciferase Luciola mingrelica

Lomakina G.Yu.[1]*, Ugarova N.N.[1]
[1]*Lomonosov Moscow State University;*
* lomakinagalina@yahoo.com

Firefly luciferase catalyzes the oxidation of D-luciferin by oxygen in the presence of ATP and magnesium ions, which is accompanied by bioluminescence in the region of 450–600 nm. In our laboratory, various mutant forms of the thermal stable firefly luciferase Luciola mingrelica, differing in their bioluminescence spectra, were obtained, which were successfully used as highly sensitive protein markers for studying specific intermolecular interactions in molecular diagnostic methods. The advantage of bioluminescent systems is the absence of a background signal and the ease of detection - they do not require a source of exciting radiation. The use of thermostable luciferase makes it possible to conduct studies at 37°C and above without changing the activity of the enzyme inside and outside cells for a long time, which opens up great opportunities for working with living cells.
Luciferase as a marker of intracellular processes. Test systems based on living prokaryotic and eukaryotic cells producing thermostable firefly luciferase can be used to study any external factors of a physical and chemical nature that affect their functioning. Bioluminescent cells make it possible to register the initial stages of changes in cell membrane permeability and assess the degree of cell damage in the presence of membrane-active compounds, which is one of the criteria for their viability. Recombinant Escherichia coli cells expressing luciferase were used to study the effect of colistin, a polypeptide polycationic antibiotic of the polymyxin series. Damage to the cell membrane leads to a proportional increase in extracellular luciferase (Aex) activity. The residual concentration of living cells is proportional to the activity of the enzyme inside the cell (Ain). To study the effectiveness of the action of membrane-active saponins (digitonin and its analogs dioscin and protodioscin), we developed a test system for continuous recording of the process of cell membrane permeabilization in real time based on HEK293 cells expressing luciferase, based on the accumulation of luciferase activity (Aex). It was shown that the lytic activity of saponins depends not only on the concentration of the agent and the duration of incubation, but also on its structure - digitonin exhibits high lytic activity due to the formation of complexes with cholesterol, which leads to the formation of pores. However, slight changes in the structure of its analogues - a decrease in the number of carbohydrate cycles in the dioscin molecule or the presence of a bulky substituent in the aglycone part of the protodioscin molecule - leads to a sharp decrease in their lytic activity. Thus, the developed test systems based on living cells can be used for screening drugs and studying the mechanism of their action.
Firefly luciferase as a marker in bioluminescent immunoassay. We have developed a universal method for obtaining highly active, stable and functionally active conjugates of thermostable luciferase with biospecific proteins (albumin, avidin from chicken eggs and antibodies) and demonstrated the possibility of their use in various enzyme immunoassay schemes. To do this, target proteins were attached to the surface SH groups of luciferase cysteine residues using a heterobifunctional crosslinking agent, N-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP), which is specific for luciferase SH- groups and NH2-groups of the protein. The presence of a spacer between the conjugated proteins and the remoteness of the attachment points from the active site made it possible to preserve the mobility of molecules, enzymatic activity, and the ability to bind affinity. The obtained conjugates were successfully tested in bioluminescent immunoassay. Thus, the Luc-Alb conjugate was used in competitive ELISA for the detection of microalbuminuria, which made it possible to detect free albumin in the analyzed sample in the concentration range from 5 to 300 μg/mL. The Luc-Avi conjugate is a versatile affinity tag that can detect any biotinylated macromolecule, such as a biotinylated secondary antibody. Luciferase conjugates with avidin and antibodies were used for enzyme immunoassay detection of Salmonella cells with a cell detection limit of 5 104 CFU/ml. To reduce the detection limit of Salmonella paratyphi A cells, we obtained Luc-AntiSal conjugates and developed a pseudo-homogeneous bioluminescent enzyme immunoassay of bacterial cells using a new matrix for analyte capture - polystyrene microparticles coated with Pluronic F108-PDS, covalently labeled with Sal antibodies. The use of this matrix made it possible to effectively trap the analyzed Salmonella cells in solution and detect them with a detection limit of 2.7 x 103 CFU/ml without prior concentration of the sample. This approach can be used to analyze bacterial cells in biological samples, food and other analyzed objects.

### S6.372. Biological Activity of Nanoparticles. Comparison of Toxic, Antioxidant and Radiomodifying Properties via Bioluminescent Bioassays

Kudryasheva N.S.[1]*
[1]*Institute of Biophysics SB RAS, FRC KSC SB RAS, Krasnoyarsk, Russia;*
* n_qdr@yahoo.com

Presently, the biological effects of nanostructures are of significant interest for biomedicine and environmental technologies. The modifiability of nanoparticle surfaces diversifies their interactions with surrounding media and organisms. This complexity prevents the prediction of nanoparticle bioeffects based solely on physico-chemical characteristics; integral and nonspecific biological assessment methods should be involved. Bioluminescent assays are prospective candidates for the comparison of the biological activity of various nanoparticles (NPs) due to simplicity and high-throughput capacity. In current study, bacterial bioluminescence assays were adapted to monitor and compare bioeffects of different NPs. Cellular and enzymatic assays (luminous marine bacteria Photobacterium phosphoreum and their enzymatic reactions, respectively) were developed and applied to monitor toxicity, antioxidant activity and radioprotective properties of nanocompounds; bioluminescence intensity was applied as a signaling physiological parameter. We studed NPs differed in core structure and surface modification: (1) fullerenols with different cage size, a number of oxygen substituents, involvement of endohedral and exohedral metal, (2) iron oxide NPs with different surface modificators, (3) gold NPs, as well as (4) humic substances - nanostructures of natural origination. Two additional methods were used: reactive oxygen species (ROS) content was estimated with a chemiluminescent luminol method, and bacterial infrastructure was monitored using electron microscopy. The peculiar bioeffects of NPs were explained with hydrophobic interactions involving cellular membranes, electron affinity and disturbing of ROS balance in the bioluminescence systems. Thus, the bioluminescence bioassays, cellular and enzymatic, are appropriate express tools for studying and comparing the bioeffects of NPs and nanostructures.

### S6.373. Bioluminescence of Pleurobrachia pileus (O. F. Müller, 1776) in summer

Temnykh A.V.[1], Silakov M.I.[1], Mashukova O.V.[1]*
[1]*A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (IBSS), Russia;*
* olgamashukova@yandex.ru

The ability to emit light as a result of a biochemical reaction is called bioluminescence and, unlike other types of luminescence, is inherent only in living organisms that have a specialized enzymatic apparatus or

biosubstrate. Bioluminescence is an important element in the functioning of pelagic communities. This is due to the ecological role of light in the life of hydrobionts. Bioluminescence is used for intraspecific and interspecific communication, to attract prey and scare away predators. The ability to bioluminescence, found in many marine aquatic organisms, was also found in representatives of gelatinous macroplankton - Ctenophora. Three species of ctenophores, which play an important role in the Black Sea ecosystem, are also species dominating in plankton biomass. The bioluminescence of two invasive species, Mnemiopsis leidyi A. Agassiz, 1865 (Lobata) and Beroe ovata Bruguière, 1789 (Beroida), has been most thoroughly studied. The luminosity of the third - Pleurobrachia pileus (O. F. Müller, 1776) (Cidippida) is a controversial point. Many aspects of P. pileus bioluminescence are currently unknown.

Biological material for the study was collected at 26 stations on the scientific cruise No. 102 of the R/V Professor Vodyanitsky in the period from 10.06 to 01.07.2018. Samples were collected using the Bogorov-Ras macroplankton net (mesh 400 μm, inlet 0.5 m2). A total of 498 objects 0.4–2.0 cm in size (oral-aboral length) were studied.

The study of the luminescence parameters was carried out on the laboratory biophysical complex "Svet" by the method of mechanical and chemical stimulation of a separate object. As a mechanical stimulator, we used sea water with a volume of 2 ml, injected using a piston device with the creation of a turbulent flow of liquid in the cuvette. Chemical stimulation was carried out by adding 2 ml of 96% ethanol in a laminar flow.

The main energy parameters of the bioluminescent signal are the amplitude (A), the total signal energy (E), the total signal duration (L) is chosen as the time parameter.

It was found that the bioluminescent response of P. pileus in summer to mechanical and chemical stimulation is exhibited by all size groups of this species.

A bioluminescent signal of this type could consist both of separate sharp pulses, unevenly spaced from each other, and represent a single long-duration pulse with multiple peaks-vertices. A clear relationship between the shape of the impulse, the size of the species, and the season was not found. In addition, during chemical stimulation, after which the individual died, the last impulse often had gentle and long rise and fall fronts.

The amplitude values of the bioluminescent signal differ with mechanical and chemical types of stimulation. The average amplitude for all size groups during mechanical stimulation varied from 0.02 to 0.14•10-3 μW•l•cm-2. During chemical stimulation, the minimum mean amplitude of Aav in groups was 0.12•10-3 μW•l•cm-2, the maximum was 0.23•10-3 μW•l•cm-2. The amplitude of the bioluminescent signal in all groups during mechanical stimulation both in the morning and at night was lower than with chemical stimulation. The largest amplitude values were noted in groups with sizes of 12 mm (in the morning) and 18 mm (at night) during chemical stimulation.

In the daytime during mechanical stimulation, the average amplitude for all groups was 0.08•10-3 μW•l•cm-2. In the dark time of the day, it was slightly higher than the morning value and amounted to 0.09•10-3 μW•l•cm-2. With the chemical type of stimulation in the morning and at night, the average amplitude for the groups was 0.15•10-3 μW•l•cm-2, while the maximum values were observed in the size group of 12 mm. For almost all groups in the morning, the signal duration significantly (2-4 times) exceeded that at night, both during mechanical and chemical stimulation.

The duration and energy of the signal at night in most size groups were lower than morning values; this pattern was sometimes violated only for groups with sizes of 14 and 16 mm. The average signal duration Lav for all size groups varied from 1.25 to 2.82 s during mechanical stimulation and from 0.97 to 2.39 s during chemical stimulation. With the chemical type of stimulation, the maximum duration of the signal in different groups reached Lmax=5-6 s.

Comparing the values of the main parameters of the bioluminescent signal of P. pileus with those of B. ovata and M. leidyi, one can notice the similarity in the daily rhythm of the bioluminescent signal amplitude. Thus, in B. ovata, the minimum falls at 10 am and the maximum at 01 am; amplitudes in P. pileus. However, in P. pileus in the summer season, such a clear dependence of the signal amplitude on the size of an individual was not revealed, as in B. ovata and M. leidyi.

In the summer season, with the chemical type of stimulation, on average, in all size groups, the amplitude was approximately two times higher than with the mechanical one. No general regularity was noted for the duration of the signal.

The work was carried out within the framework of the state order No. 121041400077-1 "Functional, metabolic and toxicological aspects of the existence of aquatic organisms and their populations in biotopes with different physicochemical regimes."

**S6.374. Bioluminescent saliva analysis for monitoring body fatigue**

Stepanova L.V.[1]*, Kolenchukova O.A.[1,2], Zhukova G.V.[1]
[1]*Siberian Federal University, Krasnoyarsk, Russia;*
[2]*Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russia;*
* slyudmila@mail.ru

The problem of preventing fatigue of students is relevant for any age and different educational institutions. An express assessment of the state of health is proposed - enzymatic bioluminescent testing of salivary fluid. A change in the intensity of luminescence in the blue-green region of the spectrum is recorded due to the inhibition of the enzymatic reaction when the biochemical composition of saliva is exposed to the substrates of bioluminescent reactions and the activity of enzymes of luminous bacteria: NADH:FMN-oxidoreductase and luciferase. An important characteristic of the biotest is noninvasiveness and expressiveness, which allow painlessly and quickly monitoring the state of the body by changing the level of luminescence when loads appear on the body. Detection of the body's reaction using an integral bioluminescent saliva indicator can be presented as a reliable tool for determining the fatigue of the body.

The purpose of the work is to assess the degree of fatigue of the body of students and to identify the causes of its occurrence after the training load.

The study involved three groups of subjects from Krasnoyarsk: Group 1 – students of the 8th grade of Gymnasium No. 13 "Akadem" (n=15), group 2 - students of the 10th grade of Lyceum No. 1 (n=22), group 3 – students of the 1st year of the Federal State Educational Institution named after Prof. V.F. Voino-Yasenetsky (n=30). Among the subjects there were girls and boys who had different academic performance.

The functional state of the body of the participants was examined using bioimpedance analysis of body composition (Tanita BC-587, Japan), the physical development of the body was examined by employees of the Laboratory of Sports and Tourism of the Siberian Federal University, visual acuity - by employees of the Eye Center of Krasnoyarsk.

All participants were tested before and after the academic load, i.e. for schoolchildren – on one of the school days, for students – in one semester exam. The material of the study was salivary fluid, which was collected by self-spitting.

Bioluminescent testing was carried out using the author's platform technology of bioluminescent enzymatic biotesting (RF Patent No. 2017106705). Testing was carried out on a flatbed luminometer (TriStar LB 941, Germany) using a complex of reagents "CRAB" (IBF SB RAS, Krasnoyarsk).

The biotest reaction was determined by the level of quenching of the bioluminescent glow in comparison with the control - an integral bioluminescent indicator. The indicator before the training load was considered as the norm, after the training load – the indicator with the

load. Bioluminescent saliva indicators before and after the training load were compared and the difference in the bioluminescent saliva index was determined (the indicators after and before the training load were subtracted). The magnitude of the difference in the bioluminescent saliva index showed the degree of overload (fatigue) of the body. A small difference in the bioluminescent index indicated less fatigue in the body of students.
Statistical data processing was performed in the Statistic program. The reliability of the differences was calculated by the Wilcoxon criterion, the correlation was calculated by the Spearman criterion.
The results of bioluminescent testing showed that the bioluminescent saliva index of all students after the training load is significantly lower than at the beginning ($p>0.05$), i.e. the general condition of the students has changed. The higher the academic status of the institution, the lower the bioluminescent saliva index of students
Students (regardless of gender) with low academic performance had a reduced bioluminescent indicator after the academic load and an increased one for students with high academic performance.
Analyzing the difference in the bioluminescent saliva index after and before the training load, two groups of trainees with varying degrees of fatigue were identified.
The first group included students with a positive difference (when the bioluminescent indicator is higher after the training load, than before the load) or a negative difference (when the bioluminescent indicator is lower after the training load, than before the load), where the magnitude of the difference showed the overload of the body. The second group consisted of students with a zero difference in the bioluminescent index, which indicated the absence of fatigue of the body.
It was revealed that the difference in the bioluminescent saliva index is interrelated with the physiological data of the body. There is no difference in the bioluminescent saliva index for students with physiological and functional indicators within the normal limit. The positive difference is characteristic of the students who had a low fat content ($r=-0.5$; $p<0.05$), a normal dynamic strength index ($r=0.5$; $p<0.05$) and low visual acuity ($r=0.5$; $p<0.05$). The negative difference is characteristic of students with a lack of muscle mass ($r= 0.4$; $p<0.05$), high blood pressure ($r=-0.5$; $p<0.05$) and low visual acuity ($r=0.5$; $p<0.05$). Thus, it is shown that a positive or negative difference in the bioluminescent saliva index of students, determined by a comparison after and before the training load, revealed the presence of overload in the body, and the magnitude of the difference in the bioluminescent saliva index indicated the degree of fatigue of the body. The degree of fatigue of the students' body depended on the state of functioning of the body. The academic load at school or university did not tire at all or tired least of all the students who had normal functional and physical development.
1. RF Patent No. 2017106705. A method for determining the level of stress resistance of a person. Kratasyuk V.A., Zhukova G.V., Kolenchukova O.A., Sutormin O.S., Esimbekova E.N., Gulnov D.V., Stepanova L.V. Publ. 28.08 2018.

### S6.375. Biosensors and enzymatic bioassays based on enzymes of luminous organisms

Kratasyuk V.A.[1,2]*
[1]*Institute of Basic Biology and Biotechnology;*
[2]*Institute of Biophysics, Siberian Branch of Russian Academy of Sciences;*
* VKratasyuk@yandex.ru

Historically, the application of bioluminescence in toxicology began with the usage of luminous bacteria and they are still widely used. As opposed to other test objects such as paramecia, algae, and so on, the luminous bacteria assay is faster (< 30 min). However, as with other organisms, luminous bacteria is petulant. The failure to maintain the stable state of bacterial culture during measurements and storage results in low accuracy of measurement, a clear disadvantage of this method caused by the "petulance". The bacteria react to the toxic substances either by decreasing or by increasing the luminous intensity, often leading to ambiguous interpretation of results. Because of these shortcomings the luminous bacteria assay didn't show reliable results. The new approach to develop the bioluminescent enzymatic biosensors, toxicity bioassays and reagents has been described. To solve the problem of how to detect, identify, and measure the contents of the numerous chemical compounds in environmental monitoring, food product monitoring, and medical diagnostics, the bioluminescent enzymatic toxicity assays were proposed, wherein the NAD(P)H:FMN-oxidoreductase +luciferase substitutes for living organisms. The immobilized reagent Enzymolum was introduced to facilitate and accelerate the development of cost competitive enzymatic systems for use in biosensors. Prototype biosensors offer cost advantages, versatility, high sensitivity, rapid response, extended shelf and flexible storage conditions. Due to the coupling with luciferase, it is possible to design new enzymatic bioassays in toxicology and combine them into a set. The set includes key enzymes of metabolic processes such as LDH, trypsin and others. The set of bioluminescent enzymatic toxicity assays was used for monitoring natural and laboratory aquatic ecosystems, soil contamination, as well as for toxicity analysis of pesticides and sanitary assessment of nanomaterials. The new possibilities of enzymatic bioassays are discussed. We are ready to say and discuss the problems which were arising during developing this new approach. At the beginning it was very hard to discuss with our peers use the new perspective as key to our idea. But even now when we win we have problems with understanding of the new approach and wish to show the way and history of developing bioluminescent bioassays from idea to commercial products.

### S6.376. Chemiluminescence analysis of the radical-scavenging capacity of arabinogalactan-stabilized silver nanoparticles

Zvereva M.[1]*, Hiteva T.[1], Zhmurova A.[1]
[1]*A.E. Favorsky Irkutsk Institute of Chemistry SB RAS;*
* mlesnichaya@mail.ru

Supporting homeostasis is one of the most effective methods of preventing the development of a number of dangerous diseases of non-infectious and infectious nature. The search for new highly effective and harmless radical-neutralizing systems continues, and is due to the fact that in high concentrations or due to their excessive reactivity, known organic antioxidants induce free-radical reactions, exhibiting pro-oxidant properties. In this regard, the use of nanoparticles, in particular silver nanoparticles (Ag(0)NP), as potential antioxidants attracts particular attention. Due to their small size and high value of the interphase surface and, consequently, the excess surface energy, nanoparticles are characterized by high physical and chemical activity combined with their extremely low toxicity. Combining the antimicrobial properties of Ag(0)NPs with their antioxidant properties, as well as the water-solubility and biocompatibility of their stabilizing polysaccharide arabinogalactan (Ar-Gal), will allows the development of modern nanomaterials to combat both infectious agents and to neutralize the effects of excess free radical generation in the conditions of the natural immune response. Whereas the determination of potential mechanisms of the antiradical action of nanoparticles, including highly sensitive chemiluminescent method, will be possible to vary its expression by changing their characteristics [1].
This work is aimed at the synthesis and chemiluminescence analysis of the radical-scavenging capacity of Ar-Gal-stabilized Ag(0)NPs.
Synthesis of water-soluble organic-inorganic nanocomposites consisting of Ar-Gal-stabilized Ag(0)NPs was carried out by reduction of silver nitrate in an aqueous solution of Ar-Gal, which simultaneously serves as a silver ion reductant to Ag(0) and stabilizer of the formed

nanoparticles. The reaction was initiated by increasing the pH of the reaction medium to 10-11 and the temperature to 60 C°. The synthesis time ranged from 10 to 50 minutes depending on the amount of silver introduced, which was varied by changing the cations Ag/Ar-Gal ratio from 0.005 to 0.05. The phase analysis of the nanocomposites was performed on the basis of diffractograms characterized by the presence of intense reflexes in the region of 38.1, 44.2 and 64.3 2θ ° corresponding to (111), (200) and (220) planes of the Ag(0) crystal lattice. According to the data of transmission electron microscopy and X-ray phase analysis, nanocomposites are formed in the form of particles distributed in the Ar-Gal polysaccharide matrix with the shape similar to spherical and the average size of Ag(0) nanocrystallites 3.0 nm, 3.5 nm, 4.0 nm and 5 nm for samples containing 0.5 %, 1.0 %, 1.9 % and 3.6 % Ag respectively.
Using the well-known radical generating system "horseradish peroxidase - H2O2" with luminol as a chemiluminescence activator, as well as by processing the obtained kinetic curves of chemiluminescence by TAR (Total Antioxidant Reactivity) we characterized the antioxidant properties of silver-containing nanocomposites.It was found that their introduction into the radical generating system "horseradish peroxidase - H2O2" is accompanied by a pronounced quenching of chemiluminescence, comparable to the value of the quenching degree (q) with q of the standard antioxidant - ascorbic acid. In this case, the value of q depends on both the concentration of aqueous solutions of nanocomposites, varying in the range of 0.19-0.98, and the quantitative content of silver nanoparticles in the composition of the composite.
Study of chemiluminescence kinetics in the presence of nanocomposites showed that in low concentrations (0.1-0.4 mg/ml) of their aqueous solutions chemiluminescence quenching is identified exclusively by plateau intensity reduction. Whereas increasing the concentration of nanocomposite aqueous solutions up to 2.5-5.0 mg/ml is accompanied by a partial decrease of the chemiluminescence intensity at the initial stage (0-6 min) with subsequent slow increase of the luminescence intensity to the control level [2]. Our data demonstrate a concentration-dependence of the action of polysaccharide-stabilized Ag(0)NPs, in particular, at low concentrations they exhibit themselves as typical weak antioxidants, whereas at high concentrations they are medium strong antioxidants.
Thus, we obtained a number of water-soluble nanocomposites consisting of polysaccharide-stabilized Ag(0)NPs with an average size varying in the range of 3.0-5.0 nm. Using the method of activated chemiluminescence for the first time quantitative assessment of antioxidant properties of polysaccharide-stabilized Ag(0)NPs was performed, and also a pronounced dependence of q value on the concentration of their aqueous solutions and the percentage of silver in the composition of the composite was determined.

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### S6.377. Chlorophyll dimers harbored by Water Soluble Chlorophyll-binding Proteins as photosensitizers of redox reactions

Neverov K.V.[1,2]*, Obukhov Yu.N.[1,2], Maleeva Yu.V.[2], Kritsky M.S.[1]
[1]*A.N.Bach Institute of Biochemistry, Biotechnology Research Center RAS;*
[2]*M.V.Lomonosov Moscow State University;*
* neverovk@mail.ru

Water Soluble Chlorophyll-binding Proteins (WSCP) of higher plants, in contrast to the membrane bound pigment-protein ensembles of the photosynthetic apparatus, are water-soluble complexes that are localized outside the chloroplast membranes and do not take part in photosynthesis. They have a tetrameric structure and bind up to four molecules of chlorophyll (Chl) [1].
Chl molecules in WSCP holoforms are packed as molecular dimers, analogues of the “special pair” of chlorophylls (bacteriochlorophylls) in photosynthetic reaction centers (RCs), i.e., there is a convergent similarity between WSCPs and RCs. Since the “special pair” of Chl (BChl) in RC serves as an electron donor in the primary photochemical event, the question arises about the ability of Chl dimers in WSCPs to exhibit photochemical activity. Despite the described photogeneration of the excited triplet state by Chl molecules in WSCP [2], the ability of WSCP to photoinduce electron transfer reactions has not been studied yet.
To elucidate whether Chl in WSCP could contribute to photochemical processes, we studied the interaction of the in vitro assembled WSCP holoforms with electron acceptors and donors upon irradiation of their solutions with red light that could be absorbed only by Chl. For these purposes, proteins BoWSCP and LvWSCP were selected. These proteins represent the subclasses of the WSCP IIa family (bind only Chl a) and IIb (bind, in addition to Chl a, up to 30% of Chl b), respectively.
Irradiation of BoWSCP in the presence of cytochrome c with red light ($\lambda \geq 650$ nm) in a deaerated solutions led to cytochrome c reduction, which was detected by an increase in its absorption peak at 550 nm. The cytochrome c photoreduction rate constant depended linearly on the cytochrome concentration, which indicates a direct interaction of cytochrome c with the photoexcited Chl dimer in WSCP. Present in the samples as a buffer base Tris (tris(hydroxymethyl)aminomethane) was found to serve as the electron source for cytochrome reduction in our experiments [3].
In air-saturated WSCP solutions irradiated with red light, oxidation of electron donors, NADH or ascorbate, was observed, while in an oxygen-free environment, photooxidation of these donors did not occur. A decrease in the rate of donor photooxidation upon the addition of NaN3, a quencher of the Chl triplet state and inhibitor of singlet oxygen (1O2) generation, indicated that the reaction proceeded according to a type II mechanism, i.e., through the Chl-mediated 1O2 photogeneration. The rate constant of NADH photooxidation did not noticeably differ for BoWSCP and LvWSCP, which is consistent with the similar quantum yields of singlet oxygen generation for Chl a and Chl b.
During the photooxidation of NADH and ascorbate, the amplitude of the Chl absorption band, as well as the amplitude of the circular dichroism signal corresponding to Chl dimers, decreased insignificantly (up to 10% in 15 min of exposure). This indicates a significant resistance of both Chl itself in WSCP and its dimeric structure to reactive oxygen species. The rate constant of BoWSCP-driven photooxidation of ascorbate was higher than that of NADH, although the latter is a stronger reducing agent. This is obviously due to the different efficiency of the interaction of electron donors with singlet oxygen: the 1O2 quenching constant by ascorbate ($kq = 4x108$) is twice as high as its value for NADH ($kq = 2x108$).
The discovered photosensitizing activity of the Chl dimer in the WSCP holoproteins sheds light on establishing the mechanisms of the photoprotective function of the proteins of this family in the plant cell. The detection of the photochemical redox activity in Chl dimers in WSCP may serve a prerequisite for constructing water-soluble models of reaction centers of photosynthetic systems in order to study the evolution of the photosynthetic apparatus, as well as to develop a new nanobiosensors, artificial solar energy converters, and phototherapeutic medicines.

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### S6.378. Chromophoric group modification as method for fine tuning the microbial rhodopsin optical properties

Khodonov A.A.[1]*, Belikov N.E.[1], Safinova A.Ya.[1], Demina O.V.[1], Petrovskaya L.E.[2], Kryukova E.A.[2], Dolgikh D.A.[2], Lukin A.Yu.[3], Kirpichnikov M.P.[2], Varfolomeev S.D.[1]
[1]*N.M. Emanuel Institute of Biochemical Physics RAS;*
[2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS;*
[3]*MIREA — Russian Technological University;*
* khodonov@gmail.com

Retinal-containing proteins play a key role in a number of important biological and physiological processes - in vision and tissue differentiation in higher animals and in light-dependent transport of protons, chloride ions, or phototaxis in some types of microorganisms.
The purpose of this work was to develop and put into practice an integrated approach to the study of structure-function relationships in microbial rhodopsins by chemical modification of functionally significant structural elements of the chromophore group molecule.
As we have shown earlier, for the formation of a covalent bond between the retinal analogue and the apoprotein - opsin, the presence of a polyene chain fragment containing from two to four conjugated multiple bonds with the terminal formyl group is necessary in the retinal analogue molecule, while the trimethylcyclohexene ring can be easily replaced by more massive residue.
Based on the results of our own research and literature data, the database "Properties of artificial bacteriorhodopsin analogs. From 1975 to 2019", version 2.0, 2020, summarizing information about the ability of apoprotein (bacterioopsin - BO) to form covalent and non-covalent complexes with various polyene compounds and about their photochemical and functional properties. It includes the following structural, spectral and photochemical parameters and other information about the products of interaction of more than 440 polyene compounds with bacterioopsin (BO).
The structure of all polyene compounds was classified based on their differences from the natural chromophore (all-E-retinal).
The following series of modifications of retinal analogs are considered:
A - Natural chromophore - retinal and its isomers
B - Terminal polar group modification
C - Polyenic chain modification
D - Alteration of the bond types and its disposition in the chromophore polyenic chain
E - Alteration of the polyenic chain length and bond disposition and terminal group types
F - Alteration or locking of the bond configuration. Non-isomerizable analogs
G - Alteration of the trimethylcyclohexenic ring. Ring modification
H - Alteration of the trimethylcyclohexenic ring. Replacement of the ring with aromatic or heterocyclic fragments
I - Alteration of the trimethylcyclohexenic ring. Acyclic analogs
J - Miscellaneous modifications
K - Labelled retinal derivatives (radioactive, photoaffinic, fluorophoric, heavy-atom, paramagnetic (SL), ionophoric and photochromic probes)
The main descriptors were: the structure of the specific isomer of the polyene compound tested; λmax of the initial compound; of models (Schiff bases with n-butylamine in methanol, in non-protonated and protonated forms); of non-covalent complex with bacterioopsin; of pigment in aqueous buffer (light- and dark-adapted (LA and DA forms); presence and type of photocycle, its main intermediates; efficiency of proton transport; isomeric composition of the chromophore (ratio of all-E- and 13Z-isomers); "opsin" shift; stability of interaction products to hydroxylamine and all-E-retinal and other additional data.
A comparative analysis of our database showed that by diversifying the nature of the chromophore, it is possible to directly change λmax in the spectra of bacteriorhodopsin analogues in a fairly wide range (from 412 to 830 nm), although not all of these new pigments are capable of cyclic photochemical reactions.
The work was partly supported by the Russian Foundation for Basic Research (project no. 20-03-00139a).
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### S6.379. Comparative study of the impact of iron and palladium on the photobiological properties of cyano(aryl)porphyrazines containing these metals as central cations

Shestakova L.N.[1]*, Belotelov A.O.[1], Lyubova T.S.[2], Klapshina L.G.[2], Lermontova S.L.[2], Balalaeva I.V.[1], Shilyagina N.Yu.[1]
[1]*Lobachevsky State University;*
[2]*Razuvaev Institute of Organomettalic Chemistry;*
* lshn1998@yandex.ru

The pursuit of the "ideal" photosensitizer (PS) is a major focus in the development of photodynamic therapy (PDT). One approach to develop an "ideal" PS's is to modify the chemical structure of PS's used in clinical practice or known in pre-clinical applications. Both the macroheterocycle itself, for example by including a metal cation, and its peripheral frame are modified. It has been shown that the presence and nature of the central atom has a significant influence on the photochemical, photophysical and photobiological properties of PS. In additional, a number of studies have reported an increased antitumour effect through Fenton reactions if iron cation is present in PS.
We have synthesized tetra(aryl)tetra(cyano)porphyrazines as potential PS in the form of free bases and metal complexes containing iron or palladium atoms in the macrocycle centre, with thiophene, benzothiophene and naphthyl groups in the macrocycle periphery. To evaluate the Fenton reaction contribution, we have used a noble metal complex that does not catalyse Fenton-type reactions and an iron complex for comparison.
We have shown that the free bases and their metal complexes under study are characterised by absorption and fluorescence peaks in the red and far-red region of the spectrum. We have confirmed that the metal complexes under investigation are belonging to the class of fluorescent molecular rotors by registering a pronounced dependence of the quantum yield of fluorescence on the viscosity of the environment. It has been shown that the addition of a metal cation to the macrocycle changes the photophysical properties of tetra(aryl)tetra(cyano)porphyrazines. Thus, for iron complexes we have noted a higher viscosity sensitivity and increased photostability in comparison to a free base.
We have demonstrated intense accumulation of free bases and iron and palladium complexes by human epidermoid carcinoma A431 cells. We have shown a rise in photodynamic indices reflecting how many times more toxic in the light than in the dark for iron and palladium complexes compared to free bases. This might indicate a potential reduction of side-effects on healthy organs and tissues. It was important to note that the photodynamic index for the iron complexes was higher than for the palladium complex. We also registered an elevated level of singlet oxygen generation for compounds containing iron cation compared free bases. We have demonstrated an extension of the excited state lifetime of porphyrazine metal complexes, localized in the cytoplasm of A431 cells, after photodynamic treatment. We hypothesise that

this may be evidence of a photoinduced local increase in intracellular microviscosity.
Altogether, the results demonstrated that the presence of iron and palladium cation has a major impact on the photophysical and photobiological properties of the cyano(aryl)porphyrazines. The investigated compounds in the forms of free bases and metal complexes can be considered as potential photosensitizers and highly sensitive fluorescent sensors of cell viscosity, particularly for photodynamic therapy.
This work was financially supported by the Russian Science Foundation (project no. 23-23-00515) and state assignment of the Ministry of Science

### S6.380. Construction of experimental growbox for potato microclonal reproduction

Burdysheva O.V.[1], Solgin E.S.[1], Lisina T.N.[1]*, Tsema L.G.[1]
[1]*Perm Research Institute of Agriculture;*
* atea2@yandex.ru

Potato (Solanum tuberosum L.) is a significant crop for the economy of many countries, including Russia. The key to obtain a successful high yield of potato is the quality of the planting material. To get healthy potato planting material, reproduction is carried out in vitro culture, where lighting conditions are very important [1]. The potato productivity in light culture is influenced by such characteristics of illumination as intensity, duration of the photoperiod, pulsed/continuous radiation, and spectral composition [2,3]. Different potato varieties have a specific response to illumination with different spectral characteristics [4, 5], therefore, when working with potato at the stage of micropropagation, it is important to choose the optimal illumination, taking into account varietal characteristics. The aim of the work is to design an experimental grow box to identify lighting with optimal spectrum characteristics for different potato varieties.
The employees of Perm Research Institute of Agriculture have designed a grow box. To form a sufficient number of experimental sectors, the growbox shelves were separated by reflective screens. The microclimate in the growbox is maintained with the help of ventilation holes and through the installed climate control systems in the laboratory rooms. Thermocouples TP-A-2488-10-4-XA were installed to measure the temperature inside the growbox sectors. The collection and storage of data from temperature sensors is implemented using an Orange Pi Zero 2 V1.3 microcomputer.
The required 16-hour photoperiod inside the grow box is implemented using a Systec mechanical timer. OSRAM L 30W/77 T8 Fluora fluorescent phytolamps were used as the main lighting. To add an additional spectral component to the main spectrum, LED strips are implemented. 3W SMD diodes were used, which were installed on radiators in a line. LED strips were fixed near the main lamps. The LED strips were powered from the power supply unit "PM(P4) 350W" with 12V output to 7 strips connected in parallel within one shelf and in series between the shelves. LED strips are fixed next to the main lamps. The LED strips are powered from the power supply unit "PM(P4) 350W" with 12V output to 7 strips connected in parallel within one shelf and in series between the shelves. In the study of the influence of light source, the following additional spectra were used for the OSRAM L 30W / 77 T8 Fluora phytolamp: in the visible region - 660 nm, IR - 850 nm, 730 nm, 940 nm, 395-400 nm, 420-430 nm, UV- 365nm, control - without additional spectrum.
After analyzing the spectra, it can be seen that the lines of LEDs 365 nm, 395-400 nm, 420-430 nm did not make a significant contribution to the total photosynthetic photoflux. The 660 and 730 nm rulers gave a significant increase in the total photo flux. The 850 and 940 nm rulers are commensurate with the main spectrum of the phytolamp in terms of intensity.
Thus, in the Perm Research Institute of Agriculture, PFRC, Ural Branch, Russian Academy of Sciences, a grow box was designed to experimentally determine the optimal spectral composition of illumination for microclonal cultivation of potatoes in vitro. The spectral characteristics of lighting in the sectors of the growbox are selected taking into account the published results of experimental work on the study of lighting effect on potato plants.
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### S6.381. Dependence of red blood cell aggregation on endogenous and exogenous factors: study by laser techniques in in vitro experiments

Ermolinskiy P.B.[1]*, Maksimov M.K.[1], Muravyov A.V.[2], Korneev K.N.[1], Umerenkov D.A.[1], Lugovtsov A.E.[1], Priezzhev A.V.[1]
[1]*Faculty of Physics, Lomonosov Moscow State University, Moscow, Russia;*
[2]*Yaroslavl State Pedagogical University, Yaroslavl, Russia;*
* ermolinskiy.pb15@physics.msu.ru

Human blood consists of liquid blood plasma and blood cells, most of which are red blood cells (RBCs). RBCs are cells that deliver oxygen to body tissues. In large arteries and veins (low shear stress forces), RBCs can reversibly interact with each other producing linear or more complex structures called RBCs aggregates. This interaction is called RBCs aggregation process. It strongly influences the microcirculation of blood and impacts human health in general [1,2]. The mechanisms of RBC aggregation are still unclear and contradictory [1]. It is well known that RBC aggregation can occur only in the solution containing macromolecules with a large molecular weight (usually over 70 kDa). Since the middle of last century, two main hypotheses of RBC aggregation mechanism have been coexisting: the "depletion" theory and the "bridging" model [1]. In the "depletion" theory, RBCs interact due to the osmotic forces that arise in the solution of macromolecules (e.g., proteins or synthetic macromolecules) surrounding the cells. In the "bridging" model, the RBCs interaction is described by the forces of adsorbed macromolecules at the surface of RBCs membranes and producing the "bridges" between RBCs membranes. To this day, there are strong arguments in favor of both models and there are some assumptions that both mechanisms influence the RBC aggregation [3].
Aggregation properties of blood can change due to many cell factors (pathological cell properties, RBC aging, RBC deformability, etc.) and medium factors (alterations of blood plasma composition, changes in blood temperature, osmolarity of medium, etc.) [1]. To

date, the influences of these factors on RBC aggregation are not fully understood. In this work, the effects of different endogenous and exogenous factors are demonstrated using laser techniques, such as laser aggregometry and laser tweezers [4].
It is shown that RBC aggregation is strongly dependent of blood plasma proteins, such as fibrinogen and gamma-globulin. Moreover, the proaggregant effect is synergetic. Interestingly, the influence of gamma-globulin on RBC aggregation is almost insignificant in the normal concentration range. The effect of c-reactive protein at different concentrations has been shown to be negligible. Also, the effect of RBC aging was studied. Based on the results [5], the more the age of interacting RBCs, the stronger they interact. Additionally, other RBC aggregation factors were investigated. These results are important for a complete understanding of RBC aggregation mechanisms.
This work was supported by the Russian Science Foundation (Grant No. 22-15-00120) and performed according to the Development program of the Interdisciplinary Scientific and Educational School of Lomonosov Moscow State University «Photonic and Quantum Technologies. Digital medicine».
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**S6.382. Diagnosis and eye radiation**

Egorov V.V.[2], Kruglova L.V.[1]*
[1]*PFUR;*
[2]*Federal State Budgetary Educational Institution of Higher Education «Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin»;*
* kruglova_lv@pfur.ru

The use of human radiation parameters for monitoring and diagnosing his state is described in [1]. In particular, the human eye is associated with other organs and systems of the body, the dysfunctions and structures of which are reflected in the visual system [2-5]. The assumption of the presence of human visual radiation based on the results of indirect experiments: chemiluminescence, measurement of seed germination and using an electrochemical cell was made in the works of Zhuravlev A.I., Egorov V.V., Tsetlin V.V. [6,7].
In the present work, direct registration of such radiation in the IR range is carried out for the first time. The studies were carried out using a KM infrared thermometer (OOO Timol, registered spectral range 8-14 μm) connected to a personal computer at room temperature at a distance of 5 cm from the eyes. The measurements were recorded at a frequency of 1 Hz. The experiments were carried out at different illumination: 100 lx (daylight), 10 lx (darkness). A total of ten experiments were done with different subjects, seven experiments each. Infrared radiation of closed and then open eyes was measured. A significant difference was found between the radiation of open eyes and closed eyes under light and dark conditions.
As noted, eye radiation parameters are an important diagnostic factor that can be used both during the initial examination to diagnose a person's state and during treatment to control the quality of the procedures and methods of therapy.
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**S6.383. Direct excitation of dissolved mol oxygen at 800-1300 nm. Mimicking of theurapeutic action of laser radiation**

Kozlov A.S.[1], Zhuravlev S.G.[2], Egorova O.N.[2], Medvedkov O.I.[2], Krasnovsky A.A.[1]*
[1]*FRC of Biotechnolgy RAS;*
[2]*FRC "Prokhorov General Physics Institute" RAS;*
* phoal@mail.ru

Therapy based on the application of low- and medium-intensity IR laser radiation sources in the region of 800-1300 nm is actively employed for the treatment of various diseases. Since it is known that oxygen has absorption bands in the near-IR region of the spectrum, it has been proposed that molecular oxygen dissolved in living cells and tissues can be a photoreceptor of laser radiation. To find out the viability of this mechanism of laser therapy, we investigated the effect of IR laser radiation in the range of 800-1300 nm on the oxygen in aerobic media at room temperature and atmospheric pressure. The data were obtained using a set of diode and fiber IR lasers, while using cuvettes with an optical path of 1 cm. The singlet oxygen generation rate during the excitation of oxygen molecules by laser radiation was measured using a chemical trap of singlet oxygen – 1,3-diphenylisobenzofuran. Carbon tetrachloride, hexafluorobenzene, freon-113, acetone, ethanol and heavy water were used as solvents. It was found that in all solvents in the action spectra of the trap oxidation, the most intense band coincide with the main absorption band of oxygen with a maximum of 1273 nm ($1\Delta g(0) <- 3\Sigma g-(0)$ transition). In addition, another band with a maximum at 1070 nm and a half-width of 8-18 nm (depending on the solvent) corresponding to the first vibronic transition of molecular oxygen ($1\Delta g(1) <- 3\Sigma g-(0)$) was reliably detected. The intensity of this maximum is about 100-fold less than that of the maximum at 1273 nm. Addition of singlet oxygen quenchers (acetone in hexafluorobenzene and sodium azide in heavy water) reduced the rate of trap bleaching under the action of lasers at 1070 and 1273 nm. The molar absorption coefficients for oxygen at these maxima were measured in all solvents ($\varepsilon 1273 = 1.6 \div 5.1 \times 10\text{-}3$ M-1cm-1, $\varepsilon 1070 = 1.1 \div 4.9 \times 10\text{-}5$ M-1cm-1). Under the action of IR radiation at 800-1060 nm, the rate of trap photooxidation was lower by an order of magnitude than for 1070 nm in all solvents, and it did not depend on the excitation wavelength and almost did not exceed the rate of dark bleaching of the trap in the absence of irradiation. It indicates that the oxygen absorption coefficients in this spectral interval are much smaller than even for the band at 1070 nm. Thus, in our work absorption coefficients corresponding to the 1070 nm absorption band of molecular oxygen dissolved under natural conditions in polar and non-polar media have been obtained for the first time. In addition, our data suggest that the therapeutic effects of IR radiation

of 800-1100 nm, are not related to the direct excitation of oxygen. Part of the results of this investigation was published in the article [1]. This work was partially supported by RFBR grant No. 19-04-00331 (A) and the state assignment of the FRC of Biotechnology of the Russian Academy of Sciences.

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### S6.384. Effect of hydrogen peroxide on the structural and functional reorganization of the light harvesting complex of the antenna of photosystem 2

Balashov N.V.[1]*, Vetoshkina D.V.[1], Borisova M.M.[1]
[1]*Laboratory of Photosynthetic Electron-Transport Chain;*
* kbalashovv@mail.ru

The most important factor in the functioning of photosynthetic organisms is their ability in adaption to various lighting conditions, and one of these regulatory mechanisms is the phosphorylation and dephosphorylation of photosystem 2 antenna proteins. This process occurs when the night period changes to daytime, as well as when the light spectrum changes. In the english literature such protective mechanism is called state transitions. State transitions is characterized as a reversible phosphorylation and dephosphorylation of proteins of the external light-harvesting antenna of photosystem 2, which include proteins Lhcb1 and Lhcb2.

Reactive oxygen species (ROS), including hydrogen peroxide, play an important role in the development of adaptive responses occurring at the level of the photosynthetic apparatus of higher plants and green algae. Although our proposed hypothesis of the involvement of hydrogen peroxide in the reversible phosphorylation of proteins of the light-harvesting antenna of photosystem 2 was proposed in some of our works, it is still not proven.

In this work we studied the effect of hydrogen peroxide on the regulation of the state transitions process. The accumulation of phosphorylated Lhcb1 and Lhcb2 proteins was assessed in isolated thylakoids of Arabidopsis thaliana ecotype Columbia-0 plants at low light intensity (about 60 μE) in the absence and presence of hydrogen peroxide. The accumulation of phosphorylated Lhcb1 and Lhcb2 proteins was also evaluated at high light intensity (about 900 μE) in the presence and absence of catalase, which decomposes hydrogen peroxide.

During the addition of hydrogen peroxide to the thylakoid suspension, the amount of phosphorylated proteins of the light-harvesting antenna of photosystem 2 decreases, while during the addition of catalase, which decomposes hydrogen peroxide, the decrease of the amount of phosphorylated proteins of the light-harvesting antenna of photosystem 2 was not observed.

The results thus obtained the regulatory role of hydrogen peroxide in the the state transitions process.

The work was supported by the Russian Science Foundation grant No. 22-74-10088.

### S6.385. Effects of a viscous microenvironment on the bacterial bioluminescent reaction: the role of diffusion restriction and intermolecular interactions

Nemtseva E.V.[1,2]*, Lisitsa A.E.[1], Sukovatyi L.A.[1], Gulnov D.V.[1], Kratasyuk V.A.[1,2]
[1]*Siberian Federal University;*
[2]*Institute of Biophysics of the Siberian Branch of the Russian Academy of Sciences;*
* enemtseva@sfu-kras.ru

Revealing the mechanisms of the influence of media with different physicochemical characteristics on enzymatic catalysis is a key task for understanding the principles of regulation of biochemical processes in a living cell (= in a dynamic microenvironment of a complex composition). The activity of enzymes in a cell is determined by many factors, among which a significant role is played by the viscosity of the medium. The addition of various co-solvents to traditional buffer solutions is used to mimic intracellular conditions in vitro, allowing for the reduction of co-regulatory effects. The bioluminescent reaction catalyzed by bacterial luciferase is a multistage process that includes the formation and degradation of at least four intermediates. The mechanisms of the influence of the medium viscosity on such a biochemical process have not yet been fully established.

The aim of this study was to determine the diffusion-controlled steps of the reaction catalyzed by the bacterial luciferase P. leiognathi. To achieve this, we analyzed the rate constants of individual reaction steps in media with increased viscosity (up to 6 cP) created by adding cosolvents of various molecular weights (ethylene glycol, glycerol, glucose, sorbitol, sucrose, dextran with a molecular weight of about 70 kDa and polyethylene glycol with a molecular weight of about 4 kDa). The rate constants were calculated based on the non-stationary reaction kinetics measured by the stopped flow method using a mathematical model that takes into account the sequence of reaction stages. The model was developed in the Laboratory of theoretical biophysics of the Institute of Biophysics of the Siberian Branch of the Russian Academy of Sciences. Also, the calculation of the molecular dynamics of bacterial luciferase surrounded by water and cosolvents was performed to detect a possible change in the structure of the protein in viscous media. In addition, intermolecular interactions of luciferase with cosolvents were studied by fluorescent spectroscopy technique.

It was found that the rate constants of binding of reduced flavin mononucleotide and long-chain aldehyde (formation of intermediates I and IIA) depend on the viscosity of the medium according to a power law, that is, they exhibit the properties of diffusion-controlled processes. At the same time, the rate of oxygen binding (formation of intermediate II) does not depend on the viscosity of the medium. The rates of the "dark" stages of the reaction (oxidation of the reduced flavin and decomposition of intermediate II) also exhibited diffusion limitations in viscous media, but this did not lead to an increase in the overall quantum yield of the reaction. The catalytic constant of bacterial luciferase turned out to be independent of the viscosity of the medium: it increased in media with glucose, sorbitol, and sucrose, and decreased in media with ethylene glycol and dextran.

The molecular dynamics of bacterial luciferase in the environment of low molecular weight cosolvents showed that in the presence of ethylene glycol, the solvent accessible surface area of the protein increases, while in other cases it does not change. In addition, the preferential binding of glucose, sorbitol and sucrose to luciferase, but not of ethylene glycol and glycerol, has been established.

It was found that the fluorescence spectra of bacterial luciferase upon excitation at 295 nm showed a hypsochromic shift in the presence of glucose and sucrose, which indicates the transition of tryptophan residues to a less polar environment than in the buffer. Ethylene glycol and polymer cosolvents did not affect the luminescence spectrum of the protein.

Thus, based on the results of experiments and molecular modeling, a complete picture of the effect of viscous media on the bioluminescent reaction catalyzed by P. leiognathi luciferase was obtained: (i) slowing down the diffusion of the reaction components leads to a decrease in the rate of binding of the flavin and aldehyde substrates, but also helps to slow down the competing "dark" stages of the reaction; (ii) there is a preferential binding to the surface of the luciferase of some cosolvents, which correlates with an increase in the catalytic constant of the enzyme in their presence; (iii) biopolymer solutions are affected according to their low viscosity and molar concentration, no additional effects due to the macromolecular crowding are observed.

**S6.386. Evaluation of the binding of zinc phthalocyanines to nanodiamonds for application in photodynamic therapy**

Gudkova V.[1]*, Dolmatov V.Yu.[2], Maksimov E.G.[1]
[1]*Lomonosov MSU, Faculty of Biology, Biophysics Department;*
[2]*FGUP SKTB Tekhnolog;*
* gudkova.v.r@gmail.com

Photodynamic therapy (PDT) is a promising method for the treatment of cancer[1]. First-generation photosensitizers (PS) are characterized by an intense absorption band in the region of 400 nm (Sore band). Their disadvantages include low yield of reactive oxygen species (ROS) and low absorption capacity in the range of 650-850 nm. Second-generation PS are much better at absorbing light in the range of 600-700 nm (Q-band absorption), which ensures greater PDT efficiency not only on the surface, but also deep in the tissue. Nevertheless, such PS lack selectivity to cancer cells, and also tend to interact with blood proteins, which reduces their effectiveness. Therefore, third-generation PSs, which combine second-generation PS into a complex with a platform for their delivery, are now being developed. Carbon nanoparticles can serve as such a platform. Nanodiamonds (ND) are promising nanoparticles. Their advantages include high stability, the possibility of chemical modification of the surface, and a high surface area to volume ratio.

We compared the efficiency of the formation of ND complexes of different types with FS from the zinc phthalocyanine line. ND obtained in FGUP SKTB Technolog (Russia) and having commercial names TAN and STP were used. ND synthesized from the explosive tetril were also characterized, followed by treatment in air at 430°C. FTIR spectroscopy was used to show differences in the chemical structures of ND. Chemical modification of ND by TAN carboxyl groups was carried out, which was confirmed by FTIR spectroscopy and dynamic light scattering techniques. To assess the efficiency of ND binding to phthalocyanines, a procedure of titration of various solutions of phthalocyanines with a suspension of ND followed by registration of the Q-band absorption peak position of the phthalocyanines was carried out. A method for evaluating the adsorption constant of phthalocyanine on ND was also developed based on the construction of an adsorption isotherm.

According to the results obtained, the most effective formation of ND-phthalocyanine complex occurs between phthalocyanine with choline substituents and negatively charged ND. Phthalocyanines with pyramidine substituents showed lower binding efficiency with ND, which is explained by spatial factors. Phthalocyanines with carboxyl substituents did not interact with either positively or negatively charged ND.

Our work allows us to evaluate the prospects for the use of various ND and phthalocyanines for the development of third-generation photosensitizers.

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**S6.387. Femtosecond laser spectroscopy and femtosecond laser micromachining in biophysics**

Nadtochenko V.A.[1,3]*, Cherepanov D.A.[1,2], Semenov A.Yu.[1,2], Aybush A.V.[1], Gostev F.E.[1], Shelaev I.V.[1], Titov A.A.[1], Osychenko A.A.[1], Zalessky A.D.[1], Martirosyan D.Yu.[1], Syrchina M.S.[1]
[1]*Federal Research Center for Chemical Physics N.N. Semenov Russian Academy of Sciences, Kosygina 4, Moscow, Russia, 119991;*
[2]*Research Institute of Physical and Chemical Biology A.N. Belozersky, Moscow State University named after M.V. Lomonosov, Leninskiye Gory 1 building 40, Moscow, Russia, 119991;*
[3]*Department of Chemistry, Moscow State University named after M.V. Lomonosov, Kolmogorova, 1, Moscow, Russia, 119991;*
* nadtochenko@gmail.com

The report is devoted to the applications of femtosecond pulsed laser for studying ultrafast biophysical processes in photosynthesis reaction centers, for 2D and 3D chemical mapping of biological cells and tissues using femtosecond Raman microscopy-spectroscopy, as well as for femtosecond laser nanosurgery of cells, embryos and biological tissues. The application of femtosecond laser spectroscopy to the study of PS I and PS II photosystems containing Chl d, Chl f chlorophyll molecules made it possible to reveal new features in the mechanism and kinetics of light quantum transformation into chemically active radical ion states. The spectral features of Chl d and Chl f, which differ significantly from Chl a, as well as the known structural data on the localization of Chl d and Chl f in photosystems, made it possible to determine the kinetics and sequence of elementary acts of energy and electron transfer in PS I and PS II photosystems. A feature of femtosecond laser pulses is their high peak intensity and large spectral width. The first circumstance makes it possible to effectively observe nonlinear optical processes, incl. processes of highly directed coherent Raman scattering: CARS (coherent anti-Stokes Raman scattering), SRS (stimulated Raman scattering). The second circumstance makes it possible to simultaneously obtain a spectral range of sample vibration frequencies of hundreds of reciprocal centimeters. Compared to traditional Raman microscopes, the use of a unique femtosecond microspectrometer developed at the FRC CF RAS has advantages for solving the following problems:

- In tasks where it is necessary to have the highest possible spatial resolution when scanning a sample (up to 200 nm). This resolution is achieved due to the nonlinearity of the generated optical processes in the sample under study.
- In problems where the determination of combination frequencies in the Stokes region is very difficult. In this case, in a femtosecond microscope-spectrometer, the signal is taken from the anti-Stokes region (CARS), which is free from sample luminescence.
- In tasks where the fastest possible 2D/3D display is required in a narrow range of vibration frequencies (~10 1/cm). An increase in the scanning speed up to 3 orders of magnitude is achieved through the use of high-speed galvanic mirrors and PMTs. This configuration is particularly suitable for specimens where mechanical movement of the test specimen is undesirable. Spectral focusing of laser pulses can be used for samples with low concentrations of substances or low intensity of Raman lines. In this case, the scanned range of oscillation frequencies can be reduced to 2-3 1/cm.
- In tasks where fast 2D/3D mapping of a given range of vibrational frequencies (up to 1200 1/cm wide) with a spectral resolution of 10 1/cm is required. The scanning speed is ten times higher than in traditional Raman microscopes.

The technique has been tested in studies:

- GFP proteins, rhodopsins, xanthophylls, lipid-protein mixtures, pigment-protein complexes (including chlorophyll-containing), invertebrate ommochromes.;
- Cell cultures, tissue sections of animals and humans (liver, brain, adipose tissue, lipid drops, lipofuscin granules).
- Living biological objects: oocytes, spermatozoa, stem cells.

The use of highly focused femtosecond laser pulses makes it possible to create micro- and nanocuts in biological material without thermal heating. femtosecond laser in the near infrared range; cw lasers in the visible and near infrared ranges; optical microscope with a motorized 2D platform; spatial light modulators (SLMs) allow for minimally invasive micro- and nanosurgical operations, manipulations with individual cells, embryos and tissues. The combined use of a continuous-wave laser and SLM makes it possible to obtain complex distributions of electromagnetic fields in the sample volume, for example, to create multiple independent laser foci. In particular, each of these laser foci can be an optical trap for cells or cell organelles. With the right choice of experiment parameters, it is possible to hold, rotate or independently move these objects, incl. disconnection or connection of several objects at the same time (optical multiplexer). This provides rich information

about the forces between organelles, the elastic properties of biological samples. This installation allows you to solve the following tasks:
• Laser dissection of tissues and cells.
• Optical transfection (introduction of external genetic material into cells through channels created in the cell membrane).
• Artificial laser fusion of two or more cells.
• Laser inactivation of cell chromosomes.
• Therapeutic laser cloning.
• Study of the elastic properties of biological objects at different stages of development; interaction forces between individual parts of a biological system (organelles, organelles and membranes, etc.).
A fundamentally new technology has been developed and the necessary material and technical equipment has been developed for minimally invasive nanosurgery of mammalian embryos using lasers with radiation in the transparency window of biological tissue. A promising technology has been developed for obtaining genetically modified preimplantation mammalian embryos.

### S6.388. High intensity of FAD autofluorescence as an indicator for detecting cellular pathology

Bryanskaya E.O.[1]*, Dolgikh A.I.[1], Vinokurov A.Yu.[1], Dunaev A.V.[1]
[1]*Cell Physiology and Pathology Laboratory, Orel State University, Orel, Russia;*
* bryanskayae@mail.ru

Optical imaging using endogenous fluorescence (FAD), which is involved in such processes as fatty acids oxidation, Krebs cycle and other redox reactions, is one of the promising ways to study the metabolic status of cells. The possibility of simple and non-invasiveness FAD determination is based on its autofluorescence, with an excitation spectrum in the wavelength range of 350-500 nm with two peaks - at 370 and 450 nm, and the emission spectrum falls in the region of 500-600 nm with a maximum at 525 nm.
According to literature sources, cells in different physiological states have different levels of FAD intensity in green-blue spectrum. To determine the physiological state of cells by the difference in the FAD intensity, in this work, a culture of skin fibroblasts was studied after after 20-day cultivation in DMEM-based growth medium (Gibco, UK) containing 4.5 g/l glucose, 10% fetal bovine serum (Biological Industries LDT, Israel), penicillin (100 units/ml) (Gibco, USA), streptomycin (100 µg/ml) (Gibco, USA), in a CO2 incubator (Thermo Scientific) at 37°C, 100% relative humidity and 5% CO2 content (Eppendorf AG). Studies were performed using a ZEISS LSM 900 laser scanning confocal microscope with Airyscan 2 system (Carl Zeiss AG, Germany) at a wavelength of 488 nm.
The first study stage, fibroblasts were planted on 0.5 mm thick coverslips with pre-applied mesh to locate the individual cells. As the performed analysis shows, cell culture contained cells with high and low fluorescence intensity. This could be explained by the difference in metabolic status of cells. This made it possible to divide the cells into two subgroups based on the intensity of the autofluorescence signal: with a high autofluorescence signal (presumably senescent or pathological) and with a low signal.
After 24 hours, the proportion of necrotic cells in the culture was analyzed by double staining with Hoechst 33342 (5 µM) and propidium iodide (20 µM) for 30 min at 37 °C. To count the total number of cells, Hoechst 33342 was used, which stains the nuclei of cells in any physiological state. Propidium iodide, unable to penetrate entire membranes and stain viable cells, was used for staining and counting necrotic cells. The proportion of necrotic cells among cells with a high FAD autofluorescence intensity was 47.4%, whereas among cells with a low autofluorescence signal – 27.3%. Intense FAD signal can be associated with highly oxidized state of a coenzyme included in the structure of redox enzymes.
At the next stage of the study, to determine the structure of the FAD signal, different solutions were alternately introduced: adrenaline (10 µM) to activate MAO-A, selegiline (20 µM) to inhibit this enzyme, FCCP (2 µM), which is a protonophore and leads to the separation of mitochondrial respiration, and KCN (1 µM), which is an inhibitor of complex IV electron transport chain. This made it possible to determine the level of the FAD signal associated with MAO, as well as the total pool of FAD complex II of mitochondria. It was found that in cells with a high autofluorescence intensity, the pool MAO was 12.3% ± 2.1 of the total autofluorescence signal, whereas in cells with a low intensity this value was 6.4% ± 0.9. The combination in cells of a higher level of the MAO pool and a reduced level of the mitochondrial FAD pool may be a consequence of the formation of the corresponding aldehydes during oxidative deamination of monoamines, which can inhibit succinate dehydrogenase. As a result, the expression of this enzyme decreases, thus, the functioning of the complex II electron transport chain is impaired.
This study shows the possibility of early diagnosis of various diseases by detection of the FAD autofluorescence signal and by finding the cells with high fluorescence intensity, which are mostly necrotic. Low level viability of cells with high fluorescence intensity in a green-blue spectrum can be a marker for early diagnosis of various diseases, determining the exact localization and prevalence of pathology in the tissue. At the same time, the increased FAD signal is due to high MAO activity, which makes it possible to further find tools for influencing this enzyme, preventing the development of a pathological state of cells. The proposed approach has important advantages, including non-invasiveness, high sensitivity, safety and can be used for early diagnosis of various diseases and monitoring of patients' response to therapeutic interventions, including in real time.
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### S6.389. Influence of fermentation on prolonged hydrogen photoproduction by Chlamydomonas reinhardtii cells under mineral starvation

Petrova E.V.[1]*, Volgusheva A.A.[1], Kukarskikh G.P.[1], Antal T.K.[2]
[1]*Lomonosov Moscow State University;*
[2]*Pskov State University;*
* eslepova@list.ru

Lack of macroelements such as nitrogen, phosphorus, sulfur, iron and magnesium is one of the key factors that play an essential role in the processes of phytoplankton life activity. The shortage of these minerals has a particular impact on the process of long-term photogenesis of hydrogen in some green microalgae that are part of phytoplankton communities, which is their specific response to stress conditions. The basis of this response of algae is the interaction between aerobic and anaerobic processes inside the cell which provide microorganisms with energy under conditions of mineral starvation. At the same time, the participation and role of primary processes of photosynthesis as well as starch storage (and consumption) during hydrogen production in light by green microalgae cells have been studied in sufficient detail so far. At the same time, the influence of fermentation reactions on hydrogen photogenesis in starved cultures of eukaryotic microorganisms has been studied to a lesser extent.
The aim of this work was to study the effect of fermentation processes in the cells of green microalgae Chlamydomonas reinhardtii of wild type and mutant strain on continuous photogenesis of molecular hydrogen under sulfur deficit conditions. For this purpose, the following measurements and comparative analysis were performed in photoreactors under constant light and absence of sulfur in the medium: hydrogen content in the reactor gas phase, starch content in cells, measurement of photosynthetic activity and respiration rate in cultures of mutant

Ch.reinhardtii pfl 1-1 without pyruvateformylase activity and in corresponding control Ch.reinhardtii SS-125.
It was shown that, in contrast to the wild type, the cells of the mutant strain Ch.reinhardtii pfl 1-1 are characterized by a much more pronounced ability to form hydrogen under prolonged mineral deficiency, although its release is later relative to the control organism. Nevertheless, pfl 1-1 showed much greater stability in hydrogen photogenesis under conditions of mineral stress. The dynamics of starch content and consumption within the cells of the control (wild type) and mutant showed their relative similarity due to the experiment. However, the amount of starch stored by Ch.reinhardtii pfl 1-1 during the first 48 hours was almost 2 times higher relative to Ch.reinhardtii CC-125. This result indicates a significantly more pronounced ability of the mutant strain to accumulate and consume starch. It is known that starch metabolism is one of the main factors influencing the ability of microalgae to secrete hydrogen. Another such factor is the activity of primary photosynthetic reactions. Experiments evaluating photosynthetic activity as well as respiration of culture cells showed the preservation of much higher FS activity in the late stages of starvation of Ch.reinhardtii pfl 1-1 culture relative to the wild type. Cell respiration changed in a similar manner, but quantitatively the rate was 20% higher in the mutant. Thus, the mutant is characterized by more intensive accumulation and expenditure of stored starch, higher photosynthetic activity in the late stages of sulfur starvation, which has a stimulating effect on long-term hydrogen photogenesis, and reflects, in general, higher stress tolerance of the genetically modified strain.
The data obtained as a result of the studies allow us to conclude that the fermentation process has a significant effect on the duration of molecular hydrogen formation in the light by Ch. reinhardtii culture under sulfur starvation conditions, especially at the late stages. The mutant strain Ch.reinhardtii pfl 1-1 may be a promising target for biotechnology as a producer of hydrogen, an alternative, environmentally friendly energy source.
Keywords: Hydrogen photoproduction, Fermentation, Photosynthesis, Green microalgae, Stress adaptation

### S6.390. Influence of physical properties of thylakoid membranes and morphological features of chloroplasts on the rate of state transitions in higher plants

Vetoshkina D.V.[1]*, Kozuleva M.A.[1], Proskuryakov I.I.[1], Terentyev V.V.[1], Berezhnov A.V.[2], Naydov I.A.[1], Ivanov B.N.[1], Borisova-Mubarakshina M.M.[1]
[1]*Institute of Basic Biological Problems of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences";*
[2]*Institute of Cell Biophysics of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences";*
* vetoshkina_d@mail.ru

The photosystems of higher plants differ in their spectral characteristics, which under certain conditions may be the reason why only one of the two photosystems (PS) is preferentially excited. A rapid adaptation mechanism called state transitions (ST) is triggered to rebalance the excitation energy distribution between PS. ST represents a reversible transition of a part of the PSII light harvesting complex (L-LHCII) between photosystem II (PSII) and photosystem I (PSI) in response to the change in light spectral composition. This work demonstrates a slower development of the state 1 to state 2 transition, i.e. L-LHCII transition from PSII to PSI, in the leaves of dicotyledonous arabidopsis (Arabidopsis thaliana) than in the leaves of monocotyledonous barley (Hordeum vulgare) plants that was assessed by the measurement of chlorophyll a fluorescence at 77K and of chlorophyll a fluorescence at room temperature. It is known that the first step of the state 1 to state 2 transition is phosphorylation of Lhcb1 and Lhcb2 proteins, however we detected no difference in the rate of accumulation of these phosphorylated proteins in the studied plants. Therefore, the parameters, which possibly affect the second step of this transition, i.e. the migration of L-LHCII complexes along the thylakoid membrane, were evaluated. Spin-probe EPR measurements demonstrated that the thylakoid membranes viscosity in arabidopsis was higher compared to that in barley. Moreover, confocal microscopy data evidenced the different size of chloroplasts in the leaves of the studied species being larger in arabidopsis. The obtained results suggest that the observed deference in the development of the State 1 to State 2 transition in arabidopsis and barley is caused by the slower L-LHCII migration rate in arabidopsis than in barley plants rather than by the difference in the Lhcb1 and Lhcb2 phosphorylation.
When the effect of light intensity was investigated, it was shown that in arabidopsis leaves the state 1 to state 2 transition was inhibited at light intensities above 300 μmol of quanta m-2 s-1, whereas in barley plants this process was still observed at intensities up to 1000 μmol of quanta m-2 s-1. The functioning ST in barley leaves at higher light intensities is probably determined by inhibition of STN7 kinase only at light intensities above 1000 μmol quantum m-2 s-1, since at lower light intensities a significant accumulation of phosphorylated Lhcb1 and Lhcb2 proteins occurs in barley leaves.
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### S6.391. Influence of the absence of thylakoid α-carbonic anhydrase 2 on state transitions in Arabidopsis thaliana plants

Nadeeva E.M.[1]*, Ignatova L.K.[1], Rudenko N.N.[1], Ivanov B.N.[1], Vetoshkina D.V.[1]
[1]*Institute of Basic Biological Problems, Federal Research Center «Pushchino Scientific Center for Biological Research of the RAS;*
* zhurikova-alena@yandex.ru

«State transitions» (ST) is an acclimation mechanism in which the absorbed excitation energy is redistributed between two photosystems (PS) due to the reversible migration of a part of the PSII light-harvesting complex (LHCII) between PSII and PSI. Under illumination, binding of the reduced plastoquinone in the cytochrome complex activates STN7 kinase. STN7 kinase phosphorylates the Lhcb1 and Lhcb2 proteins, which leads to the dissociation of these proteins from PSII and migration to PSI (state 2). In the dark, dephosphorylation of Lhcb1 and Lhcb2 by TAP38/PPH1 phosphatase occurs, which leads to dissociation of these proteins from PSI and return to PSII (state 1). We have previously shown that the absence of α-carboanhydrase 2 (α-CA2), which catalyzes the CO2+H2O=H++HCO3- reaction, in Arabidopsis thaliana mutant plants leads to a lower reduction of the plastoquinone pool and a higher rate of electron transport through PSII compared to plants of wild type (WT). Based on this, it seems interesting to evaluate the transitions of LHCII from state 1 to state 2 and back in these mutant plants.
The transition from state 1 to state 2 was evaluated by the classical approach, i.e., by measuring the low-temperature fluorescence of chlorophyll a. Under illumination with excitatory light, preferably PSII, in WT plants, the ratio of PSI/PSII fluorescence peaks changed by 22%, and in the α-CA2 mutant only by 8-13% compared with the values obtained after dark adaptation. This indicates a less pronounced transition of LHCII from PSII to PSI. The reverse transition of part of LHCII from PSI to PSII was evaluated at room temperature by measuring the non-photochemical quenching of chlorophyll a fluorescence, expressed by the NPQ parameter. Between the 15th and 24th minutes (ST-dependent relaxation) after turning off the effective red light in the α-CA2 mutant, the NPQ value was 2 times lower than in WT plants. Thus, a lower ability of mutant plants with blocked α-CA2 synthesis

to carry out the reverse transition from state 2 to state 1 was found compared to WT plants.
A possible reason for the observed differences in the course of state transitions may be a change in the activity of STN7 kinase and the accumulation of phosphorylated proteins Lhcb1 and Lhcb2 in mutant plants. Using Western blot analysis, it was shown that the content of phosphorylated proteins Lhcb1 and Lhcb2 in the α-CA2 mutant was lower than in WT. Interestingly, the total size of LHCII in the mutant is larger than in WT due to the higher content of Lhcb1 and Lhcb2 proteins.
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**S6.392. Influence of the photosensitizer structure on its adsorption and photodynamic properties on bilayer lipid membrane**

Konstantinova A.N.[1]*, Zykova D.D.[1], Markinsky K.I.[1], Urodkova E.K.[1], Sokolov V.S.[1]
[1]*The Institute of Physical Chemistry and Electrochemistry RAS (IPCE RAS);*
* anna.n.gavrilchik@gmail.com

The development of the photodynamic therapy method is associated with the research and directed synthesis of new photosensitizers (PS), as well as the development of methods to evaluate their effectiveness. Currently, the effectiveness of new potential drugs is tested mostly in vitro. Despite the enormous advantages of this testing technique, it does not provide definitive answers to questions about the interactions of the studied photosensitizer with the cell membrane (the first barrier on the way of PS into the cell) or the factors influencing the photodynamic activity of membrane-bound PS.
In earlier research we have developed the original procedure for the estimation of the adsorption and photodynamic activity of PS on bilayer lipid membrane (BLM) [1,2]. It is based upon the measurements of the changes in the membrane/water boundary potentials (BP) by three methods. Comparison of obtained values allowed to define the capability of PS to penetrate membrane, the position of PS charge group in it and to estimate the PS surface density in membrane. The photodynamic activity of membrane-bound PS was estimated by the destruction rate of singlet oxygen target (TO), which was adsorbed on the membrane and its destruction was monitored by measuring the boundary potential change during illumination. This procedure was used to study the commercially available compounds: sulphonated complex of phthalocyanines with aluminum (AlPcSn, n-number of sulphogroup) [1] and meso-tetra-(4-sulfonatophenyl) porphyrin [2]. The main investigation result was a proof of a connection between an increase in PS immersion depth into the membrane results and an increase in PS efficiency and a confirmation of the effect of PS binding to the membrane.
The goal of this research is to determine how PS structural elements affect the position of PS in the membrane and the effectiveness of PS. This work studies two distinct series of similar compounds synthesized in our institute:
1. phosphorus(V) porphyrins with different number of pyridyl peripheral groups (0, 1, 2) and axial ligands (hydroxy or ethoxy)
2. octa-cationic phthalocyanines as free-base, magnesium(II) and zinc(II) complexes (8bMePc, Me=H2, Mg, Zn) [3].
Adsorption of all compounds except porphyrins without pyridyl peripheral groups can be detected by BP change. The results show that independently of the analyzed PS structure, all PS possessing the charged peripheral groups have them immersed into the BLM below the phospholipid polar heads.
The structure of the axial ligand of porphyrins significantly influences their position in the BLM but not the binding affinity to the membrane. Porphyrins with hydroxy ligands P(OH)2 can penetrate the membrane, whereas porphyrins with ethoxy ligands P(OEt)2 cannot. However, the boundary potentials for P(OEt)2 was larger than the ones for P(OH)2. This fact is most likely associated with different orientations and positions of porphyrins with different axial ligands in the BLM, which explains the ability of P(OH)2 to penetrate through the membrane.
The phthalocyanines (Pc) adsorption potential is almost independent on the Pc macrocycle ion structure. Pc are unable to penetrate the membrane as well. The Pc adsorption on the membrane surface was detected by the Pc fluorescence spectra. These compounds aggregate in an aqueous solution, quenching their fluorescence. The introduction of liposomes consisting in DPhPC into the solution initiates the PS monomerization with complete restoration of fluorescent properties. The association constants of these Pc with lipids have been evaluated to be 4*10^3 M^-1, 5.5*10^3 M^-1 for 8bZnPc and 8bMgPc, respectively.
PS photodynamic activity is determined by the rate of the TO destruction and is observed for all compounds even those with adsorption not detected by the BP measurements. This fact indicates that porphyrins without pyridyl peripheral groups also adsorb on the BLM. The efficiency of porphyrins has been found to increase linearly with their concentration in solution. The effectiveness of phosphorus porphyrins with ethoxy axial ligands was higher compared to the hydroxy ones. This difference is most likely associated with different positions of the porphyrins with P(OEt)¬2 and P(OH)¬2 in the BLM. The self-destructions of the phosphorus porphyrins in BLM under illumination was observed, the rate of which increases with the number of pyridyl substituents, thus decreasing their efficiency as PS.
In case of phthalocyanines, the concentration dependencies of activity are more complex: the rate of TO oxidation increases linearly while PS concentrations are low, before it reaches a plateau. These differences in concentration dependence for phthalocyanines can be explained by the well-known ability to quench the singlet oxygen just by phthalocyanines. Furthermore, the significant difference in TO damage due to the influence of the PS macrocycle internal ion wasn't observed.
These results confirm that the PS efficiency mainly depends on their location and the effectiveness of their binding with BLM which strongly depends on the nature both of the axial ligands as well as the peripheral groups of the macrocycle.
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**S6.393. Inhibition mechanism of the oxygen-evolving complex of photosystem II by lanthanide cations**

Lovyagina E.[1]*, Loktyushkin A.[1], Semin B.[1]
[1]*Faculty of Biology, Moscow State University;*
* Elena.Lovyagina@gmail.com

La3+ cations and other lanthanides (Ln3+) were used as analogues of the Ca2+ cation to investigate the role of this cation in the operation of the oxygen-evolving complex (OEC) of photosystem II (PSII). Ghanotakis et al. (1985) found that La3+ inhibits the OEC activity, displacing the Ca2+ cation from the Mn4CaO5 catalytic center. The reaction of oxygen evolution in the PSII membranes containing the Ln3+ cation instead of Ca2+ is not reduced by the exogenous Ca2+ cation, i. e. the Ln3+ cation bound to the Ca-binding site is not displaced by the Ca2+ cation. In the Ca-depleted PSII membranes (PSII(-Ca)), Ln3+ cations compete with Ca2+ cations for the Ca-binding site (Ono 2000).

In addition to the Ca-binding site, lanthanides effectively bind to the Mn-binding site, namely, the high-affinity Mn-binding site of the PSII from which manganese cations have been previously extracted (Lovyagina et al. 2021). The high-affinity Mn-site is localized in the native crystal structure PSII at the Mn4 position according to the numbering of Umena et al. (2011). The ligands of this manganese cation are amino acid residues of Asp170 and Glu333 in polypeptide D1 (Asada and Mino 2015). Interestingly, the D1-Asp170 residue is also involved in the binding of the calcium cation, being a bidentant ligand. This feature suggests the possibility of interaction of the Ca-binding site with the high-affinity Mn-site of OEC when binding Ln3+ cations. In this work, a similar possibility has been investigated.

PSII membrane preparations from which calcium cation was extracted by treatment with high ionic strength medium (2M NaCl) were used. Previously it was shown that La3+ and Tb3+ cations bind irreversibly to the Ca-binding site (the bound cation is not removed by centrifugation and sample washing). Further, after appropriate sample treatment, the reduction kinetics of the artificial electron acceptor 2,6-dichlorophenolindophenol was measured in the presence of exogenous electron donors - a pair [Mn2+ + H2O2] that donates electrons only through a high-affinity Mn-binding site, or 2,5-diphenylcarbazide that donates electrons through two sites - high-affinity and low-affinity. The following samples were examined. 1) Manganese was extracted from the PSII(-Ca) particles by treatment with hydroxylamine or hydroquinone. 2) PSII(-Ca) membranes were incubated with La3+ or Tb3+ cation, after which unbound lanthanide cations were removed by centrifugation and manganese was extracted from the membranes. 3) Manganese was extracted from the PSII(-Ca) membranes after incubation with the La3+ or Tb3+ cation and reprecipitation, after which a lanthanide cation was added, and kinetics were measured in the presence of the Ln3+ cation. The results showed that the Ln3+ bound to Ca-site significantly inhibits oxidation of the donor pair [Mn2+ + H2O2] through the high affinity Mn-binding site (65-80% inhibition after complete extraction of manganese with hydroxylamine, sample 2). This means that Ln3+ cation bound to Ca-site effectively inhibits binding of the Mn2+ cation to the high affinity site. Two mechanisms of modification by the Ln3+ cation the coordination sphere of the high-affinity Mn region are possible - before manganese extraction or after. Substantial inhibition of the water oxidation by Ln3+ cation in PSII(-Ca) preparations without an exogenous electron donor (by about 50%) means that modification of the high affinity Mn-binding site by the lanthanide cation occurs when it binds to the Ca-binding region prior to extraction of manganese from the OEC.

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## S6.394. Investigation of the PI3-kinase role in the mechanism of Ca2+ signaling mediated by acetylcholine

Dymova E.A.[1]*, Rogachevskaja O.A.[1], Kotova P.D.[1]
[1]*Institute of Cell Biophysics RAS, FRC PSCBR RAS, Pushchino, Russia;*
* dymova.ek.a@gmail.com

In non-excitable cells, IP3-driven Ca2+ release plays a pivotal role in agonist-induced Ca2+ signaling. The efficiency of the phosphoinositide cascade, which couples diverse cell surface receptors to Ca2+ mobilization, is modulated by a number of kinases, including phosphoinositide 3-kinase (PI3K) that phosphorylates PIP2 to generate the phospholipid PIP3. We have previously shown that the PI3K inhibitor wortmannin does not affect acetylcholine-induced Ca2+ signaling in HEK-293 cells, while PI828, a PI3K inhibitor of distinct chemical nature, completely suppressed cellular responses to the agonist. As a possible reason for the different effectivity of wortmannin and PI828, PI3K isoforms functioning in HEK-293 could be much more sensitive to PI828. To clarify this issue, we generated a monoclonal line of HEK-293 cell, which expresses two genetically encoded sensors, namely, the cytosolic Ca2+ sensor R-GECO1 and the PIP3 sensor PH(Akt)-Venus. The cells of this line allowed for simultaneous monitoring of Ca2+ signals and PI3K activity. While R-GECO1 fluorescence is directly stimulated by Ca2+ binding, generation of PIP3 by PI3K initiates the translocation of PH(Akt)-Venus from the cytosol to the plasmalemma. It turned out that acetylcholine initiated a transient increase in the intracellular Ca2+ but did not affect the distribution of the PIP3 sensor in the cell cytosol. This indicated that acetylcholine did not stimulate PI3K activity. At the same time, insulin, which stimulates PI3K through tyrosine kinase receptors, caused the cytosol/plasmalemma translocation of PH(Akt)-Venus, thus demonstrating insulin-induced PI3K activity. This insulin-evoked translocation of PH(Akt)-Venus was canceled by wortmannin and PI828, suggesting that the inhibition of PI3K activity by these compounds was rather effective. Thus, being capable of stimulating intracellular Ca2+ signaling in HEK-293 cells, acetylcholine did not stimulate the PI3K pathway, which, therefore, was not involved in cholinergic transduction. Although the inhibition of PI3K by wortmannin and PI828 was undoubtable, the results of the present work suggest that PI828 suppressed acetylcholine-induced Ca2+ signaling nonspecifically, that is, not involving PI3K, but acting on some other cellular target.

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## S6.395. Isolation and characterization of carotenoid-protein complexes from thylakoid membranes of cyanobacteria Arthrospira platensis

Telegina T.A.[1], Vechtomova Yu.L.[1]*, Aybush A.V.[2], Borzova V.A.[1], Gevorgiz R.G.[3], Buglak A.A.[4], Kritsky M.S.[1]
[1]*Research Center of Biotechnology of the Russian Academy of Sciences;*
[2]*N.N. Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences;*
[3]*A.O. Kovalevsky Institute of Biology of the Southern Seas of the Russian Academy of Sciences;*
[4]*Saint Petersburg State University;*
* vechtomova@inbi.ras.ru

In the thylakoid membranes of photosynthetic organisms, along with the superfamily of chlorophyll-binding proteins of light-harvesting complexes (LHC) and the reaction center, there are low-molecular-weight light-inducible proteins [1]. It is believed that these proteins function in

photoprotection and assembly of thylakoid pigment-protein complexes, are expressed constitutively, and their expression levels increase in response to stress and primarily to light stress. In mutants lacking these low molecular weight proteins, photosynthetic electron transport is practically not detected. In cyanobacteria, which are the evolutionary precursors of chloroplasts, these proteins are called Hlips (high light-inducible proteins) or SCPs (small Cab-like proteins, small chlorophyll-binding proteins). These proteins are single stranded membrane low molecular weight proteins and are believed to be able to bind chlorophyll, carotenoids, and lipid molecules and participate in the assembly of photosystem I and II [1-2].

We believe that such a structuring role of SCP/Hlip proteins may be related to the cis-configuration of carotenoids in the carotenoid-SCP protein complexes present in thylakoid membranes, when carotenoids in the cis-configuration are like a "gluing" principle. In order to elucidate the structuring role of carotenoids in the photosynthetic apparatus, a method has been developed for obtaining carotenoid-protein complexes from the thylakoids of the cyanobacterium Arthrospira planensis (spirulina) using ultracentrifugation in a stepwise sucrose gradient (20-50% aqueous solution). Solubilized with the detergent n-dodecyl-β-D-maltoside (DDM), the thylakoids were separated by ultracentrifugation (220,000 g, 3 hours, 4°C) into six fractions containing chlorophyll-carotenoid-protein and carotenoid-protein complexes. The obtained complexes were characterized by UV/visible spectroscopy, circular dichroism, vibrational IR spectroscopy (FTIR), linear (including resonance mode) and nonlinear (BCARS) Raman scattering. Additionally, the isolated carotenoid-protein complexes were separated by electrophoresis in PAAG and separated in the transverse asymmetric flow field (AF4) to assess the content of low molecular weight proteins. The Raman spectra for carotenoids in the composition of complexes were compared with the theoretical spectra of cis-carotenoids obtained by quantum chemistry methods using non-stationary density functional theory (TD-DFT) and the CAM-B3LYP functional.

In general, the presence of cis-configuration carotenoids in the thylakoid membranes of spirulina in the composition of carotenoid-protein and chlorophyll-carotenoid-protein complexes containing β-carotene with an absorption maximum of 340 nm and xanthophylls (mixoxanthophyll, oscillaxanthin and zeaxanthin) with an absorption maximum in the region of 383 nm. CD spectrometry has shown that in aqueous conditions, carotenoid-protein complexes solubilized with DDM detergent are prone to aggregation, probably due to aggregation of the carotenoid component of the complex. At the same time, a strong CD signal is recorded in the cis-carotenoid region.

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### S6.396. Laser plasmon resonance photothermal therapy of model transplanted kidney cancer in rats

Genina E.A.[1,2]*, Genin V.D.[1,2], Bucharskaya A.B.[3,1], Kirillin M.Y.[4], Zarkov S.V.[5], Navolokin N.A.[3,1], Terentyuk G.S.[3], Khlebtsov B.N.[6,1], Khlebtsov N.G,.[6,1], Maslyakova G.N.[3], Tuchin V.V.[1,2,5]

[1]*Saratov State University;*

[2]*Tomsk State University;*

[3]*Saratov State Medical University;*

[4]*Institute of Applied Physics of RAS;*

[5]*Institute of Precision Mechanics and Control of RAS;*

[6]*Institute of Biochemistry and Physiology of Plants and Microorganisms of RAS;*

* eagenina@yandex.ru

Currently, along with many already traditional methods of treatment, such as surgery, chemotherapy, photodynamic therapy, radiation therapy, etc., methods based on the use of modern nanotechnology and, in particular, laser plasmon resonance photothermal therapy (PPTT) are attracting increasing attention. The effect of PPTT is based on the accumulation of plasmon resonance nanoparticles in tumor tissues and their local heating by laser irradiation with the appropriate wavelength, which allows to reduce the dose of laser radiation and reduce the damage to healthy tissues surrounding the tumor.

In this study, a kidney cancer cell culture obtained from the bank of tumor strains of the N.N. Blokhin Russian Cancer Research Center, transplanted subcutaneously to male albino rats, was used as a model. Gold nanorods (GNRs) with a maximum absorption wavelength of 800 nm were injected intratumorally. The irradiation was carried out percutaneously using a diode laser. The optical properties of separate layers (capsule, periphery and central part) of mature tumor and rat skin in the spectral range of 350-2200 nm were measured, and the absorption, scattering, scattering anisotropy and reduced scattering coefficients were calculated by using the inverse adding-doubling method. The simulation of photon propagation in the skin and tumor before and after the introduction of a suspension of GNRs was carried out by the Monte Carlo method and used to assess the absorption of laser energy in the tumor. The results of numerical simulation of heat distribution in the tumor and surrounding tissues are obtained. The simulation results correlate well with the experimentally measured kinetics of the skin surface temperature over the tumor during PPTT. Changes in the optical properties of the studied biological tissues as a result of PPTT have been experimentally studied.

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### S6.397. Led lamp for laboratory cultivation of chlorella microalgae (chlorella)

Burdysheva O.V.[1]*, Sholgin E.S.[1]

[1]*Perm Agriculture Research Institute division of PFRC UB RAS;*

* burdyshevaolga@gmail.com

In spite of the fact that chlorella microalgae have been widely used in many fields of human activity [1-3] research works aimed at the improvement of cultivation methods are carried out. An important condition for algae growth is lighting. Photobioreactors [4-6], oriented to produce large amounts of biomass, are mainly used as illumination units. Such units have large dimensions and are unsuitable for laboratory studies in which several low-volume vessels with samples differing in the conditions of culture development created in them (for example, composition of nutrient medium) are used, since they do not provide uniformity of irradiation. This factor reduces the validity of test results, complicates their interpretation and reproducibility.

The aim of the presented work was to design a LED-light for cultivation of chlorella microalgae in laboratory conditions, providing uniformity of illumination of experimental samples.

In this work the basic requirements for a radiation source for cultivation of microalgae in the laboratory were considered. The calculation by the method of successive approximations with summation of the contribution to the illumination of all diodes of the installation to assess the degree of homogeneity of illumination of experimental samples was carried out in the work. Three types of points located on the glass, characterized by their spatial position relative to the diodes, were distinguished. The relative divergence between the points of the three considered types is not more than 6% at the third iteration (i.e. when taking into account the neighboring 3rd order diodes), in connection with what we can say about sufficient uniformity of illumination of the working area. Calculation showed that in the central part of the working area relative discrepancy δ=(Emax-Emin)/<E>

of total illumination for points of three types did not exceed 0.3%. Illumination of points on the periphery of the working area, of course, is lower than in its center.

For approbation a specialized light fixture for chlorella microalgae was realized taking into account specifics of laboratory cultivation on the basis of Laboratory of Ecology and Immunology of the Institute of Ecology and Genetics of Microorganisms, Ural Branch of Russian Academy of Sciences. It seems optimal to place experimental samples on the luminaire itself. The specialized luminaire in this realization provides a uniform illumination of the working table with the samples installed on it, which allows to conduct a reliable comparative analysis of the results of cultivation of chlorella.

Specialized lamp contains a power unit, power regulator and a cascade of diodes, the dimensions of the lighting installation 340x220x50 mm. Through the power regulator is possible to control diode voltage, which allows to vary photosynthetically active flux (PAR) in the range of 150 - 350 µmol/(s•m2).

Conclusion

Developed specialized lamp provides a high degree of uniformity of illumination on its working surface, and also corresponds to the specifics of laboratory cultivation of chlorella and can be recommended for use in biological experiments.

The work was carried out within the framework of the state task, state registration number R&D 122031100058-3. The implemented lamp is used in the experiments of the Laboratory of Ecology and Immunology of the Institute of Ecology and Genetics of Microorganisms UB RAS.

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### S6.398. Long-distant transport as a photosynthesis regulation mechanism in characean algae

Alova A.[1]*, Bulychev A.[1], Cherkashin A.[1], Krupenina N.[1], Shapiguzov S.[1], Eremin A.[2], von Rueling F.[2], Rubin A.[1]
[1]*Lomonosov Moscow State University, Faculty of Biology;*
[2]*Otto von Guericke University Magdeburg, Institute of Physics;*
* annaalova@gmail.com

Photosynthesis is closely related to the long-distant transport and intercellular distribution of assimilates and signaling molecules. Long-distant transport of substances and permeation through intercellular barriers can become a bottleneck in the overall process and limit photosynthetic production. The presence of multiple intercellular barriers in the leaves of higher plants limits the lateral exchange of intermediates and reaction products between mesophyll cells. For this reason, the initial stages (at $t < 10$ s) of induction changes in the fluorescence (FL) of chlorophyll (Chl), measured in a limited area of the leaf, do not depend on the illumination or darkening of the surrounding areas. At the same time, Chl FL measurements at times of 100–400 s with a wide and narrow illumination field of the specimen make it possible to reveal the induction processes of photosynthesis associated with long-distant intercellular interactions. In the giant cells of Characeaen algae, the effect of long-distant transport of metabolites on photosynthesis and Chl FL should be especially noticeable, since the transfer in the lateral direction (parallel to the layer of immobile chloroplasts) is facilitated by the rapid rotational flow of the cytoplasm.

However, the distances over which photometabolites can propagate with the flow of the cytoplasm have not yet been determined, and there is no sufficient information about the role of long-distant interactions of chloroplasts in the induction of Chl FL. Little is known about the selectivity of metabolite transport through the intercellular pores of the plasmodesmata.

Long-distance interactions in photosynthesis can be studied by tracking the transients in Chl FL under local illumination of sample sites located away from a small region of FL detection (area of interest, AOI). The other approach is that the induction changes of Chl FL in AOI are compared under narrow-field and wide-field illumination of the sample. The divergent parts of FL induction curves in these light treatments reflect the exchange of metabolites between AOI and surrounding areas. These approaches were applied to study long-distant intra- and intercellular interactions in internodal cells of Chara australis and Nitelloposis obtusa algae.

It was shown that photometabolites exported from chloroplasts in areas of bright local light (LL) are entrained by cytoplasmic streaming to distances d of at least 10 mm along the cell length. Their delivery to AOI (at $d = 10$ mm) produces a sharp transient rise in Chl FL in about 120 s after the end of LL stimulus. Since N. obtusa algae are more resistant to salinity than C. australis, we assume that microfluidic signaling operates in both sensitive and salt-resistant charophytes.

The induction changes in Chl FL caused by illumination of the whole cell differed greatly from those induced by local illumination of AOI and adjacent areas; however, these distinctions disappeared after the arrest of cytoplasmic streaming in the presence of cytochalasin D. To elucidate the role of light-dependent transporters of the chloroplast envelope in the induction changes of Chl FL, transitions from zonal (local) lighting to general illumination of the internodal cell were employed. The S-M-T stage in the FL induction arising after such a transition was found related to both the internal processes in chloroplasts of AOI and the metabolite exchange between AOI and the areas located outside AOI. The amplitude and peak position of S-M-T stage depended on the condition of light-activated enzymes that transport the photometabolites across the chloroplast envelope. The results show that the export of photometabolites from illuminated chloroplasts starts 50–60 s earlier than the entry of metabolites from the cytoplasm into the stroma. The results prove the involvement of long-distant interactions in the photosynthetic induction.

Permeation of photometabolites through plasmodesmata of Chara cells was quantitatively assessed. It was shown that the mobile cytoplasmic package produced after exposure of cells to an intense LL at low and high background irradiance contains two types of metabolites that cause opposite changes in Chl FL. The reducing metabolites were found to freely permeate through intercellular barriers, while the products acting as FL quenchers were unable to overcome them. Apparently, plasmodesmata act as a selective filter that restricts the passage of potentially destructive agents generated at high-intensity light.

The effect of action potential (AP) generation on plasmodesmal conductance was studied in noncalcified cell regions in which Chl FL is insensitive to plasmalemma excitation. The AP generation in Chara internodes was found to have a stronger inhibitory action on intercellular transport of metabolites than on intracellular transport. The

results indicate the existence of specific mechanisms for plasmodesmal conductance regulation that are mediated by an increase in cytoplasmic Ca2+ concentration during the AP generation.
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**S6.399. Mechanisms of hydrogen peroxide formation and diffusion in chloroplasts of higher plants**

Borisova-Mubarakshina M.M.[1]*, Kozuleva M.A.[1], Naydov I.A.[1], Vetoshkina D.V.[1], Rudenko N.N.[1], Ivanov B.N.[1]
[1]*Institute of Basic Biological Problems, Russian Academy of Sciences;*
* mubarakshinamm@gmail.com

It is known that in the chloroplasts of higher plants hydrogen peroxide (H2O2) is formed in the superoxide anion-radical disproportionation reaction (O2•−) catalyzed by superoxide dismutase (SOD) in the chloroplast stroma (Asada, 1999). We have shown that part of H2O2 molecules is formed in the thylakoid membranes of chloroplasts and that the increase of H2O2 formation with increasing light intensity occurs mainly due to the formation in the membrane. There is no SOD in the membrane; moreover, the disproportionation reaction of O2•− in the aprotonic medium of the membrane with low dielectric permittivity is hampered, suggesting that another, different from disproportionation, pathway of H2O2 formation in the thylakoid membrane is taking place. We found that the formation of the "membrane" H2O2 occurs as a result of the reaction of O2•− with plastohydroquinone (PQH2): PQH2 + O2•− → PQ•− + H2O2; the reaction is thermodynamically favorable because of the large difference between the values of the redox potentials of the PQ•−/PQH2 (Em7 370 mV) (Hauska et al., 1983) and O2•−/ H2O2 (Em7 940 mV) pairs (Asada, 1999); the calculated thermodynamic equilibrium constant of the reaction ~ 5×10^9 (Mubarakshina, Ivanov, 2010) According to these potentials, the reaction of PQH2 with O2•− proceeds at the thylakoid membrane/stroma phase boundary.
H2O2 is one of the most important signaling molecules among reactive oxygen species (ROS). A fundamental factor for the implementation of retrograde signal by ROS (signal from organelles to the nucleus) is the ability of ROS to diffuse from the site of formation to the site of signal induction. We investigated i) the possibility of H2O2 to diffuse through chloroplast envelope membranes and ii) the mechanism of this diffusion using the EPR method and hydrophilic spin trap POBN, as well as confocal microscopy and Amplex Red dye. The results showed that some of the H2O2 molecules formed inside the chloroplasts are able to diffuse through the chloroplast membranes even at low light intensities. To elucidate the mechanism of H2O2 diffusion, the role of aquaporins, proteins that form pores in the chloroplast membrane, was investigated. Using the aquaporin inhibitors AgNO3 and acetazolamide (AZA), it was shown that H2O2 diffuses through the chloroplast membrane via aquaporins. In experiments with AZA, which in addition to inhibiting aquaporins is also known as a carboanhydrase (CA) inhibitor, enzymes that catalyze the reversible reaction of carbonic acid formation from carbon dioxide and water, the chloroplast envelope was found to have CA activity, indicating the presence of CA(s) bound to the chloroplast envelope. Considering that passive diffusion of H2O2 molecules through membranes is unlikely, the hydrophobic phase (benzene)/water distribution coefficient being 0.005 (Leo et al., 1971), the participation of aquaporins in the transport of H2O2 through the chloroplast envelope allows us to consider these proteins as an important element of intracellular signaling. The data we obtained on the presence of CA(s) bound to the chloroplast envelope indicate that the regulation of aquaporin activity appears to be related to the functioning of chloroplast membrane envelope CA(s).

**S6.400. Mitogenetic effect of ultraweak photon emission: the sleeping beauty or the alchemical past? (to the centenary of discovery of ultraweak photon emission from biological objects by A.G. Gurwitsch)**

Volodyaev I.V.[1], Naumova E.V.[2]*
[1]*Faculty of Biology, Lomonosov Moscow State University;*
[2]*Rzhanov Institute of Semiconductor Physics of SB RAS;*
* naumova@yandex.ru

As is known, the first work on ultraweak photon emission (UPE) of biological objects was published in 1923 by A.G. Gurwitsch [1], and the research on UPE has been widely developed in the following years (see, for example, monographs [2,3]). Apart from a direct continuation in Gurwitsch's laboratories, this research has attracted the widespread attention of biologists, physicists and chemists throughout the world and resulted in over 1000 publications from major laboratories in the USSR, Germany, France, Italy, the Netherlands, USA and other countries (see reviews [4-7]), including over a dozen papers in "Nature" and other highly ranked journals (e.g. [8-11]). Significant contributions to the research of the time were made by eminent scientists: Nobel laureate D. Gabor [3], Acad. G.M. Frank and Acad. J.B. Hariton [12,13], the eminent chemist R. Audubert [14], the famous microbiologists O. Rahn [15] and L.K. Wolff [11] and others.
The authors of this period attributed the UPE they observed to the UV range according to a set of experimental data: transmission by materials with different transparency windows, spectral studies, measurements with UV-sensitive gas-discharge counters. A considerable part of works of that time on UPE was devoted to its influence on mitosis rate in some biological objects (bacteria, yeast fungi, plant meristems, etc.). This "type of UPE" was called mitogenetic radiation (MGR).
With the outbreak of World War II, publications on the topic of MGR almost completely ceased, and the subsequent rediscovery of UPE [16-18] led to the successful development of the field of the intrinsic ultraweak chemiluminescence in the visible and infrared bands. At the same time, references to the "early works" of 1920-x-1940-x remained mostly in historical reviews.
At the same time, a number of issues raised in the early publications have remained without an unambiguous solution. Thus, in works [19-21] the quenching effect of human and animal blood MGR was used for diagnostics of oncological diseases. The effectiveness of this diagnostics was verified in the leading oncological clinics of Moscow, Leningrad and some other cities of the USSR, as well as on experimental animal models, and showed 95-97% coincidence with the diagnosis [19-21]. Moreover, the very fact of the authors' claimed stimulation of mitosis in tissues by their exposure to MGR of other bioobjects is unexpected (in the context of the current knowledge about the nature of UPE in longer wavelength ranges) and its verification is of fundamental importance.
We were fortunate to have access to the unique library of A.G. Gurwitsch and his family and to have seen the original works. Most of them, for various reasons, are practically inaccessible to the modern reader, and are considered by us in a series of reviews on this topic [4-7] and a forthcoming monograph on endogenous biophotonics (Springer-Nature, 2023). Here we analyze the methodological aspects of the works on MGR and compare their results with modern data on the physics, biophysics and physiology of the research objects. Although some of the work of the time was performed at an uncontemporary level of evidence and in some cases, there are outright errors, a number of in-depth careful experimental works (e.g. [3,11-13,19]) deserve serious attention and verification by modern investigators.

There have been no meaningful attempts to replicate and verify these works, observing the conditions described by the authors, with few exceptions (e.g. [22]), apparently due to their low availability.
We are convinced that an accurate, well-conducted test of the MGR phenomenon will be of great scientific value - both the fundamental (the study of signaling cascades and regulatory processes) and the applied one (diagnosis of cancer, a non-invasive method of assessing the state of various systems). Significant progress in the methods of cell biology, biochemistry and biophysics, physical methods and experimental techniques allow us to hope that this issue, which has important fundamental and applied significance, will be solved at the present level.

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### S6.401. Multichromophore photoactive proteins for optogenetic applications

Maksimov E.G.[1]*, Borshchevskiy V.I.[2], Sluchanko N.N.[3], Yaroshevich I.A.[1], Tsoraev G.V.[1], Lukashev E.P.[1], Petrovskaya L.E.[4], Bogachev A.V.[1]
[1]*Lomonosov Moscow State University;*
[2]*Moscow Institute of Physics and Technology;*
[3]*Federal Research Center "Fundamentals of Biotechnology", Russian Academy of Sciences;*
[4]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences;*
* emaksimoff@yandex.ru

Many photosynthetic organisms use carotenoids as additional chromophores in pigment-protein complexes to ensure efficient light absorption in the blue-green part of the spectrum, regulation of energy transformation and protection of cell membranes from photodamage. The investigation of the principles of modular organization of water-soluble orange carotenoid protein in cyanobacteria allowed us to develop methods for the assembly of photo-controlled split-domain systems and ways to deliver carotenoids into cell membranes. Protein-mediated delivery opens up new opportunities for solubilization of hydrophobic carotenoids, controlled enrichment of cell membranes with natural antioxidants, and allows investigation of a specific family of proton and sodium pumps capable of binding carotenoids as an additional chromophore on cellular models. The report will reveal the structural and functional features of a number of carotenoid proteins and their modifications optimized for optogenetic applications.

### S6.402. Optical properties, energy transfer and charge separation in photosynthetic reaction centers

Pishchalnikov R.Y.[1]*
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences, GPI RAS;*
* rpishchal@kapella.gpi.ru

Among the great diversity of photosynthetic pigment-protein complexes, reaction centers are of the greatest interest for theoretical study because both oxygen and non-oxygenic photosynthetic organisms have similar spatial arrangements of pigment molecules. The main feature of any reaction center is the presence of two branches of pigment molecules, consisting of chlorophylls, pheophytins (bacteriochlorophylls and bacteriopheophytins), carotenoids as well as quinones. There are two types of reaction centers, classified according to the type of terminal electron acceptor. The first type includes reaction centers in which iron-sulfur clusters (photosystem I, green-sulfur bacteria, heliobacteria) are electron acceptors; the second type (chlorophlexia, purple bacteria, photosystem II) has quinones as acceptors. The theoretical modeling of the results of various spectroscopic techniques makes it possible to analyze the observed optical properties of the reaction centers and describe the mechanism of efficient conversion of solar energy into electronic excited and charge-separated states. Moreover, the use of X-ray data of pigment-protein complexes as well as modern computational and optimization methods allows the construction of universal quantum-mechanical models necessary for the description of photosynthetic processes in different organisms.
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### S6.403. Photocytotoxicity of upconversion nanoparticles complexes with a photosensitizer in vitro

Anisimov R.A.[1], Verkhovskii R.A.[1], Lomova M.V.[1], Navolokin N.A.[3], Kochubey V.I.[1,2], Yanina I.Yu.[1,2]*
[1]*Saratov State University;*
[2]*Tomsk State University;*
[3]*Saratov State Medical University;*
* irina-yanina@yandex.ru

The unique photoluminescent properties of upconversion nanoparticles (UCNPs) are promising for bioimaging due to an increase in image contrast due to the absence of tissue autofluorescence and photostability, which allows long-term imaging at the level of individual nanoparticles [1]. These properties make UCNPs attractive as contrast agents or biological sensors for biomedical imaging and theranostics of various diseases [2].

Due to the contradictory data on the cytotoxicity of UCNPs, particles are often encapsulated in various carriers, such as vaterite particles, and based on its core-shells and polyelectrolyte capsules [3]. It was previously shown that the formation of hollow microcapsules or core-shell microcapsules helps to remotely control the permeability of the shells [4]. Of interest is the development of a complex containing UCNPs and a photosensitizer (PS).
Fibroblasts L-929 and 4T1 were used in the experiments in vitro. Cells were seeded separately in special cell culture plates and cultured in DMEM medium with 1% penicillin-streptomycin cocktail, 2 mM L-glutamine, and 10% fetal bovine serum. Cultivation was carried out in a special incubator containing 5% CO2 at 37°C. The media were replaced with fresh ones every 2 days. Cell cultures with the formation of 75-85% of the monolayer were removed using 0.25% trypsin and the number of cells was counted using a hemocytometer.
In the work, we used UCNPs of our own production NaYF4: Yb3+, Er3+. UCNPs (about 80 nm in size) were synthesized by the hydrothermal method. The concentration of UCNPs was 5 mg/mL. Cy3 and Cy5 were used as PS.
Cell viability was studied by adding a suspension of AKNP and subsequent IR irradiation (exposure time from 5 to 45 minutes). Cytological and morphological comparison of responses of cell lines to exposure was carried out.
Submicron core-shell systems containing ANNP and PS were obtained. Submicron particles of CaCO3 were used as the core.
The presence of radiative energy transfer from ANNP to PS is shown when the luminescence of UCNPs is excited by long-wavelength radiation (980 nm).
The particles exhibited cytotoxic, cytostatic properties. The results obtained can be taken into account in the development of complex preparations for photodynamic therapy and the study of ways to increase their effectiveness during the photodynamic therapy (PDT) procedure using them.
The study was supported by the Russian Science Foundation grant No. 21-72-10057, https://rscf.ru/project/21-72-10057/
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### S6.404. Photoinduced neutrophil extracellular traps

Zakrytnaya D.S.[1]*, Demina E.M.[1], Shutikov A.A.[1], Arynbek Y.A.[1], Mamatkulov K.M.[1], Arzumanyan G.M.[1]
[1]*JINR, FLNP;*
* darya.zakrytnaya@nf.jinr.ru

NETosis is a programmed cell death which occurs in response to various types of stimuli. Nowadays, studies devoted to the investigation of photoinduced NETosis activated predominantly by ultraviolet (UV) radiation are becoming increasingly important. This work is devoted to the study of the activation of neutrophils at two wavelengths to cause photoinduced NETosis at three different doses of radiation (4, 16, 32 J/cm^2). Phorbol 12-myristate 13-acetate (PMA) used as a positive control for activation. We proposed that cytochromes are the prime photoacceptors of light sources. Cytochromes trigger the whole the descending chain, starting with the activation of ROS generation and ending with the released extracellular traps. Cytochromes are part of NADPH oxidase and granulocyte mitochondria. Application of selective inhibitors showed that under the influence of exposure of two wavelengths undergoes a mechanism of NETosis through two signaling pathways.

### S6.405. Photosystem II regulation at microalgal hydrogen production

Tsygankov A.A.[1]*, Grechanik V.I.[1]
[1]*Institute of Basic Biological Problems RAS, PNCBI RAS;*
* ttt-00@mail.ru

Separate groups of microalgae are capable, after a period of anaerobic adaptation, of light-dependent hydrogen production. However, the process is inhibited by oxygen and is therefore short-term. After lighting, the accumulating oxygen inactivates hydrogenase, and the hydrogen production ceases. An approach for the extention of hydrogen production has been developed. To do this, cultures are placed under conditions of deprivation of sulfur, nitrogen, phosphorus or carbon. After some time of cultures adaptation to a lack of a nutrient element leads to a decrease in the activity of photosystem II, the respiration rate becomes higher than the rate of photosynthesis, and the cultures pass into anaerobic conditions, where hydrogenase is synthesized. After its synthesis, hydrogen production begins, which lasts for several days. There are reports in the literature that several regulatory mechanisms may be involved in reducing the activity of photosystem II: accumulation of QB non-reducing centers, over-reduction of PQ pool, appearance of stably reduced forms of QA, state transitions, xanthophyll cycle, D1 degradation, photoinhibition (oxidative stress), accumulation of ascorbate, followed by the use of ascorbate as an electron donor instead of water. At the same time, different authors indicate one or two mechanisms of photosystem II inhibition. Surprisingly, almost all the work was done on the green microalgae Chlamydomonas reinhardtii, placed under conditions of sulfur deprivation. According to our studies, these mechanisms do not work simultaneously, and the implementation of a specific mechanism of photosystem II inhibition is determined not by nutrient deprivation but by other environmental factors, primarily by the light. The work shows that, depending on the light intensity, photoautotrophic cultures of C. reinhardtii with a deprivation of nutrients can implement various mechanisms of inhibition of the activity of photosystem II.
This work was supported by the Russian Science Foundation grant 19–14-00255.

### S6.406. Plants Photochemistry Growing Under Glass with Photoconversion Luminescent Eu Nanocomposites

Paskhin M.O.[1]*, Yanykin D.V.[1,2], Pobedonostsev R.V.[1], Gudkov S.V.[1]
[1]*Prokhorov General Physics Institute of RAS;*
[2]*Institute of Basic Biological Problems RAS, Pushchino, Russia.;*
* pashin.mark@mail.ru

Photoconversion materials have great potential for agricultural applications [1], as they convert light that is little used or even harmful to plants into photosynthetically active radiation (PAR) [2,3]. Luminescence in the PAR region is reached due to the downconversion of photons of ultraviolet radiation. Some rare-earth metals, in particular Eu-based nanoparticles, have similar properties.
Two types of nanoparticles were obtained: Eu: Eu2O3 obtained by laser fragmentation of Eu2O3 and Eu3+:LaF3 by hydrothermal-microwave

treatment. Their physical and luminescent properties were tested. After that, the nanoparticles were deposited on the glass surface, and the effect of converteded light on the morphometric, physiological, and photochemical parameters of the Solanum lycopersicum tomato culture was studied.
As a result of the research, it was found that the size of both nanoparticles types was 200 nm. Eu2O3 nanoparticles slightly reduce the light intensity in PAR. The obtaining particles have fluorescence in the red region: Eu2O3 at 612 nm and 626 nm, and Eu3+:LaF3 at 592 nm and 620 nm. Growing plants under such coatings led to changes in their morphometric parameters, in particular, the leaf surface area increased by 37%, the stem length increased by 25% in plants growing under coatings with Eu2O3 nanoparticles, than in control plants and plants growing under Eu3+:LaF3 nanoparticles, and the number of leaves did not change in any experiment. At the same time, the amount of chlorophyll fell in both experiments, compared with the control.
Statistically significant differences in photochemical experiments appear on the 43-rd day after germination. It was found that the development of the effective quantum yield of photosystem II (Y(II)) develops faster in plants growing under a coating with Eu2O3 nanoparticles, and the quantum yields of controlled (Y(NO)) and uncontrolled (Y(NPQ)) heat dissipation were smaller and plateaued faster than other samples. Similar results were obtained for photochemical (qL) and non-photochemical (qN) quenching. In addition, the electron transfer rate of photosystem II (ETR(II)) also rapidly increased in the samples growing under the Eu2O3 coating. The obtained results may indicate a higher rate of plant adaptation to light, which leads to faster growth and development of plants, as indicated by morphometric indicators.
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### S6.407. Primary photoreactions of Exiguobacterium sibiricum rhodopsin (ESR) as a function of pH

Smitienko O.A.[1]*, Feldman T.B.[1,2], Petrovskaya L.E.[3], Yakovleva M.A.[1], Shelaev I.V.[4], Gostev F.E.[4], Cherepanov D.A.[4], Kolchugina I.B.[2], Nadtochenko V.A.[4,5], Kirpichnikov M.P.[2,3], Ostrovsky M.A.[1,2]
[1]*Emanuel Institute of Biochemical Physics;*
[2]*Biological Faculty Lomonosov Moscow State University;*
[3]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry;*
[4]*Semenov Federal Research Center of Chemical Physics;*
[5]*Department of Chemistry, Lomonosov Moscow State University;*
* djolia@gmail.com

The microbial rhodopsin of the soil bacterium Exiguobacterium sibiricum (ESR) carries out a light-dependent active proton transport from the cell to the external environment, thereby converting light energy into membrane electrochemical potential. The functioning of this protein, as well as other microbial and animal rhodopsins, is based on the photoisomerization of the retinal chromophore group, which proceeds in an excited state in the femtosecond time range. Previously, we have shown that there is an additional non-reactive path of the excited state decay in the picosecond time range, which affects the overall quantum yield of the reaction [1]. It is likely that the presence of the non-reactive pathway is due to the pH-dependent heterogeneity of the initial state, which was demonstrated for the closely related to ESR proton pump proteorhodopsin [2], the proton pump bacteriorhodopsin [3], and the sodium pump KR2 [4]. To test this hypothesis, the dependence of the rate and efficiency of the ESR photoreaction on pH was studied.
The work was carried out by femtosecond laser absorption spectroscopy with 25–45 fs pump pulses and probing in the range of 400–900 nm. ESR was expressed in E. coli, purified by chromatography and extracted into 0.05-0.1% DDM at pH 5.3, 7.4 and 9.5. The characteristic times of the observed processes were estimated by model exponential curves based on experimental kinetic curves.
It was shown that, at each chosen pH value, signals from the excited state are observed, which are replaced by absorption signals of the first photoproducts containing isomerized retinal, J and K. Product J is formed in the femtosecond time range and passes into the next product K in several picoseconds. The dynamics of the excited state decay consists of two components: a fast femtosecond component characterizing the reactive pathway for the formation of product J, and a slow picosecond component characterizing the nonreactive pathway of returning some of the excited molecules to the initial state. With an increase in pH from 5.3 to 9.5 units, a decrease in the fast component from 830 to 490 fs is observed, which indicates an increase in the rate of the photoisomerization of the retinal chromophore group. In this case, the contribution of the non-reactive excited state decreases from 40 to 26%, and the quantum yield of the photoreaction increases significantly, as can be seen from the increase in the signal intensity of the photoproduct K by 2.5 times.
The results obtained indicate a significant effect of pH on the rate and efficiency of the ESR photoreaction. Using other microbial rhodopsins as an example, it was shown in [2–4] that the heterogeneity of the initial state is associated with the degree of protonation of the aspartate amino acid residue, which serves as a counterion to the retinal Schiff base (SB). The interaction with the counterion in the deprotonated form strongly affects the system of electronic levels of retinal and the dynamics of the photoreaction. In the case of ESR, this is D85, which is bound to the proton of SB by a strong hydrogen bond through a water molecule. At pH 9.5, it is completely deprotonated, resulting in the fastest and most efficient photoreaction. Thus, it can be concluded, that the photoreaction of ESR have much in common with microbial rhodopsins that act as cation pumps. Probably, these properties are associated with the adaptation of rhodopsins to environmental conditions, the pH of which is shifted mainly to the alkaline region, such as, for example, in the sea (PR, KR2) and salt lakes (BR).
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### S6.408. Processes of state 2-state 1 transitions and redox state of plastoquinone pool in algal and cyanobacterial thylakoid membranes of Scenedesmus obliques and Synechocystis PCC 6803 cells by modeling fluorescence and P700 induction kinetics

Belyaeva N.E.[1]*, Bulychev A.A.[1], Klementiev K.E.[2], Paschenko V.Z.[1], Riznichenko G.Yu.[1], Rubin A.B.[1]
[1]*Biological Faculty, Moscow State University, Moscow, 119234 Russia;*
[2]*Biological Faculty, Shenzhen MSU-BIT University, Shenzhen, 518172 China;*
* natalmurav@yandex.ru

The closed system of thylakoid membranes (TM) operates both in chloroplasts (leaves, algae) and in the cytosol of cyanobacterial cells [1]. Mitochondrial metabolism indirectly affects the processes in the

electron transport chain (ETS) in algae [1, 2]. Cyanobacterial TM separates the lumen/cytoplasm and provides both photosynthetic and respiratory electron transport (PET and RET). PET and RET are connected by participating in the processes of electron influx and outflow into the pool of plastoquinones/quinols (PQ/PQH2) [1-4].
The coherence of photosystems II and I (PS II and PS I) is important for the transition of the TM system from darkness to light conditions. The features of the regulation of light induction are determined by the structure of the light-harvesting antennae complexes of the reaction centers (RC) PS II and PS I. The role of antenna complexes is performed by movable phycobilisomes located outside of the TM in cyanobacteria. The dynamics of OJIPSMT light-induced changes in fluorescence (FL) in vivo were studied by us for two samples: 1) cyanobacterium cells Synechocystis sp. PCC 6803 (Synechocystis); 2) microalgae Scenedesmus obliques (Scenedesmus). The "Thylakoid" model [5-7] has been debugged to quantitatively describe the fluorescence induction curves (FI) of Synechocystis and Scenedesmus. The light responses of the samples were calculated at the stages of rapid OJIP increase of FL to P peak ($t < 1$ s) followed by a slow (seconds - minutes) wave of PSMT decline of variable FI. Description of the processes was obtained on a time scale from microseconds to 5-10 minutes for algae and cyanobacteria.
Description of the state transition effect was used to find the parameters of the "Thylakoid" model. It is shown that the turning on of state transition 2→1 leads increasing in SM on 2-3 minutes of the kinetic pattern in the calculations of the IF Scenedesmus. Redox cofactors of ETC were maximally reduced under conditions of light-induced charge redistribution in the lumen and stroma compartments in the calculations of IF Synechocystis and Scenedesmus during the formation of a fast OJIP pattern. The filling and outflow of electrons in the carrier pools after PS II and PS I give contributions to a slow PSMT wave. Regulation of linear electron outflow by light activation of ferredoxin–NADP reductase is important for the balance of photosystems, but qE-dependent quenching is caused by lumen acidification only for algae Scenedesmus and does not work for cyanobacteria Synechocystis. Further, it is possible to study the molecular mechanisms of regulation of state transitions 1→2 and 2→1 through the redox state of the PQ pool in the "Thylakoid" model as an effect that has been studied for leaves and algae and is assumed for cyanobacteria [1-4].
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## S6.409. Protective effect of light on oxygen-evolving complex of photosystem 2 in spinach thylakoid membranes under heat stress conditions

Lovyagina E.[1]*, Loktyushkin A.[1], Semin B.[1]
[1]*Faculty of Biology, Moscow State University;*
* Elena.Lovyagina@gmail.com

The oxygen-evolving complex (OEC) of photosystem II (PSII) is one of the most heat-sensitive components of chloroplasts. It is known that inhibition of oxygen release begins to occur in the region of 40°C as a result of dissociation of extrinsic proteins and subsequent destruction of the Mn4CaO5 cluster, which seems to involve reactive oxygen species. The sensitivity of the donor side of the PSII to heat treatment can be significantly affected by the pH of the medium, since this factor affects the protonation/deprotonation of amino acid residues and water molecules associated with the catalytic center of water oxidation - the manganese cluster. In this regard, investigation of changes in OEC resulting from modulation of the pH of the intrathylakoid environment may contribute to the discovery of new mechanisms of plant resistance under stress.
We previously found that the temperature resistance of the manganese cluster in PSII preparations without Ca2+ cation in OEC significantly depends on the pH of the medium: resistance is minimal at pH 6.5, increases with a decrease in pH to 5.7 and remains almost constant with a further decrease in pH to 4.0 (Lovyagina and Semin 2022). These results correlate well with the data we have obtained (Semin et al. 2015, 2018; Davletshina and Semin 2020), demonstrating that at pH 5.7 in OEC, a structural transition occurs, accompanied by an increase in the resistance of manganese cations to the action of reducing agents and photoinhibition.
In the present work, we investigated the thermoinactivation of OEC in spinach chloroplasts under dark conditions and under non-photoinhibiting illumination. It was shown that pronounced inhibition of oxygen evolution by thylakoid membranes is observed from 40°C - by ≈50%. However, illumination of chloroplasts (30% of the saturating light intensity in the Soret band) when heated significantly reduces the inhibitory effect of temperature. The maximum difference we observed at 40°C was a decrease in inhibition of more than 20%. We associate this effect with the acidification of lumen to pH 5.7 as a result of the formation of a proton gradient on the thylakoid membrane under illumination. To test this hypothesis, we measured the oxygen evolution curves of thylakoids after warming them up in the dark and in the light in the presence of protonophores - nigericin and ammonium chloride. Indeed, in the presence of protonophores, the photoprotective effect on the thermal inactivation of OEC was completely absent.
We assume that the increase in the resistance of the PSII to heat stress in light during lumen acidification (pH 5.7) is determined by a change in the redox potential Em of one or more manganese cations in OEC. The change in Em in turn increases the resistance of manganese cations to reducing agents, for example, active oxygen species involved in the manganese cluster degradation process. Thus, the structural transition in OEC at pH 5.7 can play the role of a built-in mechanism that protects the PSII from both photoinhibition and temperature stress.
The study was carried out within the framework of the scientific project of the state assignment of Moscow State University No. 121032500058-7.
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### S6.410. Reversibility of redox reactions in the ommochromes of the compound eye of insects

S6.410. Reversibility of redox reactions in the ommochromes of the compound eye of insects

Yakovleva M.A.[1]*, Dontsov A.E.[1], Aybush A.A.[2], Gulin A.A.[2], Gubina M.V.[2], Vasin A.A.[2], Nadtochenko V.A.[2], Ostrovsky M.A.[1,3]
[1]*Emanuel Institute of Biochemical Physics, RAS;*
[2]*N.N. Semenov Federal Research Center for Chemical Physics, RAS;*
[3]*Lomonosov Moscow State University;*
* lina.invers@gmail.com

Ommochromes belong to a large class of substances found in various tissues of invertebrates, mainly in arthropods. They perform the function of shielding excess light, antioxidant protection and coloring of the body [1]. The color change of ommochromes is usually associated with redox transitions in the pigment molecule. Under the action of oxidizing and reducing agents, ommochromes can change from the reduced to the oxidized form and vice versa, which was recorded by reversible changes in the position of their absorption maximum [2]. In this case, when ommochromes are oxidized, their absorption maximum shifts to shorter wavelengths, and when reduced, it returns to its original position [3]. The redox status of ommochromes is of great importance in the life of arthropods. For example, during puberty, in some species of dragonflies, the body color changes from yellow to red, which is associated with the appearance of a larger number of reduced ommochromes. In addition, it is known that ommochromes have antioxidant and antiglycation activities only in the reduced state [1]. Therefore, the ability of ommochromes to reversible oxidation-reduction is of great biological importance. The aim of this work was to study the physicochemical properties of the oxidized and reduced forms of the ommochromes of the compound eye of insects using FT-IR spectroscopy, CARS spectroscopy, fluorescence spectroscopy, and HPLC analysis. For the first time, reversible changes in the fluorescence characteristics of insect ommochromes during redox reactions have been shown. A reversible change in the composition of ommochromes in oxidation-reduction reactions has been demonstrated. The HPLC analysis of samples from the eye of the black soldiers fly (Hermetia illucens, family Strationyidae) showed the possibility of almost complete recovery of the oxidized forms of the pigment. The results of this work are important both for the general development of knowledge about the physicochemical properties of ommochromes and for understanding the functional significance of these compounds in insect tissues. Measurements in the framework of FT-IR and CARS spectroscopy were performed by the Central Collective Use Center/UNU FRC CP RAS (registration numbers no. 1440743 and no.

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### S6.411. Study of Danio rerio colour preferences and responses to light

Lubarets E.I.[2], Khavronyuk I.S.[2], Skuratovskaya E.N.[1], Kuznetsov A.V.[1,2]*
[1]*A.O. Kovalevsky Institute of Biology of Southern Seas, Russian Academy of Sciences;*
[2]*Sevastopol State University;*
* andrei_kouznetsov@hotmail.com

Understanding the basis of light and colour signal perception by organisms in the environment is an important task of biophysics. Most vertebrate species have a visual system based on opsins sensitive to red, green, blue and UV light [Bowmaker, 2008]. Danio rerio is a convenient model in embryology. Its genome has been sequenced [Howe et al., 2013], many opsins have been cloned and their spectral characteristics have been determined [Robinson et al., 1993; Vihtelic et al., 1999]. However, the reasons for the colour preferences of D. rerio and their change during individual development remain poorly understood [Peeters et al., 2016]. It is interesting to relate the number of coloured opsins found in D. rerio and their structural features to fish behaviour patterns under different light sources.

D. rerio was kept in a 41x25x35 cm aquarium at 25°C with daily light. Experiments were carried out in the aquarium or in channels. We used ~1 W semiconductor lasers: red (650 nm), green (532 nm) and blue (405 nm), and a miniature UV (395 nm) light source and coloured LEDs: red (620 nm), green (529 nm) and blue (470 nm), and white. The LEDs were connected to an Arduino board for pulsed illumination with random flashes of light and pauses of up to 1 s in between. The laser beam was directed at fish swimming in the aquarium in daylight or in the dark. Behavioural reactions during acute light exposure were investigated. In another variant of the experiments, D. rerio was placed in white channels (200x6x3 cm) or channels painted in different colours (100x6x3 cm). Sometimes a part of the channel was covered with daylight cover, simulating a grotto with one or a tunnel with two entrances-exits. LEDs were placed at the edge or in the centre of the channel above the water surface so that they shone downwards in total darkness. The baffles were lowered into the water after 3 minutes of exposure and the proportion of fish in the compartments was counted. Each experiment was repeated 3 times. The study lasted for 2 months and consisted of several series with 20 fishes from 1 month of age.

The amino acid sequences of D. rerio opsins were searched in the NCBI database. Multiple polypeptide sequence alignment was performed on the Clustal Omega server. Spatial models of opsins were constructed on the Phyre2 server. Retinal docking was performed on a SwissDock server.

D. rerio was found to exhibit a pronounced response to green and blue lasers in light, while in darkness it reacts vigorously to green lasers and markedly to other colours (white, red, blue, UV), which correlates with the content of green light sensitive opsins (4 homologues).

D. rerio are evenly distributed in the channel in diffused daylight, 2/3 of the fishes hide in the grotto and almost all are collected in the tunnel. This fact suggests that 4 types of opsins (red, green, blue, UV) are involved in white light processing.

Fishes concentrated in the red half and did not stay in the white part in the two-coloured channels; ~2 times fewer fishes were collected in the green part than in the blue part; D. rerio chose red when comparing red with green. Observations indicate that D. rerio prefers the main colours in the following order: red ≈ blue > green > white, with white and bright areas being the least preferred. Fish stays in these areas only for a short time, which is consistent with [Avdesh et al., 2012].

The fishes were indifferent to white and red light under constant LED illumination. In contrast, D. rerio moved away from constant green or blue light. Random flashes of white light frightened the fishes and they were distributed chaotically in the channel. Coloured random flashes had a less pronounced effect, again indicating complex processing of light inputs by the nervous system [Connaughton et al., 2021].
The correlation between experiments with location of light sources (W, R, G, B) at the edge or in the centre of the channel for constant light is r=0.62 and for random light flashes r=-0.04. This result suggests a patterned response of fishes to constant lighting of different colours and an unpredictable response to random flashes, resembling startle [Koyama et al., 2021].
A total of 145 opsin-like proteins of D. rerio were detected, of which 121 opsins were analysed. Nine clusters were identified: red, green, blue and UV-sensitive opsins, rhodopsins, melanopsins, teleost multiple tissue opsins, ancient vertebrate opsins and uncharacterised proteins. Of most interest are 4 groups of opsins sensitive to red, green, blue and UV light. The number of opsins has been reduced to 11 unique polypeptides.
The following opsins were characterized: NP_001300644.1 (R-opsin 1, 357 aa, 39.89 kDa) and NP_001002443.1 (R-opsin 2, 356 aa, 39.51 kDa), NP_571328.2 (G-opsin 1, 349 aa, 38.85 kDa), NP_878311.1 (G-opsin 2, 349 aa, 38.71 kDa), NP_878312.1 (G-opsin 3, 349 aa, 38.89 kDa) and NP_571329.1 (G-opsin 4, 349 aa, 38.72 kDa), the blue-sensitive cone opsin BAC24133.1 (354 aa, 39.49 kDa), 2 short-wave cone opsins AAH60894.1 (336 aa, 37.26 kDa) and AAH67683.1 (336 aa, 37.28 kDa), and 2 UV-sensitive opsins of cones AAD24756.1 (336 aa, 37.15 kDa) and BAC24134.1 (336 aa, 37.27 kDa).
Unique N-terminal peptides were observed, as well as a deletion in the C-terminal region in red opsins; in addition, a deletion was found downstream in UV opsins, with the deletion at the N-terminus in green opsins. A divergent evolution of the UV opsins AAD24756.1 and BAC24134.1 was detected. Retinal is incorporated into the 3D models of UV opsins with -ΔG values: 8.68 for AAH60894.1, 8.62 for AAH67683.1, 8.58 for BAC24134.1 and 8.45 kcal/mol for AAD24756.1.
It follows from experiments and calculations that D. rerio is able to distinguish at least 2 shades of red, 4 shades of green, blue and possibly no less than 2 shades of UV light during certain stages of embryogenesis, to process the received information in a neural network and to make decisions expressed in motor activity.

### S6.412. Study of EGFP fluorescence during its de- and renaturation, and with the addition of nanoparticles

Mozhaeva V.A.[1]*, Sarimov R.M.[1]
[1]*Prokhorov General Physics Institute of the RAS, Moscow, Russia;*
* 1996-racer@mail.ru

Introduction
Green fluorescent protein (GFP) was first isolated from the jellyfish Aequorea Victoria. It exhibits bright green fluorescence. The so-called enhanced GFP (EGFP) is a mutant version of GFP, with improved spectral characteristics, as well as more efficient folding at relatively high temperatures, which makes it possible to use EGFP in mammalian cells. This protein is widely used in cell biology through the method of fluorescence microscopy.
It is important to study the influence of various environmental factors on GFP and its derivatives, in particular, to study the stability of its fluorescent properties and the ability to restore the fluorescent signal during denaturation-renaturation. Previously [1], researchers were able to achieve renaturation of 90% of the GFP protein. In this work, we studied the denaturation-renaturation of the most commonly used version of this protein, EGFP, as well as the effect of the addition of nanoparticles on its fluorescence.

Materials and methods
The fluorescence spectrum was recorded using a JASCO spectrofluorimeter FP-8300 with a resolution of 1 nm in the cell with optical path of 1 mm. The measurement range was: for excitation (Ex) - 240-540 nm, for emission (Em) - 260-600 nm. The total volume of all measured samples was 1.5 ml. Denaturation of the EGFP protein was carried out during 30 min in a 6 M solution of guanidine HCl (GdHCl). Iron oxide particles (FeO, Fe2O3, Fe3O4) coated with sodium citrate (TSC-IONP) were added to EGFP. Nanoparticles were obtained by chemical precipitation of oxide with ammonium hydrate from an aqueous solution of a mixture of iron chloride salts. Nanoparticles form self-organizing stable clusters ~10 and 50–80 nm in size, consisting of NPs 3 nm in size. Stability was monitored using the dynamic light scattering method.
Results
Fluorescence spectra of native EGFP were recorded. In addition to the main fluorescence peak Ex488nm/Em510nm (major peak), an Ex277nm/Em511nm peak (minor peak) was observed, the presence of which was explained earlier by the existence of two different conformations of this protein [2]. There was also a peak due to aromatic residues with a characteristic excitation wavelength in the region of 280 nm. During denaturation, complete quenching of the main and minor fluorescence peaks was observed. At the same time, the peak due to aromatic residues became more intense (approximately 2 times). This may be due to the release of hydrophobic residues on the surface of the protein during its denaturation.
To observe the process of denaturation over time, we measured the fluorescence kinetics (interval scan measurements) of this process. We were able to observe the quenching of fluorescence with time upon denaturation of EGFP at a concentration of 0.053 g/L: the intensity of the main maximum decreased by a factor of 2 in less than half a minute. We also found that, as the protein concentration increases, fluorescence quenching during its denaturation slows down. Thus, at a concentration of 0.067 g/L, the decrease in the intensity of the main maximum by a factor of 2 occurred already in approximately 0.8 min. This is probably due to a decrease in the ratio (number of GdHCl molecules)/(number of protein molecules) with increasing protein concentration.
We also conducted a study of the recovery of fluorescence of denatured EGFP upon renaturation of this protein by diluting (with water) its solution in GdHCl by 10 and 50 times. In the first case, the fluorescence signal was not restored, while at a 50-fold dilution (in this case, the final concentration of EGFP was 0.002 g/l), we found a recovery of the fluorescence intensity in the amount of 40% of the initial signal. Based on the assumption of a linear dependence of the protein concentration on the fluorescence intensity, this number can be considered as the percentage of renaturation.
Additionally, the effect of adding different amounts of TSC-IONP (concentration of 10 nm clusters ~10^13-10^14 ml^-1) on the fluorescence spectra was studied. In this case, only the change in the position of the fluorescence maximum was considered, since the change in the fluorescence intensity was, first of all, due to a decrease in the light transmission of the solution of nanoparticles with an increase in their concentration. It was found that when TSC-IONP is added, the main protein fluorescence maximum drifts towards longer wavelengths (the red shift). This may be due to a change in the environment with which the protein chromophore interacts electrostatically. It is known [3] that a number of polar groups and structured water molecules are buried adjacent to the chromophore. It is possible that the particles, through interaction with the outer surface of the protein, change this environment of the chromophore.
Conclusions
Thus, we were able to demonstrate partial EGFP renaturation and elucidate some details of the denaturation-renaturation process. We also established the red shift of the main protein fluorescence maximum upon the addition of iron oxide nanoparticles.

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### S6.413. Study of mononucleosome conformation changes in the presence of quercetin by single particle fluorescence microscopy

Andreeva T.V.[3]*, Lyubitelev A.V.[3], Studitsky V.M.[3,1], Feofanov A.V.[3,2]
[1]*Fox Chase Cancer Center, Cottman Avenue 333, Philadelphia, 19111 Pennsylvania, USA;*
[2] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia.;*
[3]*Biological Faculty, Lomonosov Moscow State University, Moscow, Russia;*
* andreeva.tatyana.2014@post.bio.msu.ru

Quercetin is a flavonoid of natural origin. It has antioxidant, immunomodulatory, anticancer, anti-inflammatory, antiviral and antibacterial activities. Quercetin can be used to treat cardiovascular diseases and obesity [1]. It can cause epigenetic changes that suppress oncogenes and reactivate tumor suppressor genes [2]. It is known that quercetin can bind in the groove, form hydrogen bonds, intercalate, and also interact electrostatically with DNA [3-5]. Our work is devoted to the study of the effect of quercetin on the conformation of nucleosomes, because this aspect of the properties of quercetin has not been studied [6].

The study was carried out by single particle fluorescence microscopy based on the FRET effect (Förster resonance energy transfer) [7] using mononucleosomes with two DNA linkers of 20 bp. Each nucleosome contained a pair of fluorescent labels located in the nucleosomal DNA at positions +13 and +91 bp from the beginning of the nucleosome-positioning sequence. To evaluate the effect of quercetin, we measured the coefficient E (analog of FRET efficiency) for single freely diffusing nucleosomes in the absence and presence of quercetin and analyzed the obtained frequency distributions of nucleosomes by the value of E. Based on these data, we calculated the fractions of nucleosomes with $E < 0.3$ (LE). This subpopulation includes nucleosomes where compaction of DNA on the histone core is damaged.

It is revealed that conformational changes in the structure of the nucleosome occur at a quercetin concentration of 6 μM or higher. They are detected as a concentration-dependent increase of LE-subpopulation of nucleosomes from 1-5% in the absence of quercetin to ~40% in the presence of 24 μM of quercetin. These structural alterations affect 20 bp of linker DNA and at least 13 bp of DNA in the nucleosomal core and, apparently, are accompanied by unwrapping of DNA from the histone octamer.

According to the literature data, the dissociation constant of the quercetin-DNA complex is equal to 14 μM [3], therefore, the detected structural changes are most likely caused by the direct interaction of quercetin with nucleosomal DNA.

The data obtained by us may indicate that quercetin increases the availability of nucleosomal DNA for various protein factors involved in intranuclear processes and can regulate these processes.

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### S6.414. Study of physico-chemical characteristics of fluorescent probes of azolotriazines class for identification of microorganisms

Vandyshev D.Yu.[1]*, Krutskikh A.S.[1], Koltakov I.A.[1], Potapov A.Yu.[1], Antipov S.S.[1], Shikhaliev Kh.S.[1], Khmelevskaya T.N.[2]
[1]*Voronezh State University;*
[2]*Voronezh State Medical University named after N.N. Burdenko;*
* francy_2007@mail.ru

Most of the known probes are characterised by low values of Stokes shift, which makes it difficult to distinguish the fluorescent signal against the scattered excitation light, in particular, radiation in the UV-region during fluorescence navigation. In addition, most of them have a rather high cytotoxicity. Therefore, the problem of selecting and creating a nomenclature of fluorescent molecule libraries with lower excitation energy and, consequently, less harmful for living organisms, optimally suited for the identification of bacterial strains, remains a topical issue.

The starting point of the study was the screening of the spectral characteristics of azolotriazine fluorescent probes synthesised de novo. Four sample probes were used, which had multiple absorption maxima in the wavelength range 361-504 nm. The first probe (3-methyl-6-phenylimidazo[1,2-b][1,2,4]triazine-2-carboxylic acid) - hereafter FZ-1, had two maxima in the 361 and 523 nm region, The second probe (2-(6-oxo-2-phenylimidazo[1,2-b]pyrido[4,3-e][1,2,4]triazine-7(6H)-yl)ethanesulfonic acid) - hereafter FZ-2, had two maxima in the region of 379. 5 and 504.4 nm, the third ((6-oxo-2-phenylimidazo[1,2-b]pyrido[4,3-e][1,2,4]triazine-7(6H)-yl)acetic acid) - hereafter FZ-3, at 372. 5 and 495 nm, and the fourth (2-(2-(6-oxo-2-phenylimidazo[1,2-b]pyrido[4,3-e][1,2,4]triazine-7(6H)-yl)pentandioic acid) is further FZ-4, in the region of 374 and 493.5 nm. The intensity of the absorption maxima of the first two dyes was similar, the third probe had a 27% lower intensity maximum, and the intensity of the absorption maximum of the fourth probe was

~ 23% of the intensity of the first two probes. Thus, the data obtained suggest different efficiencies in the interaction with both individual protein molecules and bacterial cells. Therefore, to test this assumption, the next step was to experimentally test changes in fluorescence parameters on two model objects of different levels of organisation. A commercial preparation of lysozyme protein was used as a protein model and Escherichia coli strain BL21*(DE3) was used as a model microorganism.

Evaluation of the fluorescence efficiency of the zone-protein complex was investigated using an excitation wavelength corresponding to the absorption maxima identified experimentally. The model protein was lysozyme, an antibacterial enzyme of the class of hydrolases, whose structure and functions are well studied. Registration of fluorescence spectra of the probe-lysocim complex revealed shift of fluorescence maximum of FZ-1 and FZ-3 into shorter wavelength region of spectrum, and of FZ-2 and FZ-4 into longer wavelength region. In addition, a nearly 10-fold decrease in fluorescence intensity was observed for FZ-2 and FZ-3 in the presence of lysozyme, a 2-fold decrease for FZ-1 solution, and about 5-fold decrease for lysozyme solution and FZ-4 as compared with the initial value. Spectra were recorded immediately after preparation and after incubation of the solution for 45 min at 37°C. The fluorescence signal intensity of the freshly prepared samples was outside the sensitivity limits of the instrument, so the content of these dyes in the sample was reduced by a factor of 5 and a factor of 10. A 10-fold decrease in FZ-1 in the sample resulted in an almost two-fold decrease in fluorescence intensity, and a 45-minute incubation of the complex at 37°C resulted in a further two-fold decrease in intensity. However, a 5-fold decrease in the content of this probe, by contrast, caused an almost two-fold increase in fluorescence, which decreased within 45 minutes at 37 °C, but did not reach the initial value. The decrease of FZ-2 and FZ-3 in the sample had a similar pattern. Their dilution by a factor of 10 and 5 led to a decrease of fluorescence signal by a factor of 10 and 5, and after incubation at 37 °C for 45 min the signal was reduced by almost two times more. The use of FZ-4 resulted in signal changes similar to those in experiments with FZ-1. However, it is worth noting that the initial fluorescence intensity of FZ-4 was 27% lower than that of FZ-1.

Cytochemical staining of E.coli cells was performed using a modified and optimised protocol. A Nikon Eclipse Ni-E fluorescence microscope with an excitation wavelength of 260-380 nm was used for imaging. Cells not treated with the dye were used as a control. The data obtained indicated that no fluorescence signals were detected in the control group from objects of comparable size to E.coli cells. The results of cell staining using FZ-2 and FZ-3 gave no significant results, which is in agreement with the spectral findings. Cytochemical staining of cells using FZ-4 gave a signal, but its intensity was significantly lower and the number of objects was lower compared to the case of using FZ-1.

Thus, it can be concluded that the chemical structure and properties of the fluorescent probes differ significantly. The FZ-1 probe is optimal for the task at hand. Taking into consideration the fact, that a significant decrease of bacterial cell growth did not occur when FZ-1 probe was added, it could be considered as a potential low-toxic dye for the study of cytoarchitectonics of biosystems by fluorescence correlation spectroscopy. To improve FZ-2 and FZ-3, and partially FZ-4, the introduction of additional chemical anchoring groups (introduction of other groups, substituents, etc.) for better binding to the bacterial cell matrix, which can be associated with a more thorough synthetic processing to increase the Stokes shift into the longer wavelength region of the spectrum, is required.

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**S6.415. Study of possibilities of laser pre-sowing treatment of seeds of agricultural crops**

Burdysheva O.V.[1]*, Sholgin E.S.[1], Lisina T.N.[1]
[1]*Perm Agriculture Research Institute division of PFRC UB RAS;*
* burdyshevaolga@gmail.com

The experience of applying lasers in the agrarian sphere is quite broad, starting with the use of lasers in the design and production of agricultural machinery and ending with laser irradiation directly to biological organisms [1].

One of the practical applications of laser technologies in agriculture is seed pre-sowing treatment. Seed treatment with infrared radiation allows intensifying germination biochemical processes, resulting in increased germination energy and germinating ability. Ultraviolet treatment reduces the phytopathogenic load. To date, there are many studies proving that exposure to low-intensity coherent light increases seed germination energy, increases resistance to adverse biotic and abiotic factors [2-5]. This reduces the use of toxic protective drugs - fungicides. Recent scientific priorities in the field of agricultural policy provide for limiting the use of chemicals, hormones and pesticides in the cultivation of crops. Methods of laser pre-sowing treatment contribute to realization of this policy.

In the laboratory of Agrobiophotonics of Perm Research Institute of Agricultural Sciences, the branch of Perm Branch of Ural Branch of Russian Academy of Sciences the research on the development of experimental unit for seed pre-sowing treatment and technology of its use for different agricultural structures is being carried out. Scientific novelty of the developed technology consists in the complex effect of different optical sources. Stimulating effect of laser and infrared radiation on seed germination should be combined with ultraviolet irradiation for influence on phytopathogens. Seed treatment can be carried out simultaneously with seed dressing with chemical reagents. For the moment, tests were carried out with the laboratory illuminating unit that includes an ytterbium laser with a wavelength of 1080 nm (with the possibility of varying the optical power in the range from 0.05 to 18 W), a set of lenses for beam forming, a power unit, a PC with software to control the laser diode.

Nine different agricultural crops were investigated: 1 - spring barley Rodnik Prikam'ya (substandard seeds), 2 - spring oats Persheron, 3 - spring soft wheat Irren, 4 - spring oats Stayer, 5 - meadow clover Lobanovsky, 6 - oilseed flax Uralsky, 7 - white mustard Rhapsody, 8 - spring barley Rodnik Prikam'ya (conditioned seeds), 9 - Peas sown Ulyanovets. The seed material for the study was provided by the laboratory of agricultural technology of the Perm Research Institute of Agriculture PFIC Ural Branch of RAS.

The research confirmed the difference in the effect of laser radiation on different crops. Regardless of the radiation power, the highest increase in germinated seeds showed holo-grain oats Persheron; laser treatment for 1 minute and a power density of 113.23 W/m2 contributed to an increase in germination by 11% compared with the control group. Negative effects were recorded: for barley seeds Rodnik Prikam'ya there was a decrease in germination up to 19% when irradiated for 1 minute and power density 113.23 W/m2. For flax seeds, an increase in the percentage of seed germination by 10% was demonstrated, as well as a significant increase in their sprout length. When wheat was irradiated, a 7 % increase in germination was observed for 3 minutes of irradiation at a power density of 113.23 W/m2. In the case of irradiation of oat Styler there was an increase in germination by 6% at 1 minute of irradiation power density 509.55 W/m2. When clover Lugovskii Lobanovskii was irradiated, germination increased by 9% at 5 minutes of irradiation at a power density of 113.23 W/m2 and germination decreased by 9% at 3 minutes of irradiation at a power density of 509.55 W/m2. Irradiation of mustard seeds led to an increase in germination by 10% at 1 minute of irradiation and a power density of 509.55 W/m2. Pea seeds after

irradiation for 3 minutes showed a significant increase in sprout length, however, the percentage of germination does not change.
After the laboratory stage, it is planned to carry out studies in the protected ground to assess the possible time-delayed effect of laser treatment on seed development, on the subsequent stages of plant development.
The work is carried out within the state assignment, state registration number of R&D 122031100058-3.
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**S6.416. Study of the oxygen and metabolic status of tumors in vivo by the method of optical bioimaging**

Komarova A.D.[1,2]*, Kritchenkov I.S.[3], Shcheslavskiy V.I.[2], Shirmanova M.V.[2]
[1]*Lobachevsky State University of Nizhny Novgorod;*
[2]*Privolzhskiy Research Medical University;*
[3]*Saint Petersburg State University;*
* komarova.anastasii@gmail.com

The study of local oxygen concentrations in cells and tissues is of great interest for many areas of physiology and medicine, including oncology. Phosphorescence lifetime imaging (PLIM) is a promising optical method for assessing the oxygen content in cells and tissues in real time. At the moment, an urgent task is to search for new phosphorescent sensors that have a high sensitivity to oxygen, effectively accumulate in cells and tissues and don't have pronounced toxicity.
The aim of the study was to evaluate the possibility of using new phosphorescent sensors in tumor cells as molecular oxygen sensors.
Water-soluble organometallic complexes based on Ir(III): Ir-1, Ir-2, Ir-2a, Ir-3, and Ir-4 were studied in this work. The complexes were tested on CT26 mouse colorectal cancer cells. The cytotoxicity of the complexes was assessed by the MTT assay. Analysis of the dynamics of the penetration of complexes into tumor cells in vitro was carried out in the range from 1 to 6 hours. Subcellular distribution was assessed using a laser scanning microscope LSM 880 (Carl Zeiss, Germany) with excitation at a wavelength of 543 nm, with a laser power of 8 mW. The signal of the complex was detected in the range of 650 – 750 nm. The PLIM method was used to assessment the phosphorescence lifetime of complexes in CT26 tumor cells under hypoxic conditions. The complexes were excited in the two-photon mode at a wavelength of 760 nm, and the signal was detected in the range of 596–660 nm. The complexes were excited in the two-photon mode at a wavelength of 760 nm, and the signal was detected in the range of 596–660 nm.
According to the results of the MTT assay, it was found that the complexes Ir-2, Ir-3 and Ir-4 don't exhibit pronounced cytotoxicity. After 24 hours of incubation of CT26 cells with complexes, the percentage of viable cells at a complex concentration of 125 μM was 87.9 ± 4.5 %, 94.6 ± 0.5 %, and 92.5 ± 2.7 %, respectively. For the Ir-2a complex at a concentration of 75 μM, the percentage of viable cells was 83.2 ± 6.5%. The Ir-1 complex exhibits cytotoxicity; the Half-maximal inhibitory concentration (IC50) of the Ir-1 was 50 μM. Further in vitro studies were carried out on Ir-2, Ir-2a, Ir-3 and Ir-4 complexes, which do not exhibit cytotoxicity and are soluble in water. Using laser scanning microscopy, it was found that the phosphorescent complexes Ir-2 and Ir-2a penetrate into living tumor cells. The intensity of the luminescence of complexes in tumor cells increases in the period from 1 to 6 hours, which indicates an increase in the concentration of complexes in living tumor cells. The study of the intracellular localization of the complexes showed that the complexes are evenly distributed in the cytoplasm of cells and do not penetrate into the cell nucleus. The PLIM method recorded variations in the phosphorescence lifetime in tumor cells in the simulation of hypoxia. The phosphorescence lifetime for the Ir-2 complex under normoxic conditions was 1.48 ± 0.05 μs, under hypoxic conditions, 2.42 ± 0.09 μs, for the Ir-2a complex under normoxic conditions, 1.04 ± 0.16 μs, and under hypoxic conditions, 1.94 ± 0.32 μs. Thus, the phosphorescence lifetime of the Ir-2 and Ir-2a complexes under normoxic conditions decreased by factors of 1.6 and 1.8, respectively; therefore, the complexes are sensitive to oxygen.
Based on the results of the study, it can be concluded that Ir-2 and Ir-2a complexes are the most promising for further in vivo studies and can be used as molecular oxygen sensors.
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**S6.417. Study of the structure of the vascular bed and the level of oxygenation of tumors of different morphogenesis**

Glyavina A.M.[1,2]*, Akhmedzhanova K.G.[1,2], Kurnikov A.A.[1], Khochenkova Yu.A.[3], Khochenkov D.A.[3], Subochev P.V.[1], Orlova A.G.[1]
[1]*Institute of Applied Physics Russian Academy of Sciences, Nizhny Novgorod, Russia;*
[2]*Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
[3]*N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russia;*
* annaglyavina@gmail.com

Compared to the vessels of normal tissues, the vessels of malignant neoplasms have a number of structural and functional anomalies. They are tortuous, saccular, and their walls are highly permeable due to the uneven coverage of the basement membrane, endothelium, and pericytes. Such anomalies contribute to the formation of areas of tumor hypoxia, ultimately causing a decrease in sensitivity to treatment. It is important to note that different types of tumors differ significantly from each other in the structure of blood vessels, and these differences are considered as a prognostic criterion and determine the choice of the method of therapy. Understanding the features and characteristics of the bloodstream of the neoplasm will make possible timely predict the tumor response to treatment and correct the effect on the tissue.
In this work, a combination of optoacoustic (OA) microscopy and optical diffuse spectroscopy (DOS) was proposed to assess the structure of the vascular bed and the level of oxygenation of experimental neoplasms. Optoacoustic angiography is a convenient non-invasive method for visualizing blood vessels. It is based on the registration of ultrasonic waves induced by the expansion of a tissue area after the absorption of laser pulses by hemoglobin molecules. The OA method allows you to visualize the three-dimensional distribution of hemoglobin, giving a picture of the location of the blood vessels of tissues. Assessment of the oxygen status is possible using the method of optical diffuse spectroscopy. DOS is also based on the absorption capacity of hemoglobin. At the same time, the method makes it possible to estimate the concentration and ratio of the oxy- and deoxy-forms of a given compound, which characterize the balance of oxygen delivery to tissues and its consumption. The aim of the work was to compare the structure of blood vessels and the level of oxygenation of experimental tumors of different morphogenesis by OA and DOS methods. To verify the DOS

data, an immunohistochemistry (IHC) study of tumor tissues with the hypoxia marker pimonidazole (PM) was performed.
The study was carried out on Balb/c-nude female mice with tumors inoculated subcutaneously. Three tumor models based on cell lines SN-12C (human kidney cancer, n = 4), HCT116 (human colon cancer, n = 4), Colo320 (human colon cancer, n = 4) were selected for the experiment. The study was performed at an average neoplasm volume of 700 mm3 on day 29 of growth of HCT116 and Colo320 tumors, and on day 125 of growth of SN-12C.
For OA and DOS studies, animals under isoflurane anesthesia were fixed in a side position on a portable base plate with a hole for positioning the study area above the OA sensor of the microscope. For OA, a setup (IAP RAS) with a laser with a wavelength of 532 nm and a repetition rate and pulse duration of 2 kHz and 1 ns was used. For the ODS, we used an installation (IAP RAS) with an optical probe of four fibers, using a light-emitting diode with a wavelength of 400–700 nm as a source and a spectrometer as a detector. For IHC, PM was injected intraperitoneally, after 45 minutes the tumors were removed, and cryosections were made. Sections were stained with mouse monoclonal antibodies to pimonidazole conjugated with fluorescein isothiocyanate. The relative hypoxic fraction was calculated as the percentage of the area of PM-positive zones of the total area of the sample.
In the course of the work, it was shown that SN-12C tumors are characterized by a low growth rate compared to Colo320 and HCT116, the doubling time of the tumor volume of this model exceeds that for Colo320 by 2.5 times and for HCT116 by 2.3 times. The DOS method in Colo320 showed an increased content of hemoglobin and a reduced level of blood oxygen saturation compared to SN-12C and HCT116. The reason for reduced oxygenation is the high content of deoxyhemoglobin, which characterizes the oxygen consumption of tissues. Using OA imaging, the absence of a regular structure of the vasculature of all experimental neoplasms was shown. The Colo320 tumor is characterized by the presence of extensive hemoglobin-containing structures, presumably hemorrhages, as well as higher values of vessel size and fraction. For HCT116 and SN-12C, the values of these indicators were comparable to the norm. The IHC method revealed higher values of the relative hypoxic fraction in Colo320 compared to SN-12C and HCT116, which confirms the results of the DOS.
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### S6.418. Synuclein and singlet oxygen regulation of insulin production in mice

Zharkikh E.V.[1]*, Loktionova Y.I.[1], Dremin V.V.[1], Chaprov K.D.[2], Dunaev A.V.[1], Ninkina N.N.[2], Abramov A.Y.[1,3]
[1]*Orel State University named after I.S. Turgenev, Orel, Russia;*
[2]*Institute of Physiologically Active Compounds FRC of Problems of Chemical Physics and Medicinal Chemistry RAS, Chernogolovka, Russian Federation;*
[3]*Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK;*
* ev.zharkikh@gmail.com

Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels. The state of hyperglycemia results from two main factors: insufficient insulin production by the pancreas and a decrease in the sensitivity of cells to insulin. Diabetes is characterized by complications that affect all body systems.
It has previously been demonstrated that there is a close relationship between glucose metabolism, mitochondrial function, and insulin secretion. Loss of PINK1 function (a major cause of early onset of autosomal recessive Parkinson's disease (PD), a common progressive neurodegenerative disease) has been shown to impair glucose sensitivity, leading to increased insulin release. Based on these findings, the association of PD with type 2 diabetes was reported. There are common mechanisms in the pathophysiology of both diseases, including mitochondrial dysfunction, oxidative stress, hyperglycemia, and inflammation. The formation of neurotoxic aggregates of neuronal synuclein proteins caused by various factors, including mutations in synuclein genes, is a histopathological characteristic of PD. Under physiological conditions, monomeric α-synuclein has demonstrated the ability to increase the efficiency of ATP synthase.
Therefore, the aim of this work was to study the regulation of insulin production by synucleins. In addition, we aimed to evaluate the possible effect of 1267 nm light irradiation, which leads to singlet oxygen production, on insulin production in the animal organism.
An enzyme immunoassay using phospho-specific antibodies against insulin receptors (Rat/Mouse Insulin ELISA, Merck KGaA, Germany) was used for measurement. Blood plasma was used for the assay. Plasma was obtained by taking 200 µl of whole blood from mice, allowing it to clot at room temperature for 30 min and subsequently centrifuging it at 4 °C. Mouse insulin samples at concentrations of 0.2, 0.5, 1, 2, 5, and 10 ng/ml were used as positive controls. Transgenic mice knockout for the SNCA, SNCB, and SNCG genes and triple knockout for the α-, β-, and γ-synuclein genes were used in the study; wild-type mice were used as controls.
To evaluate the effect of low-intensity 1267 nm infrared irradiation on insulin production, two wild-type mice were irradiated. Radiation was delivered via an optical fiber of a specially designed device, which was fixed on the proximal part of the middle of the animal's tail to irradiate the caudal vein. The irradiation dose was 50 J/cm2 for one animal and 100 J/cm2 for the second animal. Five minutes after irradiation, 200 µl of whole blood was taken from each animal for further immunoassay. The results showed that the blood of wild-type mice contained 2.7±0.2 ng/ml of insulin. Almost the same value (2.8±0.3 ng/ml) was observed in mice with double knockout of α- and γ-synuclein genes. The lowest insulin levels were observed in mice with β- (0.2±0.1 ng/ml) and γ-synuclein (0.6±0.1 ng/ml) gene knockout, and in mice with triple knockout of α-, β-, and γ-synuclein genes (0.3±0.0 ng/ml). Irradiation of animals with 1267 nm laser led to an increase in blood insulin concentration, and this increase appeared to have a dose-dependent effect. When irradiated with 50 J/cm2 light, insulin concentration in blood was 0.6±0.1 ng/ml, and at 100 J/cm2 it was 1.9±0.2 ng/ml.
Thus, the present work showed that knockout of genes encoding synuclein proteins is associated with a decrease in insulin production with the most prominent manifestations in the knockout of β- and γ-synuclein genes as well as in the triple knockout. Noninvasive optical generation of singlet oxygen in the animal leads to a dose-dependent increase in blood insulin concentration.
This work was supported by Grant No. 075-15-2022-1095 of the Government of the Russian Federation (studying the connection between gene knockout and insulin production) and Grant No. 22-75-10088 of the Russian Science Foundation (research with a 1267 nm laser).

### S6.419. Tetra(aryl)tetracyanoporphyrazine free bases and their metal complexes for photodynamic therapy of oncological diseases

Shilyagina N.Yu.[1]*, Shestakova L.N.[1], Peskova N.N.[1], Plekhanov V.I.[2], Lermontova S.A.[3], Klapshina L.G.[3], Balalaeva I.V.[1]
[1]*Lobachevsky State University of Nizhny Novgorod;*
[2]*Institute of Applied Physics of the Russian Academy of Sciences;*
[3]*Razuvaev Institute of Organomettalic Chemistry of the Russian Academy of Sciences;*
* nat-lekanova@yandex.ru
Photodynamic therapy (PDT) is an intensively developing direction in the treatment of oncological diseases, which is based on the use of a photoactive compound, the so-called photosensitizer (PS), which, under local exposure to visible or near-infrared light, can enter into photochemical reactions with the formation of reactive oxygen species

(ROS), which induce the development of local oxidative stress, which ultimately leads to the death of irradiated cells.
Despite the high efficiency, selectivity, and good tolerability of PDT, the problem of creating an "ideal" PS remains unresolved, which is why the search for new drugs is a key link in improving this technology. In addition to the need to search for the "ideal" PS, the PDT method is limited by the inability to adequately and timely adjust the light dose delivered to the tumor, depending on the response of the irradiated tissue. The above difficulties largely hinder the introduction of a personalized approach to this type of therapy and the development of PDT in general.
In order to develop a personalized approach in PDT, we synthesized and studied a series of tetra(aryl)tetracyanoporphyrazine macroheterocycles in the form of free bases and metal complexes with unique photophysical properties. To date, more than thirty compounds with various side substituents in the peripheral environment of the macrocycle have been obtained and studied, including compounds with the inclusion of such metals as ytterbium, iron, copper, palladium, cobalt and others in the center of the macrocycle. The advantage of arylcyanoporphyrazines, compared to many clinically approved PS, is the simplicity and mildness of the conditions for their synthesis, as well as a high yield of target products. The most interesting feature of the studied compounds is the combination of photosensitizer and molecular rotor properties that we found. Molecular rotors are compounds that show a strong dependence of photophysical parameters, such as quantum yield and fluorescence lifetime, on the viscosity of the medium. This dependence makes it possible to use porphyrazines as sensors for changes in the local microenvironment and, in particular, microviscosity. In experiments on cell cultures, we have shown that porphyrazines are efficiently accumulated by tumor cells, being localized mainly in the membrane organelles of the cell. It has been shown that porphyrazines are capable of inducing photoinduced generation of ROS in the cell, leading to the triggering of cell death. We have shown for the first time the fundamental possibility of using porphyrazines as probes of intracellular viscosity, including during PDT. Moreover, the presence of a pronounced dose dependence of the response to photodynamic exposure, namely, an increase in the lifetime of porphyrazines, which is apparently due to an increase in microviscosity in the cell after therapy, has been shown. The established pattern points to the fundamental possibility of using the photophysical parameters of porphyrazines for dosimetry during PDT.
In order to increase the selectivity of porphyrazine accumulation in tumors, we developed and characterized delivery systems based on biocompatible nanomaterials, such as liposomes, polymer brushes, and submicron particles of calcium carbonate in the vaterite polymorph. Using vaterite particles as an example, we have demonstrated the possibility of evaluating the processes of porphyrazine release from a transport carrier in living tissue in real time. In an experiment on tumor-bearing animals, we have shown a rapid (within several hours) and highly selective accumulation of porphyrazines in tumors when administered as part of nanosized carriers. It has been established that porphyrazines accumulate and localize in the cytoplasm of tumor cells, and not in the intercellular space or vessels that feed the tumor. The ability of porphyrazines to cause biologically significant inhibition of tumor growth and complete elimination of tumors in seventy percent of cases has been proven. Moreover, some compounds from the group of porphyrazines have been shown to be capable of inducing immunogenic cell death.
For the first time, using the method of fluorescent imaging with a function of temporal resolution, we have shown the possibility of in vivo monitoring of changes in the viscosity properties of tumor tissue during photodynamic therapy with porphyrazines. The proposed approach will further improve the efficiency of photodynamic therapy due to objective optical monitoring of the ongoing treatment, namely, the selection of individual doses of light exposure based on the change in the fluorescence lifetime in the tumor tissue after irradiation.
Thus, we suggest that tetra(aryl)tetracyanoporphyrazines have great potential as drugs for photodynamic therapy of oncological diseases and can contribute to the introduction of a personalized approach to this type of therapy and the development of PDT.
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### S6.420. The activity of the photosynthetic apparatus of Solanum tuberosum depending on the structural state of the tubulin cytoskeleton

Puzina T.I.[1]*, Makeeva I.Yu.[1]
[1]*Orel State University named after I.S. Turgenev;*
* tipuzina@gmail.com

At present, the activity of the photosynthetic apparatus is increasingly judged by biophysical parameters, primarily by chlorophyll fluorescence. The fluorescence method is widely used in ecological and physiological studies of photosynthesis. Elements of the cytoskeleton interact with membranes through associated proteins, forming a continuum, the violation of which can affect membrane processes. The effect of microtubule destructuring agent oryzalin (15 μM) on the rate of non-cyclic electron flow, coefficients of photochemical and non-photochemical quenching, as well as the ratio of their rates in potato plants grown in soil culture in a growing house was studied. Registration of chlorophyll fluorescence parameters in the leaves of the seventh layer of the middle formation in intact plants, previously adapted to the dark, was carried out using a portable fluorimeter MIN PAM. A 21% decrease in the non-cyclic electron flow rate (ETR) was found in the variant with destructured microtubules, apparently, this is due to pharmacological stress caused by the action of oryzalin. At the same time, a 1.8-fold decrease in the photochemical quenching coefficient (qP), i.e., the fraction of light energy consumed by open reaction centers of PS II, was shown. On the contrary, under the action of oryzalin, the coefficient of non-photochemical quenching (qN) increased, which is associated with the processes responsible for the conversion of a part of the energy absorbed in the light phase of photosynthesis into heat. The increase was 26%. The calculation of the ratio of the reaction rates of photosynthetic and non-photosynthetic use of the excitation energy of the reaction center of PS II (Fv/F0) showed its significant decrease (by 2.7 times). The revealed changes in chlorophyll fluorescence parameters under conditions of microtubule destruction occurred against the background of a decrease in the content of auxin phytohormones. There is evidence that exogenous indoleacetic acid is involved in the regulation of chlorophyll fluorescence. Previously, we showed a decrease in the rate of the Hill reaction and the process of photophosphorylation during the destruction of the tubulin cytoskeleton in Solanum tuberosum. Perhaps one of the reasons is disturbances in the operation of the photosynthetic apparatus, as evidenced by changes in chlorophyll fluorescence.

### S6.421. The destruction of the metaphase plate in mouse oocytes by the near-infrared femtosecond laser radiation

Osychenko A.A.[1]*, Zalessky A.D.[1], Tochilo U.A.[1], Martirosyan D. Yu.[1], Nadtochenko V.A.[1]
[1]*FRCCP RAS;*
* alina.chemphys@gmail.com

The authors have developed an original method of the genetic material destruction (enucleation) by the tightly-focused 795 nm femtosecond laser radiation. Ordinary, oocytes are enucleated by the microneedle

through the aspiration of the metaphase plate within a small amount of the surrounding cytoplasm. This is the way of enucleated oocytes (recipient cytoplast) obtainment. Recipient cytoplast is required for animal cloning and mitochondrial replacement therapy in humans. The loss of this particular portion of the cytoplasm containing metaphase plate may affect the development, because exactly in this place some reprogramming factors are concentrated, for example, MPF, ORF1 and soronin. An advantage of the laser is an ability to localize the impact exactly in the area of metaphase plate and to avoid losing of reprogramming factors.

We have shown before that we are able to perform the femtosecond laser enucleation of the oocytes with a perfect preciseness and low invasiveness (Osychenko A.A. et.al, Biomedical Optics Express, 2022). To develop our technique, we tried different laser parameters for determination of non-invasive diapason of oocyte enucleation. More, we studied the cytotoxic effect of the femtosecond laser exposure.

The range of femtosecond laser radiation parameters for local destruction of the mouse oocyte genetic material has been studied. In this work we applied the femtosecond pulse repetition rate mode of 80 MHz, 1 kHz, 10 kHz, and 100 kHz. The efficiency of the mouse oocyte enucleation was studied depending on the wavelength of femtosecond laser radiation, pulse energy, and pulse train duration. Limitations of the range of parameters in terms of the pulse energy and the duration of the trains were found, due to the speed of the procedure and the frequency of formation of unwanted vapor-gas bubbles.

The cytotoxic effect of the femtosecond laser enucleation procedure was studied by fluorescent microscopy. The fluorescence lifetime of NADH was measured by FLIM (Fluorescence-lifetime imaging microscopy) in oocytes irradiated and not irradiated (control) with femtosecond laser radiation. The results indicate an approximately equal contribution of the bound and unbound forms of NADH to the metabolism of oocytes, this ratio does not depend on the presence or absence of laser irradiation, and also does not change significantly in the presence/absence of parthenogenetic activation.

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### S6.422. The effect of microenvironment dynamics of tryptophan on its fluorescence parameters at different temperatures

Gorokhov V.V.[1], Knox P.P.[1], Korvatovsky B.N.[1], Goryachev S.N.[1], Paschenko V.Z.[1]*, Rubin A.B.[1]
[1]*Lomonosov Moscow State University;*
* vz.paschenko@gmail.com

The fluorescence of tryptophanils in proteins is widely used as a natural indicator of their intramolecular conformational dynamics, which is closely related to the functional activity upon temperature changes, in particular. Temperature dependences of spectrum patterns and Trp fluorescence lifetime are the reference sources for the the intramolecular dynamics behavior of the closest environment of the excited tryptophan (Trp) molecule. The Trp fluorescence decay kinetics both in solution and in polypeptides are multicomponent. Depending on the type of solvent, pH, 2–3 fluorescence components are recorded with times of hundreds of picoseconds to about 10 ns. The nature of intramolecular microconformational dynamics of the water-protein medium, which affects the functional activity, is largely determined by the state of the hydrogen bond system in the macromolecule. Certain information for understanding mechanisms of the processes occurring in this case can be obtained, among other things, by a comparative analysis of temperature dependences of the tryptophan fluorescence parameters in solution and in the protein composition.

It is on record that an ordered system of hydrogen bonds is formed in the tryptophan molecule environment in an aqueous medium. This system is shaped as a zwitterionic complex and causes a significant effect on the excitation dynamics in the Trp molecule. Here we interrogated the change in the spectral and kinetic parameters describing the decay of tryptophan fluorescence in an aqueous medium under pulsed photoexcitation as a function of temperature in the range of -170°C to +20°C. A model is proposed and a quantitative analysis of the rates of direct and reverse electronic transitions in the tryptophan molecule from the excited state to the ground state and to the charge transfer state (CTS) is carried out. Basing on the experimental and theoretical data, three regions in the Trp fluorescence spectrum were distinguished: the short-wavelength region (300 nm < l < 386 nm), the medium-wavelength region (386 nm < l <400 nm), and the long-wavelength region (400 nm < l < 470 nm), for which temperature dependences of the CTS formation rate are different. The key role of structural transformations of hydrogen bonds in the system determining the nonlinear behavior of the change in tryptophan fluorescence parameters in the selected spectral regions is shown.

The revealed nonlinear nature of the temperature dependence of the decay time of the fast (t1, a few ns) and slow (t2, about 10 ns) components of the fluorescence of an aqueous solution of tryptophan molecules is explained by appearance of an additional deactivation channel Trp* - electron transfer from the indole part of the tryptophan molecule to hydrogen bonds of the aqueous environment and then to its amide groups. As a result, a state with charge transfer Trp^+R^- is formed (where R can denote both a system of hydrogen bonds in the environment of the Trp molecule and amide groups with electron-withdrawing properties associated with Trp). Deactivation of this CTS occurs both resulting the reverse transition from this state to the excited Trp state and either due to fluorescence, or during thermal relaxation to the ground state.

The spectral-dynamic approach used in this work to study the temperature dependences of the deactivation rates of Trp excited states also allowed detection of conformational (phase) transition occurrence in the system of hydrogen bonds in the environment of Trp* and CTS within the temperature range of -80 °C to 20 °C. In this temperature range, a nonlinear behavior of such parameters as t1, t2, the activation energy Ea, and the solvation shift rate of the fluorescence spectra is observed. It is significant that in the temperature range of -110 °C - -80 °C the Trp* molecule is solvated faster than CTS, while at higher temperatures (T > -80 °C) the solvation rate of the Trp^+R^- state becomes greater than the Trp* solvation rate. We explained this fact so that in the temperature range of -80°C - 20°C in the CTS environment, the structural (phase) transition captures a greater number of H-bonds than in the Trp* environment. In such temperature range of -80°C - 20°C, temperature dynamics of the excited tryptophan molecule transitions deviate from the standard Arrhenius behavior with the constant Ea value. Thus, the hydrogen bond system dynamics are decisive for the nonlinear change in parameters describing deactivation of Trp* and CTS. It is obvious that further comparison of these results with similar studies for tryptophanils in the composition of proteins will provide new information on the extent to which differences in the fluorescence parameters of tryptophan in the composition of a functional protein detected during thawing of samples cooled under different conditions, can be due to direct changes in the nature of its interaction with protein molecules and with the surrounding solvent.

### S6.423. The influence of wavelength, power and exposure of laser radiation on singlet oxygen generation

Makovik I.N.[1]*, Vinokurov A.Y.[1], Eratova L.V.[1], Dremin V.V.[1]
[1]*Orel State University named after I.S. Turgenev;*
* irina.makovik@gmail.com

Singlet oxygen (SO) is a highly reactive form of molecular oxygen and plays an important role in many physical, chemical, and biological processes, as well as in the therapy of different pathologies. The growing interest in SO due to its high chemical activity has contributed to the development of various approaches to SO generation in biological systems. Thus, the mechanism of direct photosensitizer-free optical generation of the singlet form of oxygen by light at specific wavelengths has been actively studied recently. Along with the study of the effect of laser-induced SO on physiological processes at the cellular and tissue levels, approaches to its detection and quantification are being actively developed and studied. This is an important task for evaluating effective doses and searching for optimal parameters of triplet oxygen excitation. In this work, the influence of a number of wavelengths, powers and exposures of laser radiation on SO generation is studied, and the advantages and disadvantages of existing approaches to the quantitative measurement of SO formation are analyzed.

To date, due to the high reactivity of SO, there are a limited number of possible approaches to its detection. The paper considers a polarographic method using an Oxytherm+R respirometer (Hansatech Instruments, UK) and a method using a Singlet Oxygen Sensor Green (SOSG) fluorescent probe (Invitrogen, USA). The ground triplet state of oxygen has several absorption bands in the spectral range from 390 to 1300 nm, at which SO can be produced. The laser wavelengths of 1267 and 1064 nm, which have the greatest absorption by the triplet form of oxygen, are considered. In this work, 1244 and 1122 nm laser radiation sources were used as controls. According to the data from literature, they do not activate the transition of triplet oxygen to the singlet state.

The study by polarographic method included measurement of the level of dissolved oxygen in ddH2O or 5 mM L-histidine solution in ddH2O. L-histidine was used as a "chemical trap" due to its ability to interact with SO. This made possible the polarographic measurement of the decrease in the dissolved oxygen concentration. Laser-induced SO generation was carried out through the glass wall of the measuring chamber, in which the temperature was maintained at a level of 26 °C. Measurements were made using a Zeiss LSM 900 microscope (Carl Zeiss AG, Germany) with a 10× objective. To excite the fluorescence of the probe, a laser with a wavelength of 488 nm and a power of 0.1 % of the maximum was used. The delivery of laser radiation during the experiments was carried out from the opposite side of the microscope objective. The study protocol included the stages of recording the baseline signal level (3 min), the signal level in the process of SO generation by laser radiation (the duration depended on the selected dose), and recording the signal after laser exposure (6 min). The analysis of the influence of time (laser radiation exposure) and power factors on the formation of SO was carried out when exposed to different doses (50, 100, 150, 200 and 250 J/cm2) of laser radiation at a fixed power value of 50 mW, as well as for a dose of 200 J/cm2 at different powers equal to 50, 100 and 150 mW.

An analysis of the measurement results by the polarographic method showed that the content of dissolved oxygen in the L-histidine solution in the measuring chamber after 1267 nm and 1064 nm laser exposure turned out to be lower compared to the control lasers. However, this approach is characterized by insufficient sensitivity to the detection of various doses of generated SO. In addition, the sensitivity of the polarographic method to temperature changes, despite its stabilization by the design of the respirometer, makes it impossible to analyse the change in the signal at the time of laser exposure. Therefore, only an indirect estimate can be made after calculating the final decrease in oxygen concentration compared to its initial level before exposure.

Measurements using SOSG fluorescent probe showed that 1267 and 1064 nm laser exposure leads to an increase in the green fluorescence intensity of the probe oxidized form. The increase in the signal indicates the production of SO and its selective interaction with SOSG. At the same time, the wavelength of 1267 nm has a more significant influence. An increase in fluorescence intensity was also registered under laser irradiation at a wavelength of 1244 nm. This result may indicate the formation of SO and the incorrect use of this radiation source as a control laser. An analysis of the results under the influence of different doses of laser radiation showed the sensitivity of the approach to differences in the amount of SO generated. Exposure with a power of 50, 100 and 150 mW at a dose of 200 J/cm2 revealed a greater influence of exposure time than power on the amount produced SO. This is most likely due to the short lifetime of the SO.

The considered approaches for the detection of SO generation are characterized by different sensitivity to its detection. These methods allow one to realize only an indirect estimation and cannot provide SO-generation control during the laser exposure. In the case of the polarographic method only SO generated in solution or diffused from the tissue can be measured. But the last seems impossible due to the low SO lifetime. The use of SOSG is significantly limited by its poor penetrating ability through the cell membrane. Therefore, the use of chemical traps or specialized fluorescent probes is suitable only for a narrow type of tasks. This indicates the need to develop new highly sensitive SO detection methods.

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### S6.424. The mechanism of age melanin concentration decrease in the retinal pigment epithelium cells of the eye

Dontsov A.E.[1]*, Yakovleva M.A.[1], Vasin A.A.[2], Gulin A.A.[2], Aybush A.V.[2], Nadtochenko V.A.[2], Ostrovsky M.A.[1]
[1]*Emanuel Institute of Biochemical Physics, RAS;*
[2]*N.N. Semenov Federal Research Center for Chemical Physics, RAS;*
* adontsovnick@yahoo.com

It is known that in the process of aging there is a significant decrease in the number of melanosomes in the retinal pigment epithelium (RPE) cells of the human eye [1, 2]. However, the exact mechanisms of this phenomenon are unknown. Previously, we showed that the interaction of melanin melanosomes with superoxide radicals results in its oxidative destruction with the formation of water-soluble fluorescent products [3] containing highly active carbonyl compounds [4]. In the present study, using fluorescence analysis, HPLC, and mass spectrometry, it was shown that when melanolipofuscin granules isolated from human eye RPE cells are irradiated with visible light, water-soluble fluorescent products are formed. The formation of these products occurs as a result of oxidative degradation of melanin caused by superoxide radicals, which are generated by the lipofuscin part of the melanolipofuscin granule. It is important to emphasize that when the fractions of melanosomes and lipofuscin granules are irradiated, the formation of water-soluble fluorescent products does not occur. Destruction of melanosomes under the action of light is also possible; however, this requires significantly higher irradiation intensities than when the melanolipofuscin granules are irradiated. This is explained by the fact that in melanosomes, in contrast to melanolipofuscin granules, there is no lipofuscin, a light-dependent generator of superoxide radicals. Fluorescent products of light-induced melanin decay were identified both in the melanosomes and in the melanolipofuscin granules fractions. Statistical analysis by principal component analysis (PCA) for mass spectrometric data obtained by ToF-SIMS allowed us to identify the peaks characteristic of these products of light-induced melanin degradation. It was shown for the first time that water-soluble melanin degradation products caused by superoxide radicals are light-sensitive generators of reactive oxygen species. From this, it follows that the process of light-induced melanin degradation in the melanolipofuscin granule can be enhanced with the accumulation of melanin degradation

products. It is concluded that the decrease in the concentration of melanin in RPE cells of the human eye with age is due to its oxidation by reactive oxygen species generated by lipofuscin in the composition of melanolipofuscin granules under the action of light.

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### S6.425. The role of optogenetics in the regulation of neuron-glial interactions in the hippocampus and in the restoration of cognitive functions in mice with Alzheimer's disease model

Gerasimov E.I.[1], Erofeev A.I.[1], Bolshakova A.V.[1], Bezprovanny I.B.[1,2], Vlasova O.L.[1]*
[1]*Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, Russia;*
[2]*Department of Physiology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA ;*
* olvlasova@yandex.ru

Nowadays, Alzheimer's disease (AD) is one of the most common and incurable neurodegenerative disease, which affects the processes of memory formation, its subsequent storage and leads to a progressive decrease in cognitive functions. There are various hypotheses of etiology and pathogenesis, as well as different approaches to the treatment of this disease. In this work, we used the advantages of optogenetics for precise activation of astrocytes in hippocampal slices in order to study its effect on synaptic function and cognitive abilities on the in vivo level, since it is known that astroglia plays an important role in maintaining and regulating the work of neural networks of the brain.
By releasing specific gliotransmitters, astrocytes act on neuronal receptors, modulating neuronal excitability, synaptic transmission and plasticity. Astrocytes respond to an external stimulus with intracellular calcium waves [Ca2+]. During the propagation of this wave, serine, cytokines and lactate are released, which further modulate the activity of neurons. The ability of astrocytes to release glutamate allows to regulate the function of NMDA receptors, thereby regulating excitation in the neural network. Astrocytes are closely related to the pathogenesis and pathological processes of neurodegenerative disorders, so the ability to control their activity becomes an urgent and necessary task of therapy [1].
Results. Conducted experiments have shown an increase in the activity of pyramidal hippocampal neurons in response to optical stimulation of astroglial cells transduced by the AAV5_GfaABC1D_opto-a1AR-EYFP virus (encodes a metabotropic Gq- coupled receptor). An increase in field excitatory postsynaptic potentials (fEPSP) was recorded in hippocampus after light activation of astrocytes expressing Opto-a1AR [2]. Significant activation of "immediate-early" gene expression in hippocampal slices was also observed [3]. Preliminary results were obtained on the restoration of cognitive abilities in mice with an Alzheimer's disease model after optogenetic activation of the metabotropic receptor in vivo. Conclusion. The positive reaction of hippocampal neurons to the light activation of astroglial cells ex vivo and in vivo using a metabotropic construct was demonstrated in the study.
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### S6.426. UV-induced structural modifications of serum albumin and ribonuclease A

Basharina O.V.[1]*, Sekretareva U.S.[1], Artyukhov V.G.[1]
[1]*Voronezh State University;*
* bov-bio@yandex.ru

The study of absorption spectra of biomolecules remains one of the most effective and accessible methods for analyzing the structural properties of biomolecules. The spectral properties of protein macromolecules are studied mainly in the absorption region of the side groups of aromatic amino acids in the wavelength range 240 - 320 nm. Often detected changes are associated with the unfolding/folding of the protein globule, accompanied by exposure/screening of its chromophore groups relative to the surface of the protein macromolecule.
The objects of the study are solutions of bovine serum albumin (BSA) and bovine pancreatic RNase A at a concentration of 1●10-5 mol/l in 0.1 mol/l Na–phosphate buffer (pH 7.4).
Solutions of BSA and RNase were irradiated with UV light using a Bio-Link-BLX irradiator (Vilber Lourmat, France) in narrow wavelength ranges: with λmax 254 nm. The irradiation intensity of 1 lamp out of 6 is 0.3 J/ (cm²●min). Absorption spectra of serum albumin solutions were recorded on a UV-2401 PC spectrophotometer (Shimadzu, Japan) in the wavelength range from 240 to 400 nm, spectral slit width 0.5 nm, scanning step 0.5 nm, scanning speed corresponded to the Slow mode. The measurements were carried out in a standard quartz cuvette with an optical path length of 10 mm. The resolution increase in the absorption spectra of the samples was carried out by calculating the second derivative.
The complex absorption band of the studied proteins in the region of 250-300 nm is due to the light absorption of phenylalanine (the short-wavelength shoulder of the absorption band), tryptophan and tyrosine. From the analysis of the derivative of the absorption spectrum of the native BSA, it follows that local maxima (transitions in the absorption band) are observed at wavelengths: λ = 242,9; 255,8; 262,5; 267,0; 271,2; 282,1; 289,2 and 295.2 nm; the absorption maximum is most clearly expressed at 282.1 nm, it is due to the presence of tryptophan in albumin. The absorption spectrum of RNase has an absorption maximum at λmax = 276.6 nm, which is due to the presence of tyrosine in the ribonuclease. This type of spectrum, in which only one maximum is well observed, is explained by significant differences in the amino acid composition of the studied proteins: in the BSA molecule there are 30 phenylalanine, 21 – tyrosine and 3 – tryptophan residues, whereas in the RNase there are only 3 phenylalanine and 6 – tyrosine residues. The main contribution to the absorption spectrum of RNase is made by tyrosine, which is due to the absence of tryptophan, which is why the shorter-wavelength position of the absorption maximum (compared to BSA) is associated with this. The low molecular weight, fewer aromatic amino

acids and the absence of tryptophan are the reason for the lower optical density, and, accordingly, the low extinction coefficient of ribonuclease. Under the influence of UV light, the optical density of protein solutions increases throughout the studied wavelength range. At the same time, the increase in optical density relative to the native protein for RNase was 22.5% under the action of UV light at a dose of 755 J/m²; at 1510 J/m² - 37.5%; 3020 J/m² – 32.3%. For BSA, an increase in the optical density relative to the native protein when irradiated at a dose of 3020 J/m² is - 6%, which indicates its greater photostability.

At the same time, the position of characteristic points in the spectra of UV-irradiated proteins does not change in comparison with the native sample, therefore, there is no formation of oxidized products of aromatic amino acids, a possible change in the microenvironment of chromophore groups does not lead to a shift in the wavelengths of electronic transitions in aromatic amino acid radicals.

The measured optical density is the sum of the true value of the optical density and the optical density due to light scattering. The contribution of light scattering can be taken into account by extrapolating the optical density values from the region where there is no contribution of true absorption by chromophores (in this case, 340 – 400 nm). To account for the contribution of light scattering, the formula Dls=10^a·λ^n was used, where a and n are empirical coefficients characterizing light scattering. The table shows the values of these coefficients depending on the radiation dose.

Table

Estimation of the light scattering contribution using empirical coefficients

| Coefficient | n | | a | |
|---|---|---|---|---|
| Object | BSA | RNKaza | BSA | RNKaza |
| Native protein | 1,54 | 1,33 | 2,71 | 1,22 |
| 755 Дж/м² | 1,93 | 0,96 | 3,69 | 0,8 |
| 1510 Дж/м² | 2,17 | 2.18 | 4,36 | 3,95 |
| 3020 Дж/м² | 2,44 | 1,29 | 5,06 | 1,82 |

The dependence of the obtained coefficients for BSA is linear, for RNase we see non-directional changes in this parameter, which, apparently, indicates non-directional changes in the volume of the protein globule. The data obtained by us show that, using the spectrophotometric method, it is possible to correctly describe the structural changes in the protein molecule.

### S6.427. Using chlorophyll fluorescence parameters to predict biomass accumulation and drought tolerance in wheat

Sherstneva O.N.[1]*, Gromova E.N.[1], Kior D.S.[1], Abdullaev F.F.[1], Vodeneev V.A.[1]
[1]*Lobachevsky State University of Nizhny Novgorod;*
* sherstneva-oksana@yandex.ru

Breeding new varieties of agricultural plants with the desired economically significant traits requires the use of a complex of studies that ensures high reliability in the selection of plant lines sent to the subsequent stages of selection tests. In addition, at present, one of the key aspects in the development of methods for selecting promising lines is its acceleration by reducing the duration of the test cycle. To achieve this goal, genotyping methods are widely used, as well as phenotyping, which makes it possible to obtain information about the state of the studied plants at an early age and to find a relationship between phenotypic parameters and economically significant traits at a later age. Optical methods occupy a special role among phenotyping methods. Due to the high speed of data acquisition and non-invasiveness, they allow massive research of a large number of plant variants without removing them from the further selection process. The aim of this work was to search for parameters of chlorophyll fluorescence that have the potential to predict the accumulation of biomass and tolerance to drought in wheat plants at a later age.

The objects of the study were seedlings of common spring wheat (Triticum aestivum L.) of 11 varieties. Cultivation of plants was carried out under controlled conditions of the growing room (temperature 24ºC, relative humidity 50%, lighting mode light/dark 16/8 h). At the age of two weeks, chlorophyll fluorescence parameters were recorded in the studied plants using the PAM fluorometry method, for which an Open FluorCam FC 800-O/1010-S fluorescent imaging system, Photon Systems Instruments, Czech Republic, was used. After that, the watering of the experimental group was stopped; then, every five days (on the 5th, 10th, and 15th days of drought), chlorophyll fluorescence and morphometric parameters were recorded in the experimental and control groups. To assess the activity of photosynthesis, we analyzed the quantitative parameters of the time dynamics of the quantum yield of photosystem II photochemistry (ΦPSII) and non-photochemical quenching of fluorescence (NPQ); among the morphometric parameters, fresh and dry weight, as well as the length of roots and shoots were evaluated. To assess the relationship between the fluorescent parameters of young seedlings and the accumulation of biomass and drought tolerance of plants at a later age, a correlation analysis was carried out. For this, the Pearson correlation coefficient of the values of the chlorophyll fluorescence parameters of two-week-old wheat seedlings was calculated with the value of the dry weight of plants at the age of 30 days (for connection with the accumulation of biomass) and with the value of the residual dry weight of 30-day-old plants (for connection with drought tolerance). Residual dry weight as a measure of drought tolerance was calculated as the ratio of the dry weight of drought-exposed plants to the dry weight of control plants. A statistically significant correlation ($p < 0.05$) was shown between the effective ($r = 0.74$) and dark ($r = 0.69$) quantum yields of photosystem II (ΦPSIIef and ΦPSIId) and half-yield time per ΦPSIIef after turning on the actinic light (t1/2( ΦPSIIef)) ($r = –0.65$) in two-week-old wheat seedlings with the dry weight of wheat plants at the age of 30 days, which indicates a rather high predictive potential of these parameters when predicting the accumulation of wheat biomass.

In the case of studying the analyzed fluorescent parameters as predictors of wheat drought tolerance, only the dark level of the quantum yield of photosystem II ΦPSIId ($r = –0.63$, $p < 0.05$) was noted as promising. It should be noted that this parameter correlated both with the accumulation of biomass and with drought tolerance in wheat; however, the dependences were oppositely directed.

The use of the identified predictors of wheat traits can serve as a tool to increase the efficiency and speed up the breeding process. Due to the non-invasiveness and high speed of analysis, the applied method can complement other research methods without excluding promising lines from the next stages of testing.

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## S7. Mechanisms of action of physical and chemical factors on biological systems

### S7.428. Effect of low-frequency magnetic field onto the physical endurance of laboratory animals under stress load conditions

Lopatkina N.V.[1]*, Devjatkova N.S.[1]
[1]*RFNC-VNIIEF;*
* natali.lopatkina.80@mail.ru

The most important area in solving the problem of reducing the functional disturbance risks which are caused by acute stress load is searching for prevention and rehabilitation means which stimulate

mobilization of body's own functional reserves. Low-frequency magnetic field (LFMF) could be one of these means.
The paper presents the results of experimental researches of the vortex low-frequency (up to 110 Hz) magnetic field action with maximum magnetic induction value of (Bmax) up to 3,15 mT onto physical endurance of laboratory animals under stress load conditions.
Simulation of acute stress conditions was carried out using the «Forced floating with load until total exhaustion» method with triple load floating of animals. Performance criterion was the floating time of animals until "total exhaustion" for 180…1200 seconds [ - ].
The experiment was carried out on mature white outbred (non-linear) rats – males of 260 to 320 grams weight in quantity of 64 individuals, which were distributed for experimental and control groups. Experimental groups differed by versions of LFMF influence: before simulation of stress load – five-day (once a day) pre-condition action with Bmax=3,5 mT; during the loading between the second and third trial – single action with Bmax=1,4 mT; combined action, i.e. both before and during the loading with maximum magnetic induction values of 3,5 mT and 1,4 mT, respectively. In control group the LFMF action was imaginary.
The obtained results in control group confirm that the "exhaustion-recovery" state under acute stress load conditions passed according to the standard scheme: reliable decrease (for 2,2 times, $p \leq 0,05$) of the floating time of animals during the second trial of floating in relation to the first one with the following growth (for 1,2 times, $p \leq 0,005$) of the investigated factor during the third trial in relation to the second one, as well as the absence of reliable differences between the floating time during the third and the first trials.
In experimental group with pre-condition action of LFMF, the floating time of animals in all three trials didn't differ significantly. It can be assumed that LFMF is an "actoprotector" which increases the physical endurance of animals under unfavorable stress load conditions.
The change of the investigated factor in the group of animals using LFMF during the loading between the second and third trials was close to that one of the control group: decrease (for 2,4 times, $p \leq 0,005$) of the floating time during the second trial in relation to the first one and growth (for 1,1 times, $p \leq 0,05$) during the third trial in relation to the second one. However, the floating time of animals during the third trial was reliably lower (for 2,1 times, $p \leq 0,05$), than during the first trial. It can be assumed that single action of magnetic field onto the exhausted animals was an additional loading factor, "decreasing" the recovery processes of the body.
In the group of animals with combined action of LFMF between the first and the second trials the reliable decreasing (for 2,4 times, $p \leq 0,002$) of the investigated factor was marked, i.e. magnetic field effectiveness for "moderation" of development of the exhaustion state after the first floating loading was not sufficient enough. Hence, pre-condition action of LFMF may show unstable "actoprotector" effect. Additional application of LFMF during the loading between the second and the third trials led to the increasing (for 1,8 times, $p \leq 0,002$) of the floating time during the third trial in relation to second one. Besides, the floating time of animals between the third and the first trials didn't differ significantly. The obtained results prove that low-frequency magnetic field depending on the application enables keeping physical resistance of animals under stress load conditions.
Thus, vortex low-frequency (up to 110 Hz) magnetic field with maximum value of magnetic induction up to 3,15 mT is a mobilizing factor of keeping functional reserves and physical endurance of animal's body under acute stress conditions. Though, the obtained effects will be reasonably stable under combined action including pre-condition and additional action of LFMF with maximum values of magnetic induction of 3,5 mT and 1,4 mT, respectively.

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## S7.429. A survival of A549 cells under proton beam irradiation in flash and standard mode

Rzyanina A.V.[1,2]*, Agapov A.V.[1,2], Gritskova E.A.[1,2], Mytsin G.V.[1,2], Uglova S.S.[1,2], Shipulin K.N.[1,2]
[1]*Joint Institute for Nuclear Research;*
[2]*JINR;*
* rzjanina@mail.ru

The main problem with radiation therapy is that it can harm healthy tissues surrounding the tumor. Therefore, the dose applied to the tumor is limited by its toxicity to nearby healthy tissues. This can lead to a decrease in the effectiveness of radiation therapy and incomplete destruction of the tumor. Therefore, the methods of radiation therapy are based on principles that take into account the differences in the response to radiation between normal and tumor tissues, as well as approaches that ensure the preservation of healthy tissues at an acceptable level. To achieve this goal, the irradiation parameters can be varied: the amount of absorbed dose, dose rate, dose fractionation and methods of dose delivery to the tumor. The amount of dose delivered to the tumor is defined as the maximum tolerated dose that can be delivered to the tumor safely for healthy tissues. Dose fractionation is used to reduce the frequency of radiation-induced side effects. The most promising method of radiation delivery is proton therapy, which provides high conformality of dose distributions, which reduces the radiation load on normal tissues. Along with the improvement of the methods of the method of dose delivery to the tumor and fractionation modes, until recently, insufficient attention was paid to the possibility of dose rate regulation. More and more research is being conducted in a new field called flash therapy, which involves ultra-fast dose reduction at high power (almost 3 orders of magnitude higher than with standard therapy). Data from numerous studies show that this makes it possible to effectively reduce toxicity in normal tissues and at the same time effectively affect tumor cells [1].
Aim: To compare the survival rate of A549 cells under proton beam irradiation in flash and standard modes.
Materials and methods: Cell culture: Human lung carcinoma cells A 549.
Proton irradiation: Irradiation of cells was carried out on a proton beam of 660 MeV of the JINR phasotron by the "overflight" method in two modes: standard at a dose rate of 0.1 G/s and in flash mode at a dose rate of 70 G/s. The other parameters of the beam were the same.
Determination of cell survival. After irradiation with protons at doses of 0, 1, 2, 4 and 6 Gy, cells were seeded at the rate of 50 cells/ml of medium. The cells were cultured for the time required for colony formation. To determine the survival rate of cells 12-14 days after sowing, the number of grown colonies was calculated.
Results and conclusions. As a result of the experiments, there is a difference in the survival rate of A549 cells irradiated in flash and standard modes. The survival rate of cells irradiated in flash mode was higher. For a dose of 1 Gy, the ratio was 1.1; for 2 Gy -1.2; for 4 Gy - 1.3 and for 6 Gy -1.6.

### S7.430. AICAR as a potential radioprotective agent

Abdullaev S.A.[1,2]*, Saleeva D.V.[1]
[1]*State Research Center – Burnasyan Federal Medical Biophysical Center of FMBA, Moscow, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* saabdullaev@gmail.com

The present work is devoted to the study of the effect of the AICAR compound on the survival rate of mice, the frequency of micronuclei in mouse bone marrow cells, and the urinary excretion of extracellular nuclear and mitochondrial DNA in rats exposed to X-rays.
The search for ways to modify radiosensitivity is the most important fundamental problem, both from the standpoint of reducing the effects of ionizing radiation (IR) on the body, and from the standpoint of increasing the effectiveness of tumor radiotherapy. The efficiency of the functioning of repair systems depends not only on their usefulness, but also on the amount of induced DNA damage, their complexity, as well as the energy supply for the development of a response to DNA damage (DNA). At the same time, 5'-AMP-activated protein kinase (AMPK) plays a key role in maintaining energy homeostasis in cells irradiated with low and sublethal doses of IR. AMPK is a polysubunit (heterotrimeric) complex, the main regulator of cellular and systemic energy homeostasis. A number of studies have shown that additional activation of AMPK and mitochondrial biogenesis in cells can be achieved using pharmacological compounds of various classes. Among them, of considerable interest is 5-aminoimidazole-4-carboxamide-ribose (AICAR), an AMP analog that is transported into cells and is widely used in experiments.
The aim of our work was to study the effect of the AICAR compound on the survival of mice and on the frequency of formation of micronuclei (MN) in bone marrow cells, as well as on the excretion of cell-free nuclear DNA (cf-nDNA) and cell-free mitochondrial DNA (cf-mtDNA) with the urine of rats irradiated X-rays.
Materials and methods
The study used male Balb/c mice 2 months old and male Fisher-344 rats 3 months old, obtained from the nursery of the branch of the Institute of Bioorganic Chemistry, Russian Academy of Sciences (Pushchino, Moscow region). To determine the survival of mice, irradiation was performed at a dose of 8 Gy, and to analyze the occurrence of MR in bone marrow cells, at a dose of 2 Gy. Rats were irradiated at a dose of 5 Gy. AICAR (Merck, Darmstadt, Germany) was administered to animals intraperitoneally at 400 mg/kg of body weight. The drug was administered 30 min before and 20 min, 6 h, 24 h after irradiation. Survival curves were generated for 30 animals per curve in each independent experiment. Statistical differences in survival experiments between groups of mice were compared using the Kaplan-Meier method. Differences between data obtained before and after treatment of rats were analyzed using the Mann-Whitney U test or unpaired Student's t-test. Data are presented as mean (for 8 animals) and standard error of the mean (±SEM). A $p<0.05$ value was considered statistically significant.
Results
The results showed that AICAR has a radioprotective effect, both in mouse survival and in reducing the frequency of micronuclei. It has been shown that AICAR has a significant radioprotective effect only when it is administered to mice immediately after irradiation. The results of the analyzes indicate that the radiomitigatory effect of AICAR on irradiated animals may be manifested through a mitochondria-targeted mechanism. Data on the analysis of cf-nDNA and cf-mtDNA excretion with the urine of irradiated animals suggest that AICAR also promotes the accelerated removal of damaged cells and dysfunctional mitochondria from the tissues of irradiated animals through the activation of autophagy (mitophagy).
Conclusion
Thus, the results of our studies show that AICAR acts as a radiomitigator effector and has a high potential as a radioprotective agent for active practical use.

### S7.431. Adaptation of microcirculation to the action of low intensity mm-radiation in animals under the stress of different duration

Ravaeva M.Yu.[1]*, Cheretaev I.V.[1], Chuyan E.N.[1], Mironyuk I.S.[1], Dzeldubaeva E.R.[1], Nagorskaya M.V.[1]
[1]*V.I. Vernadsky Crimean Federal University;*
* ravaevam@yandex.ru

The study is aimed at solving an urgent fundamental problem related with establishment animal's tissue microhemodynamics adaptation mechanisms to the action low-intensity electromagnetic radiation of the millimeter (mm) range to the conditions of acute (AS, forced swimming test, once for 60 minutes) and chronic stress (ten-day restriction of mobility, hypokinesia, CS), as well as their various combinations. This makes it possible to deepen modern ideas about body's stress reactions development; to identify early "markers" of pathological changes accompanying to the stress reaction development (a non-specific component of any disease, and to determine the possibilities to modify and / or level the stress reaction development.
A microcirculation was studied by laser Doppler flowmetry using a laser blood flow analyzer "Lazma-MC" (manufactured by NPP "Lazma", Russia), oscillatory and non-oscillatory indicators of skin microhemodynamics were calculated. The wavelength of the radiation is 7.1 mm, the power flux density is 0.1 MW / cm2; localization is the occipital-collar region, exposure is 30 minutes, duration is 10 days.
The results of the study showed that microcirculatory reactions on effects of stress factors with different duration had pronounced specificity. Thus, a feature of the tissue microhemodynamics reaction in animals to AS conditions is a certain pattern: non-nutritive hyperemia (one hour after the action of stress factor) is replaced by nutritive (24 hours after the action of stress factor) and returns to non-nutritive (48 hours after the action of stress factor). The opposite Mc reaction was formed to the CS action, in which vasoconstriction developed, disruption of blood inflow and outflow and dominance of shunt blood flow, a decreasing in the number of functioning capillaries.
The study of Mc under the action of a combination of stress factors allowed us to establish that the preliminary effect of CS modifies the Mc response to the action of acute stressful factor, leveling the development of hyperemia accompanying the isolated action of AS. This is supported by the dynamics of the modification coefficient of microcirculatory indicators, the values of which gradually increased in the negative part of the graph, which indicates an increase in the effect of modification. Preliminary exposure to AS in animals makes it possible to reduce the level of manifestations of stress-induced vasoconstriction accompanying CS and, as a consequence, tissue ischemia. It can be assumed that the use of AS is a kind of training that prepares Mc and the body as a whole for the action of adverse factors, one of which is prolonged restriction of mobility.
With isolated 10-fold mm exposure in animals, a significant change in the activity of all microvascular tone regulation components was observed, which was expressed in an increasing in endothelium-dependent vasodilation, a decreasing in peripheral resistance, an increasing in blood flow to the nutritive microvascular bed, and an improvement in venular outflow.
The combined effect of low-intensity MM radiation, AS and CS led to a significant decrease in microhemodynamic disorders developing under stress, as evidenced by the absence of significant differences in most Mc indicators in animals of all studied groups compared with animals in a control group.

Thus, one of the main MM radiation mechanisms of action is its ability to limit the stress reactions development at the level of Mc, which is one of the main manifestations of the physiological effects of low-intensity mm radiation underlying its antistress effect. Almost all regulatory components of vascular and extravascular genesis are involved in the Mc reaction to MM exposure. It is likely that such a systemic nature of the body's response is due to a large number of targets for mm waves, including skin microvessels, blood cells, diffuse neuroendocrine system, as well as nerve endings and peripheral nerves of the skin, the activation of which changes the functional activity of the nervous, immune, endocrine systems of the body with changes in the content or synthesis of biologically active substances (hormones, cytokines, neurotransmitters), which plays an essential role in the mechanisms of regulation of blood Mc processes.

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### S7.432. Adaptive response in mice induced by indirect action of steam from a high-voltage discharge chamber

Panchelyuga V.A.[1]*, Panchelyuga M.S.[1], Zaichkina S.I.[1], Dyukina A.R.[1], Potselueva M.M.[1]
[1]*Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences;*
* victor.panchelyuga@gmail.com

Currently, most of the world's leading countries have government programs aimed at researching Low-Energy Nuclear Reactions. (LENR). Typical phenomenology of such reactions usually includes excess energy release, isotopic changes in reaction products, absence of ionizing radiation, as well as some not yet identified radiation, first described in [1], where it was called 'strange radiation' (SR) because of unusual tracks it leaves on the track detectors. A wide range of potential applications of LENR reactions makes it especially important to study the biological effects of the SR radiation.

The beginning of such investigations was given in [2], where an experimental system was created that made it possible to separate the action of SR and electromagnetic radiation of electric discharge. In [2], a high-voltage discharge in steam was used as an SR generator, as well as the fact that water steam can bind SR particles and transport them through steam pipelines [3] to a box with experimental animals. As a control, we used a similar box filled with 'clean' steam from a separate steam generator. Both boxes were at equal distances from the source of electromagnetic radiation - the shielded discharge chamber. Such a design made it possible to equalize the electromagnetic effect on the experimental and control groups of animals, although, in this case, the steam from the discharge chamber could have slightly different chemical properties compared to the 'pure' steam. To eliminate such a 'chemical' inequality, the experimental setup was updated to exclude the contact of animals with the steam. To do this, the experimental and control boxes were made in the form of large glass cylinders, on which the 'experimental' and 'control' steam pipelines were wound from a flexible silicone hose.

In total, our approach is based on three assumptions: 1) SR is transported by steam; 2) SR penetrates through the walls of the silicone hose; 3) SR has a high concentration in a small vicinity of the SR generator and steam pipelines (about 20–30 cm), as follows from our experiments [3] and from the experiments of other groups. At distances exceeding the above values, a sharp (by about two orders of magnitude) decrease in the SR concentration occurs. By virtue of 3), the experimental and control glass cylinders separated by a distance of about two meters turn out to be reliably separated with respect to the action of the SR. At the same time, the diameters of the experimental glass cylinder (18 cm) were chosen such that the SR concentration inside it was maximum.

Thus, the geometry of the experiment makes it possible to equalize the electromagnetic and acoustic influence on the control and experimental groups of laboratory animals and to separate these groups in relation to the SR-influence. At the same time, in the updated version of the experimental setup, the influence of the inhomogeneity of the chemical composition of 'experimental' and 'control' steam on animals is eliminated.

During the experiment, laboratory mice were placed in the control and experimental cylinders for 60 minutes per day for one and two days. Simultaneously, a separate group of mice was irradiated at a dose of 0.1 Gy of X-ray radiation, and a day later, all groups were irradiated with a revealing dose of 1.5 Gy of X-ray radiation and after 28 hours mice were taken out of the experiment by decapitation and bone marrow cytological preparations were prepared. Cytogenetic damage was estimated by counting the number of polychromatophilic erythrocytes with micronuclei. At least 5 mice were used in each experimental group.

In the experimental group, after SR-exposition 60 min per day, once, and irradiation at a dose of 1.5 Gy, a decrease in cytogenetic damage is observed, i.e. there is an induction of an adaptive response. At the same time, SR-exposition in 60 min per day for 2 days no longer protects against the action of a dose of 1.5 Gy, which indicates that the threshold of the adaptive dose has been overcome and leads to damaging consequences. For X-ray radiation, such a dose starts from 0.5 Gy. In all experiments at various exposures in the control group, no adaptive response was detected.

Thus, as a result of the conducted studies (three series of experiments were performed), we found a weak damaging factor, the effect of which on the body of experimental animals is similar, according to the mechanism of cross-adaptation, to the effect of X-ray radiation at a dose of 0.05-0.4 Gy. Based on the geometry of the experiment, its source is presumably SR-particles transported by steam from the discharge chamber. A similar 'clean' steam circulating around the control cylinder does not have such an effect.

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### S7.433. Analysis of the contribution of genetic regulation to the effects of ionizing radiation on stress signals of plants

Pirogova P.A.[1]*, Zdobnova T.A.[1], Ladeynova M.M.[1], Grinberg M.A.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhni Novgorod;*
* poly.h@mail.ru

The significant influence of ionizing radiation (IR) on living organisms is realized, in many respects, due to the modification of stress signals and changes in the status of signaling systems. In natural conditions, the effects of IR on stress signals, which in turn form adaptations to natural factors, can become of particular importance. One of the

significant types of stress signals of plants is electrical signals (ES). ES are propagating changes in the membrane potential that occur in response to various stimuli (heating, cooling, mechanical action, etc.). Other signaling systems, including hormonal ones, take part in the transformation of ES into a functional response. Stress hormones — abscisic (ABA), jasmonic (JA) and salicylic acid (SA) — are involved in the ES-mediated change in photosynthesis activity, transpiration, and gene expression. To date, it has been shown that IR is able to have an effects on the parameters of ES and the functional responses they cause. The effects arising from the action of IR can be caused by a change in the level of gene expression, which is realized mainly due to reactive oxygen species (ROS), capable not only of damaging action, but also of regulating various systems.

The studies were carried out on 15-day seedlings of soft wheat (Triticum aestivum L.) of the "Daria" variety. The experimental group was irradiated with a $^{90}Sr$-$^{90}Y$ β-emitter with an activity of 0.1 MBq and a dose rate of approximately 31.3 mGy/h. The duration of irradiation of plants was 15 days. The maximum accumulated dose was about 11.3 mGy. The level of gene expression was assessed by real-time PCR. The work analyzed the genes of proteins that can potentially cause the effects of IR on electrogenesis, and the genes of proteins of biosynthesis of stress phytohormones. The development of primers of the genes of interest for real-time PCR was carried out in compliance with the necessary parameters. β-actin (ACTB) and the homolog of the vacuole fusion protein (MON1) were used as reference genes. The obtained results were analyzed using the ΔΔCt method.

In irradiated plants, the ES parameters change: the amplitude and propagation speed increase. Theoretical analysis of potential IR targets made it possible to identify the key components of signaling systems, the impact on which can explain the experimentally observed modification of the ES and the signal conversion process in response. These include, first of all, $H^+$-ATPase, NADPH-oxidase, ion channels of various types and phytohormones. In the course of the work, primers were selected for the genes of proteins involved in electrogenesis: $H^+$-ATPase (HA1), NADPH oxidase (RBOHs), anionic (CLC1 and ALMT1), potassium (SKOR and AKT1) and calcium (TPC1) channels. Also for the genes of phytohormone biosynthesis proteins: ABA — 9-cis-epoxycarotinoid dioxygenase (NCED3), xanthoxin dehydrogenase (ABA2), β-glucosidase (BG1); JA — lipoxygenase (LOX6), allenoxide synthase (AOS), 12-OPDA-reductase (OPR2), jasmonoyl-L-amino synthetase (JAR1); SA — isochorismate-synthase (ICS1). Based on the results of real-time PCR, the relative level of expression of genes of interest in irradiated plants was determined. A decrease in the expression of the potassium channel gene SKOR and multidirectional changes in the expression of genes for the biosynthesis of various phytohormones were found. The analysis of the expression of genes of interest allows us to determine the contribution of genetic regulation to the effects of IR on stress signaling.

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### S7.434. Antioxidant and neuroprotective effects of meconic acid in model systems

Kozin S.V.[1,2,3]*, Kravtsov A.A.[1,2], Moiseev A.V.[4], Levchenko A.A.[2], Ivashchenko L.I.[2]

[1]*South Scientific Center of the Russian Academy of Sciences, Rostov-on-Don;*

[2]*Kuban State University, Krasnodar;*

[3] *Kuban State Technological University, Krasnodar;*

[4] *Kuban State Agrarian University, Krasnodar;*

* kozinsv85@mail.ru

The development of new effective and safe neuroprotectors remains an urgent task in pharmacology. With regard to comenic acid, an antioxidant and neuroprotective effect has been established, and chelidonic acid has a pronounced anti-inflammatory effect. With regard to meconic acid, the neuroprotective and antioxidant effects have not been studied. The aim of this work was to establish the neuroprotective and antioxidant potential of meconic acid in model systems.

The protective effect of meconic acid on the culture of cerebellar neurons was studied using the model of glutamate toxicity and oxygen–glucose deprivation (OGD)[1]. The antioxidant activity of meconic acid was studied by quantum mechanical calculations in the ORCA 5.0.1 software package and experimentally by chemiluminescent analysis in model systems: citrate-phosphate-luminol (CPL) and yolk lipoproteins (YLP)[2]. The chelating properties of meconic acid with respect to Fe3+ in solutions were studied by Job's method [3].Meconic acid (MA) was obtained from kojic acid in the Department of Biologically Active Substances of the Kuban State University.

According to the calculated data, meconic acid has the lowest value of the enthalpy of one-electron transfer in the trianionic form and is 318.7 kJ/mol, while for the dianion form this value is 421.3 kJ/mol. The calculated values of the electron transfer energy in the aqueous phase for MA are commensurate with the natural antioxidants β-catechins, and the experimentally obtained values of inhibition of the development of free radical reactions in model systems are higher than those of ethylmethylhydroxypyridine (EMHPS). In the model CPL system, MA showed a dose-dependent decrease in the intensity of the luminescence of the chemiluminescent reaction. At a concentration of 0.2 mM, MA reduces chemiluminescence by 73% versus 48% of EMHPS. The decrease in the integral luminescence index at antioxidant concentrations of 0.3 and 0.6 mM was 80 and 87% for MA versus 55 and 73% for EMHPS ($p < 0.05$). MA and EMHPS also had a dose-dependent decrease in the intensity of development of the free radical reaction of yolk lipid oxidation in the model system of yolk-lipoproteins. The experimental data obtained in two model systems and the calculated data indicate a high antioxidant potential of MA. MA has been found to have a protective effect in in vitro models of ischemia. Its action leads to a decrease in the level of intracellular calcium and the restoration of the membrane potential of mitochondria in a culture of cerebellar neurons under glutamate exposure, and an increase in the percentage of living cells under OGD. Under the action of OGD in the absence of MA, the number of living cells was 12%.The addition of MA to the culture of cerebellar neurons subjected to OGD contributed to an increase in cell survival. There was no statistically significant difference in the number of living cells between the studied concentrations, however, the maximum effect was observed at a concentration of 1 mM and amounted to 56%, and the minimum effect was observed at a concentration of 0.001 mM and amounted to 31%. A ten-minute incubation of glutamate resulted in 60% death of cerebellar neurons compared with the control, while the addition of MA at all concentrations contributed to a decrease in neuronal death. There was no significant difference in the number of living cells between the studied concentrations, however, there was such a tendency that the maximum concentration of 1 mM MA had the least effect (55% of living cells), while at a concentration of 0.01 and 0.001 mM, the number of surviving cells was maximum and amounted to 65 %. Exposure to glutamate led to an increase in the level of cytosolic calcium by 69% relative to the control.On the other hand, in cultures with the addition of MA, the increase in the level of calcium was significantly less and amounted to 38%, 46%, 46%, 48% of the control, respectively, for concentrations 1; 0.1; 0.01 and 0.001 mM. The action of the excitatory amino acid on brain cells led to a decrease in the membrane potential of mitochondria by 17% relative to the control. MA contributed to a smaller decrease in the value of the membrane potential of mitochondria by 9%, 8%, 7% and 10% compared with the control for concentrations 1; 0.1; 0.01 and 0.001 mM. The spectroscopic results of studies of MA with Fe3+ showed that at physiological pH, the complex with a meconic acid/Fe3+ ratio of 3:1 of ~ 97% with a high stability constant prevails, while the complex with a composition of 2:1 accounts for ~ 3%. These data allow us to

speak about the possible chelation of iron ions by MA in the brain tissues and a decrease in the intensity of oxidative processes due to the inhibition of the Fenton reaction,and,as a result, a decrease in damage and death of neurons.
The obtained antioxidant, chelating, and cytoprotective action of meconic acid provides a basis for further study of the possible neuroprotective properties of this compound in in vivo experiments, and the data obtained in the work on its physicochemical properties can be useful for the synthesis and study of new coordination compounds based on meconic acid.
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### S7.435. Approaches to studying the fungistatic action of the plant peptide nigellin from Nigella sativa at the cellular and molecular levels

Barashkova A.S.[1], Bocharov E.V.[1], Rogozhin E.A.[1]*
[1]*Shemyakin And Ovchinnikov Institute Of Bioorganic Chemistry Russian Academy Of Sciences;*
* rea21@list.ru

Plants are a good source of various biologically active compounds, including proteins and peptides. The latter, as components of the innate immunity of plants, implement a variety of functions aimed at counteracting damaging environmental factors, in particular, microorganisms that cause diseases, as well as insect pests. Despite the fact that over the past three decades, an impressive amount of data has been accumulated on the structural diversity of such molecules, the spectrum of their biological activity, and the elucidation of a specific role in plant immunity, very little information has been obtained in terms of elucidating the mechanisms of their action at the molecular level. Membrane-active methods of antimicrobial activity for plant peptides have been studied in most detail, as a rule, leading to cell lysis or apoptosis of pathogenic microorganisms. At the same time, a whole group of biologically active peptides that penetrate the cell and perform their function through association with an intracellular target remains practically unexplored.
In the framework of this work, we studied the interaction of a hairpin-like antimicrobial peptide, nigellin, from Nigella sativa seeds with conidia and mycelium of a model mycelial fungus, Aspergillus niger, at the cellular and molecular level. Previously, it was shown that the main antifungal effect of this molecule is to delay the physiological growth and development of fungi, which is a priority function for many representatives of all hairpin-like plant defense peptides (alpha-hairpinins). Later, using laser scanning confocal fluorescence microscopy, it was found that this peptide is a typical representative of the so-called cell-penetrating molecules, while a similar effect is observed in a wide range of effective concentrations (0.5-32 μM). According to the ratio of the signals of green (SYTO GREEN) and red (propidium iodide) fluorescent dyes, it was revealed that the action of the studied peptide does not lead to permeabilization of the plasma membrane. This effect was recorded in temporal dynamics (8-24 hours) against the background of observing a fungistatic effect compared to the control variant. In order to understand the probable molecular determinant associated with the cell membrane and being the probable "site" of the primary binding of the peptide, the interaction of the molecule with artificial lipid bilayers was studied by calorimetric titration. For this purpose, a comparison pair was used based on phosphatidylcholine (100%) and phosphatidylcholine/phosphatidylserine (70/30%), which causes differences in the total charge of the membranes. The results obtained also indicate that the adsorption of nigellin on more negatively charged membranes (phosphatidylcholine/phosphatidylserine) is stronger, which, apparently, determines the differences in the action of this peptide on prokaryotic and eukaryotic cells.
This work was supported by a project of the Russian Science Foundation (No. 18-74-10073-P).

### S7.436. Biocidal properties of nanoscale potentially membrane-active 1,10-phenanthrocyanines (bi-1,10-phenanthrolylenes) of redox-sensitive Co(II) and Mn(II)

Demidov V.N.[1]*, Bogomolova E.V.[2], Badalyan A.G.[3], Pakhomova T.B.[4]
[1]*I.V. Grebenshchikov Institute of Silicate Chemistry of RAS, St. Petersburg, Russia;*
[2]*V.L. Komarov Botanical Institute of RAS, St. Petersburg, Russia;*
[3]*A.F. Ioffe Institute of Physics and Technology of RAS, St. Petersburg, Russia;*
[4]*St. Petersburg State Technological Institute (TU), St. Petersburg, Russia;*
* vndemidov@mail.ru

Currently, due to the progressive bacterial and fungal antibiotic resistance, the development of fundamentally new methods for the targeted synthesis of coordination compounds of d-elements with pharmacophore ligands and the study of the possibilities of their use as metal drugs [1], in particular, fungistatics [2], as well as eco-friendly biocides in the composition of protective coatings. Metallo-drugs do not have such a long history as organic antibiotics and the development of significant resistance of microorganisms has not been recorded in relation to them. One of the promising approaches to the synthesis of new metallo-drugs and biocides (the so-called new paradigm) is the creation of binuclear structures [LnMz+(μ–L)Mz+Ln] X–m (Mz+– metal ion, L - ligands, μ–L - bridging ligand, X- anions).
Paramagnetic (low spin, with strong field ligands) nanoscale binuclear complexes Co2+ [Ar]3d7 and new Mn2+[Ar]3d5 (phen)nM2+(μ-σH,πH-PCH)M2+(phen)n(–OAc)4 complexes with pharmacophore N,N have been synthesized in this work'–N',N"-bis-chelated 1,10-phenanthrocyanine (bi-1,10-phenanthrolylene) bridging ligands μ-σH,πH-PCH (phen–1,10-phenanthroline, –OAc –acetate anions, n=0, 1), their initial purple-violet (as well as derivatives yellow-brown chromophore forms, μ-σH, πH-PCH'), soft colored colloidal glasses, and their fungi-, bacterio-, viro- and cytostatic properties were studied. The synthesis of the complexes was carried out on the basis of a thermal metal-assisted non-dehydrogenative C(sp2)H coupling of 1,10-phenanthroline [3] in the precursors of M(phen)n(OAc)2 (n=1,2). The compounds were characterized by IR spectroscopy, ESR, and elemental analysis data.
Primary data on the fungistatic properties of yellow-brown forms of compounds (phen)M(μ-PCH')M(phen)(OAc)4 in relation to fungi from the genera Aspergillus, Penicillium and Trichoderma show that the Mn(II) complex can be attributed to moderate (mild) fungistatics. The activity of the Co(II) compound is significantly lower. Nevertheless, the ICmin found for it for Ulocladium chartarum fungi was 89.06 μg/ml, which indicates a significant fungistatic effect [4]. The study of the behavior of 1,10-phenanthrocyanins (PC) Co(II), Zn(II) and Cd(II) in relation to Mycobacterium tuberculosis, Herpes virus, Candida albicans, as well as HeLa cells in vitro indicates their pronounced

bacterio- (IC50 0.1-3.0 μg/ml), viro- (IC50 0.1-10.0 μg/ml), fungi and cytostatic activity.
In connection with the discovered biocidal properties of compounds, it is important to study the mechanisms of their biostatic action. Several possible variants (modes) of the "metal complex–target" interaction are considered. An increase in the reduced viscosity of DNA solutions in the presence of Co(phen)2(OAc)2 suggests that intercalation of the phen ligand into the DNA double helix is not excluded for it [5]. Due to the propensity of PC Co(II) and Mn(II) associates (as volumetric chromophores, with properties approaching organic dyes) to IMI of the dispersion ("surface–to–surface") type [6], it can be expected that the most likely primary biological targets for them will be cell membranes and cellular organelles, in particular, the kernel. The proposed effect of Mn2+ redox sensitivity on the biocidal properties of its complex is considered. Temperature-accessible lower electronic biradical triplet states have been identified for compounds by the ESR method. Photoactivation also leads to the appearance of similar forms. In this regard, we investigate the probable thermo- and photo-activation of the biostatic action of the complexes.
The study of ESR compounds was carried out in the IPT RAS and RC MRMI St.-Pt. St. Univ., the study of their fungistatic properties – in the BIN RAS, under the theme «Biodiversity, ecology and structural and functional features of fungi and fungi-like protists» (AAAA19-119020890079-6).
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### S7.437. Biological activity of fullerenols of various structure. The role of reactive oxygen species. Bioluminescent monitoring

Sushko E.S.[1,2]*, Sachkova A.S.[3], Vnukova N.G.[2,4], Churilov G.N.[2,4], Stepin E.A.[4], Kicheeva A.G.[1,5], Kudryasheva N.S.[1,4]
[1]*Institute of Biophysics SB RAS, FRC KSC SB RAS, Krasnoyarsk, Russia;*
[2]*Institute of Physics SB RAS, FRC KSC SB RAS, Krasnoyarsk, Russia;*
[3]*Division for Nuclear-Fuel Cycle, Tomsk Polytechnic University, Tomsk, Russia;*
[4]*Siberian Federal University, Krasnoyarsk, Russia;*
[5]*FRC KSC SB RAS, Krasnoyarsk, Russia;*
* kkovel@yandex.ru

Fullerenes are bulk hollow molecular compounds, allotropic forms of carbon. Fullerenes are practically insoluble in polar solvents, including water, but water-soluble derivatives of fullerenes can be obtained. Fullerenols are water–soluble polyhydroxylated derivatives of fullerenes, nanoscale biologically active compounds, perspective agents for drug development. Especially promising in radiomedicine are: (1) redox fullerene derivatives influencing redox processes in biochemical cycles (for example, iron atoms); (2) candidates for use as new contrast agents for magnetic resonance imaging (for example, fullerenols containing gadolinium). Gadodiamide, dimeglumine gadopentetate, meglumine gadoterate, etc. are widely used Gd-containing compounds in medicine; their toxicity reduces the prospects for their use in this area. Metallofullerene derivatives retain toxic Gd3+ ions in an inert but strong carbon shell, preventing their release and reducing their toxicity. We analyzed the toxicity and antioxidant activity of fullerenols of various structures – with different amount of oxygen substituents, carbon cage size, involvement of endo- or exohedral metal atom: C60,70Oy(OH)x, where x + y = 10-12; Gd@C82Oy(OH)x, where x + y = 20-24; C60Oy(OH)x, C60,70Oy(OH)x, where x + y = 24-28; C60,70Oy(OH)x, Fe0,5C60Oy(OH)x, Gd@C82Oy(OH)x, where x + y = 40-42. To monitor the toxicity of fullerenol solutions, luminescent cellular and enzymatic bioassays were used (luminous marine bacteria Photobacterium phosphoreum and their enzymatic reactions, respectively). The main parameter of physiological activity of these bioassays is the intensity of bioluminescence. To characterize the toxicity of fullerenols, their concentrations inhibiting the bioluminescence of test systems by 50% were determined. Antioxidant activity of fullerenols was studied in solutions of model oxidizers (1,4-benzoquinone and K3[Fe(CN)6]); reducing of toxicity of the model oxidant solutions was monitored, coefficients of antioxidant activity were calculated. The content of reactive oxygen species (ROS) was evaluated in experimental solutions by chemiluminescent luminol method, correlations between the toxic/antioxidant fullerenol' characteristics and the content of ROS in solutions of fullerenols were revealed, with this indicating the active role of ROS in the bioeffects of fullerenols.
The paper demonstrates the high potential of luminescent bioassays for comparing the biological activity of fullerenols. All fullerenols demonstrated toxicity at high concentrations (>10-3 g/L) and antioxidant activity at lower concentrations. The toxicity and antioxidant activity of fullernols were associated with a decrease in the content of ROS in the studied solutions. Fullerenols were characterized with lower toxicity and higher antioxidant activity if the number of oxygen-containing groups is close to ½ of the number of carbon atoms in the cage. This regularity is probably due to involvement of the hydrophobic π-system and the polar (hydrophilic) oxygen-containing groups into the biological activity of fullerenols. It was shown that fullerenols inhibit the bioluminescence of bacterial bioassay more intensively than the enzymatic one, which is probably due to the additional ways of influencing the hydrophobic fullerenol fragments on the bacterial cell membrane. The Gd-containing fullerenols Gd@C82Oy(OH)x (x+y=20-24; x+y=40-42) are characterized by the least toxicity for the bacterial bioassay ,which is probably due to the large size of their carbon cage and the tendency to aggregation. The antioxidant effect of fullerenols depends on the amphiphilic characteristics of the medium and is maximal in solutions of an organic oxidizer – 1,4-benzoquinone. Fullerenols accelerate enzymatic NADH-dependent reactions, leading to acceleration of bioluminescent reactions and contributing to their antioxidant effect.

### S7.438. Biophysical characteristics of uromodulin in vitro in verification of the role of metamorphosis of its isoforms in vivo

Iakovleva A.V.[1]*, Zaleskij M.G.[1], Shapovalov V.V.[2]
[1]*St. Petersburg State Medical University named after I.P. Pavlova;*
[2]*Saint Petersburg Electrotechnical University "LETI";*
* gi_ns@mail.ru

Actuality. Urolithiasis is a significant medical and social problem, since it is widespread among the able-bodied population and occupies one of the leading places in the structure of urological pathology.
Objective: to study the biophysical phenomenon of the phase transition "sol-gel" isoforms of uromodulin using various technologies.
Materials and methods of research: In the course of the work, 100 patients were examined: men - 48 (48%), women - 52 (52%). The study group overwhelmingly included patients with oxalate urolithiasis, the results of the «Lithos-System» were taken into account. Determination of the hydrodynamic dimensions of biological objects was carried out on the analyzer LKS-03, INTOX (Russia) by the method of

hydrodynamic light scattering. Study of the Z-potential for measuring electrophoretic particle mobility using the Doppler effect, determination of molecular weight from the intensity of scattered light using the Debye graph and evaluation of the dynamics of these characteristics when pH and ionic strength change with metrological traceability to NIST standards (Zetasizer Nano ZS analyzer, Malvern Instruments).
Results. On model solutions simulating the composition of urine (density 1.010 (300 mosm / l); 1.017 (650 mosm / l); 1.030 (1000 mosm / l)), the dynamic light scattering method revealed the predominance of the oligomeric form of UMD in healthy individuals both at low osmolality (95% at 300 mosm / l) and at very high osmolality (75% at 1000 mosm / l). Uromodulin from the urine of a patient with urolithiasis is overwhelmingly represented by a polymeric form: at 300 mosm / l to 60% in the polymer form of UMD (28), and in concentrated urine (1000 mosm / l) - UMD (28) is found in 95%.
When measuring the Z-potential of the UMD pool, it was found that uromodulin from the urine of patients with urolithiasis forms a curve with two peaks. The second peak is represented by a part of macromolecules that are actually devoid of negative charge. When studying the size of uromodulin molecules in the process of cooling the sample to 4 ° C, uromodulin from the urine of patients with urolithiasis progressively increases the hydrodynamic size measured by dynamic light scattering. The formation of crystalline mineral components occurs when the colloidal stability of the biological environments of the body is violated (decreased). The unique biophysical properties of uromodulin provide colloidal-osmotic properties of urine and prevent the formation of stones, preventing the adhesion of crystalloids.
Detection of gel-like precipitate-cryogel during cooling of urine confirms the presence of high-molecular compounds in the urine. The phenomenon of the phase transition of "sol" to "gel" was recorded on the developed model of the medical device "Uroscrin". The electro-optical device allows you to fix the formation of "urine cryogel" when performing a "cold urine test". Approbation of the analyzer allows it to be used in the future to ascertain the effect of pharmacological modification of the crystallogenesis process.
Conclutions. Stability and steadiness of the colloidal system is provided by the oligomeric form of uromodulin (7 MDa) due to the pronounced electronegative properties of this form, which significantly prevails in the population in healthy people over the oligomeric form (28 MDa). "Uroscrin" is a new medical technology for studying the diagnostic effectiveness of screening for assessing the state of colloidal urine homeostasis as a criterion of renal dysfunctions.

### S7.439. Cellular respiration of mouse ovaries after 4-day antiorthostatic hindlimb suspension

Gorbacheva E.Yu.[1], Sventitskaya M.A.[1]*, Biryukov N.S.[1], Ogneva I.V.[1]
[1]*Cell biophysics laboratory, Institute of Biomedical Problems RAS;*
* ma_sventitskaya@mail.ru

The influence of space flight factors on the female reproductive system is still practically unexplored, although more and more women are participating in the space programs. However, studies involving humans and, especially, in real flight conditions, are quite difficult to implement. Therefore, it is common practice to use ground-based models and conduct animal experiments. The purpose of this work was to determine the cellular respiration of the ovaries of mice after simulating space flight conditions.
Antiorthostatic suspension of female mice was carried out according to the method of Ilyin-Novikov in the Morey-Holton's modification during the full estrous cycle – 96 hours. The experiment used 14 mice, which were randomly divided into two groups: control (C) and antiorthostatic suspension (HS). The weight of animals in group C was 27.9±0.6 g, in group HS – 27.1±0.9 g. Animals were euthanized with an overdose of Isoflurane inhalation anesthesia, and the ovaries were immediately isolated, one of which was frozen for subsequent determination of the content of proteins and mRNA, and the second was used to assess cellular respiration by polarography. Substrate-inhibitory analysis was carried out in accordance with the protocol of Kuznetsov A.V. et al. (2008). After the sample was transferred to a polarographic cuvette, V0, the rate of oxygen uptake by permeabilized cells, was recorded. Then 10 mM glutamate and 5 mM malate, substrates of the first respiratory chain complex, were added and the respiratory rate Vglu+mal was recorded. Next, 2 mM ADP was added and the maximum respiratory rate Vmax was recorded. Then, inhibitors and substrates of the following complexes of the respiratory chain were added in turn to analyze their functional activity: 0.5 μM rotenone (complex I inhibitor), 10 mM succinate (complex II substrate) – the rate of oxygen uptake V(II) was recorded, 5 μM antimycin A (complex I inhibitor III), 0.5 mM TMPD + 2 mM ascorbate (artificial substrates of complex IV) – the oxygen uptake rate V(IV) was recorded. After the substrate-inhibitory analysis, each sample was tested for intactness of the outer mitochondrial membrane by adding 10 μM cytochrome c: if the membrane is intact, then the respiratory rate does not change or increases by a maximum of 15%. The rate of cellular respiration was expressed as pmol O2/mL/min/mg dry weight of the ovary. All experiments with animals were approved by the Commission on Biomedical Ethics of the State Scientific Center of the Russian Federation - IBMP RAS (protocol No. 622 of October 12, 2022).
The results obtained indicate a significant increase in V0, Vglu+mal and Vmax by 81%, 169% and 133% ($p<0.05$), respectively, in the HS group in comparison with the control. The substrate-inhibitory analysis showed that V(II) and V(IV) remained unchanged after modeling the effects of weightlessness. This may indicate, first of all, that the increase in the maximum rate of ovarian respiration after suspension is due to the complex I of the respiratory chain.
However, the content of mRNA of the genes encoding cytochrome c and one of the subunits of H+-ATP synthase increased by 272% and 182% ($p<0.05$), respectively, in the HS group compared to the control, although the relative content of mRNA of the gene encoding cytochrome -c-oxidase remained unchanged.
The results may indicate that after 96 hours of antiorthostatic suspension, intensification of cellular respiration of the mouse ovaries took place. It should be mentioned that we observed a similar situation during the exposure of Drosophila melanogaster under simulated weightlessness (Ogneva I.V., Usik M.A., 2021; Usik M.A. et al., 2021). It can be assumed that an increase in cellular respiration may mediate the formation of an adaptive pattern of expression of genes encoding proteins that form complexes of the respiratory chain.
It should be noted that the complex I of the respiratory chain is one of the most significant sources of reactive oxygen species in the cell, the accumulation of which, in turn, can have a number of negative consequences. However, there are no data to support such a scenario in mouse ovaries after antiorthostatic suspension, which requires further research.
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### S7.440. Changes in the content of deuterium in the blood and brain of rats with the intake of deuterium-depleted water

Kozin S.V.[1,2,3], Kravtsov A.[1,2,3]*, Lyasota O.M.[2]
[1]*Kuban State University;*
[2]*Southern Scientific Center of the Russian Academy of Sciences;*
[3]*Kuban State Technological University;*
* aakravtsov@mail.ru

The content of deuterium in natural water is 150 ppm. Water in which the deuterium content is below the natural value is deuterium-depleted water (DDW). To date, scientific literature contains data on the effect

of low deuterium concentrations on metabolic processes in cells and tissues of mammals. A decrease in the concentration of deuterium in the body of animals enhances its antioxidant and antitoxic functions. A change in the balance between deuterium and protium in the internal environment positively affects the stress resistance and anxiety level of laboratory animals under the action of prolonged stress factors [5], improves reference memory [4], changes the electrophysiological activity of the hippocampus [2], increases resistance to hypoxia and increases cognitive abilities of laboratory rats [1, 3] However, data on how changes in the content of deuterium in consumed foods affect the balance of hydrogen isotopes in the body is not enough.

The purpose of this work was to investigate the effect of DDW intake by laboratory animals on the level of deuterium in the blood and brain of rats.

Deuterium-depleted water was obtained using a setup developed at the Kuban State University [6]. The deuterium content in the DDW was 50 ppm. The resulting deuterium-reduced water and distilled water with natural deuterium content (150 ppm) were then subjected to additional purification in the Milli-Q system. After that, OFA and distilled water with a natural content of deuterium were mineralized. Water mineralization was carried out by adding mineral salts to it to obtain a physiologically complete mineral composition (mineralization 314–382 mg/l: hydrocarbonates 144–180, sulfates <1, chlorides 60–76, calcium - 6, magnesium - 3, sodium 50–58 , potassium 50–58).

The experiment was performed on 66 male Wistar rats. All animals were kept in the same vivarium under the same conditions and had free access to food and water.

The animals were divided into two groups:

group 1 (n=33) - rats that intake water with a natural content of deuterium;

group 2 (n=33) - rats that intake DDW.

From each group of animals, 3 rats were taken out every day for a week, then on days 10, 15, 21, 42 for blood sampling by decapitation. Serum was prepared from the obtained blood and the concentration of deuterium in it was determined on an NMR spectrometer (JEOL JNM-ECA 400 MHz). On the 42nd day of the experiment, the brain was removed and subjected to freeze drying for further determination of the deuterium content in it using a DELTAplus mass spectrometer (Finnigan, Germany).

An analysis of the results showed that in rats in the diet of which there was DDW (group 2), there was a noticeable decrease in the concentration of deuterium in the blood. The greatest substitution of deuterium for protium in the blood was observed during the first week of drinking. So after the first day of drinking, the deuterium content decreased by 5%, on the second day by 12% compared with the initial level at the time of the start of drinking. On the eighth day of the experiment, the concentration of deuterium in the blood decreased by 23% and amounted to 113 ppm. The curve, the curve reflecting the dynamics of changes in the concentration of deuterium, reached a plateau on the 10th day and in the next two weeks the dynamics did not change significantly. The concentration of deuterium in the control group (group 1) did not change during the entire experiment. The concentration of heavy hydrogen in the brain tissues on the 42nd day of the experiment decreased by 19% and amounted to 119 ppm. The plateauing of the graph may mean saturation of the blood and lymph with protium and the beginning of active substitution of deuterium for protium in tissues and organs under the conditions of the created isotopic gradient. A decrease in the concentration of deuterium in the extracellular fluid and tissues of the body occurs due to the isotopic D/H exchange in proteins, lipids, and nucleic acids that form cells. It is known that such substitution occurs most actively in functional groups that have an unshared electron pair and are capable of forming hydrogen bonds. Such atomic groups include hydroxyl (-OH), carboxyl (-COOH), amino groups (-NH2). The transition of protons and deuterons from one biomolecule to another is realized along the chains of hydrogen bonds according to the Grotthuss mechanism. Also, the active substitution of deuterium for protium in the body is facilitated by isotope exchange, which is realized between these groups of biomolecules and the hydrate shell through hydrogen bonds.

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### S7.441. Changes in the content of regulators of differentiation of germline stem cells in the ovaries of D. melanogaster after 79-hour exposure under weightlessness simulation

Golubkova M.A.[1]*, Ogneva I.V.[1]

[1]*Cell biophysics laboratory, Institute of Biomedical Problems RAS;*

* ma_golubkova@mail.ru

One of the priority tasks in the exploration of deep space in the future may be the maintenance of the species. Weightlessness, as a factor that has a continuous effect on the human body during space flight, prevents the solution of this problem. The impact of weightlessness can induce rearrangements of the cytoskeleton and changes in gene expression, which, in particular, may result in a violation of the regulation of proliferation and differentiation of stem cells observed in microgravity (Ogneva I.V., 2022). In the event of abnormalities in oocyte progenitor cells, the fertility of individuals, presumably, may decrease.

D. melanogaster is a widely used model organism suitable for studying the control of self-renewal and stem cell differentiation due to the conservatism of most of the mechanisms that carry out this process. Germline stem cells (GSCs), which serve as precursors of oocytes, are localized in Drosophila in the proximal part of the ovary, the germarium. In the germarium, niches of self-renewal and differentiation are distinguished, successively replacing each other. The Dpp morphogen, a human BMP homologue, secreted by somatic cells of the self-renewal niche, appears to be one of the key players in determining the cell fate of GSCs in Drosophila ovaries (Xie T, Spradling AC, 1998). Dpp directs daughter GSCs along the path of self-renewal by repressing genes such as bam, the expression of which is necessary for GSC differentiation (Song X. et al., 2004; Kirilly D., Xie T., 2007).

In experiments carried out under 1g conditions, it was found that as a result of Dpp overexpression, stem cell differentiation in the germarium does not occur (Xie T, Spradling AC, 1998). Ectopic expression of the Bam protein, on the contrary, leads to the depletion of the germline stem cell pool (Ohlstein B, McKearin D, 1997).

We determined the relative content of the Dpp morphogen and the Bam protein in the ovaries of 2-day-old D. melanogaster exposed under simulated weightlessness on a random positioning machine for 79 hours, which corresponds to the full cycle of fruit fly oogenesis.

According to the results obtained, in the group whose 79-hour exposure took place under conditions of simulated weightlessness, the content of the Dpp morphogen was reduced relative to the control group, but the level of the Bam protein in the group was comparable to the control.
The results, reflecting the absence of changes in the content of Bam, with a decrease in the relative content of Dpp, in the ovaries of flies after 79 hours of exposure to weightless conditions, may indirectly indicate an accelerated depletion of the germline stem cell pool under these conditions and be important for understanding the mechanisms, participating in its maintenance in conditions of altered gravity.
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### S7.442. Changes in the water state in plant leaves under controlled artificial drought

Gall I.R.[1]*
[1]*Institute for Analytical Instrumentation RAS, Saint-Petersburg, Russia;*
* ivan.gall@mail.ru

The structural state of the water component in a plant plays an important role in its water regime, determining the development and adaptability of the plant to environmental conditions.
In accordance with predictions of theory [1], the water system in a living cell is present in two different states: the water component of the cytoplasm, electrostatically strained by the ions and cellular elements dissolved in it, and the core, close to fractal and crystalline, energy-stressed structures of water molecules. However, it is difficult to obtain experimental confirmation of such water structure in living cells, since most of the methods used to study the chemical composition of the water component are not suitable for working with living systems without destroying them. We have previously shown that low-frequency L-dielcometry, which carries out measurements in the frequency mode characteristic of structural processes in aquatic and aquatic-molecular systems of living things, makes it possible to obtain the required information for plants without their destruction [2]. In this method, the presence of water structures is determined by the peaks in the spectrum of the dielectric loss tangent obtained by changing the frequency of the EMF of the oscillatory circuit in which the plant under study is placed in the inductor. While using this method, when artificial drought was applied to various phytotest objects, one could see, by changes in the peaks intensities in the tgδ spectrum, that water in living plants is present in the form of two independent structures: free water and bound water, and when the plant dries, the spectrum peaks related to different structures , change in different ways, which makes it possible to restore the water regime of the plant [3]. At the same time, “free” water, the water component of the cytoplasm, dries up first in the plant, while “bound” water, which makes up the network of intermolecular and intercellular communications in the plant, collapses and dries out much later, when the cytoplasm water has long evaporated. This situation is illustrated by the sequence of spectra in the below figure.
These results fully correlate with the data obtained by the pulsed NMR method, published in the works of the Kazan Scientific School of Phytophysiologists [4]. The results of studies of the water state in chlorophytum leaves dried by various methods show that drying regimes change the ratio of free and bound water in different ways during the drying of plants.
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### S7.443. Character of the Influence of Exogenic Phytohormones on the Starting Growth of Seedlings Depends on the Intensity of Stress Factors

Fedulov Yu.P.[1]*, Degtyarev E.A.[2], Podushin Y.V.[1], Mikhailova M.K.[1], Baghdasaryan M.N.[1]
[1]*Kuban state agrarian University, Krasnodar, Russia ;*
[2]*Institute of Basic Biological Problems of RAS, Pushchino, Russia;*
* fedulov.ju@kubsau.ru

The influence of heteroauxin (10-8M), kinetin (10-8M) and gibberellin (in the form of GA-4.10-5M) on the start growth was studied on soybean variety Vilana.
The experiment was planned according to the scheme of a full two-factor experiment, where one factor was the temperature of seed germination (T1 = 12oC, T2 = 20oC, T3 = 32oC), the other was the humidity of the growing substrate - sand (B1 = 30%, B2 = 50%, B3 = 80%). The seeds treated with phytohormones were germinated in sand of a given humidity [2] in thermostats that allowed maintaining the set temperature with a maximum deviation from the set one of no more than 1,5oC.
After germination, until the leaves emerged from the coleoptile, the seedlings were washed from sand and weighed with an accuracy of 1 mg. The experiment was repeated three times.
The activity of the antioxidant system was assessed by the catalase activity of plant tissues [1].
Both stress factors, lowering the temperature and moisture deficiency, inhibited the accumulation of biomass both in control plants and those treated with hormones. Depending on the intensity of the stress factor, phytohormones either enhanced or inhibited the growth of seedlings.
Regardless of the temperature and methods of seed treatment, the biomass of seedlings increased with increasing substrate moisture. On the other hand, at all given substrate moisture levels, seedlings formed a larger biomass at a higher temperature.
At a germination temperature of 12oC, all phytohormones inhibited the accumulation of the total biomass of seedlings at all studied substrate moisture levels. The greatest growth inhibition by phytohormones was noted at 12oC in the variant with a substrate moisture content of 30%. In this variant of the experiment, heteroauxin and kinetin inhibited growth to the same extent, while the inhibitory effect of gibberellin was almost twice as weak. With an increase in humidity at this temperature up to 80%, the inhibitory effect of phytohormones was preserved, although the degree of growth inhibition decreased.
At a substrate moisture content of 30%, heteroauxin and kinetin retained their inhibitory effects on growth at all temperatures, although the degree of growth inhibition decreased with increasing germination temperature.
In contrast to heteroauxin and kinetin, seed treatment with gibberellin under conditions of water deficit (substrate moisture 30%) stimulated the growth of seedling biomass even at a temperature of 20oC.
Comparison of data on growth inhibition and catalase activity of seedling tissues showed that these parameters change in opposite directions with changing growing conditions: the more growth is inhibited, the higher the catalase activity. The maximum catalase activity of soybean seedling tissues after presowing seed treatment with phytohormones was observed when seeds were treated with heteroauxin and kinetin in plant tissues germinating at a substrate moisture content of 30% and a

temperature of 12oC. It should be noted that it was in this variant that the maximum inhibition of seedling growth was observed.
To obtain a quantitative estimate of the degree of change in the growth-stimulating activity of phytohormones under changing environmental conditions, multiple linear regression equations were calculated that relate the rate of biomass accumulation with changes in environmental parameters. The regression coefficients of these equations allow a quantitative comparison of the effect of individual phytohormones on the growth parameters of seedlings under the action of various stress factors.
The results obtained are in full agreement with the data obtained on winter wheat [2]. However, it should be noted that soybean plants respond much more strongly to a similar change in the levels of the same stress factors than winter wheat plants, due to profound differences in their biology.
The obtained results show that presowing treatment of seeds with phytohormones enhances the response of plants to stress factors, and with a high degree of probability it can be assumed that the nature of the stress factor does not matter, due to nonspecific resistance [3].
It can be assumed that the revealed dependence of the response of seedlings to presowing treatment with phytohormones on the intensity of stress factors reflects the reorientation of the main metabolic pathways of developing seedlings from ensuring growth processes to the activation of protective reactions [4].
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### S7.444. Combined effects of radiation and photodynamic therapy on human epidermoid carcinoma cells A431

Soroko S.S.[1]*, Molodtsova D.S.[1], Skamnitsky D.V.[2], Seriev I.R.[1], Balalaeva I.V.[1], Vodeneev V.A.[1], Shilyagina N.Yu.[1]
[1]*Lobachevsky State University of Nizhny Novgorod;*
[2]*Nizhniy Novgorod Regional Oncology Hospital;*
* kastarashan@gmail.com

Radiotherapy is one of the main methods of cancer treatment in modern medical practice and is used as an adjuvant, neoadjuvant or palliative agent at certain points in the clinical course of the disease. In this case, various variants of ionizing radiation are used for therapy, differing in the nature of the radiation and/or the dose rate delivered. The most common and classic option is radiation therapy, however, to date, beta-emitters, which are part of targeted radionuclide drugs, and are characterized by a low dose rate, are gaining more and more popularity. In turn, most of the studies were carried out using methods of radiation therapy with gamma and X-ray sources, and knowledge about the mechanisms of action of corpuscular beta radiation of different intensity is fragmentary. Another acute issue of clinical radiobiology is the need to reduce the total dose load on the body and increase the sensitivity of tumor cells to radiotherapy. One of the approaches to increase the radiosensitivity of tumor cells is the use of combination therapy in order to achieve additive or synergistic effects.
In general, understanding the mechanisms of action of ionizing radiation of various nature and dose rate, as well as the development of approaches aimed at increasing the effectiveness of therapy, are significant tasks of radiobiology.
We used a cell culture of human epidermoid carcinoma A431, since radiotherapy is widely used in clinical practice for carcinomas.
Irradiation was carried out in two exposure modes: high and low dose rate. Irradiation in the high dose rate mode simulated external beam or electron beam therapy used in the clinic and was carried out using a Novalis Tx linear accelerator by irradiating cells with high-energy electrons or photons.
Irradiation in the low dose rate mode was used to simulate contact or radionuclide therapy, and Sr-Y-90 beta-emission closed preparations were used as a source.
Irradiation was carried out in the range from 4 to 80 Gy. Cell viability after exposure was assessed using the MTT test. To analyze the contribution of cytotoxic and cytostatic effects, we studied the mechanisms of cell death and analyzed the distribution of cells by phases of the cell cycle by flow cytometry. The evaluation was performed every 24 hours after irradiation for three fission cycles.
The production of reactive oxygen species in cells after irradiation was monitored using dichlorodihydrofluorescein diacetate H2DCF-DA; the study was performed by confocal laser scanning fluorescence microscopy.
Photosensitizers of chlorin and porphyrin nature were chosen as radiosensitizing agents. For combination therapy, concentrations of photosensitizers and radiation doses not exceeding IC50 and LD50 were used. Irradiation with ionizing radiation was carried out after treatment with a photosensitizer. The severity of the combined effects was determined using the synergy coefficient.
It was shown that the percentage of viable cells depends on the time elapsed after irradiation, which indicates the presence of cytostatic effects. Thus, the percentage of viable cells is significantly lower when assessed 72 hours after irradiation. After 24 hours, the decrease in cell viability does not exceed 20%. The percentage of viable cells after irradiation at a dose of 5 Gy at a high dose rate was 50%. At low dose rate irradiation, 50% of viable cells were observed after irradiation at a dose of 18 Gy. Thus, in both variants of exposure, there are delayed effects of the action of radiation.
One of the main mechanisms of action of radiation is the formation of reactive oxygen species (ROS). When studying the dynamics of ROS generation in tumor cells after irradiation, we registered a 4-fold increase in ROS concentration in cells after exposure to doses close to semi-lethal for each of the exposure modes.
An analysis of the mechanisms of the cytostatic effect of radiation showed an increase in the number of cells undergoing arrest or radiation block of mitoses in the G2/M phase after high dose rate irradiation. In the case of low dose rate beta radiation, no such regularity was found. At the same time, 72 hours after exposure, a dose-dependent increase number of dead cells. At 24 and 48 hours after exposure, the number of dead cells was noticeably lower, which may be due to the fact that cell death occurs during one of the first four postradiation mitoses.
During combined therapy with ionizing radiation and photosensitizers of chlorin and porphyrin nature, a significant decrease in the number of viable cells was observed in comparison with monotherapies. We have registered additive and synergistic effects with a combination of photodynamic and radiotherapy.
Thus, we assume that the comparative analysis of the mechanisms of action of ionizing radiation of different nature and dose rate, as well as the presence of additive and synergistic effects in the combination of radiotherapy with photosensitizers, potentially opens the way to the development of new, improved protocols for radiotherapy of oncological diseases.

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### S7.445. Conductivity of cardiomyocyte monolayer under the influence of cardiotoxic substances

Podgurskaya A.[1,2]*, Agladze K.[1,2]
[1]*Moscow Institute of Physics and Technology (National Research University);*
[2]*M.F. Vladimirsky Moscow Regional Research and Clinical Institute ;*
* Alisapodgurskaya@mail.ru

Conductivity characteristics were studied under the influence of heptanol, ethanol, erythromycin, diphenhydramine, cyclophosphamide by optical mapping in vitro, as well as a connection with their effect on voltage-gated ion channels and gap junctions. Research methods: optical mapping of a monolayer of cardiomyocytes with a fluorescent calcium-dependent dye, immunocytochemistry, confocal microscopy. Results: 1. Heptanol (0.1–0.9 mM) and ethanol (17-614 mM) dose-dependently reduce the conduction velocity and have different effects on the value of the critical radius of curvature of the excitation wave front in the monolayer of neonatal rat cardiomyocytes. 2. Erythromycin (15-45 μM) reduces the conduction velocity slightly, by a maximum of 12± 9%, reduces the maximum capture rate by a maximum of 28± 12% and causes re-entry at the sharp end of the linear obstacle in 33% of cases in a monolayer of human cardiomyocytes obtained from the IPSC of a healthy donor of the m34Sk3 line. The absence of the listed effects of erythromycin was found when exposed to a monolayer of neonatal rat cardiomyocytes. 3. Diphenhydramine (0.3–16 μM) reduces the conduction velocity by a maximum of 47±18%, reduces the maximum capture rate by a maximum of 48± 12% and causes re-entry at the sharp end of the linear obstacle in 50% of cases in a monolayer of human cardiomyocytes obtained from the IPSC of a healthy donor of the m34Sk3 line. 4. Cyclophosphamide (213-852 μM) does not affect the conduction velocity within the margin of error, reduces the maximum capture rate by a maximum of 25 ± 7%, does not cause re-entry at the sharp end of the linear obstacle in the monolayer of human cardiomyocytes obtained from the IPSC of a healthy donor of the m34Sk3 line. Cyclophosphamide does not affect the conduction velocity within the margin of error, reduces the maximum capture rate by a maximum of 33 ± 9% on a model of neonatal rat cardiomyocytes. A dose-dependent decrease in the conduction area of the cardiomyocyte monolayer was detected under the influence of (213-852 μM) cyclophosphamide and the disruption of α-actinin under the influence of 213 μM cyclophosphamide on isolated IPSC-cardiomyocites from lines m34Sk3 and neonatal rat ventricular myocytes for up to 30 minutes. Novelty and application of the results: for the first time, the dependences of the conduction velocity and the critical radius of curvature of the excitation wave front on the concentrations of heptanol and ethanol and the values of the conductivity characteristics (velocity, maximum capture rate, probability of re-entry formation at the sharp end of a linear obstacle) under the influence of erythromycin, diphenhydramine and cyclophosphamide were obtained. The monolayer of cardiomyocytes obtained from the IPSC of a healthy donor is close to the real heart tissue of the patient, which made it possible to expand the known data on the cardiotoxicity of these drugs and reveal the mechanism of arrhythmia formation under their influence in vitro. This research can be used in medicine and drug development.

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### S7.446. Coupling of dye discolorization and exoelectrogenic activity of Shewanella oneidensis MR-1 during electrical stimulation of an external circuit of a bioelectrochemical system

Samkov A.A.[1]*, Chugunova Y.A.[1], Kruglova M.N.[1], Volchenko N.N.[1], Khudokormov A.A.[1], Viktorovna E.V.[1]
[1]*Kuban State University;*
* andreysamkov@mail.ru

The effect of the polarity of electrical stimulation of the external circuit of the bioelectrochemical system, as well as immobilization on the anode of Shewanella oneidensis MR-1 cells containing the DyP peroxidase gene, on the rate of discoloration of different types of dyes was found. The experimental model was a microbial fuel cell (MFC) of a membrane two-chamber air cathode type, to the electrodes of which an external current and voltage source was connected, in different polarity, or a resistor. Dyes were introduced into the anode chamber in different concentrations, measuring the dynamics of the decrease in OD, and bacterial cells in a free and immobilized form. For the triphenylmethane crystal violet dye, the maximum discolorization rate by suspended S. oneidensis MR-1 cells was 2,05±0,07 μM/h and was observed when a 1,2 V direct polarity DC voltage source was connected ( the positive pole of the ionistor was connected to the MFC cathode, having a similar charge sign). The minimum speeds were observed in the case of reverse polarity of the connection. During the immobilization of S. oneidensis MR-1 cells at the anode, the specific discoloration rate was higher, reaching 2,91±0,09 μm/h and did not decrease with increasing substrate concentration. The lowest values were also noted for the reverse connection of the voltage source. When the congo red azo dye was discolorized, the maximum speed values were noted for a source with a direct ionistor connection and with an open circuit (0,26±0,01 and 0,29±0,02 μM/h, respectively), the minimum value of 0,11±0,02 μm/h was observed for reverse connection. For the products of bioelectrocatalytic discolorization of crystall violet, a significant decrease in the intensity of the main absorption peak corresponding to the 590 nm band was found, with no significant hypsochromic shift. The qualitative changes in the composition of the discoloration products are indicated by the appearance, for the option with a direct polarity of the ionistor connection, of a new absorption maximum in the region of 360 nm.

Thus, during the discolorization of crystal violet triphenylmethane dye and Congo red azo dye by S. oneidensis MR-1 culture under

anaerobic conditions of the anode chamber of the bioelectrochemical system, the influence of the polarity of the connection to the electrodes of an external current and voltage source was detected. An increase in the specific reaction rate with a stepwise increase in the concentration of the substrate was noted for the experimental variants, where the polarization of the electrodes occurred, as natural, up to 0,59 V, due to the electrogenesis of S. oneidensis MR-1, and more pronounced artificial – under conditions of positive stimulation by a DC source and voltage (ionistor) of 1,2 V direct connection (negative pole to the anode, positive – to the air cathode). When the voltage source was connected back with the positive pole to the anode, a decrease in the specific discoloration rate was observed in all cases, both for crystal violet and congo red, which was accompanied by a characteristic increased consumption of the electric charge used as a current source and voltage of the ionistor. Immobilization of cells on the surface of the anode significantly increased the rate of discoloration of crystalline violet, compared with suspended cells, while the negative effect of the reverse connection of the ionistor was preserved. Thus, artificial external polarization of the electrodes of the bioelectrochemical system allowed both positive and negative effects on the reactions carried out by the electrogenic culture, leading to discoloration of dyes of various types in the anaerobic environment of the anode chamber. The effect of stimulating or inhibiting discoloration was manifested in conditions of low redox potential of the anode chamber of the bioelectrochemical system. Due to the natural polarization of the electrode surface (an open circuit and a 1 kOhm resistor) or, more pronounced, with positive stimulation by a direct connection of ionistor, the process of N-demethylation, splitting of one of the aromatic rings and other key discoloration reactions could accelerate.

It is assumed that the exoelectrogenic activity of S. oneidensis MR-1 cells capable of transferring charge externally, using cytochrome systems and special cellular structures, can provide coupling of the external electrical circuit of the bioelectrochemical system and energy-intensive biochemical processes occurring in the volume adjacent to the surface of the electrodes, including the restoration and breaking of the double bond between nitrogen atoms in the congo red molecule, N-demethylation and cleavage of the conjugated chromophore structure of crystalline violet.

### S7.447. Cryoprotective effects of carnosine dipeptide in long-term cryopreservation of brain explants of warm-blooded non-hybernate animals

Mokrushin A.A.[1]*
[1]*Pavlov Institute Physiology RAS;*
* mok@inbox.ru

Dipeptide L-carnosine (β-alanyl-L-histidine), an endogenous dipeptide synthesized by the enzyme carnosine synthase 1 (CARNS1) from the amino acids β-alanine (synthesized in the liver) and L-histidine (obtained from food). The content of this dipeptide is highest (millimolar order of concentration) in cardiac and skeletal muscles, as well as in the brain of mammals, especially in the olfactory bulb and olfactory cortex (1–2 mM).

L-carnosine has been found to cause pleiotropic positive effects in organisms. The dipeptide exhibits antioxidant properties, neutralizes reactive oxygen species, and also prevents lipid peroxidation, while maintaining the structure of cell membranes. L-carnosine prevents the glycation process, i.e. oxidation of proteins by glucose and binds the protons formed during glycolysis. It acts as an intracellular pH buffer.

The protective potential of L-carnosine has been demonstrated. Under conditions of development of ischemic and hemorrhagic lesions of brain structures, the dipeptide proved to be an effective protector of nerve cells. The positive effects of carnosine have been observed in the treatment of neurodegenerative diseases (Alzheimer's, Parkinson's, etc.).

Based on the presented data that L-carnosine is a multipotent protector in the structures of the nervous system, we studied its cryoprotective properties during long-term cryopreservation (CP). In order to test this hypothesis, the effects of L-carnosine application were studied in an experimental model –slices of the olfactory cortex of the rat brain. Changes in NMDAR activity were analyzed upon registration of NMDA potentials induced by electrical stimulation of the lateral olfactory tract. NMDARs are the most vulnerable mechanisms under long-term CP.

The protocol of CP slices with L-carnosine was carried out in the following sequence. Slices were perfused for 20 min with artificial cerebrospinal solution (ACS) (mM: 124.0 NaCl; 5.0 KCl; 2.6 CaCl2; 1.24 KH2PO4; 1.2 MgSO4; 3.0 NaHCO3; 10.0 glucose; pH 7.3) in the flow chamber of the electrophysiological setup and recorded the amplitudes of NMDA potentials (μV). The obtained values of the amplitudes of the NMDA potentials were considered as control before freezing and were taken as 100%. Then the slices were perfused with ACS with the addition of L-carnosine at a concentration of 20 mM for 20 min, and NMDA potentials were recorded. Further the slices were frozen in an ACS solution with L-carnosine at slow speed (0.1°C/min) down to –10°C and stored in a thermostat freezer. After 30 days of CP, the slices were warmed to 37°C at the same rate (0.1°C/min), while the salt composition of the medium and the concentration of L-carnosine did not change. The NMDA potentials were registered in slices and expressed in % relative to the values before CP.

In a separate series of experiments, were studied changes in the pH of the ACS with a brain slice before and after CP with L-carnosine using a pH meter. In a special series of experiments, the effect of carnosine on the hydration/dehydration of brain slices was studied using measurements of their weight before and after CS.

The results of the studies showed that the dipeptide L-carnosine optimized the pH of the solution to pH 7.4 after CP (–10°C, 30 days) and retained the activity of NMDAR, determined by the amplitude of NMDA potentials. Note that without the use of L-carnosine, the pH of the solution with slices after CP was strongly acidified to pH 6.5 instead of the normal pH values of 7.2–7.7. Under these conditions, NMDAR activity was irreversibly blocked. These data indicate that L-carnosine is a proton trap and thus acts as a cryoprotectant.

The process of dehydration/hydration of nerve cells and intercellular space is essential in maintaining the viability of the structures of the nervous tissue during CP. L-carnosine after CP caused a decrease of the free water in slices. This effect of the dipeptide contributed to the preservation of NMDAR activity after CP equal to that before CP. We believe that L-carnosine promotes the transfer of free water from nerve cells to the intercellular space and thus becomes an ice nucleator, protecting cells from destruction during freezing/CP/warming. Therefore, exogenous L-carnosine exhibits cryoprotective properties.

Registration of NMDAR activity, determined by the amplitude of NMDA potentials, during rewarming after CP showed that L-carnosine reduced twice the level of hyperexcitability of these receptors. This fact indicates that the dipeptide inhibits the development of glutamate excitotoxicity, which develops when slices are rewarmed after CP, and contributes to the preservation of the normal functioning of NMDA mechanisms as the most vulnerable mechanisms to freezing/thawing.

Thus, the obtained results prove that the dipeptide L-carnosine exhibits the properties of an endogenous (non-toxic) cryoprotectant in brain explants of warm-blooded non-hibernating animals.

### S7.448. Cytotoxicity and Genotoxicity of Dinitrosyl Iron Complex with Mercaptosuccinate in MCF-7 cell line assessed using comet assay

Tronov V.A.[1,2]*, Tkachev N.A.[1,2], Nekrasova E.I.[1,2], Vanin A.F.
[1]*Institute of Chemical Physics RAS, Moscow, Russia;*
[2]*Institute of Biochemical Physics RAS, Moscow, Russia;*
* vtronov@yandex.ru

Some common diseases (diabetes mellitus, glaucoma, hypertension, some types of cancer) are associated with a deficiency of nitric oxide (NO) in the tissues of patients. It has been shown that synthesized dinitrosyl iron complexes (DNIC), providing NO delivery to tissues, open up the possibility for therapy of such diseases. On the other hand, DNIC also has a cytotoxic effect. This cytotoxicity is due to NO and the nitrosonium ion (NO+), both the breakdown products of DNIC in the cell, and the DNA repair mechanism. In this study, using MTT-survival assessment and DNA comet method, we evaluated cytotoxic (CT) and genotoxic (GT) effects and assessed the contribution of the DNA repair mechanism to the cytotoxicity of the synthesized complex DNIC with mercaptosuccinate (DNIC-MS) on human tumor cells MCF-7. We show that CT effect of DNIC-MS in MCF-7 cells was 2-fold higher compared to CT of mixture of precursors (MS+Fe2+) for the complex synthesis. Using alkaline comet assay we show that the mixture induced DNA single-strand breaks (SSBs) in MCF-7 cells. SSBs were repaired completely by 24h. Although the complex induced less number of SSBs compared with the mixture, they remained in 19% of the cells by 24 h after the treatment. We found by neutral comet assay that these DNA lesions are double-strand breaks (DSBs). The correlation between genotoxicity by yield of unrepaired DNA SSBs and MTT-cytotoxicity indicates that CT of DNIC-MS may be, in part, associated with DSBs induced in DNA. Lethal DSBs could be formed as a result of cellular endonuclease attack on single strand sites and AP-sites in DNA, that produced as intermediates by the nucleotide excision repair (NER). We conclude that the cellular mechanism of DNA repair, transforming non-lethal or potentially-lethal DNA damage into lethal double strand breaks, has impact into cytotoxicity of the DNIC-MS complex in human tumor cell line MCF-7.

### S7.449. Determination of durations of cryopreservation of brain slices using an agar-based freezing solution

Mokrushin A.A.[1]*
[1]*Pavlov Institute Physiology RAS;*
* mok@inbox.ru

In regenerative medicine, organ transplantation, and drug development, the protective effects of low temperature on biological objects and their cryopreservation (CP) are used. In the clinic, to restore large areas of the brain tissue of the recipient, the use of integrated nervous tissue of the corresponding brain structures is required. It is these brain explants that are needed for transplantation in neuropathologies such as stroke, epilepsy, and trauma. Obviously, brain slices are optimal experimental objects for the development of CP protocols and the creation of a nervous tissue cryobank.
The duration of the CP of neuronal and synaptic mechanisms in the nervous tissue, at which the activity values equal to the values before the CP are preserved, is an important criterion for a cryobank. The purpose of this study is to determine the duration of the preservation of the activities of glutamatergic ionotropic AMPA- and NMDA-dependent mechanisms during long-term (1-3 years) CP of brain slices of non-hibernating animals - Wistar rats.
We studied the protective effects of CP of olfactory cortex slices in a specially designed freezing solution consisting of artificial cerebrospinal solution (ACS) (mM: 124.0 NaCl; 5.0 KCl; 2.6 CaCl2; 1.24 KH2PO4; 1.2 MgSO4; 3.0 NaHCO3; 10.0 glucose; pH 7.3 ) and agar. Freezing solution for cryopreservation of slices was prepared in the following sequence. Difco Bactor agar (USA) (3 g) was filled with 100 ml of 1 M NaCl and incubated for 10 days in a thermostat at +32–+35°C. The resulting gel solution was centrifuged at 2000 rpm for 10 min. The light fraction was aspirated from the solution and used to prepare the medium for cryopreservation of slices: – 1.0 ml of the light fraction of agar and 1.0 ml of ACS (final agar concentration 50%). The brain slices were placed in glass vials with this medium. Then, the vials with slices were gradually frozen to –10°C at a slow speed (0.1°C/min) in a freezer with a thermostat and stored for: 52 days, 1 year, 2 years, 3 years. After the specified time intervals, the vials with slices were warmed to +37°C at a slow rate of 0.1°C/min. The slices were removed, placed in the perfusion chamber of the electrophysiological unit, and the amplitudes of AMPA and NMDA potentials were recorded. The obtained values were expressed as percentages relative to the values before CP.
Studies on cryopreservation of the activity of AMPA and NMDA mechanisms in the freezing solution showed that the optimal concentration of agar in the gel solution was 50%. Preservation of activity of AMPA and NMDA mechanisms was 87 and 99%, respectively, to the values before CP. Then, using this gel solution (50% + ACS), the preservation of amplitudes of AMPA and NMDA potentials at various time intervals after the start of CP was investigated.
It was found that AMPA and NMDA mechanisms exhibit different cryostability to CP duration. AMPA mechanisms were the most stable and retained their activity for 3 years (average 97% compared to pre-CP). NMDA turned out to be less stable, and their activity persisted for 1 year (average 95% in relation to the values before CP).
A decrease in the activity of AMPA and NMDA mechanisms during prolonged CP of brain slices after the detected time intervals indicates that the agar gel solution undergoes aging - the process of syneresis. This is due to a violation of the structure of the agar gel and the loss of water from the gel itself and, accordingly, from brain slices.
To strengthen the gel structure and prolong the CP time intervals of brain slices, various substances of endogenous origin were tested: mystixin (synthetic CRF-like peptide), heat shock protein with a molecular weight of 70 kDa, and dipeptide L-carnosine. The most effective was the dipeptide L-carnosine.
The addition of L-carnosine (20 mM) to freezing solution and the subsequent freeze-thaw procedure led to the prolongation of the CP time of NMDA mechanisms up to 3 years, AMPA - more than 4 years.
The developed and studied protocol for CP of brain explants (slices) of warm-blooded animals in a freezing solution with agar will be used to create a cryobank for long-term storage of nervous tissue explants.

### S7.450. Developmen of radioresistant rectal cancer model by sequential fractionated irradiation of CT26-WT cells

Burdakov V.S.[1,2]*, Verlov N.A.[1]
[1]*Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute»;*
[2]*St. Petersburg Clinical Scientific and Practical Center for Specialised Types of Medical Care (Oncological);*
* burdakov_vs@pnpi.nrcki.ru

Radiation therapy (RT) is a widely used adjuvant therapy for various types of cancer, with up to 80% of cancer patients receiving radiation therapy for either curative or palliative purposes. However, despite the success of RT in cancer, some patients still relapse after completion of RT. Although tumor recurrence after RT may be associated with residual disease or aggressive tumor biology, it may also be associated with the survival of a population of cells that are either more intrinsically resistant to RT (eg, hypoxic or cancer

stem cells) or develop de novo. These radioresistant cells can then repopulate the tumor site, leading to recurrence and treatment failure. Previous studies have shown that multiple factors are involved in the development of radioresistance, including dysregulation of signaling pathways, overproduction of oncogenic miRNAs, DNA damage responses, the presence of cancer stem cells and changes in cancer metabolism, as well as the influence of the tumor microenvironment itself (including hypoxia). Many studies have focused on isolated pathways in studying radioresistance, but it is likely that these pathways are interrelated, for example, hypoxia may induce a more undifferentiated cellular phenotype characterized by increased expression of stem cell markers, which may also affect the expression of genes and stem-like pathways such as like the Yamanaka factors. Compared to chemoresistance research, the mechanisms underlying the development of radioresistance are poorly understood, partly due to the lack of radioresistance model systems. A deeper understanding of these molecular mechanisms underlying acquired radioresistance is required, and most importantly, the development of strategies to circumvent this clinical problem. The use of global approaches to study the mechanisms of resistance is of increasing interest, as it will allow the study of several pathways at the same time and provides insight into complex biological systems and response to treatment.

In this study, we developed novel radioresistant cell lines derived from fractionated irradiation from the CT26-WT(ATCC-CRL2638) colorectal cancer cell line. The parental cell line was chosen on the basis that it is a simple and widely used animal model of colorectal cancer. The existence of an available animal model offers promise not only for the genotypic and phenotypic characterization of the obtained lines in order to search for the main molecular pathways that cause radioresistance, but also for in vivo functional studies, which may allow the development of more successful therapeutic approaches for the treatment of patients with relapses, after previous treatment. using LT. To obtain radioresistant lines, we chose two modes of fractionated irradiation with a total final absorbed dose of 40 Gray (Gy); The first included 20 fractions of 2 Gy each with a dose rate of 0.95 Gy/min, irradiation was carried out daily, then a resistant clone (CT26-WT-RR2) was selected. The second regimen consisted of 4 rounds of irradiation with a dose of 10 Gy and a dose rate of 8.5 Gy/min, after which a surviving clone was selected, which was irradiated in the next round (CT26-WT-RR10). In the second mode, the dose of 10 Gy per fraction was chosen based on the fact that when irradiated with high doses, we did not succeed in obtaining a surviving clone from the maternal line. Two approaches were chosen to establish whether one of them leads to greater radioresistance than the second and whether the mechanism of resistance acquisition is universal.

We performed a comparative analysis of the sensitivity of the resulting cell lines to the effects of ionizing radiation with respect to the maternal cell line by assessing the survival and proliferative activity of cells of all three lines after a single irradiation with a dose range from 2 to 10 Gy with a step of 2 Gy. Cell growth curves were plotted using data from the MTT test and direct measurement of the cell growth index xCELLigence Real-Time Cell Analysis (Agilent Technologies, USA). The half-lethal dose (LD50) was estimated as the dose at which, 10 days after irradiation, the number of living cells is two times less than the number of cells of the same line that were not irradiated. LD50 for CT26-WT, CT26-WT-RR2, and CT26-WT-RR10 cell lines were 2.4±0.4, 3.2±0.5, and 4.5±0.6 Gy, respectively. As a result of both approaches, we have obtained cell lines that are characterized by increased radioresistance compared to the maternal cell line. Different irradiation protocols, namely, varying the number of fractions, dose per fraction, and dose rate while maintaining the total absorbed dose unchanged, make it possible to obtain cell lines with different characteristics.

## S7.451. Divergence of nascent chemoautotrophic CO2 fixation pathways under hydrothermal conditions

Marakushev S.A.[1]*, Belonogova O.V.[1]
[1]*Divergences of nascent chemoautotrophic CO2 fixation pathways in hydrothermal conditions;*
[2]*Divergences of nascent chemoautotrophic CO2 fixation pathways in hydrothermal conditions;*
* shukaram@yandex.ru

The fixation of inorganic carbon into organic material is the chemical basis for the functioning of the first self-reproducing C–H–O systems in underwater hydrothermal systems on ancient Earth. Favorable thermodynamic and kinetic conditions for this process were created there due to the optimal composition of minerals - catalysts and thermal and chemical gradients resulting from pulses of hydrogen degassing of the liquid core and rock-water interaction. According to modern concepts, under the hydrothermal conditions of the Archean, was created a pool of carboxylic and keto acids, autocatalysts [for example, Braakman and Smith 2012; Goldford et al., 2017; Marakushev, Belonogova, 2009, 2021], which in paragenesis with hydrocarbons on the surface of minerals transformed into an autocatalytic network of CO2 fixation.

The present work provides evidence for the existence of reversible metastable equilibria between non-methane hydrocarbons and their oxidation products (carboxylic acids, alcohols, and aldehydes) under hydrothermal conditions of volcanic eruptions. It is assumed that on the ancient Earth, under these conditions, a pool of proto-metabolites of the three-component C-O-H system was formed, which are in reversible metastable equilibria with each other and have the ability to assimilate CO2 from the environment. The evolution of this pool of intermediates of nascent metabolism was regulated by reversible shunts - bifurcation nodes that determine alternative directions of development in networks of chemical reactions and lead to the formation of various proto-metabolic pathways of chemoautotrophic CO2 fixation. Paragenetic analysis of the C–H–O system in a hydrothermal environment based on the method of thermodynamic potentials [Marakushev and Belonogova, 2009] was applied to study the central chemical switches (shunts) of the electron bifurcations of the proto-metabolic network that determine the chemical evolution of five nascent bacterial paths: the reductive pentose-phosphate (RPP) cycle, acetogenic and methanogenic Wood-Lundgwala (WL) pathway, reductive citrate (RC) cycle, 3-hydroxypropionate (3-HP) bi-cycle. Hydrogen pressure-driven equilibrium fumarate (C4H4O4) + H2 = succinate (C4H6O4) regulates the development of the proto-metabolic network towards the formation of the RC cycle or 3-HP bi-cycle. The evolution of cycles was also driven by the chemical potential of CO2, which is shown in the work on the scheme of divergence of proto-metabolic cycles modules. Such a CO2 partial pressure-dependent reversal of the direction of electron flow in the RC cycle was recently demonstrated for the first time in the case of the thermophilic sulfur-reducing delta-proteobacterium Hippea maritima as an example [Steffens et al., 2021].

An interesting feature of bacterial autotrophic metabolism, the ability to switch CO2-fixing metabolism from the WL pathway to the RPP cycle and vice versa, was recently discovered in the thermophilic anaerobic bacterium Ammonifex degensii. Depending on the chemical potential of hydrogen, this bacterium organizes its autotrophic carbon fixation as a WL pathway or as an RPP cycle [Berg et al., 2022], which is regulated by another efficient hydrogen shunt, the equilibrium pair pyruvate (C3H4O3) + H2 = 1.5 acetate (C2H4O2). This switching mechanism, inherited by modern microorganisms, apparently also existed in proto-metabolic networks, in which the reversibility of the phase transition through the pyruvate ↔ acetate equilibrium creates the possibility of choosing the development between these CO2-fixing systems. In addition, the chemical potential of molecular hydrogen in the surrounding hydrothermal environment determines the development of the network

in the direction of two CO2 fixation clusters - pyruvate: (RC and RPP cycle) or acetate (acetogenic and methanogenic WL pathway and 3-HP bi-cycle). Thus, pyruvate (the central hub or metabolic replicator), forming a paragenesis with acetate, creates the most important shunt of the metabolic pool of intermediates, with the participation of which the origin and evolution of all known bacterial autotrophic metabolic pathways of CO2 fixation occurred.

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### S7.452. Donor's blood haemoglobin analysis of the spectral characteristics after exposure to its solutions with the antibiotic "Amphotericin B"

Sokolova L.O.[1]*, Litvinov N.V.[1], Kalaeva E.A.[1], Sveklo L.S.[2], Artyukhov V.G.[1]

[1]*Voronezh State University;*

[2]*Blood transfusion station at the Voronezh Regional Blood Transfusion Station;*

* lyudmila.sokolova.94@mail.ru

The polyene antibiotic Amphotericin B is used for systemic infections caused by fungi such as Aspergillus fumigatus or Candida albicans (2). It should be noted that the molecular mechanisms of interaction of this drug with biological objects are complex, have not been sufficiently studied to date and thus are subject to further development.

In view of the above, the aim of this work was to investigate the effect of "Amphotericin B" on the spectral properties of human hemoglobin. In the experiments heparinized blood of donors received at the branch of BSMC of the Voronezh Regional Blood Transfusion Station was used. All procedures were performed in accordance with the Declaration of Helsinki of 1964 [1]. Haemoglobin from donor blood was obtained according to the standard technique [3]. Then, Amphotericin B, at concentrations of 2.5 - 10-5 and 5.4 - 10-5 M, was added to 4 ml of protein, at a concentration of 10-5 mol/L. Registration of electronic absorption spectra (EAS) of tested samples was done on UV-2401 PC spectrophotometer (Shimadzu, Japan) in the wavelength range from 230 to 700 nm. Optical densities of the solutions were recorded over the whole investigated range in 1 nm with the spectral slit width of 1 nm.

Two maxima in the UV region (273-275 and 342-346 nm) and three absorption bands in the visible part of the spectrum (414, 541 and 576 nm) were detected on the ESP of native hemoglobin solution. After incubation of hemoglobin solutions with Amphotericin B for 15 min, the positions of absorption maxima in both UV and visible regions of the spectrum were found to change: after exposure to the antibiotic at a concentration of 2.5 - 10-5 M, the absorption bands in the UV- and visible parts of the EAS fell at 275-276; 325-331; 411-412; 541-542 and 576-577 nm, while at a concentration of 5.4 - 10-5 M they fell at 273-276; 327-331; 411-414; 540-541 and 576 nm. The ESP of the antibiotic-modified hemoglobin solutions showed an increase in optical density at 325-331 nm and 327-331 nm maxima compared to the intact solution.

Thus, Amphotericin B induces partial oxidation of heme iron, as indicated by the shift of the Sore band into the short-wavelength region, and disrupts heme-protein interactions, as indicated by changes in the EAS of hemoglobin at 330-346 nm.

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### S7.453. EPR study of biomagnetism in dividing cells of tatar buckwheat Fagopyrum tataricum

Yurtaeva S.V.[1]*, Yatsyk I.V.[1], Valieva A.I.[2], Akulov A.N.[2], Rumyantseva N.I.[2]

[1]*E.K.Zavoisky Physical Technical Institute, FRC Kazan Scientific Center of RAS ;*

[2]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS;*

* svetlana.vish@rambler.ru

The phenomenon of biomagnetism in dividing cells has been known since the early 1960s. This effect consists in the appearance of broad lines in the EPR spectra in the region g = 2.2–2.3, which was found in fast growing yeast cells with a simultaneous change in their static magnetic susceptibility; and was called the phenomenon of "wide EPR signals" [1–3]. It was found that magnetism in a semi-synchronized culture of yeast cells occured in the period immediately preceding the budding. After cell division, the signal disappeared [1–3]. It was assumed that the signal could be associated with clusters of iron emerging at a certain stage of the cell cycle. However, the nature of these signals in dividing cells is not completely understood and is still of great interest.

In this work, the mentioned phenomenon of magnetism during cell division was detected in intensively dividing plant cells for the first time. Culture of intensively dividing non-morphogenic cells of Fagopyrum tataricum was used as an object of study. The aim of the research was to study magnetic resonance characteristics of signals, to define their origin (physical nature) and to know if it is connected with cell division.

Frozen and freeze-dried samples of cells were studied by EPR spectroscopy. Cell culture samples were taken every day after transfer to a fresh nutrient media during its growth for 14 days. It was found that the EPR spectra of cell culture samples depend on phase of growth. The results of the EPR study showed that during the growth of the cell culture, strong change in the magnetic properties occurs. It consisted

in the appearance of additional intense EMR (FMR) signals depended on the orientation of the magnetic field during the acceleration phase of culture growth (3-4 days after passage). The integral intensity of the EPR spectra had a maximum on the 3rd or 4th day. More than 10 series of independent experiments were made. The increase of the signal was due to the appearance of the EMR signal in the region of g-value 2.1÷2.2. The temperature and angular dependences of EMR signals were studied.

As a result of the study, it was found that the characteristics of EMR signals (Hres(T), ΔH(T) and integral intensity) in the temperature range 100K-260K corresponded to the behavior of magnetite nanocrystallites (Fe3O4) described earlier in [4] with a characteristic Verwey phase transition. The presence of an angular dependence indicates the ferrimagnetic properties of Fe3O4.

The dependence of integral intensity of the EPR spectrum on the time of cultivation was plotted. Simultaneously with EPR data, a curve of cell mass increase was obtained, as well as a dependence of mitotic index on the time of cultivation since the moment of culture transfer. It has been determined that the moment of appearance of the maximum EMR signal correlates with the moment of registration of the maximum mitotic index value, that demonstrates the relationship between magnetic properties and the process of cell division.

Cultivation of cells was made in KIBB FRC KazSC RAS as part of the government assignment.

EPR spectroscopic studies of the samples were done in KFTI FRC KazSC RAS as part of the government assignment.

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### S7.454. Effect of Cadmium on the Activity of Mitochondrial Isolated from Wheat (Triticum aestivum L.) Seedlings

Antonyan A.P.[1], Poghosyan G.H.[1]*, Sargsyan E.G.[1]
[1]*Yerevan State University;*
* g.poghosyan@ysu.am

The heavy metal cadmium (Cd) is one of the most toxic anthropogenic pollutants that adversely affect the growth and development of plants. The phytotoxicity of a heavy metal manifests itself through the inhibition of a number of physiological and biochemical processes of plant life, including the formation of reactive oxygen species (ROS), which cause protein denaturation, damage to nucleic acids, and lipid peroxidation [1]. Mitochondria play a central role in all aerobic eukayotic cells, being the site of respiration and synthesis of ATP via Oxidative phosphorylation [2, 3].

Cadmium is a relatively new biosphere pollutant, the content of which in the environment is primarily associated with human activities. From this point of view, the general mechanisms of plant protection from the toxic effects of heavy metal have not yet been fully studied. In this regard, the purpose of this work was to study the influence of low concentrations of CdCl2 (10 μM, 20 μM, 30μM at 250C, 5 min.) on the intensity of lipid peroxidation (LPO), ATPase activity and volume of mitochondria isolated from wheat seedlings to reveal the possible involvement of cadmium in the induction of oxidative stress in mitochondria.

Mitochondria were isolated from 7-day-old seedlings of winter wheat (Triticum aestivum L.), cv. Bezostaya, grown in Petri dishes on filter paper in a thermostat (at 23°C) in the dark. Mitochondria were obtained by differential centrifugation in a percoll gradient (18, 23, and 35%) prepared on the basis of a medium (300 mM sucrose, 1 mM EDTA, 0.1% BSA, 10 mM K-phosphate buffer, pH 7.5) [4]. The intensity of LPO processes was determined by the accumulation of malondialdehyde-MDA, according to the method [5]. ATPase activity was determined by the concentration of isolated inorganic phosphate according to the method [6]. Changes in the volume or swelling of mitochondria were measured at 250C, using the UV-visible Spectrophotometer (model SF-46, USSR) by changing the absorption of of mitochondrial suspension at λ=520 nm. [7].

According to the data obtained, in the presence of cadmium, whit increasing heavy metal concentration, the LPO activity, that is, the content of MDA, increases. In the control samples of mitochondria, high ATPase activity was registered. The addition of cadmium solutions of various concentrations to the mitochondrial suspension resulted in a statistically significant ($P < 0.05$) inhibition of ATPase activity up to 60%. And at a cadmium concentration of 30μM, the rate of ATP hydrolysis was practically reduced to zero. Similar changes in the agreement of the mitochondrial suspension at λ=520 nm, associated with a change in the volume of organelles, were observed at a concentration of cadmium ions of 20 μM and30 μM.

Thus, according to our results obtained, cadmium ions induce concentration-dependent oxidative stress in isolated wheat mitochondria, which is expressed in an increase in the MDA content, inhibition of ATPase activity, and swelling of mitochondria.

### S7.455. Effect of Millimeter Electromagnetic Waves on Lipid Peroxidation and Chemiluminescence of Human Blood Erythrocytes Membranes In Vitro

Vardevanyan P.H.[1], Poghosyan G.H.[1]*, Mikaelyan M.S.[1]
[1]*Yerevan State University;*
* g.poghosyan@ysu.am

Electromagnetic waves (EMW) of biosphere are an essential component of external factors affecting living organisms [1]. Experiments conducted have shown that EMW in millimeter range (MM EMW) interacts with biosystems of different levels of organization. Nowadays, because of technogenic human activities, intensity of artificial electromagnetic irradiation produced by operation of commercial frequency generators and different devices have been significantly increased. So, the problem of electromagnetic safety of organisms as sums is extremely important.

The key role in metabolism of the organisms belongs to the blood and, above all, to the erythrocytes. Previously, it was shown that the influence of MM EMW leads to a change in various physicochemical parameters of blood - lipid peroxidation of red blood cells, surface charge and mobility of erythrocytes etc. [2, 3]. However, information about the effect of EMW MM on the processes of lipid peroxidation (LPO) of the blood cell system, in particular erythrocytes, is rather contradictory [4].

At the same time, until recently, the primary targets for the perception of MM EMW and the mechanisms of its action in biological systems have not been clearly elucidated. A number of studies have shown that weak physical effects can change the properties of aqueous solutions and, at the same time, the activity of oxygen-dependent reactions in cells can change with the formation of reactive oxygen species (ROS) – including superoxide radicals (O.2), hydrogen peroxide (H2O2) and hydroxyl radicals (OH.). The interaction of the last ones with non-saturated fatty acid radicals of membrane lipids induces the formation of lipid peroxide radicals RO.2, which recombine to form an non-stable tetroxide , which decomposes with the release of a chemiluminescence light quantum (CL) [5].

In the present work, we studied the effect of MM EMW on LPO - the content of malondialdehyde (MDA, using thio-barbituric acid-TBA-test) and the intensity of H2O2-induced CL of erythrocyte

membranes isolated from human whole blood. Human blood from a blood bank was used in the experiments. To the blood 5 % solution of heparin prepared by physiological solution was added, then blood was divided into experimental and control samples, which were kept under the same temperature conditions. Blood samples were irradiated with EMW at 41.8 GHz and 51.8 GHz for 5, 10, and 20 min. The choice of the frequency of 41.8 GHz is based on the fact that EMW in the 41.8-42.2 GHz range have a pronounced biological effect, and the frequency of 51.8 GHz is resonant for water molecules [1]. After irradiation, the blood was centrifuged during 10 min with 1500 g acceleration (Electronic Centrifuge Capacity). Erythrocytes were isolated in a ficoll-verografin density gradient followed by washing with buffered saline. Erythrocyte membranes were obtained according to the method [6]. The exposure source was a G4-141 generator (Istok, Russia) with 37.5–53.5 GHz interval of frequencies and 0.64 μWt/cm2 power flux density. The CL registration of the sample was carried out on the Junior LB 9509 luminometer (Berthold Technologies, Germany).
As a result of the experiments, a change in the concentration of MDA-rate and the intensity of H2O2-induced CL of erythrocyte membranes was established depending on the frequency and duration of EMW exposure.
It is also shown that under irradiation with a frequency of 41.8 GHz, the effect of MM EMW is much weaker than in the case of frequency of 51.8 GHz, which confirms the role of water in the influence of MM EMW, since the frequency of 51.8 GHz is the resonant frequency for water. Irradiation of whole blood by 41.8 GHz and 51.8 GHz frequencies for 5 min. does not invoke statistically significant differences in the content of MDA and CL intensity in erythrocyte membranes compared with the control. Exposure to EMI with a frequency of 41.8 GHz for 10 and 20 minutes led to a slight increase in MDA and CL intensity, but no statistically significant differences were observed. 10- and 20-minute exposures of MM EMW at a frequency of 51.8 GHz, resonant for water, induced a statistically significant increase in MDA amount ($P<0.05$) and the light sum of H2O2- induced CL in erythrocytes.
The results presented in this paper show that the effect of MM EMW of non-thermal intensity induces changes in cell metabolism, which indicates the stress effect of the factor on human red blood cells in vitro.

### S7.456. Effect of dehydrothermal crosslinking on physical, structural and biological properties of Corneoplast

Gorshkova Yu.E.[1,2]*, Anisimov S.I.[3,4], Popov I.A.[3], Naumenko M.V.[2,1], Vinogradov I.I.[1], Nechaev A.N.[1], Anisimova N.S.[3,4], Poziabin S.V.[5], Orlova M.N.[5], Shilkin A.G.[5]
[1]*Joint Institute for Nuclear Research;*
[2]*Institute of Physics, Kazan Federal University;*
[3]*Private Eye Center Vostok-Prozrenie;*
[4]*Federal State Budgetary Educational Institution of Higher Education "A.I. Yevdokimov Moscow State University of Medicine and Dentistry" of the Ministry of Healthcare of the Russian Federation;*
[5]*Federal State Budgetary Educational Institution of Higher Education «Moscow State Academy of Veterinary Medicine and Biotechnology – MVA named after K.I. Skryabin»;*
* Yulia.Gorshkova@jinr.ru

Tissue engineering is one of the latest achievements in the field of molecular and cell biology, which is aimed at designing and growing living, functional tissues or organs outside the human body for subsequent transplantation to a patient in order to replace or stimulate the regeneration of damaged organs or tissues. Thus, the tissues are regenerated, and not simply replaced with synthetic materials, as in the case of using implants made of inert materials, which eliminate only physical and mechanical defects of damaged tissues. The problem of creating an artificial retina in medicine is of crucial importance, since there are pathologies (injuries, tumors, hemorrhages, degenerative processes) that cause irreversible changes in the retina, as a result of which a person goes blind.
The mammalian corneal stroma has a pronounced tendency to passive hydration. In distilled water, the moisture content of the corneal stroma can reach 96% by weight. Under physiological conditions, however, the moisture content of the cornea is within 75-78%, which is due to the action of the endothelial layer of corneal cells, whose main function is to regulate the flow of fluid into the corneal stroma from the anterior chamber. It is known that the basis of the corneal stroma is type I collagen. Crosslinking of collagen (formation of cross-links between polypeptide chains) reduces the ability of the latter to hydrate. Dehydrothermal crosslinking (DTC) is the formation of cross-links in biomaterials when they are heated under vacuum. Despite the fact that the DTC method is widely used in tissue engineering, its effect on the properties of the corneal stroma is practically unexplored, and so far no attempt is known to evaluate the prospects of using such materials in keratoplasty.
The possibility of controlling physical, structural and biological properties in the process of dehydrothermal crosslinking of stromal corneal grafts based on the Corneoplast material was studied. Despite the identical surface morphology of the samples treated by DTC at different temperatures, as indicated by the surface topology obtained by atomic force scanning, the internal structure at the nanolevel is different. Structural studies were carried out using small-angle X-ray scattering (SAXS) on the USAXS/SAXS/WAXS XEUSS 3.0 station. The obtained results allowed us to draw the following conclusions: 1) shortening of fibrils along the axis by 3 nm in the intersection zone, which occurs at 140°C, should be recognized as already critical, leading to a loss of strength; 2) a decrease in the average distance between triple helices in the quaternary structure may be due to the thermal degradation of polysaccharides. All this results in a decrease in elasticity, strength of the graft, an increase in hydrophobicity, a decrease in biocompatibility and water permeability. As a consequence, samples treated at 140°C are unsuitable for use in ophthalmic surgery. In addition, Corneoplast grafts failed the epithelialization test after treatment at 140°C. One reason for this is the extremely low ability of pig grafts treated by DTC at 140°C to hydrate, as confirmed by Raman spectroscopy (RS). Corneoplast treated at a temperature of 100°C and below retains biointegration properties.

### S7.457. Effect of fizetine on the intensity of POL processes and phospholipase C activity in the regeneration of peripheral nerves

Zaitseva J.V.[1]*, Ignatyeva O.V.[1], Gromova N.V.[1]
[1]*National Research Mordovian State University named after N.P. Ogarev, Saransk, Russia;*
* zaitsevaiuliia.v@gmail.com

Peripheral nerve damage as a result of trauma is one of the most important problems of neurosurgery. The relevance of this problem is related to the high proportion of peripheral nerve injuries mainly in young and middle-aged patients. This requires paying close attention to the problems of surgical treatment of posttraumatic lesions of peripheral nerves. One of the promising areas is the study of the influence of various physiologically active compounds, including flavonoids (in particular - fizetine), on the regenerative processes.
The study was to study the effect of fisetin on the intensity of POL processes and phospholipase C activity in the regeneration of peripheral nerves.
During work on the topic of the study we used methods such as determination of diene conjugates amount and chromatographic separation of diacylglycerol with preliminary obtaining of lipid extract, and we also carried out determination of malonic dialdehyde using thiobarbituric acid.

The test objects were white Vistar rats weighing 200-300 g and aged 3-4 months divided into a healthy group and 2 groups subjected to sciatic nerve ligation, one of which was subjected to intramuscular injection of fizetine at a dose of 50 mg/kg rat. The animals were removed from the experiment on 7, 14 and 21 days.
The detected effects of fizetine are multicomponent and are determined, on the one hand, by its antioxidant ability to reduce the intensity of lipid peroxidation processes, on the other hand, the stabilizing effect of fizetine can be mediated by an increase in the activity of lipolytic enzymes, such as phospholipase C.
According to the results obtained, it can be argued that fizetine has demonstrated a positive effect, having a beneficial effect on nerve fiber regeneration, and can shorten the healing period in sciatic nerve injuries.

**S7.458. Effect of helium plasma modification on morpho-mechanical and adhesive properties of magnetoelectric substrates for bone tissue engineering**

Antipova V.[1]*, Sobolev K.[1], Omelyanchik A.[1], Korepanova E.[1], Pshenichnikov S.[1], Levada E.[1], Rodionova V.[1]
[1]*Immanuel Kant Baltic Federal University;*
* valya.antipova24@gmail.com

Bone tissue regeneration is a long and complex process with a high risk of complications, which requires the search for new effective therapies. For example, the use of functional substrates capable of influencing cellular behavior, including the rate of proliferation and differentiation of stem cells, through biophysical stimulation is of increasing interest in tissue engineering. For bone tissue engineering, the most interesting is the use of biocompatible materials whose mechanical and piezoelectric properties are similar to those of bone tissue, such as polyvinylidene fluoride.
Poly(vinylidene fluoride) (PVDF) is a fluorine-containing semi-crystalline polymer that has at least five different crystalline phases, of which the β-phase has the greatest piezoelectric response. However, the low surface energy of PVDF and its copolymers leads to high hydrophobicity and poor wettability of the polymer surface, which has a poor effect on cell adhesion to the surface of substrates based on them and limits their use in biomedical applications. There are various methods for modifying the surface of PVDF-based films (chemical etching, defluorination-sulfation, etc.), but plasma treatment is the most optimal, since it allows preserving the basic physicochemical bulk properties of the substrates.
In this work, the effect of plasma treatment on the morpho-mechanical and adhesive properties of magnetoelectric substrates was investigated. The nanocomposites were fabricated by the doctor blade method and then treated with helium plasma. PVDF modified with magnetic nanoparticles (CoFe2O4) was used as the base for the nanocomposites. The structural and magnetic properties of the obtained samples were characterized using X-ray powder diffraction (XRD) and a vibrating magnetometer (VSM). The morpho-mechanical properties of the nanocomposites were investigated before and after plasma treatment using atomic force microscopy (AFM). The nanocomposites were additionally tested on human mesenchymal stem cell culture (assessment of cell viability and adhesion).
In this study, helium plasma modification was demonstrated to improve the wettability and surface roughness of PVDF-based magnetoelectric substrates (longer treatment leads to a more pronounced effect) without altering the magnetic and structural properties of the samples. In addition, plasma treatment was found to improve the adhesive properties of PVDF-based nanocomposites, which makes them interesting for use in various biomedical applications, such as bone tissue engineering.
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**S7.459. Effect of low-intensity pulse or continuous laser radiation and chemical agents on mice in vivo**

Dyukina A.R.[1]*, Zaichkina S.I.[1], Potselueva M.M.[1], Yusupov V.I.[2]
[1]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
[2]*Institute of Phonon Technologies, FSRC "Crystallography and Photonics" of RAS, Troitsk, Moscow, Russia;*
* Dyukina@rambler.ru

Studies of the regularities of the impact of low-intensity laser radiation on a living organism are one of the priority areas of science. In parallel with the introduction of laser therapy into medical practice, research is being carried out to elucidate the primary mechanisms of the interaction of laser light with living matter. However, it is known that various ionizing and non-ionizing radiation, both natural and man-made, is environmental risk factors. The search for ways to reveal the action of these factors is an urgent problem of modern radioecology. Currently, intensive research is underway to find non-pharmacological methods for the treatment of various diseases by activating the biological reserve of resistance to various damaging effects that can lead to genetic disorders, malignant cell transformation and developmental defects in offspring.
Based on our numerous studies of the effect of low doses of ionizing radiation on various objects in vitro and in vivo, we assume that the most promising way to identify and study this stability reserve is to use the phenomenon of radiation adaptive response, which consists in the fact that preliminary irradiation of an object in small adaptive doses of gamma or X-ray radiation leads to a decrease in sensitivity to the subsequent revealing effects of large doses of the same effects (0.1 Gy + 1.5 Gy). The problem of searching for various agents of a physical or chemical nature capable, like small doses of ionizing radiation, of activating the body's natural defenses, is still relevant.
The purpose of this work is to reveal cellular reactions in whole blood and lymphoid tissues of mice under irradiation with various physical and chemical agents: by analyzing the cellular composition and the level of production of reactive oxygen species in whole blood, as well as the cellularity of lymphoid organs (thymus and spleen). The work was carried out on male mice of the SHK line. As physical agents, we used low doses of ionizing (X-rays (0.1 Gy, 0.5 Gy) and non-ionizing radiation (femtosecond laser (AVESTA, RF) (525 nm, 200 fs, 70 MHz) at dose rates of 0.05, 0.5, and 5 mW, and irradiation exposure from 1 to 50 s, which corresponds to an energy flux density of 0.1 – 52 mJ/cm2), a continuous green laser (532 nm, 3.7 mW, 1, 5, and 10 s, which corresponds to an energy flux density of 4.8, 24, and 48 mJ/cm2)) and chemical agents - the immunomodulators CaCl2 (0.4% solution for 6 days) and dibazol (0.002% solution for 6 days). A day later, according to the previously developed scheme for inducing a radiation adaptive response, all groups of animals were additionally irradiated with X-ray radiation at a dose of 1.5 Gy (60 mJ/cm2). At least 5 mice were used for each experimental point.
Using standard methods, a hematological analysis was performed, the level of ROS production in whole blood was determined by the method of luminol-dependent zymosan-induced chemiluminescence (CHEMILUM-12, Russia) and the index of the relative mass of the thymus and spleen.
It was found that in all variants of treatment of mice with chemical and physical agents, the cellular composition of blood, the level of ROS production in whole blood, and the cellularity of lymphoid organs did not differ from the spontaneous level.
An analysis of the cellularity of lymphoid organs showed that pretreatment of animals with all studied agents led to a decrease in radiosensitivity upon subsequent exposure to X-ray radiation at a dose of 1.5 Gy compared with unpretreated animals, i.e. the mass index of lymphoid

organs remained within the control values, except for the groups pre-irradiated with X-ray radiation at a higher dose of 0.5 Gy, femtosecond laser radiation at a dose of 2.1 and 52 mJ/cm2 (0.5 mW), 21 and 52 mJ/cm2 (5 mW) and continuous green laser radiation at a dose of 48 mJ/cm2 (3.7 mW) did not protect against subsequent irradiation at a dose of 1.5 Gy, i.e. resulted in a decrease in the organ mass index compared to the non-irradiated control.

Determining the level of ROS production showed that the activation index calculated from the ratio of induced light area to spontaneous light area was significantly higher in all pretreated groups of mice, which indicates the activation of the natural defense reserve compared to the group of animals irradiated only at a dose of 1.5 Gy. At the same time, preliminary exposure of animals to X-ray radiation at a dose of 0.5 Gy or femtosecond laser radiation at a dose of 2.1 and 52 mJ/cm2 (0.5 mW), 21 and 52 mJ/cm2 (5 mW) and continuous green laser radiation at a dose of 48 mJ/cm2 cm2 (3.7 mW) did not increase the activation index compared to untreated animals. A change in the cellular composition of the blood was found depending on the dose and radiation power. The protective effect of all studied agents is revealed in the same narrow dose range and correlates with the formation of ROS, which indicates a similar mechanism of its induction and the possibility of activating the body's natural defense reserve.

Thus, the obtained results on the study of the protective properties of various agents in mice irradiated with X-rays depend on the magnitude and quality of the activating dose, selected tissues and methods, and can serve as an additional sensitive test to detect damage from various environmental factors. According to the number of factors triggering the adaptive response processes, indicating the activation of the body's natural defenses and the number of body reactions in the same area of energy flows, it seems to us that the trigger mechanism of the body's natural defenses is nonspecific.

### S7.460. Effect of modulated magnetic field on biopolymers

Tekutskaya E.E.[1], Shpakov I.A.[1]*, Panchenko I.A.[1]
[1]*Kuban State University;*
* hpdefender@yandex.ru

Studying the effect of electromagnetic radiation on various biological objects is an important task. The electromagnetic field surrounds a person throughout his life. The study of the mechanisms of the effect of this radiation on biopolymers will give an understanding of the effect on the entire body.

We have investigated the effects of a modulated electromagnetic field on solutions of ready—made commercial drugs: an aqueous solution of DNA — the drug "Derinat", an aqueous solution of human serum albumin (CHSA) - a drug manufactured in Moscow.

The concentration of the initial DNA solution is 2.5 g /ml, and the initial CHA solution is 20%.The final concentration of an aqueous solution of DNA is 1.25 g / ml, and for an aqueous solution of CHSA – 2%. To achieve these concentrations, the solutions of the studied substances were diluted with distilled water.

The treatment of aqueous solutions of DNA and CHSA EMF in the frequency range from 1 to 60 Hz was carried out. Irradiation of aqueous solutions of DNA and CHSA with a modulated electromagnetic field was carried out. Fluorescence spectra were obtained using a Hitachi F-2700 spectrophotometer. Low-frequency electromagnetic field processing took place in a shielded chamber, the radiation was generated by the AKIP 3408/3 signal generator.

The analysis of the obtained dependences showed the frequency of irradiation of the electromagnetic field, demonstrating the maximum effect on the fluorescent characteristics. Such frequencies during the processing of EMF CNF for an aqueous DNA solution are 25 Hz for all excitation wavelengths studied in this work, and for an aqueous CHSA solution – 40 Hz for excitation wavelengths of 290-295 nm, and for 300-305 nm – 5 Hz. When treated with modulated EMF - for an aqueous DNA solution – at a carrier frequency of 1 kHz, and a modulating frequency of 1 Hz. For an aqueous solution of CHSA – at an excitation wavelength of 290 nm – 1 kHz, modulating 1 Hz, at 295 nm – 3 kHz, modulating - 35 Hz, at 300 nm – 3 kHz, modulating – 5 Hz, and at 305 nm – 3 kHz, modulating 15 Hz.

### S7.461. Effect of ultra-short weightlessness simulation on mouse sperm motility

Zhdankina Y.S.[1]*, Ogneva I.V.[1]
[1]*Institute of Medical and Biological Problems RAS;*
* juliaszd@yandex.ru

The motor activity of mammalian spermatozoa is a necessary condition for maintaining the species in animals. Despite the development of various assisted reproductive technologies, including those applicable to humans, maintaining the motility of male germ cells under the influence of various environmental factors has not ceased to be an urgent task. Studies of the effect of gravity changes on the motor ability of mammalian spermatozoa may not have direct practical application, however, they make it possible to understand the fundamental mechanisms of regulation of motility and, in addition, can be useful in developing measures to protect the male reproductive system during deep space exploration. Previous results indicate that the speed of movement of mouse spermatozoa decreases after 1 hour in 2g conditions and after 6 hours in 0g conditions (Ogneva I.V. et al., 2020). Changes in motor activity are accompanied by changes in the content of cytoskeletal proteins and the level of its phosphorylation (Ogneva I.V., 2021). However, it can be assumed that the initiation of such changes takes place in the earliest period of exposure under changed gravitational conditions (Ogneva I.V., 2022). In connection with the foregoing, the purpose of this work was to determine the content of cytoskeletal proteins in mouse spermatozoa after 30 minutes of exposure under conditions of weightlessness simulation.

Spermatozoa were isolated from the caudal epididymis of male mice. All procedures with animals were approved by the Commission on Biomedical Ethics of the SSC RF IBMP RAS (protocol No. 521 of September 25, 2019). Microgravity conditions were created using a random positioning machine (RPM), which allows one to reproduce weightlessness in an average of 15 seconds. Cultivation was carried out at a temperature of 370C for 30 minutes. Motility was analyzed using video files of sperm movement obtained with a Basler puA1600-60uc camera at a frequency of 60 frames/s and a resolution of 2 megapixels (Basler AG, Germany). Determination of protein content was carried out by Western blotting using specific primary antibodies to various cytoskeletal proteins.

As expected, after 30 minutes of exposure to RPM, the speed of movement of mouse spermatozoa did not change relative to the control, however, changes in the content of cytoskeletal proteins were noted. Thus, in the RPM group, the content of beta-tubulin significantly increased by 36.5% ($p < 0.05$), although the content of CKAP5, which binds microtubules, decreased by 24% ($p < 0.05$). On the contrary, the content of beta-actin tended to decrease by 13.3% ($p < 0.1$), and the relative content of SVILL binding actin filaments increased by 16% ($p < 0.1$). At the same time, the relative content of the focal adhesive kinase FAK, which plays an important role in the remodeling of the cytoskeleton of motile cells, increased by 31% in the RPM group ($p < 0.05$).

The results obtained indicate that even a 30-minute exposure of mouse spermatozoa under simulating weightlessness leads to rearrangements of the cytoskeleton, which, in turn, can lead to the triggering of signaling pathways and the formation of an adaptive structural-functional pattern.

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### S7.462. Effects of hypoxia on chromosomal apparatus organization and cognitive function in Drosophila

Nikitina E.A.[1,2]*, Medvedeva A.V.[2], Rebrova A.V.[1], Safarova D.D.[1], Karovetskaya D.M.[1,2], Savvateeva-Popova E.V.[2]
[1]*Herzen University;*
[2]*Pavlov Institute of physiology RAS;*
* 21074@mail.ru

All living organisms are in continuous interaction with the external environment, being exposed to adverse factors that cause various injuries in the cell. The need to coordinate reactions within the body and in response to external influences mediated the emergence of a temporal communication mechanism that transformed into an adaptive reaction - a conditional reflex that binds the body and the environment. Forming a conditional reflex, the body adapts not only to the external environment, but also to extreme influences. Hypoxia as an extreme exposure is one of the most common damaging factors. Because the brain is the most oxygen-dependent organ, the sensitive target of hypoxia represents cognitive functions inextricably linked to the integrity of the genetic apparatus. In this regard, it is of great interest to study the level of double-stranded breaks (DSB) of DNA and the ability to learn and form memory in Drosophila, a model organism widely used in studies of the molecular mechanisms underlying cognitive functions in higher eukaryotes. Studies were conducted using the wild-type strain Canton-S Drosophila melanogaster. A flow-type pressure chamber was used to simulate the physiological level of hypoxia. The frequency and profile of chromosomal rearrangements were determined by anaphase analysis. The ability to learn and form medium-term memory was evaluated in the conditioned courtship suppression paradigm. Cognitive behavior analysis was performed using different exposure patterns: intact control, exposure to hypoxia before, after, or during training. A two-factor randomization analysis was used to process the results. A significant increase in the rate of appearance of bridges resulting from errors in repair of DSB during interchromosomal interactions is shown. This suggests that the action of hypoxia leads to the formation of DSB. Since DNA breaks accompany intense matrix processes in neurogenesis and are an indicator of physiological activity of neurons, it is possible that DSBs are necessary in chromatin remodeling and expression of genes involved in memory formation and learning processes. The obtained cytogenetic data are in line with the provision on uniform mechanisms underlying the body's stress response and learning. Apparently, an increase in the frequency of breaks in hypoxia contributes to the spatial reorganization of the chromosomal apparatus in the nucleus. Analysis of cognitive behavior did not reveal memory impairment in any of the options for exposure to hypoxia. However, hypoxic exposure prior to training resulted in a significant learning impairment compared to intact controls and other exposure options. Hypoxia appears to have a short-term effect on learning, but does not affect the mechanisms of memory formation. The obtained data suggest that triggering a cellular response to hypoxia entails the activation of cascades significant for the implementation of cognitive functions.

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### S7.463. Effects of weak magnetic fields on the production of reactive oxygen species by neutrophils

Novikov V.V.[1]*, Yablokova E.V.[1], Fesenko E.E.[1]
[1]*Institute of Cell Biophysics RAS;*
* docmag@mail.ru

We have shown that exposure of murine peritoneal neutrophils to magnetic shielding in hypomagnetic conditions (HMC) causes the decrease of intracellular ROS production recorded by change of fluorescence intensity of the products of 2,7-dichlorodihydrofluorescein and dihydrorhodamine 123. In these experiments, we found that the effect of the hypomagnetic field is apparent on neutrophils without their additional stimulation by the chemical activators of respiratory burst, and, consequently, the mechanism of such action can be not related to the impairment of the response of neutrophils to these stimuli. We also used another method, activated chemiluminescence with selective probe for superoxide anion, lucigenin, to estimate the radical producing ability of the neutrophils after exposure to HMC. Attention was paid to determination of residual static magnetic field (SMF) values at which the effects of HMC can be reproduced. Pre-incubation of the neutrophil suspension in "zero" magnetic field (<0.02 μT) led to significant decrease of the intensity of lucigenin-dependent chemiluminescence (by around 30%). When the constant field was increased to 2.5 μT, this effect vanished, but at SMF value = 7 μT it appeared again; at 30 and 44 μT, the effect was absent (the value corresponded to SMF value in control). Such polyextreme character of the dependence of response to weak SMF was also noticed in the experiment on neutrophils during ROS production registration by fluorescence spectroscopy. We have shown that the addition of diphenyliodonium, a NADPH-oxidase inhibitor, to the incubation medium leads to the decreased intensity of chemiluminescence in both experimental (hypomagnetic conditions) and control (geomagnetic field) samples. The differences between the groups caused by action of "zero" magnetic field were observed in a wide range of concentrations of this inhibitor (2.5-100 μM) to the similar extent. Contrary to that, addition of an agent uncoupling oxidation and phosphorylation in mitochondria, 2,4-dinitrophenol, from 5 μM and up to 200 μM, almost completely leveled the differences between control and experimental group, that were observed in absence or at lower concentrations of this inhibitor. These data show the prospect of studying neutrophil mitochondria as potential targets reacting on changes of SMF values.

The effects of combined magnetic fields (CMF) are different from the effects of a "zero" field and a weak static magnetic field. As our results showed, the respiratory burst in neutrophils is sensitive to variations of magnetic parameters preceding this event. Priming effect (pre-activation of respiratory burst in neutrophils) of combined weak static (42 μT) and collinear low-frequency alternating (sum of frequencies 1, 4.4 and 16.5 Hz, total amplitude 0.86 μT) was shown as more significant enhancement of chemiluminescence of neutrophil suspension in response to application of a bacterial peptide fMLF, or a phorbol ester PMA, in presence of luminol. On the contrary, when using other parameters of CMFs, for example, the frequency tuned to the cyclotron resonance of Fe3+ ion, deactivation of respiratory burst was demonstrated. In this case, 60 μT static magnetic field and the collinear alternating low-frequency magnetic field, 49.5 Hz and 60-180 nT, caused, after 40 min pretreatment, significant alleviation of respiratory burst intensity in suspension of neutrophils in response to the activator fMLF, recorded by luminol-dependent chemiluminescence assay.

### S7.464. Electrochemical study of the behavior of toluidine blue in alginate hydrogel with immobilized peritoneal lavage cells

Novakovsraya M.V.[1]*, Cherenkov I.A.[1], Ryabov E.I.[1]
[1]*Udmurt State University, Izhevsk, Russia;*
* mariya.98@inbox.ru

Currently, there is a need to develop a model for the activation, differentiation, and evaluation of the functional activity of cells of the immune system.Such a model with a heterogeneous cellular composition can become the basis of the "inflammation-on-a-chip" microphysiological system and be used both to deepen our understanding of the cellular mechanisms of inflammation and its causative factors, and for applied purposes – to test anti-inflammatory medicinal substances.

The basis of the work was a series of electrochemical measurements on planar graphite electrode systems (working and auxiliary electrodes - graphite, reference electrode - silver chloride), which are designed to work with small volumes.
The values of the current strength, potentials for the main electrochemical processes (oxidation and reduction of the dye), as well as the characteristic properties of the current-voltage curves of cyclic voltammetry (CV) were measured.
Before measurements, the electrodes were cycled in the potential range from –1000 to 1000 mV with distilled water to stabilize the characteristics. A 0.9% sodium chloride solution (pH = 6.8) was used as a supporting electrolyte. All subsequent solutions were prepared on its basis. To model the intercellular matrix, 2% (wt.) freshly prepared sodium alginate (ρ = 997 kg/m3) was used. Its polymerization was carried out for 5 minutes with a 0.1 M CaCl2 solution (ρ = 1018 kg/m3), which was applied directly to the working electrode, the excess solution was removed with filter paper. Toluidine blue (TB) at a concentration of 0.1 mM was chosen as an electroactive label giving an analytical signal.
Leukocytes were isolated from peritoneal lavage of mice. The cell suspension was washed with sterile saline (S). The suspension was mixed with 4% sodium alginate (wt.) in a ratio of 1:1 and studied by the CV method in the potential range of 0…+500 mV (rel. Ag/AgCl) with a potential sweep rate of 35 mV/s.
To assess the diffusion of the dye in cell-free and cellular systems, measurements were carried out for 50 minutes with an interval of 5 minutes.
In the presence of 2% alginate, TB is characterized by close to reversible electrochemical behavior. Qualitative changes in the current-voltage curves of the CV are observed. Prior to the start of measurements, the dye forms two peaks: at a potential of +280.2 mV, the reduction peak (Epc), and at +203.9 mV, the oxidation peak (Epa), ΔE = 76.3 mV. As for the values of peak currents, they correspond to +0.11 μA for reduction (Ipc) and -0.14 μA for oxidation (Ipa). The excellent potential values and peak currents similar in absolute value indicate a quasi-reversible process, which is due to the presence of a polyanionic hydrogel in the system, which prevents the deposition of charged dye molecules.
Within 30 minutes, we observed a gradual increase in the peak values of the current strength. At the last cycle, they amounted to Ips = 0.23 μA, Ipa = -0.15 μA. The peak current ratio is 0.65 μA, which indicates the partial reversibility of the process. The potential values of the corresponding peaks were of interest: Epc = +316.2 mV, Epa = + 196 mV, ΔE = 120.2 mV. We assumed that this behavior of the dye is due to its partial absorption in the hydrogel, since low concentrations of CaCl2 ensured incomplete polymerization of the hydrogel. Because of this, the structure of the drop was loose, and there were enough reactive free groups inside it and on the surface.
Based on the Rendles-Shevchik equation, the diffusion coefficient of the dye through the alginate hydrogel was calculated and amounted to D = 1.18 * 10-9 m2/s. This result is lower than the estimated values of the diffusion coefficient TS for aqueous media, obtained from the calculations based on the Stokes-Einstein approximation, given in the literature (1.42 * 10−9 m2/s). Alginate hydrogel stabilized with calcium ions slows down the diffusion of TS.
After adding a cellular component to the electrochemical cell, we recorded visible changes. In the presence of cells, the system initially formed 2 peaks: Ipc = + 0.02 μA at Epc = +272.3 mV, Ipa = -0.01 μA at Epa = + 239.9 mV. The current strength values decreased by more than 10 times compared to the control, which indicates the presence of a cellular component in the system that actively interacts with the dye.
After a 30-minute TB diffusion, a significant increase in the peak current strength is observed Ipc = + 0.2 μA, Ipa = -0.28 μA, Ipa/Ipc = 1.4. The range of potentials in which dye transformations take place also shifts (Epc = 308.3 mV, Epa = 183.8 mV, ΔE = 124.5 mV).
The dependence of the peak values of the current strength on time was linear and described by the equation: Ipc = t + 0.31, R2 = 0.99. Diffusion coefficient D = 2.47 * 10-11 m2/s, which is significantly lower compared to the cell-free model.
In both systems, the predominance of the cathode current over the anode current was also noted, which is explained by the presence of recovering electroactive molecules. In the presence of cells, the values of electrooxidation currents increased, which indicates the presence of oxidized molecules, which can be reactive oxygen species released by leukocytes.
Thus, we have established the functional activity of leukocytes, expressed in terms of catalytic currents of redox transformations of TS. The interaction of peritoneal lavage cells with the components of the electrochemical model triggers a biochemical cascade of reactions that are successfully recorded by the CV method. This makes it possible to simulate the process of cell interaction with the extracellular matrix and subsequently to trace the stages of electron transfer in tissue systems.

### S7.465. Enhancement of the biological effect of ionizing radiation using gold nanoparticles

Morozov V.N.[1]*
[1]*IBCP RAS;*
* morozov.v.n@mail.ru

Nowadays, radiation therapy is used for radical and palliative treatment of a wide range of neoplasms, as well as of the non-tumor nature diseases. However, despite the intensive development of this method, there is still a significant potential for increasing the efficacy of radiation therapy: the radioresistance of several tumors and the dose load on surrounding normal tissues can significantly limit the application of radiation therapy.
So, various methods of modifying the radiosensitivity of the cells, in particular, the use of chemical radiomodifiers (primarily radiosensitizers), may be used to increase the efficacy of radiation treatment. Recently, some products of nanotechnology have attracted a lot of attention in this capacity. For example, nanoparticles containing the chemical elements with a much higher atomic number relative to soft biological tissues are a promising class of antitumor radiosensitizers (e.g., metal nanoparticles based on silver or platinum, as well as particles of more complex compositions, such as metal oxides - HfO2, Bi2O3, etc.). However, he most attractive and widely studied are gold nanoparticles due to their outstanding physico-chemical properties, controlled biocompatibility, low toxicity, and wide possibilities of synthesis and modification of functional design.
This report summarizes the main results of studying the possibilities of the enhancement the effect of radiation exposure of the biological objects using gold nanoparticles. In particular, the principles of the selection of the optimal combinations of nanoparticle parameters for given irradiation conditions are proposed. Also, we demonstrate the experimental results of the study of the radiosensitization effect of gold nanoparticles in various biological systems: solutions of biomacromolecules, tumor cell cultures, and tumor-bearing laboratory animals. These data indicate that preparations based on gold nanoparticles can be very attractive for use in combination with radiation therapy for a number of oncological diseases. In addition, we show for the first time that the combined use of gold nanoparticles and X-ray irradiation can significantly increase the efficiency of inactivation of viral particle, which is also very promising for the issue of radiation sterilization and processing.

**S7.466. Evaluation of the effectiveness of antibiotic therapy in modeling escherichiosis in mongrel mice as a result of the use of benzylpenicillin sodium salt processed with a pulsed magnetic field**

Rodenko N.A.[1,2]*, Savinkov A.V.[3], Glushchenkov V.A.[1,2], Vasilyeva T.I.[2], Ermakov V.V.[3], Belyaeva I.A.[1,2], Dmitrieva Y.V.[3], Tsay A.A.[2]
[1]*Samara Federal Research Center of the Russian Academy of Sciences;*
[2]*Samara National Research University named by academician S.P. Korolev;*
[3]*Samara State Agrarian University;*
* t.rodenko@mail.ru

Introduction. The adaptability of living organisms to existing types of antibiotics requires the development of new or activation of existing drugs. One of the ways to increase antibacterial activity is to influence an antibiotic with a high-intensity pulsed magnetic field (PMF) [1]. As a result of the conducted studies, a reliable increase in the antibacterial activity of benzylpenicillin sodium salt against bacteria Escherichia coli M17 (E.coli M17) by 12-24% was noted after PMF processing the drug [2].
The purpose of this work is evaluation of the effectiveness of antibiotic therapy in modeling escherichiosis in mongrel mice as a result of the use of benzylpenicillin sodium salt processed with a pulsed magnetic field.
Materials and methods. The experiments were carried out on 60 mongrel mice with an average body weight of 18-22 g. The experimental model of escherichiosis was obtained by injecting mice with the E.coli M17 strain by the intraperitoneal method with 0.1 ml of a suspension of microorganisms in the amount of 2•10^9 cells/ml. During the experiments, mice were divided into the following groups: uninfected (n=10), infected with the E.coli M17 strain (n=10), a control group that received injections of the antibiotic without PMF processing (n=20) and an experimental group of mice that received injections of the antibiotic processed with the PMF (n=20). Processing of benzylpenicillin sodium salt was carried out once at a PMF intensity of H=0.09•10^6A/m and at a frequency of f=40 kHz. The antibiotic (100 000 units) was administered subcutaneously 0.2 ml three times a day [3]. Treatment of the mice with the PMF processed antibiotic and unprocessed antibiotic was carried out for several days. Hematological and biochemical parameters of blood of all animals were studied [4], and the microbial contamination of the liver in mice was assessed.
Results and discussion. As a result of the conducted studies, in the experimental group of mice it was reliably found that in three days a number of white bloods increased by 34% in relation to that in three hours after infection. After three days of treatment of the mice, against the background of an increase in the proportion of lymphocytes, a decrease in the proportion of neutrophils was observed in the leukogram. As a result, after infection of mice, the level of total protein and glucose, the activity of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) in blood serum increased within physiological values, the level of gamma-glutamyltransferase (GGT) exceeds the maximum values of the norm.
When using the PMF processed antibiotic, in the mice of the experimental group on the third day of treatment, the levels of ASAT and GGT were reliably higher compared to the initial values (before infection of mice) by 34% and 73% respectively. When evaluating the effect of the PMF processed antibiotic on the quantitative composition of the culture E.coli in the liver, it was found that during the first day of treatment, the number of microorganisms decreases by 13 times in relation to the group of mice treated the antibiotic unprocessed with the PMF. On the third day of the studies, despite of the quantitative growth of the culture E.coli in the liver of both groups, the animals of the experimental group showed a decrease in the growth of the number of microorganisms compared to the control group.
Conclusion. After the administration of the PMF processed benzylpenicillin sodium salt to the animals, there is an inhibition of bacterial contamination of the liver and a higher reactive level of leukocyte protection of the body. A decrease in the level of the main parameters of red blood, as well as an increase in the values of enzyme markers of liver condition in blood serum, require additional verification as part of the assessment of the subchronic toxicity of the antibiotic on functioning bode systems.
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**S7.467. Ferroptosis depends on lipid peroxidation in mitochondria**

Lyamzaev K.[1,2]*, Avetisyan A.[1], Siminyan R.[1], Chernyak B.[1]
[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia;*
[2]*The "Russian Clinical Research Center for Gerontology" of the Ministry of Healthcare of the Russian Federation, Pirogov Russian National Research Medical University, 129226 Moscow, Russia;*
* Lyamzaev@gmail.com

Ferroptosis is a regulated form of necrotic cell death that depends on iron-catalyzed lipid peroxidation (LPO). The role of mitochondria in ferroptosis has not been elucidated. We have shown that ferroptosis in fibroblasts induced by the cystine transport inhibitor erastin or the glutathione biosynthesis inhibitor butionsulfoximine (BSO) can be prevented by the mitochondria-targeted antioxidant SkQ1. Measurements of LPO in mitochondria using a new fluorescent ratiometric probe MitoCLox showed that the protective effect of SkQ1 correlates with the prevention of mitochondrial LPO, but not with the accumulation of reactive oxygen species (ROS) in the cell. Methylene blue, a redox agent that inhibits ROS production in the electron transport chain complex I, also inhibits ferroptosis and mitochondrial LPO without reducing overall ROS levels. These data indicate the leading role of mitochondrial lipid peroxidation in ferroptosis. In isolated heart mitochondria in the presence of ferrous ions and NAD+-dependent respiratory substrates, the complex I inhibitor rotenone stimulates LPO. At the same time, the production of ROS in complex III in the presence of succinate and antimycin does not cause LPO. SkQ1 and methylene blue inhibit rotenone-dependent LPO in isolated mitochondria. We suggest that ROS formed in complex I contribute to mitochondrial lipid peroxidation and ferroptosis.

**S7.468. Hematological parameters of mice with oral administration of biogenic and synthetic ferrihydrite nanoparticles**

Biryukova E.B.[1]*, Kolenchukova O.A.[1], Kireeva A.V.[1], Stolyar S.V.[1], Loshkareva M.V.[1]
[1]*Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences;*
* helena.biryukova.1996@gmail.com

Ferrihydrite nanoparticles synthesized by chemical and biological methods were used in the experiment. After oral administration of these particles, changes in some blood parameters were noted in the group receiving biogenic nanoparticles, compared with the control.
Keywords: nanoparticles; ferrihydrite; Klebsiella oxytocin; blood parameters 1. Introduction. Iron nanoparticles (NPS) have great appeal in biomedical applications, since these particles have both magnetic and semiconductor properties [1]. Magnetic woofers are an excellent choice, for example, as a drug delivery module [2]. However, the medical use of NPS requires a comprehensive study, so the purpose of this work is to analyze the hematological parameters of mice with oral administration of biogenic and synthetic NPS ferrihydrite.
2. Materials and methods. Biogenic ferrihydrite nanoparticles were obtained from a culture of Klebsiella oxytoca bacteria grown on nutrient media containing Fe2+ oxalate and Fe3+ citrate. Ferrihydrite was isolated from the sediment and sol was obtained according to the standard procedure described earlier [3]. As a result, nanoparticle sol was obtained, which was dried at room temperature. Synthetic ferrihydrite samples were obtained by hydrolysis of iron (III) nitrate. The objects of the study are mice, males. In total, 65 individuals participated in the experiment, divided into 3 groups: Control group: 15 individuals, 16 grams of feed per day per head; 1 experimental group: 25 individuals, 16 grams of feed + 0.049 g of synthetic nanoparticle sol per head; 2 experimental group: 25 individuals, 16 grams of feed + 0.034 g of biogenic sol nanoparticles per head. The experiment was carried out in dynamics for 36 days. The 1st day, 21st day and 36th day were selected as control points. On the first day of the experiment, 5 individuals were euthanized to obtain control hematological parameters. On the 22nd and 37th days, 25 mice were euthanized (5 individuals from the control group, 10 individuals from each experimental group). Whole blood analysis with anticoagulant was performed on the "Heska Element HT5" analyzer. Statistical processing was carried out using the program Trial version of STATISTICA 10.
3. The results of the study. After processing the results on day 36 in the group of biogenic NPS, the amount of hemoglobin was 4.8% ($p = 0.001$) higher than the group of synthetic NPS, the number of leukocytes increased in 1 (122%, $p = 0.034$) and 2 (162%, $p = 0.023$) groups relative to the control measurements of 1 day, there was an increase in the number of erythrocytes by 8.75% ($p = 0.008$) in 2 groups relative to the indicators for 21 days. There was a slight decrease in the average hemoglobin concentration of 1 (1.4% $p = 0.009$) and 2 (0.4% $p = 0.009$) of the experimental groups relative to the control on day 21 and an increase in this indicator by 3.3% ($p = 0.004$) in group 2 relative to 1 on day 36. Also, in group 1, the average number of platelets decreases by 29.9% ($p = 0.01$) by the end of the experiment relative to 21 days. In group 2, on day 36, the hematocrit increased by 6.3% ($p = 0.02$) relative to 21 days and the average hemoglobin content in the erythrocyte increased by 4.6% ($p= 0.031$) relative to 1 day. In group 1, by the end of the experiment, relative to 21 days, the average platelet density decreased by 28.5% ($p = 0.01$). On the 21st day of the experiment, the average difference in red blood cells by volume increased by 6.4% ($p = 0.04$) in group 2 relative to group 1. The average platelet volume in the blood of group 2 on day 21 was 9% ($p = 0.005$) lower than the control group.
4. Discussion. The obtained data may indicate a significant effect of biogenic nanoparticles on the blood of the studied animals, since within this experimental group, when processing the results, the greatest number of significant differences were noted during the experiment. To varying degrees, an increase in group 2 indicators, such as MSNS, MCV, RBC, hematocrit, as well as an increase in hemoglobin levels on day 36 and CDV on day 21 in group 2 relative to 1, may indicate an increase in erythropoiesis activity. The effect of synthetic nanoparticles on blood platelets (decrease in their number, average density and increase in volume) requires further study. The difference in the effect of different nanoparticles on the hematological parameters of mice when administered orally in dry form with food can be explained by different degrees of bioavailability. It can be assumed that biogenic ferrihydrite nanoparticles are better absorbed during digestion. 5. Conclusion. Nanoparticles of ferrihydrite of synthetic origin have the least effect on the hematological parameters of mice. It can be assumed that when nanoparticles of ferrihydrite of synthetic origin are added to the bait, the growth of the volume of red blood cells and blood platelets slows down. Biogenic nanoparticles, when administered orally, can cause a significant increase in hemoglobin levels.
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**S7.469. Influence of chronic irradiation in low doses on the mechanisms of converting electrical signals into functional responses in plants**

Nemtsova Y.A.[1]*, Ladeynova M.M.[1], Kuznetsova D.V.[1], Ageyeva M.N.[1], Grinberg M.A.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* julnemtcova@yandex.ru

All plants grow and develop in constantly changing conditions. For the formation of adaptation to various negative environmental factors, the distribution of remote signals through the plant is required, which can regulate physiological processes, causing functional changes at the level of the whole organism. Electrical signals (ES) play a significant role in the response to a stressor; they are especially important for responding to rapidly growing environmental stressors, such as changes in temperature, illumination, attack by leaf-eating insects, etc. Along with rapidly growing stressors, plants in the natural environment can be affected by factors that modify the status of signaling systems for a long time, which affects the formation of plant adaptation and resistance. One of these chronic factors may be an increased level of ionizing radiation (IR) in the area of plant growth. According to the literature data and previous works, ES undergo changes under conditions of increased radiation load. However, the question of the mechanisms of the influence of IR on stress signals and the role of irradiation in the formation of signal-induced functional responses still remains unexplored. The purpose of this work is to analyze the mechanisms of the effect of chronic irradiation on plant ES and the process of signal conversion into a photosynthetic response.
We used tobacco plants (Nicotiana tabacum L.) expressing the fluorescent ratiometric pH-sensitive protein Pt-GFP and common wheat (Triticum aestivum L.) cv. Darya. A sealed source of 90Sr-90Y was used as a chronically active source of IR. The IR source was located above the plants at a distance of about 22 cm. The dose rate of the source was approximately 31 μSv/h. To ensure uniform irradiation, the plants were regularly moved relative to the source. Irradiation began from the day of planting and continued throughout the entire period of growing plants, the total time of irradiation of tobacco plants was 6 weeks, wheat plants - 2 weeks. The total cumulative dose was about 32 mGy for tobacco and 11.3 mGy for wheat. Control

plants were grown under similar conditions in the absence of an IR source. The leaf area, wet and dry weight of control and irradiated plants were determined as morphometric parameters. ES was induced by heating the tip of the sheet with a resistor. Registration of surface potentials was carried out using macroelectrode technology. A PAM fluorometer was used to record the photosynthesis parameters of tobacco plants. Detection of changes in pH at the level of the whole plant was carried out by Pt-GFP fluorescence using a whole plant fluorescence imaging setup, as well as using a confocal microscope. Stomatal conductance was assessed using an infrared gas analyzer. Analysis of the concentrations of phytohormones was carried out by liquid chromatography-mass spectrometry.

Stress resistance is significantly affected by the basic status of the plant - the state at rest. The results of the experiments performed demonstrate an increase in morphometric parameters, such as wet and dry weight, leaf area in irradiated plants. Under the action of IR, plants also show an increase in the activity of photosynthesis.

In control and irradiated plants, local irritation causes the spread of stress ES. In our experiment, IR did not cause changes in the amplitude of ES induced by local heating. When passing through the leaf, ES causes a temporary decrease in the intensity of photosynthesis, which, according to the literature, contributes to the formation of plant resistance to adverse conditions. In irradiated plants, an increase in photosynthetic responses caused by ES was found, and a significant violation of the correlation between the amplitudes of ES and the responses caused by them was also observed.

A decrease in the activity of the light stage of photosynthesis during the passage of ES can be associated with 1) changes in intracellular and extracellular pH, and 2) changes in stomatal conductance and CO2 availability. In the course of studying the first potential mechanism, pH shifts, it was found that more pronounced shifts in pH occurred in irradiated plants during the passage of ES. Also, there was a violation of the correlation between the amplitudes of ES and pH, similar to the effects exerted by IR on the responses of photosynthesis. H+-ATPase appears to be the key target of IR, which affects the magnitude of pH shifts. It is shown that in irradiated plants its activity increases. The study of the second potential mechanism, changes in stomatal conductance, showed that IR also enhances ES-induced stomatal responses. The influence of IR is probably realized due to changes in the concentration of phytohormones. It has been shown that the responses of jasmonic and abscisic acids are enhanced in irradiated plants.

Thus, the experimental results suggest the existence of two main pathways for modifying ES-induced photosynthetic responses in tobacco plants grown under chronic irradiation. The first pathway is associated with the influence of IR on the cell pH maintenance systems, and the second, on the stomatal conductance regulation system.

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**S7.470. Influence of chronic irradiation on resistance to unfavorable environmental factors induced by electrical signals in plants**

Grinberg M.A.[1]*, Nemtsova Yu.A.[1], Gromova E.N.[1], Ivanova A.V.[1], Vodeneev V.A.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* mag1355@yandex.ru

Ionizing radiation (IR) is one of the significant, but insufficiently studied environmental factors. The level of IR has a significant impact on the growth and development of living organisms, including plants. It is well known that the dose-response curve for IR has a complex non-linear shape, especially in the low dose range. It is noted that low doses often do not have a pronounced effect or may lead to some increase in a number of indicators. However, under the combined action of IR and other factors on plants, the effect of irradiation can become much more pronounced. One of the possible reasons for this effect is the influence of IR on plant signaling systems that are involved in the formation of a stress response and the development of resistance to a stressor. It should be noted that between the impact of a stimulus and the formation of adaptation to it lies a cascade of events, the key of which are 1) the generation and transduction of a remote signal, 2) the activation of "secondary" signal-regulatory systems and the transformation of the signal into physiological responses, and 3) the formation of resistance to stressor. To date, it is not known which of the elements of the cascade of events are responsible for the change in the final response to the stressor under the influence of IR.

15-day-old seedlings of wheat (Triticum aestivum L.) were used as the main object of experiments. To test the universality of IR effects, the most significant stages of the experiments were additionally performed using 6-week-old tobacco (Nicotiana tabacum L.) and Arabidopsis (Arabidopsis thaliana L.) plants. The source of IR was a 90Sr-90Y β-emitter with an activity of 0.1 MBq and a dose rate of about 31 μGy/hour. Irradiation of plants was carried out continuously throughout the entire growing period. The heat-induced electrical signal was recorded extracellularly using a multichannel macroelectrode setup. Photosynthesis activity and transpiration intensity were measured using an infrared gas analyzer and a PAM fluorometer. Heat stress was created by heating the vessel with plants in a thermostat.

In the course of the work, we analyzed the effect of chronic irradiation on the main stages between the action of a stressor and the formation of signal-induced resistance (signal – functional response – resistance), as well as on the basic status of plants (before the stressor). It was shown that the dose rates used in the experiments do not have a significant effect on the basic status of plants. There was a slight increase in a number of morphometric parameters, which, apparently, is due to a slight activation of photosynthetic processes. At the same time, IR has a pronounced effect on stomatal conductance, significantly increasing the level of transpiration.

Despite the weak effect of IR before the action of the stressor, irradiation has a pronounced effect on remote electrical signals caused by local heating. IR contributes to lowering the signal generation threshold, increases its amplitude, the rate of development and propagation, as well as the area covered by the signal.

II significantly enhances the functional responses evoked by electrical signals, i.e., a transient decrease in the activity of photosynthesis and the intensity of transpiration. In irradiated plants, an increase in the amplitude of responses, the rate of their development, and the area covered by the response was shown. It is noted that in irradiated plants of different species, the correlation between the signal amplitudes and the photosynthetic responses they cause is disturbed.

An experiment to study the resistance of irradiated plants to heat stress showed that IR contributes to the maintenance of a higher level of residual photosynthesis. Electrical signals in control plants also contribute to the maintenance of photosynthesis after warming up. In this case, the passage of an electrical signal in irradiated plants leads to a paradoxical decrease in the activity of photosynthesis after heat stress.

Thus, despite the weak stimulatory effect of IR on the basic status of plants, chronic irradiation can have a significant effect on signal-induced resistance to stressors. The direction of this influence is apparently determined by the nature of the stressor and the peculiarities of the influence of IR on signaling systems. IR affects all stages between the action of a stressor and the formation of resilience. The stage of converting the signal into a functional response seems to be the most significant, since it shows the greatest magnitude of IR effects, in addition, at this stage, there is a violation of the correlation between successive stages of the development of resistance.

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### S7.471. Influence of gases on the processes of freezing-thawing and survival of cell cultures during low-temperature preservation

Ugraitskaya S.V.[1]*, Fesenko E.[1]
[1]*Institute of Cell Biophysics RAS;*
* ugraitskaya@mail.ru

The preservation of biological material in a frozen state is an urgent problem due to the rapid development of medicine and the need to develop long-term cryobanks. It is required both to improve the existing approaches, developed mainly for cell preservation, increasing their efficiency, and to create fundamentally new ones in order to solve the problem of reversible freezing of such objects as tissue fragments, isolated organs.This is impossible without understanding the biological and physicochemical processes accompanying cooling, freezing, and subsequent thawing of the biomaterial, which, as is known, can be subjected to mechanical action from growing ice crystals and osmotic stress during cryopreservation.A wide range of gases has been studied during low-temperature conservation and their cryoprotective properties have been investigated. It has been shown for the first time that the nature of the gas dissolved in liquid affects the safety of HeLa and L929 cell lines during cryopreservation. The survival of HeLa and L929 cells decreases with increasing gas solubility in water in the following order: He<Ne<SF6< N2<Ar<Kr<Xe. Helium and neon have pronounced cryoprotective properties, ensuring the survival of up to 30% of HeLa cells in a medium without cryoprotective agents. Both gases can be used to reduce the concentration of classic penetrating protectants, in particular glycerol, from 10% to 3%, reducing the potential cytotoxic effects of the cryoprotective solution.According to microscopic analysis, dissolved gases affect the structure of the frozen solution due to the formation of microbubbles during water crystallization. It has been experimentally substantiated that the nature of the dissolved gas affects the number and size of gas microbubbles in the process of solution freezing. The mechanism of the cryoprotective effect of helium based on the replacement of air gases (degassing) and the reduction of the formation of gas microbubbles during crystallization by reducing the gas component of the solution, as well as the ability of light inert gases to dissolve in ice, is disclosed.The ice mass formed in this case tends to reduce the probability of cracking upon cooling to minus 50°C, which has a positive effect on the safety of the frozen biomaterial.
The results obtained are of interest as an auxiliary element for the development and implementation of effective methods for cryopreservation of cells, solving the problem of cryopreservation of tissues and organs.

### S7.472. Influence of rare earth metals on calcium-dependent processes in the myocardium

Korotkov C.M.[1], Sobol K.V.[1]*
[1]*I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry;*
* peep9@yandex.ru

Heavy metals confidently occupy the second place in terms of danger, yielding to pesticides. It is known that the level of the factor of technogenic enrichment with heavy metals has increased significantly in recent years, which has a particularly negative effect on people with cardiovascular and nervous pathologies. Acute or chronic poisoning can occur through contact with heavy metals through contaminated air, water, soil, and consumer products.
The exponential use of rare earth metals in industry and the poor management of waste disposal processes containing compounds of these metals raises serious concerns about the quality and safety of the environment. An undesirable feature of rare earth metals is their ability to bioaccumulate [1, 2], which can significantly increase their toxicity. Moreover, the concentration of rare earth metals, for example, La3+ in biological objects does not decrease after the cessation of exposure, which indicates a long biological half-life of this metal from the body [2, 3].
The goal is to investigate the effect of rare earth metals on the cardiovascular system and mitochondrial energy.
Methods - studies are carried out on isolated mitochondria, on a contractile model of the heart and blood vessels, as well as on isolated cardiomyocytes.
Results - we have shown that lanthanides (La3+, Pr3+, Nd3+, Gd3+) at high millimolar concentrations have negative inotropic and chronotropic effects, which manifests itself in a decrease in the amplitude and frequency of spontaneous contractions, respectively. At the same time, lanthanides have little effect on the respiration of isolated mitochondria in the 3 or 3PDNP state (uncoupled by 2,4-dinitrophenol, DNP). For example, unlike Cd2+, lanthanides are not capable of strong binding to the thiol groups of respiratory enzymes. Lanthanides can affect the ion permeability of the inner mitochondrial membrane and enhance the active transport of K+ into the mitochondrial matrix.
In preliminary experiments, we found for the first time that at low concentrations (less than 0.2 mM), lanthanides can stimulate cardiac activity both in normal conditions and under conditions of inhibition of mitochondrial functions with sodium azide, thereby revealing the effects of preconditioning. It is possible that this effect is associated with an increase in active transport of K+ into the mitochondrial matrix and a corresponding decrease in Ca2+ overload of mitochondria.
In conclusion, the negative inotropic and chronotropic effects of lanthanides that we found are probably largely due to the influence of these metal ions on calcium-dependent processes in cardiomyocytes, including mitochondria [4–6]. In millimolar concentrations, lanthanides can block the excitation-contraction system in the myocardium, namely the initial entry of Ca2+ into the heart cell.
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### S7.473. Interaction of DNA with coordination compounds of iridium and ruthenium

Teplukhina K.A.[1]*, Kasyanenko N.A.[1]
[1]*St. Petersburg State University;*
* tepluhina.ksenya@gmail.com

The coordination compounds used in this work can be considered as biologically active compounds interacting in vivo with the DNA molecule. Their potential antitumor activity is preliminary known. However, as a result of the aquatation reaction, coordination compounds of this type can acquire a charge of 3+ in an aqueous solution. This may contribute to the fact that, when interacting with a DNA molecule, they can induce compactization of the macromolecule in solution up to the formation of toroidal structures, as was previously shown for compounds cobalt of similar structure. Therefore, the purpose of this study was not only to consider the possibility of forming biologically significant complexes of these compounds with a DNA molecule in solution, but also to analyze their effect on the tertiary structure of a macromolecule in solution and its persistent length. The results, obtained in the study, were compared with the action of a well-known condensing agent - cobalt hexamine, which induces the formation of toroidal DNA nanostructures with preliminary structuring of a statistical coil of a macromolecule in solution.
For such studies, it is necessary to use high-molecular-weight DNA. As an object of study, we worked with a commercial preparation of high-molecular-weight calf thymus DNA (Sigma Aldrich). Studies were carried out in solutions with an excess of low molecular weight salt (NaCl), when electrostatic interactions are suppressed, and its deficiency. The stock solutions contained 0.005 M NaCl.
To study the complex formation of compounds with DNA, a set of experimental methods were used that provide information on the state of the secondary and tertiary structure of DNA: atomic force microscopy, dynamic light scattering, low molecular weight viscometry, flow birefringence, and spectral methods. We used a technique that makes it possible to study possible competition for the binding site on DNA of the studied compounds with other biologically active agents, the molecular mechanism of interaction of which with DNA is well known. Divalent metal ions, some coordination compounds (for example, trans- and cisplatin), and surfactants were used as such agents. Compounds of palladium and cobalt with different ligands were also considered, which made it possible to reveal the role of various interactions (electrostatic, van der Waals, hydrogen bonds, ion-dipole) in the formation of complexes.

### S7.474. Interrelation of self-organization, fluorescent and physicochemical properties of diluted ethanol-water systems

Ryzhkina I.S.[1]*
[1]*Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center, Russian Academy of Sciences;*
* irina.s.ryzhkina@mail.ru

In continuation of the work on establishing the physicochemical patterns of the formation and functioning of self-organizing diluted aqueous dispersed systems of biologically active compounds (BACs), whose properties, including biological ones, are determined mainly by the structure and characteristics of the water-molecular dispersed phase (nanoassociates), which are characterized by a size of hundreds of nm and a negatively charged interface [1-5], self-organization, physicochemical properties, UV absorption and fluorescence of diluted ethanol-water systems in the range of calculated ethanol concentrations of 10–1×10E-15 % vol. were studied. Similar studies were carried out for aqueous systems in the range of 1 to 15 decimal dilutions.

As an indispensable biogenic substance, ethanol is constantly (endogenously) present in the blood at concentrations of approximately 1×10E–4 M (6•10E–4 %), performing various functions, participating in the maintenance of the homeostasis of human and animal organisms. The exogenous effect of ethanol on the body is accompanied by multidirectional concentration profiles of bioeffects, mainly associated with the effect of ethanol on the central nervous and immune systems. It is known that aqueous solutions of ethanol in the range of high concentrations (20–70 %) are hydrotropic structured systems capable of solubilization of poorly soluble BAS. The process of self-organization of diluted ethanol-water systems and its connection with the properties of the systems have not been investigated to date.
Using the dynamic light scattering (DLS) method, we have shown that dilute ethanol solutions in the range from 1×10E–2–1×10E–15 % vol. are self-organized dispersed systems, which, as they are diluted, undergo a rearrangement of the dispersed phase, accompanied by a nonmonotonic increase in structuring and dimensional uniformity, reaching a maximum at 1×10E-6, 1×10E–9–1×10E–12 % vol. (monomodal distribution, decrease in the polydispersity index to 0.3, size of nanoassociates about 150–200 nm). In this concentration range, the most pronounced changes in UV absorption (A225 and A275), pH, electrical conductivity ($\chi$), redox potential (U) of the systems, as well as the maximum fluorescence intensity (I) at λem 340 nm (λex 230 and 280 nm ), which is 2-3 times higher than I340 at other concentrations. A close correlation was established between the size of the nanoassociates, I340, A225 and A275, pH, $\chi$ and the U systems in the range of 1×10E–6–1×10E–15 % vol. ethanol. The correlation coefficients, characterizing the degree of relationship between these values, ranged from 0,7 to 0,8. At ethanol concentrations of 1, 1×10E–1, 1×10E–5, 1×10E–7 % vol. no structures were detected by the DLS method, UV absorption and fluorescence are practically absent.
The study of dilute aqueous systems revealed their ability to structure formation and nonmonotonic change in properties during dilution, which are less pronounced than in the ethanol-water system, and, unlike the latter, are characterized by an almost complete absence of the relationship between the size of structures and the properties of systems.
As shown above in the example of dilute solutions of biogenic organic and amino acids [1, 4], the data obtained make it possible to predict the bioeffects of dilute ethanol-water systems, which can manifest themselves most clearly in the range of 1×10E-9-1×10E-12 % vol. ethanol.

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## S7.475. Investigation of DNA structure defects by spectral and hydrodynamic methods

Paston S.[1]*, Murzakova I.F.[1], Ivanova D.N.[1]
[1]*St. Petersburg State University;*
* svpaston@list.ru

Damage in the DNA molecule is one of the triggers for the development of pathological processes in the cell. Various types of damage are divided into two groups: single-site, such as single-strand breaks (SSB), base modifications, base release (resulting in apurine and apyrimidine sites, -AP sites). The second group is local multiple damages (LMDs), such as cluster lesions (two or more closely spaced single-site lesions), DNA double-strand breaks (DSBs), DNA-protein and DNA-DNA intermolecular cross-links [1, 2]. Single-site damages are effectively eliminated by the cell repair system using an intact DNA strand antiparallel to the damaged one as a template. Such damage occurs spontaneously in DNA, most often due to interaction with oxidative radicals that are normally formed in the cell. An excess of the normal level of reactive oxygen species (ROS) as a result of various pathological processes or under the action of ionizing radiation leads to the accumulation of a large amount of DNA damage, and densely ionizing radiation induces LMDs in DNA by a direct action of radiation [3]. The study of the mechanisms of formation and distribution of defects in the DNA structure under the influence of various physicochemical factors, the influence of environmental conditions and the structure of an intact macromolecule on this process is necessary to understand the initial stage of such important intracellular processes as repair, apoptosis, and oncogenesis. In this work, spectral and hydrodynamic methods are used to study defects in the DNA structure caused by non-ionizing UVC light and gamma radiation.

A decrease in the volume of a macromolecular DNA coil in a water-salt solution, which, in particular, is a consequence of local denaturation (occurring in places of damage in the DNA primary structure) was recorded by low-gradient viscometry. The dose dependences of the intrinsic viscosity of DNA, which is proportional to its specific volume, show that as the ionic strength of the solution decreases, the radiation effects increase. In [4], it was shown that gamma irradiation at doses up to 30 Gy does not lead to a decrease in the thermodynamic rigidity of DNA; therefore, the observed decrease in specific volume occurs due to the suppression of long-range interactions in the DNA chain. Other effects caused by irradiation (decrease in the melting point, destruction of the nitrogenous bases of DNA) also depend significantly on the ionic strength of the solution: the lower the ionic strength of the solution, the greater the radiosensitivity of DNA. It is known that with a decrease in the ionic strength of the solution, the volume of intact DNA in the solution increases, i.e., the size of the target in the irradiated sample increases. In addition, the stability of the secondary structure of DNA decreases with a decrease in the concentration of counterions in solution. It can be concluded that these factors play a decisive role in the formation of damage in the DNA structure.

The contribution of the direct and indirect action of radiation can be revealed using interceptors of active products of water radiolysis, or by varying the concentration of targets in the irradiated sample. The radiation-chemical yield G of thymidine destruction under the action of gamma radiation in aqueous (1.60 molecules/100 eV) and water-ethanol solutions (0.11 molecules/100 eV at Cet=15 vol%), as well as under the action of Fenton's reagent (0.45 mol /2.6 mol of reagent). The dependence of G of the destroyed nitrogenous bases of DNA on the dose of irradiation and on the concentration of DNA in the irradiated solution of ionic strength of 5 mM NaCl was obtained. In the range of DNA concentrations of 2 mg/dl–7 mg/dl, the constancy of G is observed, the so-called dilution effect, which indicates that all appeared products of water radiolysis react with DNA. A decrease in DNA radiation damage in the presence of ethanol in the irradiated solution is observed in a wide range of ionic strengths.

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## S7.476. Investigation of the cytotoxicity of silver nitrate and silver-cysteine nanocomplexes

Bogdanov An.A.[1,2]*, Klimenko V.V.[1], Bogdanov A.A.[1], Moiseyenko V.M.[1]
[1]*Saint Petersburg Clinical Research and Practical Centre of Specialized Types of Medical Care (Oncological);*
[2]*Institute for Analytical Instrumentation of the Russian Academy of Sciences, St Petersburg, Russian Federation;*
* vip.nasa@bk.ru

Introduction

Currently, a large number of studies are devoted to the investigation of the antitumor activity of silver nanoparticles and compounds, one of which is silver nitrate. However, silver nitrate has systemic and local toxic effects. In this work, a method was proposed for the synthesis of non-metallic complexes that do not contain toxic nitrate ions, and the cytotoxicity of silver nitrate and silver-amino acid nanocomplexes was investigated.

Materials and methods

Flow cytometry and colorimetric MTS test were used for determination of cytotoxicity of silver nitrate and silver cysteine nanocomplexes. Chemical method was developed to synthetize new silver-cysteine nanocomplexes. The physical properties of silver-cysteine nanocomplexes were studied using dynamic light scattering method.

Results

Earlier in the study of the cytotoxic and antitumor activity of silver nitrate, we showed that silver nitrate has a cytotoxic effect, as a result of the study, an IC50 value for the HeLa and K-562 tumor lines was obtained, which is 20 times higher than the IC50 for normal human MNCs. .It has also been shown that silver nitrate has an antitumor effect against Ehrlich's solid tumor, but the compound also has systemic and local toxic effects [6]. Presumably, one of the mechanisms of local toxicity is the release of nitric acid during the interaction of silver ions with sulfide-containing compounds, such as cysteine, the concentration in the blood of which is normally 166.6-249.9 μM, which, with the introduction of silver nitrate (excluding buffer capacity of blood), can cause a local decrease in blood pH to 4 at a normal value of 7.5. The nitrate ion, in turn, is also a toxic compound. A possible way to reduce the systemic and local toxicity observed with the introduction of pure silver nitrate is the synthesis of non-metallic complexes that do not contain toxic nitrate ions. The simplest, most accessible and physiological complexing substance can be the amino acid cysteine, which can effectively bind Ag + ions. In the course of the work, the development of a general synthesis scheme and the selection of the optimal conditions for the synthesis and purification of such complexes were carried out.

When an aqueous solution of cysteine interacts with silver nitrate at various ratios R = Cys/Ag, nanoscale complexes are formed. The hydrodynamic diameter of these complexes for different R was measured by the method of dynamic light scattering, and the values of the electrokinetic potential of the obtained complexes were also determined. The complexes under study have a characteristic size of several tens of nanometers

up to the ratio R = 1/2; with an increase in the mole fraction of silver nitrate in the initial mixture to 2.5, the hydrodynamic diameter increases to ~ 100 nm, and upon reaching at R = 1/4 a micron size. The electrokinetic potential of the investigated particles increases uniformly with an increase in R, when the ratio R= 1/1, its value is 35 ± 3.78 mV, with an increase in R to 1/4, the value of the electrokinetic potential increases to 52 ± 4.4 mV. When measuring the spectra of ultraviolet and optical absorption of the obtained complexes, it was shown that the absorption values increase with an increase in the ratio R over the entire investigated wavelength interval. The absence of a broad plasmon resonance peak at λ = 410 nm, characteristic of metallic silver nanoparticles, confirms the nonmetallic nature of the synthesized complexes. The measured pH value of the solution after the formation of AgCys nanocomplexes was 1.5, which makes the crude complexes unsuitable for both in vivo and in vitro studies. After the obtained nanocomplexes were purified from nitric acid as well as the residues of unreacted starting compounds, the pH value of the resulting solution was 7, which indicates the absence of nitric acid and the potential applicability of the complexes for in vitro and in vivo studies. Based on the results of the synthesis and purification of silver-cysteine complexes, we investigated the antitumor activity of these complexes in vitro on various tumor and normal, human and mouse cell cultures. IC50 for AgCys nanocomplexes were obtained for various cell cultures: K-562 - IC50 = 22.2 ± 1.61 μg / ml; HeLa - IC50 = 22.2 ± 1.61 μg / ml; 3T3b - IC50 = 22.2 ± 1.61 μg / ml; CT-26 - IC50 = 9 ± 2.17 μg / ml, no toxicity for MNCs of human peripheral blood was observed in the range of the studied concentrations, which could be the evidence of the selective action in relation to tumor cells.

Conclusion

As a result of this work, the method for the synthesis of silver-cysteine nonmetallic nanocomplexes was proposed, and the optimal conditions and concentrations were investigated and selected. The antitumor activity of silver-cysteine nanocomplexes was shown in various normal and tumor cell cultures of humans and mice.

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### S7.477. Investigation of the effect of low frequency EMF on the growth and chemiluminescence of B. subtilis

Bokareva M.A.[1]*
[1] *Kuban State University;*
* mabokar5@icloud.com

Recently, the possibility of using low-frequency electromagnetic fields (EMF) in the food industry has been actively investigated to ensure the safety and safety and quality of food products [1, 2], to maintain food, physico-chemical and organoleptic properties of food, increase stability during storage by eliminating harmful biological agents, blocking the activity of enzymes and reducing the number of microscopic, mold and yeast fungi, bacteria [3]. Low-frequency EMF is used to reduce the number of microorganisms during storage, nuts, dried fruits, spices, etc. [4]. When exposed to non-ionizing radiation, the destruction of microorganisms is carried out due to oxidative damage to DNA, proteins and lipids and leads to the death of microbial cells [5]. The issue of studying the effect of low-frequency EMF on certain groups of microorganisms of practical interest or acting as model objects remains relevant.

As such, microorganisms living on the surface of fruits and vegetables are of interest. Accordingly, Bacillus subtilis was used as a model culture.

The effects of the influence of bacteria on plants can have a different character – so the mentioned strain under normal conditions can have a growth-stimulating effect, protect plants from aggressive phytopathogens. On the other hand, when storing plant products, native microflora as a result of intensive development and assimilation of plant tissue nutrients can lead to deterioration of the commercial properties of plant products.

The aim of the work was to study the effect of an alternating electromagnetic field of low frequency in vitro on the growth dynamics, the accumulation of cell biomass and the severity of oxidative stress of laboratory microorganisms Bacillus subtilis.

The treatment of microorganisms before the start of cultivation was carried out by an alternating electromagnetic field with frequencies from 7.0 to 25.0 Hz directly in 96-well plates in a shielded chamber [6]. The circuit consisted of a generator of low-frequency signals GZ-118, which is a source of a sinusoidal signal of a precision shape, an inductor having 1200 turns. The coil was placed in a shielded chamber, which was made of structural steel with a thickness of 3 mm. The attenuation of external EMF by the camera in the range from 3 Hz to 300 kHz reached about 100 times. The resistance of the coil was 320 ohms, the voltage on the coil was 14 V. EMF treatment was carried out at room temperature (22 ± 1 ° C) for 1 min at the selected frequency. The field strength at the sample location was 550 ± 30 A/m. The field strength was monitored using the spectrum analyzer "Ekofizika-110A" and the antenna P6-70.

Treatment with a low frequency electromagnetic field of bacteria has shown that exposure to EMF with a frequency of 9 Hz leads to the greatest decrease in the intensity of growth and accumulation of biomass (compared with the treatment of samples with an electromagnetic field with other low frequencies). The maximum of the chemiluminescence flash was 39% higher than the control sample (without treatment). One of the possible mechanisms for implementing the effects of low-frequency EMF on living systems may be oxidative DNA damage [5]. The obtained chemiluminescence data of bacteria confirm this hypothesis.

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### S7.478. Lignin preparation as a potential anti-radiation agent

Raskosha O.V.[1]*, Ermakova A.V.[1], Bashlykova L.A.[1], Starobor N.N.[1], Bodnar I.S.[1], Karmanov A.P.[1], Kocheva L.S.[2]
[1]*Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences;*
[2]*Institute of Geology of the Komi Science Center of the Ural Branch of the Russian Academy of Sciences;*
* raskosha@ib.komisc.ru

The search for ways to modify the radiosensitivity of organs and tissues is the most important fundamental problem, both from the standpoint of reducing the biological consequences of exposure to ionizing

radiation on the body, and from the standpoint of increasing the effectiveness of tumor radiotherapy. Lignin are unique biopolymers of plant origin, the structural organization of which is multivariate and largely depends on the biological species of the plant. The purpose of this work was to study the radioprotective properties of natural lignin on small mammals. The experiments were carried out on mature male mice of the CBA line, obtained from the Scientific Collection of Experimental Animals of the Institute of Biology of the Federal Research Center of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (http://www.ckp-rf.ru/usu/471933/), which were kept taking into account sanitary and hygienic and bioethical aspects. Mice were irradiated on a gamma-ray unit (Researcher Rossiya) at a dose of 6 Gy (137Cs, 0.75 Gy/min). For the study, we used a water-soluble form of Pepper's organo-solvent lignin isolated from the stems of oats Avena sativa. Plant polymer animals received orally for 8 days at an average cumulative dose of 150 mg/kg of body weight. To study the anti-radiation properties of the studied preparation, animals were given lignin to drink for 8 days before (prophylactic regimen) or after (therapeutic regimen) acute gamma irradiation. At the end of the experiment, mice were decapitated and material was immediately taken for molecular-cellular analysis (neutral version of the DNA comet method and micronucleus test) of organs and tissues with different proliferative activity, analysis of the cellular composition of peripheral blood, and determination of the content of malondialdehyde in erythrocytes. The results obtained by us testify to the anti-radiation properties of the lignin preparation. This was manifested in a statistically significant decrease in DNA double-strand breaks and the level of micronuclei in bone marrow cells, as well as in a decrease in the content of malondialdehyde in peripheral blood erythrocytes of mice in both regimens of lignin administration to animals when compared with irradiated mice. The radioprotective properties of lignin are also evidenced by the results of a micronucleus test carried out on thyroid cells and data obtained from the analysis of peripheral blood - normalization of the hemoglobin concentration during the therapeutic administration of the test drug and the size of erythrocytes in both regimens of lignin administration. The use of lignin by animals before irradiation led to a decrease in the level of abnormal spermatozoa heads from epididymis and a statistically significant decrease in defects in their tail. Thus, the results of our studies have shown that the lignin preparation obtained from oat stems is capable of modifying radiation effects in the organs and tissues of mice, which is promising for further study of its antiradiation properties.

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### S7.479. Lipid determinants in the regulation of pore-forming activity of antimicrobial lipopeptides

Zakharova A.A.[1]*, Efimova S.S.[1], Ostroumova O.S.[1]
[1] *Institute of Cytology, RAS;*
* zaza2187@bk.ru

Cyclic lipopeptides (CLPs) are microbial surfactants produced by a wide variety of microorganisms. It is believed that the antimicrobial activity of CLPs is associated with a disorganization of target cell membranes due to the formation of ion-permeable pores or micelles. In light of the increasing resistance of pathogenic microorganisms CLPs are the most promising class of new antimicrobial agents. In this regard, an urgent task of pharmacochemistry is to establish lipid determinants of the regulation of the pore-forming activity of these compounds.

Using Montall and Muller technique it was found that a decrease in the boundary potential of the membrane leads to an increase in the number of channels formed by the syringomycin E (SRE) produced by Pseudomonas syringae. An increase in lateral pressure in the lipid bilayer causes the opposite effect. In turn, local anesthetics are able to block the SRE channel.

It was shown that the presence of the negatively charged lipids in the membrane is the crucial factor for ion-permeable pores induced by fengycin isolated from Bacillus subtillis. The introduction of modifiers causing disordering of membrane lipids, leads to an increase in the pore-forming activity of the lipopeptide.

The lipopeptide polymyxin B (PMB) of Bacillus polymyxa was found to interact predominantly with negatively charged membrane lipids. Phospholipids induced packing stress in the lipid headgroups region cause a decrease in the pore-forming activity of PMB. The dependences of the PMB-induced transmembrane conductance on the concentration of the antibiotic revealed a high cooperativity of binding to Kdo2-lpipd A-containing bilayers and a relatively low cooperativity to a deglycosylated analogue of lipid A. This may indicate a significant role of sugar residues of lipopolysaccharides of the outer membrane of Gram-negative bacteria in the oligomerization of PMB.

Thus, the possibility of regulation of the pore-forming activity of CLPs due to changes in the elastic and electrical properties of the membrane was demonstrated. This work was supported by The Russian Foundation of Science (22-15-00417)

### S7.480. Local action of the combination of heating and illumination causes propagation of hyperpolarization electrical signals and affects the physiological processes in wheat

Popova A.Y.[1]*, Yudina L.M.[1], Zolin Y.A.[1], Sukhova E.M.[1], Grebneva K.V.[1], Abasheva K.R.[1], Sukhov V.S.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
* SilverKumiho@mail.ru

The local action of stressors on plants causes the generation of electrical signals that ensure their adaptation to adverse conditions, affect photosynthesis and change productivity. Higher plants have three main types of electrical signals. The action potential is a short–term pulsed electrical signal of depolarization type generated in response to non-damaging effects (cooling, lighting). Variation potential is a long–term electrical signal of depolarization type, which is generated in response to damaging effects (burn, heating to high temperatures, crushing). The system potential is an electrical hyperpolarization signal that caused in response to the action of various stressors, including those that cause the generation of a variable potential. Due to the fact that in natural conditions the impact of stressors of a damaging nature on plants is quite rare, the question becomes relevant: are electrical signals generated under the action of moderate stressors (for example, bright light, moderate heating) or their combinations. The purpose of this work was to analyze the possibility of generating electrical signals under the action of a combination of moderate non–damaging stressors - local lighting and heating, and to study the parameters of electrical signals in conditions of irrigation, moderate and severe soil drought.

The study used 14-day-old seedlings of spring wheat (Triticum aestivum L.) of the "Daria" variety. The plants were grown in pots with universal soil, in a vegetation room at 24°C and 16-hour daylight. To create a water shortage, the plants were divided into two groups: with watering every two days (control) and without irrigation (drought). Extracellular $Ag^{+}/AgCl$ electrodes, an amplifier and a personal computer were used to measure surface electrical potentials (a potential shift in the more positive direction corresponds to hyperpolarization, and depolarization in the more negative direction). Measuring electrodes were located at different distances from the irritated zone to light and heating, the reference electrode was located at the base of the plant shoot on the border with the ground. To induce electrical signals, light (540 μmol $m^{-}2s^{-}1$, blue light) was simultaneously exposed to the plant leaf for 10 minutes and heated (to a final temperature of 40°C using a Peltier element) for 30 minutes. Photosynthetic parameters were

measured using Open FluorCam FC 800-O/10, the stomata conductivity index was calculated based on thermal imaging measurements using a testo 885-2 thermal imager.
During the study of electrical signals, it was shown that with the combined action of stressors, depolarization occurred near the stimulated zone, followed by hyperpolarization as it moved away from this zone. Under conditions of exposure to plants only by light, a weak hyperpolarization signal was observed, on the contrary, the signal caused only by moderate heating was close to the signals induced by a combination of lighting and heating. It has been suggested that a combination of stressors causes a depolarization signal, which, spreading through the plant, causes hyperpolarization. These signals have been identified as a system potential.
In moderate soil drought, it was shown that the shape of the hyperpolarization signal was similar to the shape of such a signal under control conditions, a significant change in the amplitude of the signal was absent in moderate drought. Signals generated by the type of depolarization were not detected. Severe soil drought led to a change in the shape and a decrease in the amplitude of electrical signals. Signals by the type of depolarization were also not detected. Thus, moderate soil drought did not significantly affect the generation of a hyperpolarization signal caused by a combination of light and heating, severe soil drought suppressed such signals.
At the next stage of the study, the influence of the detected signals on photosynthesis and transpiration was evaluated. The combination of stressors and propagating signals led to a decrease in the quantum yield of photosystem II (Y(PSII)) and to an increase in the non-photochemical quenching of chlorophyll fluorescence (NPQ) in wheat. Photosynthesis responses were not observed at large distances from the site of exposure. Combined action to light and heating led to a decrease in stomatal conductivity (Ig), while the effect decreased with increasing distance to the affected area. A study of photosynthesis and transpiration responses under conditions of single exposure to illumination showed that there were no significant changes in Y(PSII), NPQ and Ig. However, a single heating action caused a decrease in Y(PSII) and an increase in NPQ. These changes were similar in form and amplitude to the photosynthetic responses that occur under the combined action of stressors. Thus, the results obtained confirm the participation of the identified electrical signals in the formation of photosynthetic responses of the plant, since local illumination causes only electrical signals with a low amplitude, which does not lead to photosynthetic responses. On the contrary, local heating or a combination of stressors that cause more pronounced electrical responses affect photosynthesis. The generation of systemic potentials under the influence of moderate heating and lighting demonstrates that the propagation of electrical signals through the plant can be caused by stressors widespread in the environment and such potentials can play an important role in the emergence of an adaptive response in higher plants. This hypothesis requires further study, since the effect of hyperpolarization signals on physiological processes has not been sufficiently studied.
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### S7.481. Mechanisms of UV-induced cell death of human lymphocytes

Nakvasina M.N.[1,2]*, Artyukhov V.A.[1,2], Radchenko M.R.[1,2]
[1]*Department of Biophysics and Biotechnology;*
[2]*Voronezh State University;*
* nakvasina_ma@mail.ru

One of the key problems of cell biophysics is the identification of patterns of cell death as a fundamental general biological phenomenon. The study of the mechanisms of cell death, the identification of markers (indicators) of its types, the search for ways to regulate them is the key to understanding the regularities of the processes of morpho- and embryogenesis, maintaining cellular homeostasis, the biological action of physicochemical agents, the pathogenesis of various human diseases and the development of effective methods for their treatment.
The mechanisms and sequence of development and implementation of the stages of programmed cell death of peripheral blood lymphocytes of donors under exposure to UV light (240–390 nm) at doses of 151, 1510, and 3020 J/m2 were studied.
An increase in the level of expression of membrane receptors of apoptotic signals of CD95 lymphocytes suspended in the nutrient medium RPMI-1640 and Hank's solution was found to increase in relation to the control level within 1-5 hours after UV irradiation of cells at the doses used. An increase in the expression of Fas receptors of lymphocytes modified by exposure to UV light in Hank's solution is associated not only with the unmasking of previously inaccessible (hidden) CD95 molecules, but also with the synthesis of their new molecules 4 and 5 h after immunocyte irradiation.
An increase in the level of functional activity of initiating caspases-8 and -12, respectively, was found 3 hours after UV irradiation of lymphocytes at a dose of 1510 J/m2 and immediately after their photomodification. The data obtained indicate the possibility of participation of these caspases in the implementation of the receptor caspase pathway (caspase-8) and signaling pathways associated with impaired calcium homeostasis and changes in the level of second messengers - Ca2+ and cAMP (caspase-12).
An increase in the level of functional activity of the effector caspase-3 of human lymphocytes relative to that of intact samples was found 8 and 24 h and 6 and 8 h, respectively, after cell irradiation at doses of 151 and 1510 J/m2. UV modification of lymphocytes at a dose of 3020 J/m2 induces caspase-3 inactivation.
It was found that after 20 hours of incubation of lymphocytes irradiated with UV at doses of 151, 1510, 3020 J/m2, DNA fragmentation occurs, as indicated by a set of bands ("apoptotic ladder") on the electropherogram, corresponding to smaller DNA fragments compared to the control. .
DNA damage (single-strand breaks) is detected immediately after UV irradiation of lymphocytes at doses of 1510 and 3020 J/m2 (C1 type DNA comets) and reaches a maximum 6 h after cell modification (C2 and C3 comets). Probably, the accumulation of DNA single-strand breaks eventually leads to the formation of double-strand breaks. DNA double-strand breaks are a signal to trigger apoptosis, which is carried out with the participation of the transcription factor p53, the pro-apoptotic Bax protein, and other mitochondrial apoptosis factors. Changes in the structural state of mitochondrial membranes were revealed after exposure of lymphocytes to UV light at doses of 151 and 1510 J/m2.
20 hours after irradiation of lymphocytes at a dose of 151 J/m2, DNA fragments with a size of 6000 bp were found. and less than 1500 b.p. The use of the DNA comet method under these conditions made it possible to identify class C2 comets characteristic of preapoptotic cells (DNA fragments $\leq$ 50 kb).
20 hours after exposure of lymphocytes to UV light at a dose of 1510 J/m2, the formation of DNA fragments less than 1500 bp was registered. and DNA comets of the C3 class, which indicates internucleosomal DNA fragmentation, which is characteristic of dying cells.
Twenty hours after exposure of lymphocytes to UV light at a dose of 3020 J/m2, DNA fragments of approximately 5000 bp in size were found. and less than 1500 bp, comets of C3- and C4-classes. Caspase-3 inactivation was observed under the same conditions. Apparently, these results indicate the possibility of implementing the p53-dependent pathway of apoptosis, accompanied by the release of AIF (apoptosis-inducing factor) from mitochondria and the induction of a caspase-independent pathway of programmed cell death.
The assumption that p53 may be involved in apoptosis of lymphocytes was confirmed after the discovery (compared to intact immunocytes) of a higher level of this protein 6 h after cell irradiation at doses of 1510 and 3020 J/m2.

In favor of the concept of triggering the mitochondrial mechanism of apoptosis is evidenced by data on determining the level of reactive oxygen species in UV-irradiated lymphocytes. It was found that UV irradiation of immunocytes and subsequent incubation for 1 and 2 hours induced an increase in the intracellular level of reactive oxygen species compared to control samples.
Based on the results of determining the level of cytochrome c in the cytosol of lymphocytes 1.5 and 4 hours after UV irradiation of cells, it is assumed that cytochrome c may be involved in the programmed cell death of human lymphocytes induced by exposure to UV light at the minimum dose used (151 J/m2 ).
During the flow cytometric analysis of lymphocytes after their UV irradiation at a dose of 1510 J/m2, time characteristics of the implementation of apoptotic cell death were revealed. The time is 2–4 h after cell photomodification, which is sufficient for the implementation of the main events of programmed cell death, accompanied by the translocation of phosphatidylserine into the outer monolayer of the plasma membrane of lymphocytes (most of the cells in suspension are at an early stage of apoptosis).
Therefore, the death of human blood lymphocytes induced by exposure to UV light is realized with the participation of the receptor, nuclear (p53-dependent pathway), and mitochondrial mechanisms of apoptosis.

### S7.482. Modification of fish aquaculture biotechnology by modifying the redox potential of water. Biophysical aspects

Korzhov A.N.[1]*, Loza S.A.[1], Korzhova M.A.[2]
[1]*Kuban State University;*
[2]*Kuban State Technological University;*
* shtrih_ooo@mail.ru

Water is the most common substance on the planet Earth, about 71% of the globe's surface is covered with water. It is the most important substance for all living beings on Earth. Its main role is in the origin and maintenance of life on Earth, in the global circulation of substances and energy, in the chemical structure of living organisms, in the formation of climate and weather. On average, the body of plants and animals contains more than 50% water. Every year the problem of shortage of water resources becomes more acute. For successful application in economic activity, it is necessary to change its physico-chemical properties, apply various methods of modification and purification. Membrane methods and technologies successfully solve this problem. The article [1] describes a method for correcting and regulating the pH of water using bipolar electrodialysis for the needs of thermal power engineering. The work [2] is devoted to the study of the technology of reagentless electromembrane decarbonization of natural water. Also, with the help of electromembrane technology, it is possible to correct the redox potential (ORP) of aqueous solutions.
Biofeedback with modifications of the redox potential of water is described in [3]. We have described studies of the influence of water with a high negative redox potential on aquaculture. The biological feedback provided by water on the physiology of living systems is completely insufficiently studied. Fine regulation and imperceptible changes in physiological processes occurring in aqueous solutions subjected to various physico-chemical influences require a comprehensive study.
Our team conducted studies of the effect of water with a high negative redox potential (ORP) on the young of the African Clarias catfish (Clarias gariepinus). It was found that the decrease in the ORP of water from +150..250 mV to -600 .. -500 mV with the help of an electromembrane generator without changing the salt composition with a slight change in pH with an exposure of 30 and 60 minutes once a day has a positive effect on the main fish-breeding and biological indicators of growing young African clary catfish. To modify the ORP of aqueous solutions, an electromembrane installation with an ion-exchange bipolar membrane was used.
Currently, studies are being conducted on juvenile sturgeon breeds of sterlet fish (Acipenser ruthenus) and juvenile Australian red–tick crayfish (Cherax quadricarinatus). A preliminary analysis of the results of the influence of water with a high negative ORP on biological processes in the studied organisms showed significant positive effects of the process of stimulating the growth and productivity of aquaculture. Further research has great potential for the creation of new technologies for growing aquaculture in closed water supply installations.
Thus, the observed increase in the productivity of aquaculture cultivation is the result of the influence of water with a high negative ORP on the stimulation of biological processes in the body, however, the mechanism of action of this effect of influence requires more detailed and careful study. It is possible that a sharp change in the ORP of the aquaculture habitat leads to stress, which has a pronounced effect on the dynamics of biochemical, cellular, tissue and systemic regulatory processes. In addition, the ingestion of molecular hydrogen into the body leads to a change in gene expression in systemic organs, which can also affect physiological indicators.
The work was carried out with the financial support: project of the Ministry of Education and Science of the Russian Federation FZEN-2023-0006 23/ 20T.
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### S7.483. On Possible External Nature of Circahoralian Periods Spectra

Panchelyuga V.A.[1]*, Panchelyuga M.S.[1]
[1]*Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences, Pushchino, Russia;*
* victor.panchelyuga@gmail.com

In [1], the local fractal analysis of noise-like time series was presented for the first time by the all permutations method (APM). One of the first results of the APM-analysis is the study of a 329-day array of alpha-decay rate fluctuations, which revealed a stable set of periods in the range of 1–120 min [2]. For this range, not only the close connection of the found spectrum with the spectrum of the Earth's natural oscillations was shown, but also its universal character: the spectra of periods found for fluctuation processes in systems of various nature (physical, chemical, biological) always coincided with the corresponding part of the spectrum found for time series of alpha-decay rate fluctuations [2]. Due to this property of the found spectrum [2], it was later called the Universal Periods Spectrum (UPS).
The property of universality, first noted in [2], was confirmed by further studies, in particular, in the course of studying spectra in the time series of planarian chemiluminescence fluctuations [3], as well as temperature fluctuations of small mammals and birds [4–5], incl., with different levels of basal metabolism [5]. It was shown that the periods spectra found in [3] (using the APM method [1]), as well as in [4–5] (using spectral analysis, as well as cross-correlation analysis of various scales in time series of spatially separated measurements), coincide with UPS.
At the same time, the UPS in temperature fluctuations is the better expressed, the more the studied groups of animals are isolated from

each other. The condition of isolation (and, accordingly, the expressiveness of the UPS) is best satisfied in the case of spatially separated measurements, when the studied groups are separated by distances from hundreds of meters to kilometers or more. This result, in our opinion, indicates the external nature of the agent responsible for UPS obtained from time series of experimental animal's temperature fluctuations [4–5], as well as its biotropic nature. Obviously, the universal nature of the UPS also points to the external nature of the hypothetical agent: the same periods are observed both in the case of fluctuations in a physical system [2] and in biological systems [3–5].

Circachoralian rhythms (CR) - a set of periods in the range of approximately 20–120 min, have been identified in the dynamics of many biological systems. This rhythms characterize dynamics of the cell nucleus size, intensity of protein synthesis, enzyme activity, hormone and ATP concentrations, oxygen consumption, cytoplasmic pH, etc. [6- 8]. They are found in bacteria, yeast, some unicellular organisms, mollusks, and in mammalian cells. These rhythms are detected both in vivo and in vitro [6-7]. Despite the fact that most authors consider CR only as a result of internal regulatory processes of organism or intercellular interactions, the question about the nature of CR and a possible CR synchronizer remains open.

A review of numerous sources (for example, a collection of review papers [8]) made it possible to identify the most complete set of periods that various authors attribute to CR. Comparison of this set with UPS showed their good agreement. This result allows us to consider the CR, by analogy with the UPS, not as a set of independent periods, but as manifestations of a certain spectrum. At the same time, an external biotropic agent, which determines the presence of UPS in the dynamics of fluctuations in the parameters of biosystems [2–5], can also be considered as an external synchronizer of the CR spectrum.

It should be noted that the idea of an external CR-synchronizer, in fact, does not contradict the prevailing ideas about the 'internal' nature of CR, since the biological systems listed above in the dynamics of which CRs have been identified [6-8] can be considered as self-oscillatory with frequencies close to the frequencies of the UPS. As is known, the self-oscillatory nature of systems is a necessary condition for their synchronization. A number of biologically significant periods lie outside the range of 1–120 min considered in [2–5]. For this reason, the results of further studies of the UPS in the range >120 min, as well as some theoretical approaches to its description, will be considered.

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### S7.484. Pectin-based hydrogels mimic the microenvironment of the neural stem cell niche

Belousov A.S.[1]*, Shved N.A.[1,2], Grinchenko A.V.[1,2], Lanskikh D.V.[1], Malykin G.V.[2], Kuzyakova O.Yu.[1], Kovalev V.V.[2], Kumeiko V.V.[1,2]
[1]*Far Eastern Federal University;*
[2]*A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS;*
* andrei-belousov@mail.ru

Glioblastoma multiforme is the most common among adults, and also the most aggressive, of all brain tumors. Standard treatment for glioblastoma focuses only on tumor cells, ignoring their extracellular matrix (ECM). However, it has been proven that the key role in the development and progression of a tumor is played not by the tumor cells themselves, but by the extracellular matrix and the niche of tumor stem cells that it forms.

The use of materials that mimic the physicochemical properties of normal ECM and create a microenvironment for cells in the resection cavity after removal of a brain tumor can significantly improve prognosis. The required matrix material should imitate as much as possible the biomechanical properties of native tissue.

A promising application of hydrogels is their use as an implantable matrix material in regenerative medicine and transplantation for tissue structure remodeling and delivery of cells or drugs to the brain.

Carbohydrate polymers play a very important role in the ECM of the nervous tissue, forming a unique microenvironment that prevents the active proliferation and migration of nerve cells in the adult nervous system.

We have developed a set of hydrogels based on pectins with different contents of free carboxyl groups. All hydrogels are biocompatible and do not cause a significant immune response when implanted subcutaneously. The rate of biodegradation depends on the degree of esterification of pectin. The elastic moduli of hydrogels range from 100 to 1100 Pa depending on the degree of esterification, which corresponds to the viscoelastic properties of a normal human brain. All hydrogels used as matrices for cell culture maintain high viability of neural stem cells (NSCs), C6 glioma cells, and N2a neuroblastoma cells, reduce the proliferation rate, protect NSCs from differentiation, and preserve neurospheres whose morphology depends on the degree of pectin esterification. Molecular mechanisms of the action of the biomaterial on cells include suppression of the MAPK/ERK signaling pathway. Transcriptom analysis revealed changes in the cell cycle regulation pathways that maintain high levels of expression of several components necessary for maintaining the ability to proliferate, while the detected high expression of inhibitory factors may be responsible for slowing down the proliferation of NSCs and maintaining the stemness of cells cultured on pectin hydrogels.

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### S7.485. Peculiarities of spatial frequency distribution of electrostatic potential change around genomic DNA of bacteriophage T7 in the region of promoters specific for native phage and host RNA polymerase of E.coli

Glytov I.V.[1,3], Osypov A.A.[1,2]*
[1]*ITEB RAS;*
[2]*IHNA&NPh RAS;*
[3]*RSMU;*
* aosypov@gmail.com

DNA is a highly negatively charged molecule, and its electrostatic interactions with proteins play a crucial role in the implementation

of genetic information, in particular, the regulation of transcription. The charge along the DNA molecule is unevenly distributed and correlates with its biological functional elements, ensuring the regulation of biological processes.
The aim of this work was to analyze the uneven distribution of the electrostatic potential along the DNA molecule in terms of revealing the distribution of spatial frequencies of potential changes, in particular - to identify the differences between the frequency characteristics of electrostatic potential distribution around the DNA molecule of T7 phage in the area of its native and host (E.coli) promoters. A wavelet transform using the Morlet wavelet was chosen as the tool of analysis. Frequency analysis was performed using specially written Python programs. The data were taken from the DEPPDB database [1, 2].
Autocorrelation analysis of the T7 phage genome showed a correlation above 0.8 for fragments less than 100 angstroms, followed by a smooth decrease to insignificant values and did not reveal any large-scale periodicity in the potential distribution.
Comparison of the wavelet spectra showed that in the promoter regions, compared to the genome-wide average, the wavelet power of 50-70 Angstrom waves was significantly higher, i.e., about 14-20 bp, which roughly corresponds to the scale of RNA polymerase/whole protein subunit binding sites. In the host promoter region and genome-wide average, the wavelength power of 20-30 Angstrom (6-9 bp) was similar, but when comparing promoter spectrograms, it was higher in the host promoter region than in the phage region. The planting site size of the phage polymerase corresponds to the obtained wavelengths in the region of the phage promoters.
The identified features of native E. coli promoters require further investigation in view of their small sample size in T7 bacteriophage genome in comparison with the host chromosome in view of considerable difference of the corresponding RNA polymerases.
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### S7.486. Photodynamic action in pulse mode irradiation advantage for suppression of tumor spheroids growth

Bogdanov A.A.[1]*, Klimenko V.V.[1], Bogdanov An.A.[1], Knyzev N.A.[1], Moiseyenko V.M.[1]
[1]*Saint Petersburg Clinical Research and Practical Centre of Specialized Types of Medical Care (Oncological), Saint Petersburg, Russia;*
* aleks_aa@mail.ru

Background
Photodynamic therapy (PDT) has a cytotoxic effect on tumor cells due to the generation of singlet oxygen. The depletion of molecular oxygen in the tumor tissue is the limiting factor for the antitumor effect of PDT throughout the entire thickness of the tissue. Three-dimensional tumor spheroids are good model of tumor tissue with limited oxygen supply. The purpose of this work was to determine the effect of laser irradiation parameters during PDT on the efficiency of growth inhibition of tumor spheroids.
Methods
The CT26 murine colon adenocarcinoma cell line was used. The cells were cultured in 5% CO2 incubator in RPMI-1640 culture medium supplemented with 10 % FBS and penicillin/streptomycin. 3D spheroids were generated by seeding of 1000 cells in 100 µl complete medium and cultured for 5 days in 96 well round bottom plates (96 well ULA plate, Corning, USA). Tumor spheroids with a diameter of 450-600 µm were incubated with the chlorine e6 photosensitizer at a concentration of 5 µg/ml in 200 mkl complete medium. PDT was performed 24 hours later using 662 nm laser ith continuous wave (CW) mode and pulsed irradiation (Pulse) mode at fluency rate 50 mW/cm2 with irradiation dose 5, 10, 15, 60 J/cm2.
Results
It was found that a decrease in the average power density from 50 mW/cm2 to 12.5 mW/cm2 during continuous PDT with an irradiation dose of 15 J/cm2 leads to an increase in the damage area of CT26 tumor spheroids in depth from 50-60 µm up to 160 µm. The experimental comparison of the Pulse and CW modes has showed that the maximum growth inhibition rate of CT26 tumor spheroids was observed for the optimized pulsed irradiation parameters with a fluence rate of 50 mW/cm2, pulse duration of 200 ms, and repetition period of 800 ms, ratio 1/4. A further increase in the duty cycle up to 1/16 did not increase the cell cytotoxicity either in vitro. The comparison of the photodynamic cytotoxic effect on cell viability in cell monolayer and 3D spheroid models was performed. IC50(PDT) for monolayer CT26 cells was about 2.5 J/cm2 for both CW and Pulse modes. IC50(PDT) for CT26 tumor spheroids was about 7.5 J/cm2 for Pulse mode and 20 J/cm2 for CW mode. Flow cytometry data obtained for single cell suspension of CT26 spheroids at 24h after PDT with CW and Pulse mode using Annexin V Detection kit have demonstrated the increasing of Annexiv V positive cells fraction from 43,1 % at CW mode to 93,4 % at Pulse mode and the significant reduction of live cells fraction from 56,2 % at CW mode to 5,5% at Pulse mode at 50 mW/cm2 and irradiation dose 15 J/cm2.
Conclusion
Using of the optimized Pulse mode PDT has reduced mean fluency rate to 12.5 mW/cm2 and demonstrated the greater suppression of CT26 tumor spheroids growth in compare with using of CW mode due to reoxygenation and normalized efficiency of PDT in the cell proliferating region of 3D spheroids.

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### S7.487. Physico-chemical factors of the influence of low-temperature atmospheric pressure plasma on single-cellular organism Paramecium caudatum

Karpukhina O.V.[1]*, Gruzdev G.A.[1], Savinov V.P.[1], Yakunin V.G.[1], Inozemtsev A.N.[1], Timoshenko V.Yu.[1,2], Kamensky A.A.[1]
[1]*M.V. Lomonosov Moscow State University;*
[2]*National Research Nuclear University, Moscow Engineering Physics Institute;*
* karpukhina.msu@yandex.ru

We have studied the effects of low-temperature atmospheric pressure plasma generated by a plasmatron in an inert gas (argon, helium) and in a mixture of argon and air on the Paramecium caudatum cell culture [Karpukhina et al.; 2018; Savinov et al., 2019; Gruzdev et al., 2021]. The plasmatron is a high-resource arc discharge source [Ryaby et al., 2017], operating at atmospheric pressure with a constant arc voltage at the level of 20–40 V; the flow rate of the inert gas blowing the tungsten cathode was equal to 2 l/min., the

flow rate of the air supplied to the process channel was 10 l/ min., the electric arc current was equal to 50 A. The original design of the plasmatron ensures the release of an ultra-pure plasma jet into the surrounding air, which is practically free of metal particles of the electrodes. To study the effect of plasma on a biological system, Paramecium caudatum cells turned out to be a suitable test object for a quick comprehensive analysis of the simultaneous effects of such physicochemical factors as radiation of various types and fluxes of active radicals O, N, OH-, NO-, NO_2^-, O2( 1Δg). Even when pure plasma-forming inert gases are used, particles containing O and N get into the plasma jet propagating outside the discharge gap due to their diffusion into the jet from the surrounding air.

Since Paramecium caudatum is a single–celled organism that freely move in an aqueous environment, at the time of plasma treatment, most of the cells were not on the liquid'surface (h = 7mm) in a micro test tube (d= 10mm), but moved throughout the volume; the maximum allowable removal of cells from the nozzle of the plasma source was 20 cm; plasma temperature values were fixed at 28-30 ° C. The time of plasma exposure to cells varied from 0.5 to 5 minutes. One of the potentially significant factors of plasma exposure to Paramecium caudatum was a change in the ionic composition of the aqueous environment in which the cells were located. The degree of change in the pH of the medium depended on the composition of the plasma and on the time of its exposure. The result of the action of the plasma of inert gases was a slight decrease in the pH of the aqueous solution with cells, while a more noticeable effect was observed for argon plasma. After processing the micro test tube with cells with a plasma jet, which included argon and air, there was a significant decrease in the pH of the aqueous medium (by 2.5 from the initial 6.5-7), which led to serious violations of the movement of Paramecium caudatum in the initial 30 minutes, the cells moved slowly or rotated in place. The motor activity of Paramecium caudatum is formed on the basis of the work of ion channels integrated in the membrane of special organs of movement – cilia. In an acidic environment, the membranes are depolarized, which leads to a decrease in the amplitude of the beating of the cilia, a decrease in the speed and reverse movement of cells. In addition to motor disorders after a day in most cells exposed to 3 min and 5 min after plasma treatment, significant morphological changes characteristic of apoptosis appeared, such as membrane stratification, vacuolization and fragmentation of the cytoplasm before degeneration.

The reactions of the cells observed by us are a consequence of the influence of long–lived reactive oxygen and nitrogen species (NO, O, OH, ONOO -, O2(1Δg)), which are transferred from the plasma medium to the aqueous medium. The plasma source we use with a DC arc discharge in a plasma-forming inert gas creates, on average, concentrations of NO and OH radicals of at least 500 ppm, and thus, when exposed to a plasma jet in the vital environment of Paramecium caudatum, redox processes are triggered with the formation of hydrogen peroxide concentrations critical for the normal functioning of cells. In addition, the presence of free radicals in the medium disrupted the reproduction of Paramecium caudatum, which indicated the effect of plasma on the nuclears of cells, while the most expressed effect of plasma after effect (72 hours) on the reproduction of Paramecium caudatum was observed in the case of the use of helium or a combination of argon with air.

The potential effectiveness of low-temperature plasma on microorganisms demonstrates the possibility of its wide use, for example, for water purification from biocontaminants or treatment of medical instruments.

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## S7.488. Polydopamine Functionalized Composite Photothermal Scaffolds for Complex Modulation of Cellular Activity

Raikhman E.V.[1]*, Kanev I.L.[1], Kochetkova O.Yu.[1], Antonova O.Yu.[1]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
* elenaraikhman@ya.ru

The search for non-invasive remote methods for modulating cellular activity is an important trend in various fields of biomedicine. In particular, the control of directed differentiation and transdifferentiation of stem cells is of undoubted interest for regenerative medicine. One of the approaches to modulate cell activity is photothermal treatment, which has already found application in cell therapy and tissue engineering [1]. Photothermal scaffolds are three-dimensional structures or surfaces modified with components capable of absorbing light with the appropriate wavelength, converting light energy into heat. Such materials have proven themselves in the field of hyperthermic ablation of tumor cells and controlled delivery of exogenous bioactive molecules. At the same time, another promising direction is the development of integrated approaches to nanotopolog-mediated (the surface ultrastructure) and photothermal stimulation of cellular activity [2]. This report presents the results of the work on the development of composite photothermal scaffolds from aligned polyamide nanofibers, close in diameter to the components of the extracellular matrix (~100 nm). The fibers were coated with a film of light-converting polydopamine (PD) biopolymer to implement the photothermal effect. The coating technology allows us to vary the density of applying PD to the fibers. Using fibers with different diameters for modification, we obtained composite materials with different architecture and different thicknesses of the PD coating. The intensity of heating of the substrates during irradiation depended on the PD coating density and reached 79°C when irradiated with near-IR radiation on air with a wavelength of 808 nm and an intensity of 3 W/cm2. The efficiency of photoconversion of PD-coated fibrous materials is significantly higher than when copper sulfide-based plasmonic nanoparticles were used to coat the fibers [2]. The PD-functionalized scaffolds did not demonstrate any cytotoxic effect when culturing human neuroblastoma SH-SY5Y cells, which was chosen as a model object for studying the neurogenicity of the material and its ability to influence the cell differentiation process. Cultivation on the surface of composite nanofibers initiated directed growth of neurites in the absence of differentiating factors. The change in intracellular temperature, measured by fluorescent thermometry, was 15.6±2.3°C upon irradiation with an intensity of 3 W/cm2 for 5 min. Previously, it was shown that intracellular heating in this range has a stimulating effect on the differentiation of mesenchymal stem cells [3]. We also showed that photothermal heating of PD-coated scaffolds initiates the release of calcium ions, and therefore allows remote neuromodulation. The obtained results indicate the possibility of implementing a complex stimulating effect of photothermal heating and substrate architecture on cell growth, proliferation, and differentiation.

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### S7.489. Polymer-based microcarriers containing selenium and gold for combined photothermal and ROS-mediated therapy

Mitusova K.A.[1]*, Rogova A.[1,2,3], Timin A.S.[1,2]
[1]*Peter the Great St. Petersburg Polytechnic University (Spbspu);*
[2]*ITMO National Research University;*
[3]*Saint Petersburg State Chemical Pharmaceutical University;*
* mitusova.kseniya@mail.ru

Recently, multimodal combined photothermal therapy (PTT) using photoactive materials has attracted considerable attention to cancer treatment. However, drug carriers that provide effective heating at the site of the tumor have yet to be developed: this is a fundamental requirement for the widespread introduction of FTT in clinics. In this work, we design and develop hybrid carriers based on multilayer capsules integrated with selenium nanoparticles (Se NPs) and gold nanorods (Au NRs) for the implementation of combined FTT mediated by reactive oxygen species (ROS).

Polymer capsules were synthesized by the method of layer-by-layer deposition, in the core of which were Au NRs and Se NPs. For this purpose, Se NPs with a diameter of $30 \pm 5$ nm were synthesized, dispersed in water and Au NRs with an average length of $114.8 \pm 13.9$ nm and a width of $20.3 \pm 2.0$ nm. Se NPs were stabilized with polystyrene sulfonate (PSS), giving a negative zeta potential, $\zeta = -42.5$ mV, and Au NRs stabilized CTAB as a surfactant, providing a positive zeta potential in an aqueous solution $\zeta = +21.2$ mV.

We investigated the photothermal characteristics of polymer capsules with embedded in the core Se NPs and Au NRs, dispersed in water when irradiated with a laser with a wavelength of 1064 nm, using an infrared thermal imaging camera. Based on the data obtained, the photothermal conversion efficiency was 36.15%. Thus, the combination of Se NPs and Au NRs inside polymer capsules increases the efficiency of photothermal conversion.

To study the antitumor activity of the obtained polymer capsules in vitro, we varied the number of NPs inside the polymer capsules: Se NPs (5, 20, 50 and 100 μg) and Au NRs (5, 10, 50 and 60 μg). To assess the cytotoxicity and photothermal effect of all tested samples, we used three methods: calcein AM/propidium iodide, resazurin analysis and flow cytometry with 7-AAD. For all three methods, melanoma cells (B16-F10) were incubated with capsules in a ratio of 1:10 for 12 hours. Then several samples were irradiated with a laser with a wavelength of 1064 nm (2.4 W/cm2) for 5 min. The control samples were not irradiated with a laser. After 24 hours of incubation, calcein AM and propidium iodide were added to the samples to detect living and dead cells. CLSM images showed that Se NPs immobilized in polymer capsules induced cell death depending on the concentration of Se NPs and showed similar antitumor properties both under laser irradiation and without it. From the literature data, Se NPs can induce apoptosis in cancer cells due to the formation of ROS. To confirm this, we measured ROS activity in B16-F10 cells incubated with Se NPs (5, 20, 50 and 100 μg) using H2DCFDA (2',7'-dichlorodihydrofluorescein diacetate). H2DCFDA penetrates into cells, and in the presence of ROS it is oxidized to dichlorofluorescein: thus, intracellular formation of hydroxyl, peroxyl and other forms of ROS can be detected. Indeed, incubation of cells with Se NPs in the core of polymer capsules led to an increase in the intensity of cellular fluorescence, i.e. the level of ROS, in a dose-dependent manner, and control cells without Se NPs did not show pronounced fluorescence. In addition, for laser-treated samples, Au NRs showed negligible toxicity to B16-F10 cells in a wide range of concentrations. However, under laser irradiation, Au NRs demonstrated antitumor abilities due to the photothermal effect. Also, the combination of Se NPs and Au NRs in one capsule caused a more pronounced antitumor effect, which is confirmed by the data of resazurin analysis and flow cytometry-the percentage of viability of melanoma cells was less than 10% when exposed to laser radiation.

To study the antitumor efficacy of the developed capsules with Se NPs and Au NRs, B16-F10 tumor-carrying mice were used (the tumor size was about 100 mm^3 or 0.1 cm^3). After intratumor injection of the tested capsules (50 μl with 100 μg Se and 60 μg Au), the tumors were irradiated with a laser with a wavelength of 1064 nm for 5 minutes per animal. Changes in average tumor volumes were evaluated every 3-4 days. Analyzing the experimental data obtained, we can conclude that the combined effect of Se NPs and Au NRs enclosed in the core of capsules and exposed to laser irradiation (1064 nm) was much higher compared to capsules containing only one of the types of NPs (Au NRs or Se NPs). This combination leads to an increase in antitumor efficacy against melanoma B16-F10, which can be explained by two different mechanisms of action on cancer cells during such combination therapy. Firstly, Se NPs in polymer capsules induce intracellular generation of ROS, which leads to apoptosis of tumor cells. Secondly, Au NRs triggers a photothermal effect that suppresses the tumor. In addition, Se NPs enhances the heating of Au NRs, which suggests that our combined treatment method allows us to achieve synergistic therapeutic effectiveness.

Thus, in this work we have proved that the combination of Se NPs and Au NRs in the core of polymer capsules allows us to obtain hybrid carriers with promising ROS-mediated and photothermal properties for enhanced combined FTT against melanoma tumor growth. This method has high therapeutic efficacy against melanoma tumors B16-F10 without any significant side effects on healthy organs (heart, lungs, kidneys, liver and spleen). This study expands the scope of application of the Se/Au complex as a highly effective photothermal agent for the development and synthesis of multifunctional drug delivery platforms (i.e. polymer multilayer capsules) and their further use in combined ROS-mediated and photohemal therapy of malignant neoplasms.

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### S7.490. Possible mechanisms of the influence of low deuterium concentrations on living systems

Lyasota O.M.[1]*, Basov A.A.[2,3], Dorohova A.A.[1,2], Kozin S.V.[1,2], Moiseev A.V.[4], Danshin N.A.[2], Dzhimak S.S.[1,2]
[1]*SSC RAS;*
[2]*Kuban State University;*
[3]*Kuban State Medical University;*
[4]*Kuban State Agrarian University named after I. T. Trubilin;*
* artsybashevao@mail.ru

Deuterium-depleted water (DDW) has potential in antitumor therapy, especially in relation to methods that cause oxidative stress in cancer cells [1]. DDW can alleviate obesity-induced conditions and associated metabolic disorders [2].

The introduction of DDW into the diet leads to the formation of an isotope D/H gradient between blood plasma and organ tissues in the first three weeks. There is a decrease in the concentration of deuterium in the blood by 93.6% and in the tissues of the liver, kidney and heart by 37.6%, 39.9% and 37.6%, respectively, which is accompanied by a change in the adaptive capacity of the organism [3].

In order to study the mechanisms of the effect of the D/H gradient on energy metabolism in the liver, the dynamics of hydrogen peroxide production by isolated rat liver mitochondria was studied depending on the presence of preliminary adaptation to a reduced content of deuterium in the drinking diet in vivo and incubation in vitro in a medium with a reduced concentration of deuterium. When incubated in a medium depleted of deuterium, an increase of 35% in the generation of hydrogen peroxide by mitochondria isolated from hepatocytes of rats that consumed drinking water with a reduced concentration of deuterium (46 ppm) was revealed, compared with mitochondria from liver cells of rats that consumed water

with a deuterium concentration of 152 ppm. The revealed change in the functional activity of mitochondria indicates the ability of the animal body to adapt to the mode of consumption of drinking water depleted in deuterium, which may be due to the formation of a transmembrane D/H isotope gradient [4]. The results obtained correlate with the data of X. Zhang et al. They found that DDW inhibits cell proliferation, mainly by causing an imbalance between the production and neutralization of ROS in mitochondria and, thus, causing oxidative stress in cells [5]. According to the same work, DDW modulates the expression of proteins involved in the following cellular processes: the cell cycle, oxidoreductase activity, glutathione metabolism, etc. deuterium, due to which transcription processes can change, which in turn will affect expression.

To test this assumption, we carried out the following model experiments: to simulate the processes of unwinding the DNA double helix, the formation of open states and bubbles in the DNA double helix, we used a mathematical model that describes the rotational movement of nitrogenous bases around the sugar-phosphate backbone of the DNA molecule [6].

Evaluation experiments on the effect of D/H isotope exchange on base pair opening processes carried out by mathematical modeling methods show that the presence of deuterium in a nucleotide sequence can lead, depending on the values of the hydrogen bond breaking energy, both to an increase and a decrease in the probability of the occurrence of open states. [7,8]. Under natural conditions, the deuterium atom is more likely to slow down the rate of reading genetic information in transcription processes, narrowing the range of regulatory mechanisms under persistent action during the cell cycle of a low-intensity unfavorable factor and leading to a decrease in the adaptive potential of the cell.

Moreover, one cannot rule out other mechanisms of realization of isotope effects when they are included in macromolecules, for example, those associated with isotope resonance [9, 10] in living systems. Therefore, even when only one protium atom is replaced by deuterium in the DNA molecule and the average rate of DNA replication is the same, separate periodic slowdowns can occur and, in this case, equivalent to them in terms of the total severity of acceleration. Although generally leveling each other, but capable, due to a change in the intracycle pattern of reading genetic information, lead to a general accumulation of errors in its reproduction, accompanied at a certain stage by the transition of quantitative changes (the number of replication failures) into qualitative defects in the DNA structure.

Thus, the participation of deuterium atoms in the formation of hydrogen bonds in double helixes of DNA molecules can lead to a change in the time of transmission of genetic information, which can explain the effect of even small changes in the concentration of deuterium in the environment on metabolic processes in living systems.

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## S7.491. Potassium comenate increases the resistance of rat cerebellar neurons to the glutamate excitotoxicity in vitro

Kravtsov A.A.[1,2,3]*, Kozin S.V.[1,2,3]
[1]*Kuban State University;*
[2]*Kuban State Technological University;*
[3]*Southern Scientific Center, Russian Academy of Sciences;*
* aakravtsov@mail.ru

The problem of prevention and therapy of pathological conditions of the nervous system associated with excitotoxic and oxidative damage will remain relevant in the coming decades. This determines the relevance of the search for effective means of pharmacological correction and prevention of these pathologies. The aim of this work was to study the neuroprotective activity of potassium comenate (PK) under the excitotoxic effect of glutamate (Glu) in cultures of rat cerebellar neurons.

The potassium comenate compound is distinguished by the fact that the potassium cation and the comenic acid anion included in its composition are themselves biologically active. The main functions of potassium in the body are: maintaining the constancy of the composition of the cellular and intercellular fluid, acid-base balance, providing intercellular contacts, bioelectrical activity of cells, maintaining neuromuscular excitability [3]. Comenic acid (5-hydroxy-γ-pyrone-2-carboxylic acid) has a wide spectrum of biological activity: it has an antioxidant property, a mild sedative effect, anti-withdrawal, anxiolytic and antidepressant properties [4, 5]. The above properties of the potassium cation and the comenic acid anion served as the basis for studying the neuroprotective properties of PK.

In the work, we used PK synthesized in the Shurygin A.Ya. Department of Biologically Active Substances Kuban State University [1].

Cerebellar granular cell cultures were obtained from the brains of 7–9 day old Wistar rat pups by enzyme mechanical dissociation as described previously [2]. Experiments were performed after 7 days of cultivation. Cultures were treated with 100 μM Glu for 10 minutes. PK at concentrations from 1 mM to 0.001 mM was introduced into cultures after exposure to Glu. After 4.5–5 hours, the cultures were fixed, stained with trypan blue, and the number of living and dead neurons was counted using an Invertoscopes ID 03 microscope.

Intracellular calcium levels were assessed using Fluo4-AM on a FilterMax F5 multifunctional reader. Fluo4-AM was added to the cultures before Glu exposure for 30 minutes. Fluorescence was recorded at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The results are presented in %, the level of fluorescence of control cultures not exposed to Glu and PK was taken as 100%.

The antioxidant properties of PK were evaluated in vitro, in the CFL model system (citrate-phosphate buffer with luminol). The formation of reactive oxygen species (ROS) was initiated by introducing a 35 mM solution of iron sulfate. Registration of chemiluminescence was carried out with a SmartLum 5773 device for 5 minutes, the light sum was estimated. The effect of PK on ROS generation was evaluated in comparison with comenic acid. The results were calculated as % of the control, which was taken as 100%.

To assess the significance of differences between samples, Student's t-test was used.

CK in the absence of Glu, regardless of the concentration, did not affect the survival of neurons. The impact of Glu led to a sharp decrease in intact cells to 29.6%. The addition of KA to Glu-treated cultures resulted in increased survival at all concentrations tested ($p<0.05$). The highest survival of neurons in comparison with Glu (by 35.9% more) was observed at a concentration of 0.1 mM - the proportion of intact neurons was 65%.

In experiments with Fluo4-AM, exposure to Glu led to an increase in the level of calcium in neurons up to 187.3±3.7%. The addition of PK at concentrations of 1.0, 0.1 and 0.01 mM to cultures exposed to glutamate significantly reduced the level of calcium: to 158.3±9.6%, 162.3±7.2% and 153.1±7.3%, respectively. The impact of PK on cultures not exposed to glutamate did not significantly affect the level of calcium in neurons.

The results of a comparative study of the antioxidant properties of PK and comenic acid in vitro showed that PK significantly reduces the content of free radicals in the CFL model system. The level of reduction of free radicals PK has a dose-dependent effect and practically does not differ from the level of quenching of free radicals by comenic acid: at 0.1 mg / ml - a decrease by 65% and 69%, at 0.01 mg / ml - a decrease by 34% and 33 %, respectively for PK and comenic acid.

Thus, it was found that the use of PK against the background of glutamate cytotoxicity contributes to a significant decrease in the death of cerebellar neurons in culture. We guess that this effect is mediated by the antioxidant activity of PK, as well as the effect on ionic homeostasis.

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### S7.492. Potential of hydrogels based on modified pectins and tuning of their properties for brain tumor therapy

Patlay A.A.[1]*, Belousov A.S.[1], Shmelev M.E.[1], Silant'ev V.E.[1,2]
[1]*Institute of Life Sciences and Biomedicine, Far Eastern Federal University;*
[2]*Laboratory of Electrochemical Processes, Institute of Chemistry, FEB RAS;*
* patlai.aa@dvfu.ru

Mechanical signals of the extracellular environment play an important role in differentiation, metabolic activity, cell migration, adhesion, etc. [1, 2]. Therefore, when designing biomaterials for biomedicine, it is necessary to have a complete understanding not only of the chemical, but also of the structural and mechanical properties of the polymer under study. Hydrogels are promising materials for recreating the cellular environment and can be used both in the form of three-dimensional structures and in the form of functionalized coatings [3, 4].

The plant polysaccharide pectin forms biocompatible and biodegradable non-toxic hydrogels due to the ion gelling mechanism. In addition, pectin resembles hyaluronic acid in its structure, which is the main component of the ECM of the adult brain [5].

Therefore, the purpose of this work was to study the structural and viscoelastic properties of hydrogels and coatings based on modified pectins and their effect on neural cells in vitro.

We have developed variants of gels with different structures and viscoelastic properties, changing the concentration of pectin and the number of free carboxyl groups (degree of esterification, DE). The accumulation modulus of hydrogels increased exponentially with increasing concentration of pectin powder and varied in the range from 3 to 900 Pa. We selected pairs of materials with 0% and 50% DE with similar rheology over 100 Pa for remodeling the extracellular matrix of the central nervous system. The features of the swelling of hydrogels and their stability in vitro, as well as the structure studied using SEM and FTIR, differed, which may be important for biomedical applications. The mechanical and morphological characteristics of hydrogels were also studied in the coating format using AFM. Bioassays on glioblastoma C6 and U87MG cultures have shown the antigliomic potential of using hydrogels by reducing the proliferative and metabolic activity of cells and modulating their migration, while maintaining high viability of nerve cells. At the same time, materials with a DE of 50%, regardless of concentration, had a stronger inhibitory effect on the metabolism of tumor cells than materials with a DE of 0%.

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### S7.493. Properties of composite materials based on polymeric matrices of borosiloxane, poly(lactide-co-glycolide), fluoroplast, functionalized with metal oxides nanoparticles (zn, fe, al)

Burmistrov D.E.[1]*, Simakin A.V.[1], Baymler I.V.[1], Uvarov O.V.[1], Ivanov V.E.[1], Chausov D.N.[1], Gudkov S.V.[1]
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences (GPI RAS), Moscow, Russia;*
* dmitriiburmistroff@gmail.com

Today, the possibility of using metal and metal oxides nanoparticles (NPs) as antibacterial agents is being considered, due to the rapidly developing bacterial antibiotic resistance. One of the promising approaches is the creation of composite materials, including those based on polymers, which provide the possibility of using such materials in biomedical applications, the food industry, and also in domestic use.

In the course of the work, nanoparticles of metal oxides (zinc, iron, and aluminum) were synthesized by laser ablation. The maximum distributions of the ζ-potential of the obtained colloidal solutions of NPs were 20, 20, and 50 mV for NPs of zinc, iron, and aluminum oxide, respectively, which characterized the colloidal solutions of NPs as stable. The composition of the nanoparticles was determined using energy dispersive X-ray spectroscopy (EDX), the morphology was assessed using transmission electron microscopy. The nanoparticles had the required chemical purity. It was found that zinc oxide nanoparticles have a rod-like morphology, while those of iron and aluminum oxides have a spherical one. The average hydrodynamic diameter of NPs was estimated using dynamic light scattering (DLS) and was about 55, 50, and 45 nm for NPs of zinc, iron, and aluminum oxide, respectively. The obtained nanoparticles were added to polymers of poly(lactide-co-glycolide) (PLGA) and borosiloxane (BS), as well as polytetrafluoroethylene (PTFE) with final concentrations of nanoparticles in material samples of 0.001, 0.01, and 0.1%. For further studies, the materials were formed into films with a thickness of 700–900 μm (for BS and

PLGA) and 200 μm for PTFE. The surface morphology of the materials was assessed using atomic force microscopy. It was found that the surface of the samples of composite materials was defect-free. Using modulation-interference microscopy, it was found that nanoparticles are distributed in the bulk of polymers in the form of clusters, the size of which increases with increasing NP concentration. It was shown that all obtained nanocomposites exhibited bacteriostatic properties against E. coli. It is important to note that this effect was found even when using composites with a minimum concentration of zinc and iron oxide NPs in the composition (0.001%), however, significant differences during cultivation in contact with composites containing aluminum oxide NPs were observed at a concentration of these NPs in the polymer of 0.01 and 0.1%. Composite materials contributed to the formation of ROS in aqueous solutions (hydrogen peroxide and hydroxyl radicals). It was also found that the resulting materials contributed to the formation of long-lived reactive forms of proteins and 8-oxoguanine in DNA. Composite materials based on aluminum oxide NPs exhibited weaker activity compared to materials containing nanosized iron and zinc oxides. In turn, studies of the cytotoxicity of materials with respect to eucariotic cells showed that the surface of films of composite materials based on PTFE, BS, and PLGA containing NPs was suitable for the growth and development of these cells. Due to low cytotoxicity and bacteriostatic properties, the obtained composite materials are of great interest as packaging and coatings for the food industry, dry disinfectant, as well as components and coatings for biomedical applications.

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### S7.494. Protective effect of 2-hydroxypropyl-beta-cyclodextrin and osmolytes on the stability and aggregation of glutamate dehydrogenase induced by elevated temperature and freeze-thaw cycle

Borzova V.A.[1]*, Chernikov A.M.[1], Mikhaylova V.V.[1], Chebotareva N.A.[1]
[1]*FRC "Fundamentals of Biotechnology" RAS;*
* vera.a.borzova@gmail.com

The effect of 2-hydroxypropyl-beta-cyclodextrin (HPCD) and osmolytes (trehalose, sorbitol, betaine) on the stability, aggregation kinetics, and oligomeric state of glutamate dehydrogenase (GDH) from bovine liver was studied by differential scanning calorimetry, dynamic light scattering, and analytical ultracentrifugation, respectively. Two aggregation test systems were used: thermal aggregation of GDH and aggregation induced by the freeze-thaw cycle. It was shown that all the studied compounds exhibit a protective effect on the target protein. In the case of native GDH, the thermal stability of the protein increases, while after freeze-thaw cycle, trehalose and sorbitol prevent the complete loss of the native protein structure. Additionally, HPCD and betaine increased the thermal stability of freeze-thawed GDH by increasing the denaturation temperature Tmax. According to analytical ultracentrifugation, all the studied compounds protect GDH from aggregation (and in the case of freeze-thaw cycle, from complete precipitation), stabilizing the native hexameric form of GDH with a sedimentation coefficient of 14.5 S, small oligomers of the target protein and its dissociated forms. Quantification of the antiaggregation activity of HPCD and osmolytes was carried out by analyzing the kinetics of GDH aggregation at 50 deg. C and freeze-thaw-induced aggregation of GDH at 25 deg. C. From the ratio of initial aggregation rates in the presence of various concentrations of osmolytes and HPCD (v0) and in the absence of additives (v0(0)) the half-saturation concentration [L]0.5 for each compound was calculated. In the case of thermal aggregation, trehalose demonstrated the highest antiaggregation activity ([L]0.5 = 142 mM). In the case of freeze-thaw-induced aggregation, betaine had the greatest protective effect: aggregation at 25 deg. C after thawing was not observed even at the lowest concentrations of betaine studied (10-50 mM).

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### S7.495. Quantitative analysis of growth and death of tumor cells in collagen hydrogel in response to deprivation and therapeutic exposure using fluorescent imaging

Sencha L.M.[1]*, Dobrynina O.E.[1], Pospelov A.D.[1], Guryev E.L.[1], Cherkasova E.I.[1], Balalaeva I.V.[1]
[1]*Lobachevsky State University;*
* luda-sencha@mail.ru

The tumor microenvironment is currently considered as one of the most important factors in the formation of the cell phenotype, which ensures their existence and proliferation under conditions of hypoxia and nutrient deficiency, as well as cell resistance to the effects of cytotoxic compounds. Therefore, three-dimensional in vitro models, which are more relevant to the complex structure of the tumor in vivo, are becoming increasingly popular. The transition from 2D to 3D cell cultures requires modification of methods for assessing tumor growth, while the vast majority of the methods used require the destruction of the gel structure, which makes the study expensive and time-consuming. The aim of this work was to develop a method for quantitative assessment of tumor cell growth in collagen hydrogel using a fluorescent approach and to test it in the study of cell response to unfavorable cultivation conditions and cytotoxic effects.

We used SKOVip-kat human ovarian carcinoma cells with the TurboFP635 red fluorescent protein gene and A431-GFP human epidermoid carcinoma cells with the GFP fluorescent protein gene. When creating a 3D cell culture, a solution of type I collagen was used. Cells were embedded in nutrient-enriched hydrogels by mixing the cell suspension with the ingredients of the gel. The resulting gels were incubated for 8-10 days at 37°C and 5% CO2. Cell growth was controlled by direct counting of their number after gel degradation, as well as by analysis of the total DNA content in the gels. In addition, we have proposed an approach based on the registration of the integral fluorescence of gels without their destruction. To do this, images of the gels were obtained daily using a surface fluorescence imaging setup and image processing was performed using the ImageJ program. The growth rate of tumor cell cultures was compared under standard conditions, under conditions of nutrient deprivation (culture medium without FBS or glucose) and hypoxia (1% O2). In addition, the response of cells to the action of cisplatin and the targeted antitumor toxin DARPin-LoPE was analyzed. A comparison was made of the response of cells to the action of the listed factors during their cultivation in collagen hydrogel and in a monolayer culture.

The proposed approach based on real-time fluorescence registration showed good agreement with direct counting of cells isolated from the hydrogel and measurement of the DNA content in the gels. The advantage of the proposed approach is the monitoring of cell culture growth in hydrogels without their destruction, which significantly reduces the resource consumption and labor intensity of the study. Analysis of the influence of microenvironmental factors on 3D models using fluorescence visualization made it possible to evaluate differences in the sensitivity of A431-GFP and SKOVip-kat tumor cells to serum, glucose, and oxygen deprivation. At the same time, the A431-GFP culture showed high resistance to serum deficiency, but showed a strong sensitivity to glucose and oxygen deprivation. The SKOVip-kat cell line, on the contrary, was highly sensitive to the action of serum deprivation, while showing relative insensitivity to glucose and oxygen deprivation. Using the proposed approach, it was also shown that culturing in collagen hydrogel led to an increase in the resistance of SKOV3.ip-kat and A431-GFP cell lines to cisplatin. In the case of SKOV3.ip-kat, the increase in resistance was 4-fold, while in the case of A431-GFP, it was more

than 40-fold. A comparative study of the effect of various factors on 2D and 3D models showed that the presence of tumor cells in collagen gel also significantly modifies their sensitivity and can lead to both a decrease and an increase in resistance.
Thus, the proposed method of fluorescent imaging can be used to monitor the growth of tumor cells in collagen hydrogel in real time, as well as to record the response to unfavorable cultivation conditions and the action of antitumor agents. At the same time, this method can significantly reduce the cost of cell toxicity studies on 3D models of tumor growth.
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### S7.496. Radiomitigatory properties of α-lipoic acid and its use in combination with metformin and ethylmethylhydroxypyridine succinate in mice upon X-ray irradiation

Karmanova E.E.[1,2]*, Chernikov A.V.[2], Usacheva A.M.[2], Bruskov V.I.[2]
[1]*Institute of Cell Biophysics, Pushchino Scientific Center for Biological Research, Federal Research Center of RASciences, Pushchino, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* silisti@bk.ru

Introduction. α-Lipoic acid (LA, thioctic acid) is an effective antioxidant used in the treatment of diseases associated with oxidative stress and can potentially act as a radiomitigator, a radioprotective agent used shortly after exposure [1,2]. The purpose of this work was to study the radiomitigatory properties of LA and its joint use with metformin (MF) and ethylmethylhydroxypyridine succinate (EMHPS, Mexidol) upon acute irradiation of animals.
Methods. The study was performed by a 30-day test for the survival of mice in the model of acute irradiation of animals with a lethal dose (LD). Whole-body irradiation of animals was carried out at the Center for Collective Use of the Institute of Cell Biophysics, Russian Academy of Sciences, using the RUT-15 device (MosRentgen, Russia) at a dose rate of 1 Gy/min (focal length 0.375 m, 20 mA, 200 kV). Male outbred mice Kv:SHK aged 6-8 weeks and weighing 29±4 g were used for the experiments (nursery Kryukovo, Russian Academy of Sciences). The animals were kept under standard vivarium conditions. Mice were subdivided into groups of 10 to 20 animals. For injection, we used MF (1,1-dimethylbiguanide hydrochloride (Sigma-Aldrich, USA)) prepared in an injection solution or diluted samples of EMHPS ("Mexidol" solution for intramuscular and intravenous administration (Pharmasoft, Russia)) and LA ("Berlition 600" concentrate for the preparation of a solution for infusion (Berlin-Chemie, Germany)). Solutions were administered 15 minutes after irradiation intraperitoneally in a volume of 0.3 ml/mouse and orally 10 µl/mouse. Previously, a dose-response curve in the range from 5 to 7 Gy for the mice Kv:SHK was obtained. In the range from 5 to 6.5 Gy, the dependence had a shape close to linear. At a dose of 5Gy, the mortality was at the level of 35%, at 6.5Gy - 95%, LD50 being 5.4 Gy. In the interval from 6.5 to 7 Gy, the mortality reaches a maximum of 100%. In the study, LD95 was taken for experiments (6.5 Gy). 7 Gy is absolutely lethal dose.
Results. Three tests were carried out in the work: No. 1 - primary study of radiomitigatory properties, No. 2 - combination of LA with other possible radiomitigators, No. 3 - selection of a "therapeutic window". In test No. 1, mice were irradiated at a dose of 6.5 Gy, LA was administered at a dose of 40 mg/kg once [2] in 2 groups, one of which was additionally treated with LA orally once a day at 7 mg/kg for 10 days. For comparison, vitamin C (a known interceptor of free radicals) was used at100 mg/kg. In the group of control irradiated mice, the average life expectancy (ALS) was 11.8 days, and 10% survived. LA with a single injection increased survival by 40%, which is 10% lower than vitamin C, and raised the life expectancy up to 18.1 days. With the course use of LA, a radiosensitizing effect was observed: life expectancy was 9.6 days, and 100% mortality occurred on the 23rd day.
In test No. 2, LA was combined with EMHPS [3] and MF [4]. It was assumed that they enhance the radiomitigatory properties of LA and the synergism of their radiomitigatory properties would manifest itself. Then absolutely lethal dose of 7 Gy was chosen, a dose of 6.5 Gy was taken as additional control. In the 6.5 Gy group, 5% of animals survived, and 0% survived in the 7 Gy group, but life expectancy did not differ significantly: 8.5 and 8.9 days, respectively. Groups irradiated with 7 Gy were: 1 - LA 40 mg/kg + MF 30 mg/kg; 2 - LA 40 mg/kg + EMHPS 10 mg/kg per os for 10 days after exposure; 3 - LA 40 mg /kg + MF 30 mg/kg + EMHPS 10 mg/kg per os for 10 days after irradiation. The results: survival in groups 1−3 was 0, 10 and 0%, respectively. Life expectancy in groups 1−3 was also low: 8.3, 11.3 and 10.7 days. Thus, there was no synergism of the drugs.
Therefore, in test No. 3, the purpose of which was to select the "therapeutic window" of LA and re-check its compatibility with MF and EMHPS, a dose of 6.5 Gy was used. For the irradiated control, the survival rate was at the level of 10%, and the life expectancy was 16.8 days. The experimental groups were as follows: 1 - LA 40 mg/kg; 2 - LA 100 mg/kg; 3 - LA 5 mg/kg; 4 - LA 40 mg/kg + LA for 10 days at 0.4 mg/kg/day; 5 - LA 40 mg/kg + LA for 10 days at 4 mg/kg/day; 6 - LA 40 mg/kg + EMHPS 100 mg/kg; 7 - LA 40 mg/kg + EMHPS for 10 days at 10 mg/kg/day; 8 - LA 40 mg/kg + MF 30 mg/kg; 9 - LA 40 mg/kg + MF 3 mg/kg/day for 10 days. The survival rate was as follows: 1 - 30%, 2 - 50%, 3 - 60%, 4 - 60%, 5 - 40%, 6 -20%, 7 - 10%, 8 - 10%, and 9 - 0%. Life expectancy was distributed in a slightly different way: 1 - 19.2 days, 2 - 22.6 days, 3 - 24.2 days, 4 - 23.2 days, 5 - 22.6 days, 6 - 12.3 days, 7 - 14.1 days, 8 - 13.8 days, 9 - 14.7 days. Groups 3 and 4 were the best in terms of both indicators, where LA was used at low dosages. An increase in LA dosage by an order of magnitude in groups 1, 2 and 5 led to a decrease in the radiomitigatory effect. Combining LA with EMHPS and MF repeatedly not only did not give a significant positive result, but also reduced the radioprotective properties of LA (groups 6, 7 and 8) and even caused radiosensitization (group 9).
Conclusions. LA reveals radiomitigatory properties when mice are irradiated with a lethal dose of X-rays. This effect is dose-sensitive, with low doses preferred. The radiomitigatory properties of LA are weakened when it is combined with EMHPS and MF. Because all three drugs interfere with mitochondrial function, energy production in mitochondria disrupts and oxidative stress is exacerbated.
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### S7.497. Reception of the gravitational field by the cell: experimental data and a possible mechanism

Ogneva I.V.[1]*
[1]*Institute for Biomedical Problems RAS;*
* iogneva@yandex.ru

Gravity was one of the physical factors under which life appeared and developed on Earth. Remaining permanent, this force has a significant impact on the ontogenesis of all species and on some aspects of human life. Therefore, leaving the usual habitat and staying for a long time, for example, under weightlessness, can lead to negative consequences for human health and performance, which significantly reduces the possibility of deep space exploration.

The cell is formed in the field of constant gravity. Accordingly, all intracellular structures must have such mechanical characteristics in order to maintain the shape and volume of the cell in this field. Also, the speed and direction of metabolic processes should lead to the production of a sufficient amount of macroergs to carry out all the processes of the cell's vital activity in this field. In other words, the structural and functional capabilities of the cell are "tuned" precisely to the external mechanical field in which this cell was formed.
However, the key question remains how the same cell perceives opposite changes in the external field, microgravity and hypergravity. In other words, the sensor of the gravitational field must have three modalities depending on the gravity force: "E" state - the gravity in which this cell was formed; "E+" state - hypergravity relative to the initial state (if the cell was formed, for example, in weightlessness, then the gravity of the Earth will be hypergravity in this case); "E–" state is a state of weightlessness or microgravity relative to the initial state (for a cell that appeared under the conditions of the Earth's gravity, weightlessness will be microgravity; for a cell formed, for example, when rotating on a centrifuge in conditions greater than 1g, stopping the centrifuge will mean a transition to "microgravity" conditions) (Ogneva I.V., 2022).
Such conditions imposed on the sensor limit its choice. Any change in the field of the external force, in particular, the force of gravity acting on the cell, will lead to the occurrence of mechanical deformation. Indeed, during the suborbital flight of TEXUS-54, direct evidence of the onset of deformation was experimentally obtained - a change in the shape of the cells was recorded almost immediately (Thiel CS et al., 2019).
The amount of deformation of an object depends on the force applied to it and the object's own mechanical characteristics. The intrinsic mechanical properties of cells are determined primarily by cytoskeletal structures. For cells of higher animals with a developed cytoskeletal network, the concept of "tensegrity" is of great interest (Ingber D.E. et al., 2014). However, in the evolutionary series, not all cells have a developed cytoskeleton that permeates the entire cell, but all cells have a membrane and a cortical cytoskeleton associated with it. It is this barrier that separates the intracellular contents from the external environment and allows maintaining the homeostasis of the volume and shape of the cell. The conducted systematic analysis of possible gravireceptors in eukaryotes indicates different ways of responding to changes in the gravitational stimulus, but almost always mediated by the cytoskeleton. It should be noted that, in the evolutionary series, orthologs can belong to different cytoskeletal structures, which, accordingly, can determine a wide range of possible gravireceptors in different species.
Therefore, it can be proposed to consider the cortical cytoskeleton and the membrane associated with it as the primary mechanosensor (Ogneva I.V., 2022). Such a mechanosensor can be in three states: undeformed state, tensile deformation or compressive deformation with an increase or decrease in external mechanical stress, for example, with a change in gravity. It should be noted that we are talking only about the very first moments of changes in external mechanical stress. The results of mathematical modeling and comparison with experimental data indicate sufficient strain energy for dissociation, for example, from actin filaments of actin-binding proteins.
Since the cortical cytoskeleton is heterogeneous, tensile and compressive deformations can lead to the dissociation of various proteins. For example, when stretched, proteins that form the longitudinal structures of the cortical cytoskeleton can dissociate; during compression deformation, proteins anchoring the cytoskeleton to the membrane. These proteins can themselves play a signaling role, can interact with participants in other signaling pathways, leading to changes in the expression of target genes, regulation of translation and post-translational modifications, and metabolic processes in the cell. In addition, a possible change in the structure of the cytoskeleton can lead to a change in the conductivity of ion channels, the binding affinity of signaling molecules, and a change in the localization of intracellular structures (for example, nuclei or mitochondria). All this taken together will lead to the formation of an appropriate adaptive structural and functional pattern of cells.

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**S7.498. Response of HeLa cells with adenovirus to frequency- resonance influences detected using the potential sensitive fluorescent probe**

Morozova G.M.[2]*, Lopatina O.A.[1], Askarova K.Z.[2], Grinkevich O.M.[1], Firsova E.L.[1]
[1]*Epidemiology and Microbiology Research Gamaleya Institute , Moscow, Russian Federation;*
[2]*Peoples' Friendship University of Russia, Moscow;*
* gimorozova@mail.ru

An important goal of frequency-resonance effects (FRI) on a living system is to enhance its adaptive mechanisms during of pathological processes. An important meaning of the frequency-resonance influence impact (FRI) application on a living system is to enhance its adaptive mechanisms in pathological processes. Previously, it was shown that changes in electrical transmembrane potentials (TMP) in cells adequately reflect changes in their physiological and energy status [1–4]. The cervical carcinoma culture cell HeLa line is widely used in model biomedical studies to identify the physicochemical characteristics of the cancer cells vital activity under various influences. In recent years there has been an increasing interest in microbiological methods of combating oncology. On the other hand currently there is a growing interest in microbiological methods of combating oncology. In this regard, the purpose of this work is to study the coupled reactions of HeLa cells infected with adenovirus (AV) to different FRI by the potentia-sensitive probe fluorescence.
Biophysical parameters were assessed using a vital polychromatic fluorescent probe–cation DSM (4-n-dimethylaminostyryl-1-methylpyridinium) [1]. Cell staining was carried out by adding the DSM probe physiological solution to HeLa cells suspensions in Eppendorfs to the final working concentrations, followed by these cells incubation in a thermostat (37°C). Preparations with stained cells monolayer on coverslips were studied in the DSM fluorescence light on the microscope - fluorimeter «Lumam» similarly [2]. Cell preparations were exposed to the inductor of the Mini-expert-DT apparatus (Imedis) by irradiating cell preparations with F86 (mode 1) and F87 (mode 2) frequencies with an exposure of 3 min [2]. Non-irradiated preparations serve as controls. The sum values of negative TMP on the plasma and mitochondrial membranes, the positive TMP on the nuclear membrane were estimated from the DSM intensity and color fluorescence in different cell compartments in monolayer [2, 3]. Histograms of cell distribution over TMP ranges under different experimental conditions were obtained similarly to [2]. It has been established that TMP $\geq$ |-200 mV |, associated primarily with the mitochondria energization, is achieved after 24 hours of cell cultivation. A comparative analysis of our results showed that in pure cancer cell culture, both frequencies cause depolarization of membranes in cells, but to different degrees. Irradiation in mode 1 causes a decrease in the pool of cells with high TMPs by 28%, as well as mitochondria deenergization and a decrease in positive nuclear TMP in 12% of cells. While DSM cations penetrate into the nucleus. Irradiation in mode 2 only 11% of cells retain a high TMP, and the deenergized cells pool increases by 20%. Therefore, exposure to the F87 frequency is more effective for the oncocells deactivation.
Experiment with AV-infected cell preparations revealed a 35% decrease in the cells pool with high TMP and an increase in the cells pool with deenergized mitochondria by 38%. At the same time, areas with diffuse yellow-green DSM fluorescence appear in the cytoplasm and nuclei of such cells, associated with active viral accumulations, and intermembrane contacts of cells in the monolayer are disturbed. It can be concluded that the multiplication of antibodies in cell culture leads to a significant change in the electrical properties of cell membranes and

chromatin in the nuclei and, as a result, the viability of cancer cells is impaired. It was found that this effect can be weakened against the FRI background. The reaction of cells with AV has some peculiarities, namely: in mode 1 the depolarized cells pool in the monolayer increases by 25 %, the area of AV clusters decreases to the same extent; in mode 2 - 60% of the monolayer area does not contain active AV. At the same time, the cells pool (13 %) undergo membranes repolarization and mitochondria energization. Therefore, an increase in the effect of AV deactivation in cells under the influence of irradiation in mode 2 can contribute to partial resuscitation of carcinoma cells. On the other hand, this indicates a possible antiviral effect when other infected cells are irradiated with the F87 frequency.

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### S7.499. Response of hela cells with adenovirus to frequency- resonance electromagnetic irradiations influences detected using the potential sensitive fluorescent probe

Morozova G.M.[2], Lopatina O.L.[1]*, Firsova E.F.[1], Grinkevich O.G.[1], Askarova K.A.[2]
[1]*N.F. Gamaleya Institute of Epidemiology and Microbiology;*
[2]*Peoples' Friendship University of Russia - RUDN;*
* Iopatina.online@ya.ru

An important goal of frequency-resonance effects (FRI) on a living system is to enhance its adaptive mechanisms during of pathological processes. An important meaning of the frequency-resonance influences impact (FRI) application on a living system is to enhance its adaptive mechanisms in pathological processes.Previously, it was shown that changes in electrical transmembrane potentials (TMP) in cells adequately reflect changes in their physiological and energy status [1–4]. The cervical carcinoma culture cell HeLa line is widely used in model biomedical studies to identify the physicochemical characteristics of the cancer cells vital activity under various influences, in recent years there has been an increasing interest in microbiological methods of combating oncology. On the other hand currently there is a growing interest in microbiological methods of combating oncology. In this regard, the purpose of this work is to study the coupled reactions of HeLa cells infected with adenovirus (AV) to different FRI by the potentia-sensitive probe fluorescence.

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**S7.500. Reverberation influence on the location search strategy of dolphins (Tursiops truncatus) for objects at different depths of the water area**

Akhi A.V.[1]*
[1]*IEPhB RAS;*
* andrey.akhi@gmail.com

Reverberation, as a process of reflection and scattering by various inhomogeneities of a sound wave, leading to aftersound, makes it difficult to perceive acoustic information. In the marine environment, there are volumetric, surface, and bottom reverberations. Volumetric reverberation in the water column is usually small unless there are a large number of biological scatterers (fish, plankton, etc.). Surface reverberation occurs due to the scattering of sound by the oscillating surface of the sea and the large number of air bubbles dissolved in the upper layer of water. It causes a powerful interference, which is the greater, the more restless the sea. This interference masks the search object located near the surface. Bottom reverberation occurs due to the dispersion of sound energy from the inhomogeneities and unevenness of the bottom (stones, hard ground). It acoustically masks objects lying on the bottom or in a layer of marine sediments. The presence of reverberation is a problem for the auditory systems of echolocating marine animals such as dolphins. The difficulty lies in the fact that reverberation, as an acoustic response of the environment, occurs at the same frequency as the emitted echolocation pulse. Therefore, it is not enough just to switch to a different frequency range from the interference; a more complex tuning is needed. Studies have shown that dolphins use not only echolocation tuning, but also behavioural changes.
In our studies, the task was to evaluate the ability of adaptation of echolocation and behaviour of bottlenose dolphins in the search, detection, and identification of objects located at different depths of the water area, including under a layer of marine sediments, with different reverberation intensity. The experiments were carried out in a pile-net enclosure of the sea bay according to the behavioural method with food reinforcement in conditions of free swimming of animals. The task of the dolphin was not only to detect a positive target (steel cylinder 120 mm high, 120 mm in diameter, 5 mm thick walls), but also to distinguish it from a negative target of the same dimensions but made of brass.
For dolphin`s echolocation, volumetric reverberation is usually not a problem. In the water column, the dolphin uses ordinary (most comfortable for it) echolocation pulses, short in duration (10–12 μs) with a broadband energy spectrum, with an upper limit of up to 140–170 kHz [1].
Our studies [2] have shown that at the beginning of the search for a target located near the surface, the dolphin emits a conventional broadband (up to 170 kHz) signal of short duration (10–12 μs). After detecting the target and identifying it as a more complex task, the dolphin changes the signal structure: the signal spectrum is narrowed along the upper boundary to 40–60 kHz and its duration is increased to 45 μs, with an increase in the signal amplitude.
It was found [3] that when searching for a target in the near-bottom area, the dolphin switches its signals. Instead of the pulses it uses when locating a target in the water column, the dolphin uses signals with a shifted spectrum in the region of low and medium frequencies up to 100 kHz with a maximum around 50 kHz. The pulse duration increases to 15 μs. If the object is hidden by a layer of marine sediments, which makes detection and recognition even more difficult, a further change in the signal structure occurs. The effective bandwidth of the spectrum narrows to 80 kHz with the maximum around 20 kHz. The signal duration increases to 17-20 μs, and its amplitude increases by a factor of one and a half compared to the signals when searching at the bottom and four times compared to searching in the water column. In addition to the frequency tuning and the selection of a frequency range less prone to attenuation, this increases the energy of the signal to compensate for attenuation and multiple reflections.
In addition to the fact that dolphin sonar is able to adaptively change three parameters of the location pulse — duration, frequency, and intensity; it was shown [3, 4] that the dolphin also changes the behavioural search strategy, transitioning from linear movement to movement along a more complex trajectory. When identifying the target, the dolphin performs rotational movements above the target, gradually approaching it along the surface of the cone with the apex at the target point. This allows the dolphin to find the optimal position and location angle required to obtain complete information about the object in the ground necessary for its recognition.
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Key words: dolphin, location, search, classification, signal spectrum.
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**S7.501. Rheological features of the blood of ground squirrels during the non-hibernation period**

Nikitina E.R.[1]*, Katyukhin L.N.[1], Zabelinskii S.A.[1], Chebotareva M.A.[1], Pokhmelnova M.C.[1], Shukolyukova E.P.[1], Klichkhanov N.K.[2]
[1]*Sechenov Institute of evolutionary physiology and biochemistry, St-Petersburg, Russia;*
[2]*Dagestan state university, Makhachkala, Russia;*
* elena.nikitina@bk.ru

Ground squirrels are hibernating animals that prepare for wintering during the whole period from spring to autumn, increasing their fat reserves. Seasonal changes in the physiological state of animals significantly affect the hematological, biochemical and rheological properties of blood. In the active period, starting in spring, there is a change in the population of red blood cells due to the entry of reticulocytes from the bone marrow into the blood and the removal of old cells from the bloodstream. In summer, the consumption of food rich in polyunsaturated fatty acids contributes to a change in the composition of deposited lipids, as well as the composition of plasma lipids and membranes of shaped blood elements (Dark, 2005; Otis, 2011). In general, this leads to a change in the mechanical resistance of red blood cells of ground squirrels (Gulevsky, Schenyavsky, 2014). This means that the deformability of red blood cells may depend on the season of the year. However, systematic studies on the rheological and deformation characteristics of red blood cells in periodically hibernating ground squirrels have not been found in the modern literature. In this regard, the purpose of our work was to analyze the parameters of erythrocyte deformability and its relationship with the erythrocyte indices of ground squirrels in different seasons of the year during the active period of their life. Seasonal changes in the rheological properties of red blood cells of ground squirrels were investigated by ectacytometry.
It was found that in the spring, after the arousal of ground squirrels, erythrocytes have the greatest deformability, the least stiffness of the membrane and a high degree of their water permeability. In summer, compared with spring, there is a decrease in the deformability, hydration and range of osmotic stability of erythrocytes, as well as the

rigidity of erythrocyte membranes increases and their water permeability decreases. In summer, the average volume of red blood cells significantly decreases. In autumn, before hibernation, the integral deformability of erythrocytes increases relative to the summer period, but the water permeability of the membranes remains reduced, and the microviscosity of the membranes remains significantly increased. The method of ektacytometry at low shear stress revealed seasonal changes in the anisotropy of red blood cells in the form of a polymodal form of their distribution in a wide range of osmolality of the medium.

The number of red blood cells in the blood is the lowest in spring. From spring to autumn, the number of red blood cells in the blood tends to increase, as well as an increase in the volume fraction of red blood cells (hematocrit) in the blood. The Hb content in the blood is the lowest in spring, and increases significantly in autumn (by 17.8%). The volume of one red blood cell (MCV), reduced already in summer, remains reduced in autumn (by 5.8%). From spring to autumn, the average hemoglobin content in the erythrocyte (MCH) remains constant. The value of MCHC (the average amount of hemoglobin in the erythrocyte) increases slightly in summer (4.2%) and remains elevated in autumn (3.7%). The RDV-CV index (percentage of cell size distribution) does not differ significantly from spring values in summer, but in autumn it significantly increases relative to summer (5.9%). RDV-SD (the difference between the minimum and maximum cell volume) is reduced in summer (8.5%) compared to spring, and in autumn it rises to spring values. A positive correlation was found between RBC and Hb, RBC and Hct, Hb and Hct. MCHC negatively correlates with MCV and RDW-SD. A positive correlation was also found between MCV and RDW-SD, RDW-CV and RDW-SD.

Correlations were also found between the parameters of erythrocyte deformability and their hematological parameters. An important finding is the negative correlation between the degree of cell hydration (O_hyper) and the average concentration of hemoglobin in the erythrocyte (MCHC).

Thus, for the first time, we discovered seasonal variability in the deformability of red blood cells of ground squirrels, consistent with the summer activity of animals and preparation for hibernation. The approach used in this work to analyze the cell deformability index at low shear rates expands the possibilities of laser ektacytometry to assess the microviscosity of cell membranes, since it is the change in membrane deformability that is most often associated with physiological and pathophysiological changes in the rheological properties of erythrocytes. The proposed indicators significantly expand the diagnostic potential of the gradient ektacytometry method.

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### S7.502. Search for new anti-inflammatory agents among thymic hormones

Parfenyuk S.B.[1]*, Glushkova O.V.[1], Khrenov M.O.[1], Sharapov M.G.[1], Mubarakshina E.K.[1], Kuzekova A.A.[1], Novoselova T.V.[1], Novoselova E.G.[1], Lunin S.M.[1]
[1]*Institute of Cell Biophysics RAS;*
* lana_kras2@rambler.ru

Thymosin α1 is an immunomodulatory peptide belonging to the group of thymic hormones. It is known to enhance T-cell mediated immune responses by several mechanisms, including stimulation of T cell differentiation and/or maturation, activation of natural killer cells and dendritic cells, and stimulation of the release of pro-inflammatory cytokines. We used alveolar macrophages RAW264.7 cultivated in the presence of lipopolysaccharide (LPS), which is a well-known stimulator of the pro-inflammatory response of cells. In the present work, thymosin α1 was shown to exhibit anti-inflammatory properties. Thymosin α1 also modulated the production of reactive oxygen species (ROS) by LPS-stimuated alveolar macrophages. Indeed, the addition of thymosin α1 to activated macrophages reduced the ROS levels and thus prevented the cascade of pathological reactions. Furthermore, thymosin α1 reduced production of a number of pro-inflammatory cytokines in endotoxin-stimulated cells. In particular, a decrease in the production of IL-6, IL-1α, as well as a slight increase in the production of the anti-inflammatory cytokine IL-10 was shown. The effect of thymosin α1 on the proliferative activity of RAW264.7 alveolar macrophages was manifested as an increase in the number of cells in the presence of thymosin α1 both in cells without LPS exposure and in activated cells. The study of intracellular signaling in endotoxin-stimulated alveolar macrophages showed that although thymosin α1 reduced the expression of the Tlr4 gene mRNA almost to control values, NF-kB signaling cascade was unaffected. As to to the SAPK/JNK signaling pathway and AP-1 transcription factor, thymosin α1 showed previously unknown anti-inflammatory properties by inhibiting AP-1 gene overexpression in RAW264.7 cells stimulated with endotoxin. In addition, a decrease in the production of two JNK isoforms (p54 and p46) to control values was shown, although the effect of endotoxin in the cell culture medium expectedly led to a significant increase in the production of both isoforms in the absence of thymosin α1. The stabilizing effect of thymosin α1 on the redox status of RAW264.7 cells was confirmed by a sharp increase in the expression of the Nrf-2 gene, a redox-sensitive transcription factor that regulates antioxidant protection. The target genes of NRF-2 are, in particular, antioxidant enzymes such as peroxiredoxins and catalase. In the presence of thymosin α1, endotoxin-stimulated macrophages showed a higher level of catalase gene expression than untreated LPS-stimulated cells, and the expression level of peroxiredoxins 1 and 6 remained at a high level, approximately equal to the expression level of these genes in untreated LPS-stimulated cells. Particulatry, it should be noted that thymosin α1 led to a decrease in the expression of the p53 gene, indicating that thymosin α1 may reduce the level of apoptosis in cells stimulated by endotoxin. Thus, the present work showed new, previously unknown anti-inflammatory properties of the thymus hormone thymosin α1.

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### S7.503. Slow fluctuations of the magnetic field and circadian biological rhythms

Krylov V.V.[1]*
[1]*IBIW RAS;*
* kryloff@ibiw.ru

The influence of geomagnetic activity on biological objects is shown in correlation studies. However, it is extremely difficult to isolate the components from a complex signal of a geomagnetic storm and associate them with biological indicators in a sample large enough for statistical analysis under natural conditions. The use of experimental approaches for the first time made it possible to isolate time intervals and frequency components of a typical geomagnetic storm and study the influence of these factors on aquatic organisms.

We used representatives of different ecological and taxonomic groups, which had previously shown their sensitivity to magnetic effects, to study the simulated geomagnetic storm influence on aquatic organisms. In the

experiments, we simulated a typical strong geomagnetic storm recorded near the place of the experiments. The unmodified and undisturbed geomagnetic field (51.7 μT, inclination 75.05°) was used as control conditions. The simulation of geomagnetic fluctuations was carried out using a specially designed experimental setup (patent RU 108640 U1).
In a series of experiments with different types of aquatic organisms, significant effects were observed mainly after the exposure of aquatic organisms to those geomagnetic fluctuations that corresponded to the storm's main phase and the initial stages of the recovery phase. To study the frequency components of a geomagnetic storm, we utilized a broadband signal corresponding to the storm's main phase and two of its constituents: 0-0.001 Hz and 0.001-5 Hz. The results revealed that the highest biological efficiency had slow fluctuations in the range of 0–0.001 Hz, which corresponded to the storm's main phase and the initial stages of the recovery phase. I. e., slow fluctuations in the same range in which the daily geomagnetic variation registers affected aquatic organisms in our experiments.
It is suggested that diurnal geomagnetic variation can be an external zeitgeber for circadian biological rhythms, in addition to the primary night–day light cycle synchronizer. Geomagnetic storms may be perceived by an organism as a disruption of diurnal geomagnetic variation in this case. The inconsistency of biological processes synchronized with different zeitgebers may cause effects described in articles reporting correlations between geomagnetic indices and medical or biological parameters.
Following this assumption, effects similar to the influence of geomagnetic storms should manifest themselves when the diurnal geomagnetic variation shifts relative to the change of day and night. In addition, the effects of geomagnetic storms should depend on the time of day when the storm's main phase occurs. Experiments with a shift of the diurnal geomagnetic variation by 6 and 12 hours relative to the change of day and night (nighttime geomagnetic events occurred in the morning or afternoon), as well as with the reproduction of the main phase of a geomagnetic storm at different times of a day, confirmed this hypothesis.
A logical continuation of this line of research was the study of biological circadian rhythms entrainment by slow magnetic fluctuations in the absence of the primary light-dark zeitgeber. In experiments with zebrafish, the rhythms of fish locomotor activity followed slow changes in the induction of the external magnetic field under constant illumination. It resulted in a shift of the period relative to the initial rhythm. However, the preferences of different horizons in the vertical water column for these fish failed to obtain the same clear picture when analyzing circadian rhythms. Currently, experiments are ongoing on the blind cavefish Astyanax mexicanus, which, presumably, maybe more magnetically sensitive.
The role of cryptochromes in the processes responsible for the perception of slow magnetic fluctuations and the modulation of biological circadian rhythms is discussed.

### S7.504. Spectral properties of serum albumin under the influence of a high-intensity electric field of 50 hz

Dadashov M.Z.[1]*
[1]*Biophysics Institute, NAS Azerbaijan;*
* mursald@mail.ru

The effects of low-frequency electromagnetic fields (EMF), including the industrial frequency of 50 Hz, on human health have been studied for many years. And at present, many epidemiological, experimental and clinical data have been accumulated in the scientific literature, indicating both a negative and a stimulating effect on human health. It is indicated the nervous, immune, cardiovascular and reproductive systems are more exposed to their effects. Numerous works are also known that show changes in the physicochemical properties of various types of biosystems under the influence of low-frequency EMF. According to WHO, now against the background of the fact of the unconditional reaction of the human body to the impact of low frequency EMF, there is no complete clarity either on the possible consequences or on the generally accepted safety criteria under long-term exposure to modern EM sources.
Serum albumin is the main part of the blood serum, performs important functions, maintains the osmotic pressure of the blood, transports endogen and exogenous substances, and is also the protein reserve of the body.
The aim of this work was to study the ultraviolet absorption spectra of bovine serum albumin after exposure to a high voltage EF of 20 kV/m. Protein samples with a concentration of 30 mg/ml in distilled water were exposed to EF for 2 hours. The electric field was formed using an I-50 laboratory transformer (Russia). The optical density spectra of albumin were recorded on a two-beam spectrophotometer Spekord 250 (Germany) , with 10-fold dilution of samples, in a quartz cuvette with an optical path length of 1 cm in the wavelength range of 200–370 nm, with a spectral slit width of 1 nm, a scanning step of 1 nm. Scanning speed 10 nm/sec. Measurements were carried out with 3 repetitions. The spectra were processed using the OriginPro 8.5 program.
The UV absorption spectra of proteins significantly depend on the near and external environment of the absorbing molecule and provide information on the properties of macromolecules and their interactions with other molecules. Depending on the immediate environment of the same chromophore group, the position of the absorption maximum in the UV spectra may change.
After smoothing the spectra on control samples, five maxima were found with certain shifts at positions 207, 222, 232, 279, and 315 nm. The prototypes showed approximately close maxima, however, in all positions, ~ 34, 28, 18, 13 and 75% reductions were observed, respectively. It is known from the literature that the shoulder in the spectrum of a protein molecule at 200–210 nm corresponds to a low-intensity electronic transition $n \rightarrow \pi^*$, weak absorption bands at 220 nm ($n \rightarrow \pi^*$) are attributed to peptide groups, which shift to the short-wavelength region with increasing solvent polarity. The secondary structure created by hydrogen bonds determines the spectral properties of protein macromolecules depending on the type of fold and chain, the length and proportion of this fold, as well as the physicochemical parameters of the medium. Peptide bonds in the range from 230 to 320 nm practically do not absorb waves, which makes it possible to study the relationship between the structural and spectral properties of proteins, including albumin, by analyzing the absorption spectra of chromophore radicals of phenylalanine, tyrosine, tryptophan, histidine, cysteine, methionine, and cystine.
Obtaining secondary derivatives of the spectra added 4 more maxima to the existing ones, however, the intensity of the maxima in the experimental samples was lower compared to the control samples.
Interpreting the obtained data, it can be stated that an EF with an intensity of 20 kV/m and a frequency of 50 Hz in a certain way affects the structural organization, which is observed in the form of a change in the spectral properties of protein macromolecules. In other words, one of the primary targets of low-frequency EMFs can be the protein structures of living organisms. This study can be useful in studying the structural conservatism of protein molecules in the native state, as well as in assessing the boundaries of the variability of influence.

### S7.505. Strategies for the treatment of age-associated diseases

Glushkova O.V.[1,2]*, Sharapov M.G.[1], Parfenyuk S.B.[1], Lunin S.M.[1], Mubarakshina E.K.[1], Khrenov M.O.[1], Kuzekova A.A.[1,2], Novoselova T.V.[1], Novoselova E.G.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences;*
[2]*Pushchino State Natural Science Institute;*
* glushckova@mail.ru

In the present time, the proportion of the aging employable population is growing in Russia and in developed countries. This growth leads to a

number of economic, socio-hygienic, moral and ethical problems, allowing to classify aging and the age-associated diseases as a socially significant problem. It is known that the most important stage in the aging of the mammalian organism and the development of age-associated diseases is the aging (senescence) of its cells. The important feature of senescent cells is that they irreversibly lose their ability to divide, became resistant to apoptosis, and are able to secrete proinflammatory factors, which is characteristic of senescence-associated secretory phenotype, SASP. Senescent cells accumulate in almost all tissues and organs, contributing to the paracrine aging of the body. Recent experiments showed that the elimination of senescent cells in mice attenuated the progression of age-related diseases have drawn attention to this new geriatric target. Therefore, a strategy of modern cytogerontology may be a search for new agents with senolytic or senomorphic activity. These drugs are referred to as senotherapeutic agents, among which combinations of inhibitors of anti-apoptotic signaling pathways in senescent cells and antioxidants have the highest potential. Also, promising senolytics are inhibitors of heat shock proteins. Proteins of HSP90 family are regulators of many physiological and pathological functions in the cell. However, in the case of senescent cells, these proteins play the role of a paracrine aging stimulator, protecting senescent cells from apoptosis and may be a target of senolytic therapy. Inhibitors of these proteins are known, for example, geldanamycin, a pan-inhibitor that binds to the protein at the N-terminus. However, the presence of isoforms of these HSP90 proteins, such as endoplasmin Grp94, TRAP1 mitochondrial protein, and cytosolic α and β proteins, suggest isoform-specific functions. Even cytosolic forms, despite their high homology and the ability to largely compensate for each other's functions, still have a number of structural and functional differences, which allowed researchers to find a specific HSP90β inhibitor capable of inhibiting the constitutive form of this protein. Since the most promising senolytic strategies today are combinations of senolytic drugs and antioxidants, we proposed a usage of unique antioxidant protein Prdx6, which was first isolated and characterized at our institute, as a natural antioxidant. This protein is able to act as an antioxidant, limiting oxidative stress through its peroxidase activity and participate in the formation of oxidants through its phospholipase activity. These two paradoxical abilities of Prdx6 seem to indicate a regulatory role for this enzyme in oxidative stress. In the present work, we investigated the prospects for the senolytic action of a combination of HSP90 inhibitors and the antioxidant protein Prdx6. The studies were carried out both in vitro, on senescent 3T3/Balb cells, and in vivo, on models of age-associated diseases.

The report will present the results of studies on the molecular and cellular mechanisms of the senolytic action of the proposed composition and discuss the prospects for using the combination of Prdx6 with HSP90 inhibitors in the development of new drugs for the treatment and prevention of age-related diseases. The work was supported by the Russian Science Foundation grant No. 23-24-00041.

### S7.506. Study of the effect of various modes of combination of photobiomodulation and ionizing radiation on tumor cells Hela Kyoto

Belotelov A.O.[1]*, Cherkasova E.I.[1], Yusupov V.I.[3], Minaev N.V.[3], Kononova U.A.[1], Maslennikova A.V.[1,2]
[1]*N. I. Lobachevsky State University of Nizhny Novgorod;*
[2]*Privolzhsky Research Medical University;*
[3]*FSRC "Crystallography and Photonics" RAS;*
* arteom.belotelow@yandex.ru

Side effects from normal tissues that occur during radiation therapy for malignant neoplasms still represent one of the unsolved problems of modern radiation oncology. Photobiomodulation (PBM) based on the positive results of clinical practice has been used for many years for the prevention and treatment of side effects of radiation, but its effects can occur directly in the area of the location of the tumor focus (for example, with tumors of the oral cavity and pharynx). From this point of view, it is necessary to assess the possible stimulating and adaptive effect of photobiomodulation on tumor cells that may be exposed to light in parallel with radiation therapy, as well as to study the mechanisms of the combined effect of ionizing and low-intensity optical radiation on tumor cells.

The aim of this work was to study the effect of photobiomodulation of the visible red range in combination with ionizing radiation on the viability, mitochondrial potential and cell cycle of the Hela Kyoto cell line, depending on the dose of ionizing radiation, PBM fluence, as well as the sequence of these effects.

At the first stage of the experiment, tumor cells were exposed to photobiomodulation with fluences of 3 mJ/cm2, 30 mJ/cm2 and 300 mJ/cm2, as well as 0.5 J/cm2, 1 J/cm2 and 2 J/cm2, after an hour the cells were irradiated with gamma radiation at doses of 2 Gy, 4 Gy and 6 Gy. A day after irradiation, cell viability was assessed by the MTT test. At the second stage, gamma irradiation of Hela tumor cells was initially carried out at doses of 2 Gy, 4 Gy and 6 Gy, after which the cells were exposed to PBM with fluences indicated above. A day later, the viability of the cells was assessed by the MTT test. Cells after gamma irradiation in appropriate doses were used as a control. The effect of various modes of PBM in combination with AI on the cell cycle and mitochondrial potential of tumor cells after combined exposure in the modes described above was studied. The experiments were carried out on a flow cytofluorimeter FacsAriaIII (Becton, Dickinson and Company, USA). Determination of cell cycle phases for cells was carried out using a cell cycle phase detection kit (APC BrdU Flow Kit, cat. No. 552598 Becton, Dickinson and Company, USA) with bromodeoxyuridine labeled with ARS. The method is based on the joint staining of common DNA with 7-aminoactinomycin (7-AAD) and bromodeoxyuridine (BrdU). BrdU is incorporated into newly synthesized DNA by cells entering and passing through the S-phase of the cell cycle (DNA synthesis). With a combination of 7-AAD and incorporated BrdU, two-color flow cytometric analysis allows you to list and characterize cells that actively synthesize DNA in terms of their position in the cell cycle. By the intensity of 7-Aminoactinomycin D staining, the distribution of cells by phases of the cell cycle was judged and the percentage of cell separation was judged for those in the S-phase, G0/G1 phase and those in the G2/M phase.

To determine the transmembrane potential of the mitochondria of cells, the dye MitoStatus TMRE (BD Pharmingen, USA) - tetramethylrodamine ethyl ether, cationic lipophilic fluorescent dye, and 7-AAD (7-Aminoactinomycin D) (BD Pharmingen, USA) - DNA-binding fluorescent dye were used. Gating was carried out by the presence of dyes in the cells: 1) living cells with depolarized mitochondrial membrane: TMRE(-) and 7-AAD(-); 2) living cells with intact mitochondrial membrane: TMRE(+) and 7-AAD(-); 3) dead cells with depolarized mitochondrial membrane: TMRE(+) and 7-AAD(+); 4) dead cells with intact mitochondrial membrane: TMRE(-) and 7-AAD(+). During the calculations, the number of living cells with intact MM, the number of dead cells with intact MM and the total number of cells (living and dead) with depolarized MM were taken into account.

As a result of the study, it was found out that photobiomodulation leads to multidirectional effects depending on the dose of ionizing radiation, fluence and the sequence of these effects on cells. Preliminary exposure to photobiomodulation caused a slight but statistically significant inhibition of tumor cell proliferation. At the same time, in tumor cells previously exposed to ionizing radiation (stimulating effect), PBM with low fluences caused an increase in proliferative activity. And the effect of photobiomodulation with high fluences, on the contrary, in some cases caused a statistically significant inhibition of cell proliferation. When studying the potential change on the inner MM, it was shown that the increase in the number of living cells after PBM occurs precisely due to a decrease in the proportion of cells with a depolarized mitochondrial membrane, which corresponds to the data of the MTT test, which demonstrated an increase in the number of viable tumor

cells during PBM after gamma irradiation. Analysis of the cell cycle of tumor cells under various irradiation modes showed that the effect of AI causes a block of cell mitosis and their accumulation in the G0/G1 phase, in turn, additional exposure to photobiomodulation in some cases can enhance this process and increase the percentage of cells accumulated in this phase.

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### S7.507. Study of the reaction of the body of laboratory animals to a five-fold increase in the concentration of deuterium in drinking water

Kozin S.V.[1,2], Kravtsov A.A.[1,2], Dorohova A.[1,2]*, Lasota O.M.[1], Moiseev A.V.[3], Ivlev V.A.[4], Drozdov A.V.[5]
[1]*Federal State Budgetary Institution Federal Research Center "Southern Scientific Center of the Russian Academy of Sciences";*
[2]*Federal State Budgetary Educational Institution of Higher Education "Kuban State University" ;*
[3]*Kuban State Agrarian University;*
[4]*Federal State Budgetary Educational Institution of Higher Education "Kuban State Medical University" of the Ministry of Health of the Russian Federation;*
[5]*Institute of Analytical Instrumentation of the Russian Academy of Sciences;*
* 013194@mail.ru

It is known that the isotopic composition of the atmosphere of Mars differs significantly from the Earth's - it contains 6 times more deuterium (700-1000 ppm), 70% more nitrogen-15 and 5% more carbon-13 and oxygen-18. Before sending an expedition to Mars with the aim of establishing a colony, it is necessary to find out how the Martian isotope composition different from the Earth's will affect the life of mammals. It is known that a decrease in the content of deuterium in the diet can have a stimulating effect on the functional activity of living systems, while a significant increase in the concentration of deuterium can inhibit vital processes, and in some cases it has been noted that moderate fluctuations in the concentration of deuterium (both with an increase and with decrease in its content in water) can increase the functional activity of living systems [1,2].

To date, a significant number of works have been published that describe both the activating and inhibitory effects of water with a deuterium content reduced relative to the natural level on almost all levels of organization of living matter (molecular, cellular, tissue, organism) [3–5]. At the same time, of particular interest is the study of the effect of water, in which the deuterium content is comparable to that in the ice caps of Mars, on the functional systems of nonspecific defense of the body. And, first of all, on its prooxidant-antioxidant link, disturbances in which play a significant role in the development of many diseases.

In connection with the above, the purpose of this study was to study the effect of water with a modified isotope composition (750 ppm) on the parameters of the prooxidant-antioxidant system of the liver and blood of laboratory animals.

The isotope exchange of a proton for a deuteron in water of hydration or in functional groups of amino acid residues located on the surface of a protein structure helps to strengthen intermolecular bonds between the hydration layer and the macromolecule, which ultimately can lead to a change in the functional activity of the protein.

We have previously established that a medium with a deuterium concentration of 50 ppm leads to a decrease in the rate of oxidation of o-dianisidine by hydrogen peroxide, catalyzed by horseradish peroxidase [6]. Taking into account the obtained results of fluorescence and CD spectroscopy, most likely, the strengthening of hydrogen bonds leads to conformational rearrangements in the BSA (bovine serum albumin) molecule. Loss of alpha helicity percent indicates changes in the secondary structure of BSA. It is known that BSA has two tryptophan residues, one of them is located on the surface of the molecule, and the other is inside it. The main quenchers of tryptophan fluorescence are solvent (water) molecules, as well as tyrosine residues due to energy transfer by the dipole–dipole mechanism. It can be assumed that the decrease in the intensity of tryptophan fluorescence may be due to an increase in the availability of tryptophan residues to the solvent and an increase in the probability of energy transfer from tryptophan to tyrosine residues. Thus, the performed optical studies indicate a modification of the BSA structure in a medium with a deuterium concentration of 487 ppm.

On the basis of the conducted studies, it can be concluded that in rats, when water with a high deuterium content (750 ppm) is consumed, both a decrease in the intensity of free radical processes in the blood and hepatocytes and an increase in the antioxidant potential in liver tissues are observed, including due to increasing the content of low molecular weight thiol antioxidants. The data obtained in the work indicate an increase in the functional activity of the prooxidant-antioxidant link of the body's nonspecific defense system against the background of a drinking diet with a fivefold increase in deuterium.

Thus, an increase in the deuterium content to 750 ppm in the diet of mammals, i.e. level similar to Martian glaciers, has a biological effect at the tissue and molecular level, activating the antioxidant system of mammals. The results presented in the paper do not give full grounds to assert that the Martian glaciers are suitable for life on this planet. This study paves the way for further study of the biological effects of relatively high concentrations of deuterium, corresponding to its content in ice caps at the poles of Mars (from 700 to 1000 ppm), and understanding the possibility of its colonization.

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### S7.508. Study of the thermostability of the complex of bovine serum albumin with gallic acid using FT-IR spectroscopy

Fedortsov N.M.[1]*, Budkevich R.O.[1]
[1]*North-Caucasus Federal University, Stavropol, Russia;*
* fedortsov729@gmail.com

Bovine serum albumin (BSA) is known for its functional and technological properties, one of which is the ability to bind ligands [1]. Previously, we have shown the possibility of obtaining a complex of BSA with gallic acid for a food supplement with an optimal composition

[2]. The use of this complex in the food industry to impart antioxidant properties to products with functional properties involves pasteurization and preservation of stability under the influence of high temperatures. The purpose of this work is to study the change in thermal stability in terms of IR-Fourier spectroscopy of the BSA complex with gallic acid.

For the study, a complex obtained in accordance with the previously described method [2] was used. The heat treatment of dairy products (pasteurization process) was simulated in three temperature ranges with different sample holding times: 62–65°C for 30 minutes, 72–75°C for 30 seconds, and 85–90°C without holding. In the complex of BSA with gallic acid in liquid samples, the transformation of chemical bonds was studied using a Nicolet iS50 IR-Fourier spectrometer (Thermo Scientific, USA) on an attenuated total internal reflection (ATR) Smart iTR ATR (ZnSe) attachment (Thermo Scientific, USA).

The studied IR spectra contained various broadened peaks in the studied ranges, which made the analysis process difficult. For the analysis, derivatives of the initial spectra of the first order with differentiation by the Savitsky-Golay method [3] were used. Thermal stability was assessed by changes in the intensities and shifts of the bands in the region of amide I, namely, 2 main absorption waves reflecting the ongoing changes with BSA under temperature exposure - 1652 $cm^{-1}$ and 1675 $cm^{-1}$ [4]. These wavelengths are characteristic of changes in the BSA α-helix and the unfolding of the molecule in space, respectively. The spectra of BSA reacted with polyphenol and without it (control) were considered together.

In the control sample at a wavelength of 1652 $cm^{-1}$, with increasing temperature, a gradual decrease in optical density was observed (-1.4 × $10^3$, -1.42 × $10^3$ and -1.5 × $10^3$, respectively, for 62-65°C, 72-75 °C and 85-90°C), which indicates a decrease in the proportion of α-helices in the secondary structure. On the absorption band at 1675 $cm^{-1}$, a gradual increase in optical density is observed (-1.20 × $10^3$, -1.15 × $10^3$ and -1.14 × $10^3$, respectively, for 62-65°C, 72-75°C and 85-90 °C), which indicates an increase in the proportion of random structures in the BSA molecule. These changes indicated the denaturation of the original molecule under the influence of temperature.

A short-wavelength shift is observed in all studied spectra of the complex. Thus, the expected peak at 1652 $cm^{-1}$ is observed at 1653.7 $cm^{-1}$, and 1675 $cm^{-1}$ at 1676.5 $cm^{-1}$. Such shifts indicate changes in the nature of peptide bonds in the complex, which can be associated with the formation of bonds between BSA and polyphenol. At a wavelength of 1652 $cm^{-1}$ in the samples of the complex under temperature exposure, a decrease in optical density is also observed - -1.44 × $10^3$, -1.46 × $10^3$ and -1.47 × $10^3$, respectively, for 62-65°C, 72-75°C and 85-90°C. At the absorption band of 1675 $cm^{-1}$, the optical density increased - -1.23×$10^3$, -1.13×$10^3$ and -1.03×$10^3$, respectively, for 62-65°C, 72-75°C and 85-90°C. The studied complex initially has a greater influence on the proportion of α-helices in the secondary structure, which is associated with the formation of the complex itself. With further temperature exposure, it can be seen that even at high temperature ranges (85-90°C), the proportion of α-helices in the complex is retained in a larger amount (-1.47 × $10^3$) than in the control - (-1.5 × $10^3$). At a wavelength of 1675 $cm^{-1}$, a more significant increase in the proportion of random structures of the BSA molecule in the complex (-1.03×$10^3$) is observed than in the control (-1.14×$10^3$).

Thus, gallic acid makes it possible to stabilize the BSA molecule under the influence of temperature in the range of pasteurization of dairy products, and this complex can be used in the food industry under appropriate production conditions.

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## S7.509. The activity of genetic parameters in mice under the influence fractionated irradiation in small doses with the use of shielding

Saleeva D.V.[1]*, Vorobeva E.S.[1], Raeva N.F.[1], Rozhdestvensky L.M.[1], Abdullaev S.A.[1,2], Zasukhina G.D.[1,3]
[1]*State Research Center – Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency, Moscow, Russia;*
[2] *Federal state budget institution of science INSTITUTE OF THEORETICAL AND EXPERIMENTAL BIOPHYSICS Russian Academy of Sciences;*
[3]*Institute of General Genetics of the Russian Academy of Sciences, Moscow, Russia;*
* dasha_saleeva@inbox.ru

A number of complex reactions are activated in cells exposed to ionizing radiation, including DNA damage and repair, cell death, and changes in proliferation levels. The effects of high doses of radiation on mammalian cells have been studied including the activity of genes and their regulators. However, how low doses of ionizing radiation (LDR) influence these processes remains less studied. A number of authors have shown that exposure to LDR leads to various positive reactions of the cell and the organism as a whole: activation of immune system, genes and their regulators in the phenomenon of hormesis, formation of adaptive response. These observations determined the purpose of the work: the studying of the activity of genes and non-coding RNAs (long non-coding RNAs and microRNAs) in different organs of mice with transplanted Lewis carcinoma after LDR irradiation under different shielding variants.

Materials and Methods. Female mice C57Bl/6 with transplanted Lewis lung carcinoma cells exposed to total 4x fractionated X-ray irradiation at a dose of 75 mGy on the RUST M1 on 10th after tumor tissue transplantation (dose rate 0.154 Gy/min, current strength 0.7 mA, voltage 200 kV). The irradiation was performed using protective aluminum shields of different areas of mouse body: total (no shielding), tumor only (shielding of the rest of the body), the whole body without tumor (tumor shielding). Groups of mice were formed according to the screened areas. The tumor in each animal was measured every day, starting on day 11 (when the tumor volume was sufficiently pronounced) after cell injection. On 21 days after tumor tissue inoculation, all animals were euthanized. The mRNA content of protein-coding genes as well as non-coding RNAs (microRNAs, long non-coding RNAs) was assessed in bone marrow, spleen and thymus homogenate, and Lewis lung carcinoma by real-time PCR. The primer sequence and real-time PCR conditions have been published previously. The GAPDH gene was used as the constitutive gene to assess expression. The results were presented as median changes in expression of the indicators in the groups of irradiated mice, expressed relative to the median of the control group, taken as

a unit. The nonparametric Mann-Whitney test ($p<0.05$) was used to assess statistical significance.
Results. Tumor growth was found to occur exponentially in all experimental groups starting from 11th day. However, in the group of mice with total irradiation of the animal the tumor volume was larger compared to other groups, while in the group of mice with tumor shielding the volume of neoplasm was the smallest at similar measurement periods. It was shown that a statistically significant change in mRNA expression of NFkB, G-CSF, TNFα, iNOS genes was observed in the tumor homogenate during 4-fold fractionated total irradiation in the dose of 75 mGy for mice with transplanted Lewis carcinoma as compared to both the unirradiated control and the total irradiation group of the animal body. Analysis of gene and non-coding RNA expression in samples of thymus, spleen and bone marrow of mice revealed that the highest number of activated indicators (expression changed statistically significantly in response to irradiation), was found for the spleen (40% - for total irradiation - 50% for local irradiation of tumor only, 30% - for tumor shielding). These results are consistent with literature data showing that the thymus and bone marrow are more proliferative organs, whose cells are mostly in mitosis, which is reflected in reduced gene transcription, compared to the spleen. Of the 20 parameters we studied, the highest modulation of expression (3 or more times) was detected for mRNAs of G-CSF (3.12 fold), IAP (4.3 fold), P38 (3.12 fold) and long non-coding RNAs lncp21 (3.7 fold), NEAT (6 fold). However, the linearity of the effect of the magnitude of expression modulation on the biological effect still remains to be investigated. When shielding Lewis carcinoma of mice, the detected changes in the expression of NFkB(p50), G-CSF, TNFα, iNOS, lncp21 in the tumor itself were almost identical both in case of local low-dose II exposure and total irradiation without tumor compared to the control. However, only for P38 gene mRNA we showed a statistically significant change in the "irradiation without tumor" group and its absence in the case of local irradiation.
Conclusion. The gene expression results obtained in the experiments with shielding indicate different levels of immune system activation depending on the type of shielding. These findings support a reduction in tumor growth in animals exposed to total x-ray irradiation with shielded tumor compared to other experiments.

### S7.510. The effect of constant lighting on stress reactivity of the hypothalamic-pituitary-adrenal axis in nonhuman primates with various adaptive behaviors in different age periods

Goncharova N.D.[1]*, Chigarova O.A.[1], Oganyan T.E.[1], Timoshenko N.V.[1]
[1]*Research Institute of Medical Primatology;*
* ndgoncharova@mail.ru

The hypothalamic-pituitary-adrenal (HPA) axis is a key regulator of endocrine and behavioral adaptation to stress in response to threat. However, its dysfunction, accompanied by disturbances in the production of glucocorticoid hormones, contributes to the development of various stress-dependent diseases, including age-related, for example, mental, metabolic, cognitive, cardiovascular, neurodegenerative, etc. In recent decades, there has been a significant increase in both the stressfulness of the environment and the incidence of stress-associated pathology. Among stressful environmental factors, disturbances in the natural light-dark cycle are of great importance, especially with an increase in illumination at night. While the disruption in the light-dark rhythm has long been known as a powerful behavioral stressor, the function of the HPA axis under constant light (CL), especially its response to other stressors, in particular, to psycho-emotional stress exposure, taking into account age and features of stress behavior, remains extremely poorly understood. The purpose of this research is to study the effects of chronic round-the-clock lighting on the response of the HPA axis to acute psycho-emotional stress exposure (ASE), according to the age and characteristics of stress behavior on the model of nonhuman primates.
In the experiments, 35 young adults (5-8 years) and 23 old (21-33 years) female rhesus monkeys (Macaca mulatta) were used. The experiments were carried out in the summer, when ovarian cycles are not typical for this species of nonhuman primates. Half of the animals were individuals with control standard behavior (SB) and the other half were individuals with excessively anxious (restless) and depressive-like behavior (DAB). After a period of adaptation to living conditions in individual metabolic cages and the procedure for taking blood samples and also after a control basal period, half of the young and old animals (experimental) were in CL conditions (LED lamp "Navigator" 71 302 NLL-G-T8-18-230-4K-G13, Limited Liability Company "TM Navigator", Moscow, Russia; made in China – Xiamen Neex Optical Electronic Technology CO., LT; for 4 - 8 weeks, 330-400 lux), and the other half of young and old animals (control) were under standard lighting (SL) with natural light (day)-darkness (night). All animals were subjected to ASE (mobility restriction for 2 hours in metabolic cages, start at 15.00), functional tests with the administration of corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP), as well as the study of circadian rhythms of cortisol secretion and pineal hormone melatonin.
We demonstrated for the first time an inhibitory effect of CL on the response of the adrenal cortex to ASE in young mature and old female rhesus monkeys. Although the inhibition of the function of the adrenal cortex was detected in all the examined animals, regardless of age and behavior, the mechanism of this phenomenon, apparently, is age-dependent. So, young animals were characterized by an inhibitory effect of CL both on the magnitude of the rise in CORT secretion and on the magnitude of the rise in ACTH levels and its absence in control animals with normal light-dark schedule. At the same time, a decrease in the magnitude of the rise in the level of ACTH preceded a decrease in the magnitude of the rise in plasma CORT. Therefore, it can be considered that the inhibitory effect of CL on the secretion of CORT in young monkeys is due to the inhibition of ACTH secretion. In turn, the inhibitory effect of CL on ACTH secretion seems to be mediated by inhibition of the ACTH-stimulatory effects of AVP, one of the central drivers of the HPA axis, since a functional test with AVP under CL revealed an inhibitory effect of CL on ACTH and cortisol secretion, similar to ASE. In old animals an inhibitory effect of CL on the magnitude of the CORT level rise in response to ASE was noted mainly in the absence of corresponding changes in the rise of plasma ACTH. Apparently, in old animals, the inhibitory effect of CL on stress reactivity of the adrenal cortex is mediated by the transmission of light signals from the suprachiasmatic nucleus of the hypothalamus, the main regulator of biorhythms in the body, directly to the adrenals with the help of an extrahypophysial neural pathway without accompanying activation of the hypothalamic-adenohypophysial axis. This conclusion is supported by the results of tests with CRH and AVP, which did not reveal statistically significant inhibitory effects of CL on the secretion of ACTH and cortisol in old animals. A destructive effect of CL on the circadian rhythm of plasma cortisol concentration was also noted in the absence of significant changes in melatonin secretion.
The observed CL inhibition of adrenal cortical reactivity to ASE may be useful to correct increased vulnerability to ASE observed in individuals with preexisting anxiety and depression-like stress behaviors. Besides, the CL induced decrease in adrenal stress reactivity of behaviorally normal animals suggests a potential risk of reducing the adaptive capacity of the organism under conditions of continuous light exposure.
Keywords: acute stress, the HPA axis, melatonin, constant light, aging, behavior, rhesus monkeys

### S7.511. The effect of high-frequency electromagnetic radiation on the distribution of superoxide dismutase sod1 in the honeybee brain

Pribyshina A.[1], Lopatina N.[1], Zachepilo T.[1]*
[1]*I.P. Pavlov Institute of Physiology RAS;*
* polosataya2@mail.ru

A number of studies of recent years indicate the stress effect of an increase in the level of the electromagnetic background of the environment on biosystems. Organisms sensitive to magnetic and electric fields include many insects, in particular, the honeybee. Previously, a reducing of the food excitability and short-term memory in the honeybee was shown (Lopatina et al., 2019), as well as a change in the distribution of the heat shock protein hsp70 in the honeybee brain (Zachepilo et al., 2021) under the action of high-frequency electromagnetic radiation of 2.4 GHz. A possible consequence of the action of electromagnetic radiation may be the development of oxidative stress, the markers of which are an increase in the level of antioxidant enzymes (superoxide dismutase, catalase, etc.).

The aim of this work was to study the effect of high-frequency electromagnetic radiation of 2.4 GHz on the distribution of superoxide dismutase 1 (sod1) in the honey bee CNS in the bee brain. In this work, we used the exposure to electromagnetic radiation of a household Wi-Fi router (1 h) followed by immunostaining of honeybee brain sections. Next, the ratio between the level of staining of the bodies of neurons and the neuropil of mushroom bodies in the experimental and experimental groups was evaluated.

It has been shown that in the honeybee, a one-hour exposure to electromagnetic radiation from a Wi-Fi router operating at a frequency of 2.4 GHz causes a change in the ratio of staining of the neurons and the neuropil of mushroom bodies in the honeybee brain. The results obtained indicate a change in the redox state of mushroom bodies, brain structures responsible for learning and memory in insects. The development of oxidative stress in these structures can significantly reduce the level of foraging and, consequently, the pollination activity of the honeybees.

### S7.512. The efficacy of photobiomodulation during oncogenesis depending on the degree of oxidative modification of macromolecules and the features of functioning of the central nervous system

Zhukova E.S.[1]*, Shcherbatyuk T.G.[1], Chernov V.V.[2], Gapeyev A.B.[3]
[1]*Nizhny Novgorod Scientific Research Institute of Hygiene and Occupational Diseases of Rospotrebnadzor, Nizhny Novgorod, Russia;*
[2]*Federal Research Center Institute of Applied Physics of the RAS, Nizhny Novgorod, Russia;*
[3]*Institute of Cell Biophysics of RAS, Pushchino, Russia;*
* evgenya_plekhanova@mail.ru

One of the open questions on the mechanisms of realization of biological action of electromagnetic radiation (EMR) in the visible and infrared ranges is the explanation of different responses of living systems to the effect of EMR in the conditions of tumor development: stimulation of proliferation, no effect, or inhibition of neoplasia cell growth have been shown [1,2]. It is possible that the different responses are due to the different functional state of living system at the time of exposure.

It has been noted that cytochrome C oxidase, one of the main photoacceptors, has different photosensitivity depending on the degree of oxidation [1]. In this regard, we put forward a hypothesis that the efficacy of photobiomodulation at the organismal level will depend not only on the parameters of the EMR used, but also on the degree of oxidative modification of macromolecules. However, to date, there are no data on which markers reliably characterize oxidative stress in the body, therefore, an integrated approach is needed to assess the level of oxidation of lipids, DNA, proteins, and the activity of enzymes of the antioxidant system. The reflection of the internal state of oxidative processes in a complex living system may be, among other things, the functioning of the nervous system, as one of the most sensitive to oxidative stress. Some rat genes for antioxidant defense enzymes are located in the loci associated with behavior in the "open field" test [3,4]. Based on this, the behavioral features revealed using this test may probably be associated with the level of oxidative processes in the body. The purpose of the work was to evaluate the efficacy of photobiomodulation during oncogenesis, depending on the degree of oxidative modification of macromolecules and the behavior of laboratory animals in the "open field" test.

The studies were carried out on 138 juvenile outbred SD male rats. Strains of solid tumors of rats RS-1 and RA (N.N. Blokhin NMRCO) transplanted subcutaneously were used as models of neoplasia. Experimental low-intensity EMR generators (λ1=400±20 nm, 4.6 J/cm2, λ2=460±20 nm, 3.2 J/cm2, transcutaneously; IAP RAS, Nizhny Novgorod) and an AFS physiotherapeutic LED apparatus for PDT (λ3=660±10 nm; Polyronic LLC, Moscow) served as sources of EMR in the optical range. Modifiers of oxidative processes were ozone produced at a "TEOZON" medical ozonizer (RFNC VNIIEF, Sarov) administered intraperitoneally as ozonized saline [5], and the hydroxyaluminum photosensitizer trisulfophthalocyanine (FGUP "SSC NIOPIK", Moscow). Schemes of exposure and dosage were described in detail in our works [2,6]. The behavior of rats in the “open field” test, the antitumor efficacy of exposures by the tumor growth inhibition index and the tumor growth rate, the total free radical activity indirectly by a method of induced H2O2 and Fe2+ chemiluminescence, the level of lipid peroxidation by the TBARS assay, the degree of oxidative modification of proteins according to the level of carbonyl derivatives using the reaction with 2,4-dinitrophenylhydrazine, the level of DNA damage in rat blood leukocytes using the alkaline version of the comet assay, the activity of superoxide dismutase according to the reduction reaction of nitroblue tetrazolium and catalase according to the rate of decomposition of H2O2 [2,6,7] were determined.

In terms of exploratory and motor activity, emotionality and anxiety, the animals were divided according to the type of behavior: 1) passive, 2) moderately active, and 3) highly active (k-means method, $p<0.001$). Compared with moderately active rats, passive rats were characterized by a higher intensity of oxidative processes in the body, while high-active ones, on the contrary, had a low intensity [2]. For animals with passive behavior, a higher rate of proliferation of RS-1 tumor cells at the beginning of the logarithmic growth phase and subsequent spontaneous regression [2] and more aggressive development of RA with metastasis were noted [6]. The impact of violet-blue EMR on RS-1 of early stages of development in passive animals stimulated tumor progression, in moderately active animals it slowed down, in highly active animals it led to regression. The preliminary course action of ozone changed the response to the action of violet-blue EMR: in passive rats, growth was stopped followed by regression of the tumor focus, in moderately active rats, short-term inhibition and continued growth were observed, and in highly active rats, a pronounced antitumor effect was found [2]. Photodynamic exposure using red EMR and a photosensitizer contributed to the progression of RA in highly active rats. At the same time, the preliminary course action of ozone reduced the risks of stimulating the proliferation of tumor cells and increased the antitumor effect [6].

Thus, we have shown that the efficacy of photobiomodulation during oncogenesis depends on the degree of oxidative modification of macromolecules and is interrelated with behavioral features.

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### S7.513. The mechanism of action of a low-frequency electromagnetic field on aqueous solutions of biopolymers

Tekutskaya E.E.[1]*, Baryshev M.G.[2], Ilchenko G.P.[1]
[1]*Kuban State University;*
[2]*Kuban State Technological University;*
* tekytska@mail.ru

A physicochemical mechanism has been developed for the action of a low-frequency electromagnetic field (LF EMF), which has an extremely low energy, on aqueous solutions of biopolymers based on nucleic acids and proteins, associated with a change in the amount of the longest-lived form, hydrogen peroxide, in the chemical oscillator of ROS interconversions [1].
The initial stage of the cycle is the initiation of the process, i.e., the attachment of an electron from e-c Rydber excited levels of macromolecules (for example, DNA) [2] to free water protons with the formation of a hydrogen radical and the subsequent formation of a hydroperoxide radical, singlet oxygen, superoxide ion, hydrogen peroxide.
It follows from the resulting kinetic equation that in dilute solutions of biopolymers, the rate of accumulation of hydrogen peroxide depends on the pH of the solution, the concentration of electrons e-Rg from the Rydber excited levels of macromolecules, and the concentration of dissolved oxygen. Periodic accumulation and decomposition of hydrogen peroxide determine the frequency of the entire cycle of interconversions in the chemical oscillator of ROS.
If we consider the losses in the chemical oscillator to be small and the frequency of the driving force differs slightly from the natural frequency of the interconversions of hydrogen peroxide in the chemical oscillator, then after appropriate assumptions and transformations, we obtain the solution of the original equation in the following form, where is the initial phase, which, under the assumptions made, is the same for both damped and undamped oscillations, d is the damping index of hydrogen peroxide concentration fluctuations in a chemical oscillator, is the ratio of the frequency of the driving force to the natural frequency of the system . It follows from the analysis of expression (1) that the resulting fluctuation of the hydrogen peroxide content is the sum of two fluctuations having different frequencies, and the numerical ratio between the oscillation frequencies in the chemical oscillator system and the external field as a whole determines the nature of the transient process.
It has been experimentally established that oxidative damage to DNA and conformational transitions of proteins are based on a universal mechanism caused by the formation of ROS in aqueous solutions under the action of low-intensity EMF, while the quantitative content of hydrogen peroxide resonantly depends on the frequency of the acting EMF [2].
Within the framework of the developed model, the accumulation of oxidative DNA sites is possible due to the formation of ROS under the action of LF EMF. The quantitative content of 8-OHdG in DNA also resonantly depends on the frequency of the exposed EMF and enhances the already existing oxidative stress in patients with a genetically determined disease. Conformational changes in proteins are accompanied by an increase in the availability and activity of nucleophilic centers, which are potential targets for ROS; complete unfolding and denaturation of the protein amino acid chain under the action of LF MF does not occur. The increased amounts of ROS formed during the resonant interaction attack the nucleophilic centers of proteins and DNA molecules, damaging them. But if the effect of LF EMF, mediated by the generation of ROS in an aqueous medium, on DNA is mainly damaging in nature: either oxidative or structural, then proteins under the action of LF EMF mainly undergo conformational changes. In this case, redox modifications of the sulfhydryl and hydroxyl groups of proteins can occur. Being regulatory for the preservation of the structure of proteins, these groups prevent further denaturation of the protein. At the same time, the conformation of amino acid helices changes. Deformation of proteins and DNA molecules under the action of LF EMF can lead to the appearance of resonant frequencies depending on the frequency and amplitude of LF EMF.

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### S7.514. The plant tissues electrical impedance spectroscopy

Astashev M.E.[1,2]*, Konchekov E.M.[1], Kolik L.V.[1], Gudkov S.V.[1]
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences;*
[2]*Institute of Cellular Biophysics RAS, Pushchino, Russia;*
* astashev@yandex.ru

Electrical impedance spectroscopy (EIS) is a method for analyzing the electrical resistance or conductivity of materials and systems at different frequencies. The recorded impedance as a function of frequency is then associated with physical parameters or properties of materials and systems. In biology, EIS is applicable to both plant and animal systems. The vascular system of plants mainly performs two functions - delivering nutrients to the plant organs and serving as a chemical signaling network. Features of the vascular system of plants can also be studied using EIS. To solve these issues, the transport system can be represented as a combination of resistances and capacitors. The phloem and xylem conduct electricity along the stem well and provide mainly resistive properties, while the cambium separates them with low conductivity layer and provides capacitive properties. In addition, electrical impedance measurements can be carried out non-invasively on relatively young branches, which makes it possible to detect stress factors already in the early stages of plant development. As a rule, the use of EIS is associated with the need to organize special laboratory conditions for measurements. We have developed a device and the measurement procedure, which allow monitoring the condition of trees in situ, both in the field and in greenhouses. In this case, it is possible to determine the humidity, the development of the vascular system, the rate of healing of internal injuries, for example, when grafting trees.
The device is based on the AD5933 ADC chip (Analog Devices) and the ATMega328 controller (Microchip). The interface with the computer is carried out with the Bluetooth HC-05 module. The device is powered by two 18650 li-ion batteries connected in series. The device also has the function of auto-calibration, protection against

electrostatic discharges and the function of temperature compensation of measurements.
The measurement procedure was developed to obtain the dependence of the electrical impedance on the frequency, i.e. impedance spectrum. To do this, a series of complex data of flowing current was obtained for a range applied sinusoidal voltage with frequencies from 2 kHz to 10 kHz with a step of 1 kHz and from 20 kHz to 100 kHz with a step of 10 kHz. Such a frequency grid uses of the automation of the measurement procedure incorporated in the AD5933, and saves measurement time in the frequency range from 20 to 100 kHz.
Measuring the impedance of samples of tree branches with a diameter of 6-30 mm and a length of 100-500 mm was carried out by connecting the device to the bark of the studied branch through electrolytic bridges, which were used as rectangular segments of hygienic cotton pads 10x30 mm in size, 2 mm thick, which were moistened with a 5% solution sodium bicarbonate in distilled water. The quality of the contacts was evaluated by the stability of the conductivity readings, for which each sample was measured at least 5 times in a row.
We have found that the branch impedance contains both constant components and components depending on the length of the branch and their diameter. The former correspond to the connection of the measuring system to a biological object, the latter, in fact, are of interest. To separate these components, we obtained the dependence of the impedance spectrum on the length of the branches and their diameter. Measurements with a change in the length of the branches were carried out at a length of 100mm, 200mm, 300mm, 400mm and 500mm with the same branch diameter of 23mm. Measurements with a change in diameter were carried out at a constant length of 300 mm and on branches with a diameter of 5, 8, 12, 22 mm. For all measurements, branches of the Melba apple tree were used. Each point was measured at least 3 times on different branches to study the stability of the measurements. As a result, constant capacitive components of the impedance were revealed, which are probably responsible for the transitional capacitances from the electrodes to the internal tissues of the plant, formed by layers of the bark (crust, periderm) that are poorly conductive to electric current as an insulator. Additionally, we were able to estimate the total thickness of these components, which was about 9 μm for relatively young branches with a diameter of 5–23 mm. By subtracting the capacitive part of the electrodes from the total impedance, we got an idea of the impedance of plant tissues and its dependence on the diameter and length of the branches. This made it possible to build a structural electrotechnical model of a plant branch section and to evaluate the real parameters of xylem and phloem conductivity. The model satisfactorily describes the experimentally obtained data.
In addition, the temperature dependences of the impedance were measured at temperatures of 4, 23 and 36 degrees. The obtained relative value of the slope of the characteristic was 0.014–0.017 K–1, which means a decrease in the value of the impedance modulus by 30% with an increase in temperature by 20 K. This change coincides with the change in the resistance of an electrolyte solution containing relatively small ions (H+, K+, Na+, Cl - etc.) and generally corresponds to our expectations from the measurement of fluid resistance in blood vessels.

### S7.515. The process of ice crystals formation in cryoprotective media for cryopreservation of cells

Ivanova A.A.[1]*, Yakovenko S.A.[1], Mironova A.G.[2], Simonenko E.Yu.[1]
[1]*Lomonosov Moscow State University, Moscow, Russia ;*
[2]*IFV clinic "AltraVita", Moscow, Russia;*
* annetkurella@yandex.ru

Efficient freezing and storage of human sperm is an extremely urgent task for assisted reproductive technology. Despite the fact that sperm cryopreservation has been performed since the 1960s, the motility and fertilizing capacity of cells after cryopreservation is still reduced by an average of 30-70% [1]. Currently, the compositions of cryoprotective media are selected empirically, based on observations of the cell parameters without a deep understanding of the physical and chemical processes occurring in specific solutions at low temperatures and the effect of freezing rates and modes on these processes.
Therefore, the purpose of this work is to study the effect of different components of cryoprotective media and freezing conditions on the process of crystal formation in cryoprotective media, as well as to select the optimal composition of cryoprotective medium and freezing conditions that increase the survival of cells.
A PPMS (Physical Property Measurement System, Quantum Design, Inc., USA) calorimeter was used to obtain the dependence of heat capacity on temperature [2]. Evaluation of the effect on the crystal formation of the addition of individual components, as well as different modes of freezing was carried out using X-ray diffraction analysis (DESY, Germany).
The experiments resulted in temperature dependences of heat capacity for glycerol buffer solution (12% vol.) with stepwise addition of albumin (4 mg/ml) and sucrose (0.5 M). The main result of the calorimetric measurements was the conclusion that the addition of sucrose to the aqueous glycerol solution reduces the temperature gap between the melting temperature and the glass transition temperature, which may contribute to better cell survival.
The first experiments using X-ray diffraction analysis were performed for base solutions of small volumes (20-30 μl) to demonstrate the possibility of using this method to study the process of crystal formation. Diffraction patterns were obtained for water-glycerol solution (50% vol.), water (100%), and the solution under study (12% vol. glycerol). However, at present clinics when working with spermatozoa most often use the method of slow freezing of large volumes of samples. Therefore, further study of ice crystal formation in cryoprotective solutions was performed for 1.7 ml samples. In order to estimate the average size of crystals formed in solution during cryopreservation, software was written to numerically estimate the average size of crystals. Using this software, average crystal sizes were obtained for basic solutions during large volume freezing. It was shown that the average crystal size in the solution with all components was 8±1 μm, and for an aqueous solution of sucrose (0.5M) without glycerol 20±2 μm. It was also found that the addition of sucrose leads to an increase in the number of crystals in solution, but their size is much smaller. This observation correlates with the putative function of the non-penetrating components and also confirms the results obtained earlier by calorimetry.
In the course of experiments, it was found that crystal formation in 1.7 ml samples occurs inhomogeneously during classical vertical freezing. Therefore, an alternative method of sperm freezing with a horizontal position of the test tube was proposed. For this purpose, a holder was made on a 3D printer to adapt the freezing method used in clinics. It was shown that the average crystal sizes for the commercial cryoprotectant SpermFreeze when vertically positioned at the top and bottom of the tube are 13 and 8 μm, respectively. In the horizontal position, the crystal formation process is more uniform and the average size of the formed crystals is smaller, namely 10 and 8 μm at the top and bottom of the tube. The conclusion about homogeneity of crystal formation when the tube is placed horizontally was also confirmed statistically for all studied solutions.
Thus, the methods of adiabatic calorimetry and X-ray analysis show that the addition of sucrose to the aqueous glycerol solution during freezing in small volumes reduces the temperature interval between the melting temperature and the glass transition temperature, and also reduces the size of the forming crystals. In this work, using the proposed new method of diffraction pattern analysis for cryoprotective media, crystal sizes were numerically estimated for all cryoprotective media under study. It was also demonstrated that the formation of crystals when samples are placed vertically during freezing occurs

unevenly, the scatter of their size can reach 20 μm or more, while the horizontal arrangement statistically significantly leads to more homogeneous crystal formation within the sample. Based on the results obtained, a new method of sperm freezing in cryotubes with horizontal arrangement of samples during cryopreservation was developed and proposed.

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### S7.516. The role of DNA double-strand breaks in radiation-induced cellular senescence

Osipov A.A.[1]*, Chigasova A.K.[1,2], Yashkina E.I.[1,3], Ignatov M.A.[1,3], Fedotov Y.A.[1,3], Molodtsova D.V.[3], Vorobyeva N.Y.[1,3], Osipov A.N.[1,3]
[1]*N.N. Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, Russia;*
[2]*Emanuel Institute for Biochemical Physics, Russian Academy of Sciences, Moscow, Russia;*
[3]*State Research Center—Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency (SRC-FMBC), Moscow Russia;*
* a-2-osipov@yandex.ru

Among the DNA damages, the most critical for the further fate of the cell are DNA double-strand breaks (DSB). As a result of incorrect repair of DSB, microstructural aberrations of chromosomes occur, as well as various cytogenetic disorders. At the same time, the impossibility of repair leads to the activation of cell death or cellular senescence mechanisms.

Complex dynamic microstructures formed during DSB repair, consisting of hundreds and thousands of copies of proteins involved/associated with the repair process, are visualized after immunocytochemical staining in the form of bright dots, and are called DNA repair foci

DNA repair foci observed after 24 hours and later after irradiation are called residual in the literature. They are believed to be the repair sites for complex, potentially lethal DNA DSBs. It is known that an increase in the number of residual foci is associated with a decrease in colony-forming ability. The reason for the decrease in clonogenic growth and survival is not only cell death, but mainly the loss of the ability to divide due to cellular senescence.

However, the features of their post-radiation quantitative changes and their role in the processes of cell death and aging have not been sufficiently studied yet.

The aim of this work was to investigate the relationship between the changes in the number of residual DNA repair foci of key DDR (DNA damage response) proteins (γH2AX (sensor), pATM (transducer), 53BP1 (mediator), p-p53 (effector)) and the proportion of senescent cells in human fibroblasts 24, 48 and 72 hours after exposure to X-rays at doses of 1-10 Gy.

The results of the studies showed that according to the quantitative yield of residual foci, the studied proteins can be arranged in descending order γH2AX > 53BP1 > pATM ≥ p-p53. With an increase in the time after irradiation from 24 to 72 hours, the number of residual foci of all studied proteins decreases. This decrease can be explained by several parallel processes: the elimination of highly damaged cells by the mechanisms of apoptosis, autophagy, etc.; the completion of the DNA repair process and, finally, a decrease in the proliferative activity of cells, accompanied by a decrease in the amount of replicative DNA damage.

X-ray exposure also led to a dose-dependent increase in the proportion of SA-β-gal positive and Ki-67 negative fibroblasts. Irradiated cells, in response to the formation of DNA DSBs, activate cell cycle control points via the ATM/ATR signaling pathway, causing the delay or arrest of the cell cycle at certain phases. At the same time, DNA damage and slowing down (stopping) of the cell cycle are not necessarily followed by cell aging, since complete repair or cell death is not excluded.

It was also shown that there is a positive correlation between the number of residual foci and the proportion of senescent fibroblasts after irradiation in one or another studied time interval (24, 48 or 72 hours). However, if the number of foci decreases with time after irradiation, then the proportion of senescent cells, on the contrary, increases. Apparently complex, difficult to DNA repair damage triggers the process of radiation-induced aging, while in senescent cells the repair process can continue, leading to a decrease in the number of residual foci with time after irradiation, but to an increase in the proportion of senescent cells.

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### S7.517. The spin subsystem of water molecules is responsible for the dynamics of changes in its physical and chemical properties

Drozdov A.V.[1]*
[1]*Institute of Analytical Instrumentation;*
* da@biophys.ru

In the study of the physicochemical properties of water in work [1], it was revealed that the physical characteristics of water obey certain patterns. In all experiments, regardless of the method of molecular structural analysis used (IR spectroscopy, Raman spectroscopy, conductometry, microwave radiometry, NMR in the Earth's magnetic field, etc.), oscillation periods of the measured values were observed to be close in value (in our experiments, we analyzed periods from 1 to 60 minutes).

On the one hand, in order for an oscillatory total effect of intermolecular transformations to be observed in a molecular system, synchronization of transitions between different states of molecules is necessary. It is necessary to ensure that these transitions occur in all molecules of the medium (or most of it) simultaneously, in phase.

On the other hand, for the manifestation of the periodicity effect, a resonance between the states corresponding to the main energy levels is needed. In real conditions, this is unlikely, since all levels of the system are in an excited state. However, the effect of periodicity can also be observed at resonance of excited levels, but this requires a constant external action.

In nonlinear oscillatory systems, an external periodic action can lead not only to the excitation of forced or parametric oscillations, but also to the dynamic stabilization of equilibrium states. These states without external influence would be absolutely unstable, or even absent.

A well-known and studied example of such systems is a physical pendulum with a vibrating suspension point. Such a system can be stabilized in a state of equilibrium with the center of gravity above the point of suspension. In the domestic literature, attention should be paid to the work of P.L. Kapitsa [2], in which, when using the approximate solution method, an averaging method was used that has a simple physical meaning: the action of mechanical vibrations leads to the appearance of an additional ("vibrogenic") moment and to a change potential energy of the pendulum. Mechanical systems with the possibility of vibrogenic stabilization in an unstable state of equilibrium in the domestic scientific and technical literature are called Kapitsa's pendulums.

Similar phenomena of dynamic stabilization of equilibrium states of mechanical systems under the action of vibrations currently attract close attention of both experimenters and theorists working in various fields

of modern science and technology (solid state physics, hydrodynamics, chemistry, biology, genetics, elementary accelerators particles, etc.).
It was established in [3] that in an amorphous magnet with a weak random anisotropic disorder, due to the latter, a preferred direction of the ferromagnetism vector can appear. The influence of disorder turns out to be similar to the influence of vibrations of the suspension point in Kapitza's pendulum. A very interesting result was obtained in [4]. An analysis of the equilibrium state in oscillatory systems of one and two magnetic needles in the presence of an oscillating field showed that in a certain range of changes in the amplitude and direction of the oscillating field, a system of two magnetic needles has two dynamically stabilized equilibrium states.
In our NMR experiments [1], when studying the dynamics of the proton density of water samples, we obtained results that can be correlated with the phenomena of dynamic stabilization of equilibrium states described above. The observed quasi-harmonic change in the intensity of the NMR signal (intensity=proton density) indicates a change in the ratio of ortho- and para-molecules in the sample. The spins of water protons are the same magnetic arrows in the constantly oscillating magnetic field of the Earth, the action of which leads to the observed dynamics of the proton density. The facts of "partial correlation" between the strength of the Earth's magnetic field and the proton density speak in favor of the latter.
In our opinion, the dynamics of the spin subsystem of water is in good agreement with the two-component model of water, which is widely used in various biophysical models. The role of spin isomers of water was proposed by S.M. Pershin [5] and lies in the fact that the mechanism responsible for changes in intermolecular interaction in water is associated with ortho- and para-water molecules. The difference in the rotational degrees of freedom of these molecules leads to a different character of their interaction both with each other and in clusters. This idea becomes a kind of physical basis for the two-structure model of water and the structural dynamics associated with it.
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### S7.518. Transport of chloride ions through the plasma membrane of lamprey oocytes (lampetra fluviatilis)

Sherstobitov A.O., Chebotareva M.A., Shukolyukova E.P.[1], Nikitina E.R.*
[1]*Sechenov Institute of evolutionary physiology and biochemistry;*
* elena.nikitina@bk.ru

Using the radioactive isotope 36Cl, the permeability of the membrane of isolated oocytes for chloride ions was determined in the pre-spawning period of the life of river lampreys. At a physiological chloride concentration of 150 mmol/L in the incubation medium, the stationary distribution of 36Cl between the cell and the medium corresponded to the intracellular chloride ion concentration of 10.5 mmol/L in December and 18.6 mmol/L in April, with a 36Cl half-accumulation time of about 20 min. The permeability coefficient for Cl- was 1.5x10-6 cm/s (at 5°C) in December and 7.5x10-6 cm/s (at 10°C) in May.
The accumulation of chloride linearly depended on its concentration in the incubation medium (0-150 mmol/l) and on temperature with an activation energy of 24 kJ/mol at 5-20°C. Depolarization of the plasma membrane in environments with a high content of K+ in the presence of valinomycin stimulated the entry of Cl- by about 2.5 times. A decrease in the osmoticity of the medium by 50% led to a threefold activation of the Cl- input compared to iso-osmotic conditions.
Incubation of cells in the presence of 0.1-0.2 mmol/L Cu2+ and 0.2 mmol/L Cd2+ caused a noticeable acceleration of Cl- entry, while 0.2 mmol/L Pb2+, 0.2 mmol/L Zn2+ and 0.15-10 mmol/L (Ca2++ Mg2+) did not affect Cl- transport. The treatment of cells with a high concentration of the A23187 ionophore in the presence of 5 mmol/L Ca2+ to increase the level of intracellular calcium also proved to be ineffective. Bumetanide-sensitive Na-K-2Cl cotransport provided less than 5% of the total Cl- uptake. In April, Cu2+ caused an approximately 4.5-fold increase in the amiloride-sensitive component of Na+ (22Na) influx, which most likely represented the Na+/H+ exchange mechanism. In conclusion, it should be noted that during the pre-spawning period, the content of chloride ion in lamprey oocytes increases by an average of 8 mmol/L (by 77%).
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### S7.519. Use of biophysical technologies in agricultural research

Gudkov S.V..[1]*
[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences ;*
* S_makariy@rambler.ru

Climate change and the struggle to preserve the environment require the development and implementation of new solutions and approaches, including in agriculture. In the process of implementing the Large Scientific Project of the Ministry of Science and Higher Education of the Russian Federation (No. 075-15-2020-774), the following results were obtained at the VIM and GPI RAS:
A prototype of a flow sensor for milking systems has been created to determine the abnormal characteristics of milk (blood impurities, water content, etc.) and its component composition (fat, protein, lactose). For this, optical technologies have been created that make it possible to control the flow rate of highly light-scattering liquids. Optical technologies have been created that make it possible to detect objects with a size of 10-100 microns (the size of an animal cell) in highly light-scattering liquids. Optical technologies have been created that make it possible to evaluate the quantity and quality of scatterers in biological fluids. Optical technologies have been created that make it possible to evaluate the concentrations of the main biomolecules in milk. An express technology for assessing the quality of milk has been developed by analyzing the presence of staphylococcal enterotoxins and antibiotics (chloramphenicol) in it. Ecologically safe and energy-efficient solar-transforming coatings have been created to increase the productivity of protected soils in northern latitudes. For this, methods have been developed for obtaining nanoparticles with the necessary optical and magnetic properties. Technologies for obtaining nanostructured surfaces with the desired physical and biological properties have been created. A low-temperature method for incorporating nanoparticles into a polymer matrix has been developed. A technology for ordering nanoparticles in a polymer matrix has been created. A technology has been developed for applying photoconversion composites to glass and polymeric materials, including nonwovens. A number of promising structural nano-meso- and macromaterials for greenhouses have been developed. Technologies have been created to neutralize pathological processes in plants using cold plasma and plasmolites. For this, installations have been created for the production of plasmolites based on low-temperature plasma: 1. Generated by piezotransformers (small-sized portable

cold plasma installation); 2. Based on glow discharge plasma in liquid (semi-industrial/research); 3. Based on magnetron plasma generation (industrial installation). On the basis of plasmolites, technologies have been developed for disinfecting plant seeds from pathogenic microflora. For millet crops, technologies have been developed to intensify the development of the root system (increase in drought resistance). On the basis of plasmolites, a system for processing scion and rootstock has been developed that increases the likelihood of successful grafting for fruit plants of apple, pear, cherry and sweet cherry. On the basis of an unmanned aerial vehicle, a system was created for express assessment of the functional state of the fields. For this, a laser-optical classifier of seeds and biomass has been developed, which makes it possible to distinguish between infected and non-conforming parts of plants. Protocols for the use of lidar technologies, spectroscopy, raster hyperspectral imaging, and Raman scattering have been developed for the detection of phytopathogens. Technologies have been developed for scanning a set of objects from different angles, for recognizing objects, their shape and other topological parameters. Algorithms for remote monitoring and express diagnostics of the functional state of plants have been developed. A prototype of a conveyor robot has been developed that allows to separate infected and healthy grain.
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### S7.520. Using Biophysical Approaches to Reveal the Structure of Plant Resistance Trait to Stress Factors

Fedulov Yu.P.[1]*
[1]*Kuban State Agrarian University;*
* fedulov.ju@kubsau.ru

Understanding the mechanisms of plant response to stress is necessary for effective plant breeding for resistance to damaging environmental factors.
As part of solving this problem, on a large set of sample an assessment of the frost resistance of winter wheat varieties from different breeding regions was carried out using a complex of biophysical and physiological methods.
Experiments were carried out using winter wheat plants grown in the field conditions. An indicator of frost resistance was the survival percentage of plants after freezing in freezing chamber (SP).
The state of plant tissues before and after freezing was assessed by electrical conductivity methods. The stability of the photosynthetic apparatus was evaluated by the parameters of delayed fluorescence (DF) thermograms.
Simultaneously, the content of photosynthetic pigments and morphological parameters of plants were determined.
For all parameters, correlation coefficients were calculated for two sets of varieties. The first one was set of 10 check varieties of frost resistance, the second - an extended set of varieties of 35-60 samples. It was found that a number of parameters had a high correlation with SP. In all years of research, the relationship between the studied parameters and SP was higher for check varieties set than for extended sets.
The correlation coefficient between the electrical resistance of hardened plants tissues and the level of frost resistance was 0.80 for check varieties set, and 0.62 for an extended set of varieties.
Among the analyzed parameters of DF thermograms, the ratio of DF level at 20°C and at temperature 2-4°C lower than the temperature of the low-temperature maximum of DF showed the highest relationship with PS. At this temperature, the maximum differences in the levels of DF were observed between samples contrasting in frost resistance. The correlation coefficient for this parameter was 0.46 for check varieties and 0.32 for the extended set of varieties.
The processing of the collected data by the method of orthogonal factor analysis made it possible to establish that the general set of physicochemical, physiological and morphological features associated with frost resistance can be combined into components that can be used to describe frost resistance with high accuracy.
Equations were calculated for each variety, where the function is the level of frost resistance, and the arguments are the values of the obtained components. These equations can be considered as a model of a complex trait of frost resistance, reflecting its complex genetic structure [1].
An analysis of the distribution of the studied parameters among the components, as well as a comparison of the values of these components in samples with different biological characteristics and the nature of their changes during overwintering, made it possible to give them a biologically meaningful interpretation and define them as physiological systems that determine the formation of a complex trait of frost resistance.
The first four systems, with total significance 65–70%, were interpreted as nonspecific resistance due to forced winter dormancy, the ability to supercool intracellular water, the ability to grow at low temperatures, and the ability to repair damage caused by unfavorable overwintering factors.
Comparison of equations for varieties of different breeding regions showed that the optimal level of frost resistance in different breeding regions can be determined by different components.
Recently, molecular markers of various breeding traits have been increasingly used in breeding practice [2], including searching for loci of quantitative traits – QTL (quantitative trait loci). In this case, SP after freezing at a critical temperature or the temperature of semi-lethal survival LT50 is used as a parameter characterizing frost resistance.
Taking into account the presented model of the structure of the frost resistance trait and the nature of its change depending on environmental conditions, it can be assumed that it is more appropriate to search for QTLs associated not with the integral trait of frost resistance, but with individual identified components of frost resistance, which in a particular breeding region provide an optimal level of frost resistance. and do not negatively associated with productivity.
The approach used makes it possible to select in each component a biophysical, physiological-biochemical or morphological parameter that has a quantitative expression and is most closely associated with this component. This parameter can be measured for each individual of the studied population or for a group of genetically homogeneous individuals, which is a necessary condition for QTL mapping.
A similar approach can be used in breeding for resistance to any abiotic factor, since most of them are polygenic in nature. The use of such an approach in breeding would make it possible to more accurately adjust the resistance traits of interest to future crop cultivation conditions.
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### S7.521. Water as a sensor of weak impacts on biological system

Lobyshev V.[1]*
[1]*Lomonosov Moscow State University;*
* lobyshev@yandex.ru

The analysis of the problem, which has long been ambiguously accepted by the scientific community, is presented. The stimulating

effect of weak impacts on biological systems of various chemical and physical factors that inhibit these systems at high concentrations or doses was discovered a long time ago. The first experiments showing in small doses the stimulating effect of various chemical compounds on the development of various biological objects were published at the end of the XIX century. This phenomenon was called hormesis. Later, a stimulating effect of a similar nature was found for ionizing radiation and was called radiation hormesis. It was on various biological models that the dependence of the response to the decreasing concentration of the active substance or the radiation dose has a non-monotonic character and can be characterized by several extremes. When studying the effects of weak non-ionizing electromagnetic fields, non-monotonous effects were also found, depending on the intensity and frequency of the fields, including those of natural origin. Since the second half of the 1970s, there has been an increasing stream of studies in which a wide range of concentrations of active biologically active substances on various functions of biological and model systems is being investigated. Moreover, studies of the physico-chemical characteristics of aqueous solutions in the field of small and ultra-small concentrations, conducted in our country and abroad, convincingly show the appearance of similar patterns. The similarity of biological responses to weak impacts of different nature, as well as the non-classical manifestation of the physical-chemical properties of dilute solutions, forces us to search for a single cause of the observed effects, the likely candidate of which is water.

It can be considered reliably established that the non-classical behavior of dilute aqueous solutions is associated with the formation of mesoparticles of a hundred's nanometers in size. In many cases, the formation of mesoparticles is not observed when holding samples of solutions in conditions of a weakened magnetic field of the Earth. For the first time, we showed a high correlation between the physical characteristics of highly diluted solutions and the biological response of unicellular ones, which was then confirmed by other researchers. The observed effects go beyond the classical concepts of solutions, which leads to the need for careful study of the properties of water. In fact, real water is a solution even at laboratory conditions and is always nonequilibrium, which is detailed in [1]. The nature of the heterogeneity of dilute aqueous solutions and the composition of the recorded mesoparticles are still unclear. The most likely candidate for this role are nanobubbles or their complexes stabilized by ions in solution [2]. Spontaneous or induced by external mechanical and other impacts, the collapse of these bubbles entails the effects of energy accumulation, leading to high-energy processes. Because of electron generation, reactive forms of oxygen, nitrogen, and carbon dioxide appear which are recorded in the experiment even when water is kept at an elevated temperature [3]. The initiators of such processes can also serve as sources of natural ionizing radiation on Earth. As an example, the appearance of an increased amount of hydrogen peroxide is recorded in the surface waters of the ocean and in a change in the redox potential in microdroplets of fog and clouds [4]. It is clear that in the presence of biologically active substances in the solution, even more complex chemical processes essential for living organisms and model systems will occur. The technology of preparation of solutions of small and ultra-small concentrations involves an iterative procedure of dilution followed by intensive mechanical impact. Measurements of the high-frequency impedance indicate a non-monotonic complex change in the conductivity of solutions with an increase in the number of dilution iterations. The qualitative nature of the non-monotonous dependence persists at dilution steps, at which the concept of concentration loses its meaning. This result is supported by an experiment in which the first-class water dilution technology was used without adding any substances. As a result, a similar non-monotonous dependence of conductivity on the number of dilution iterations was obtained [5]. The results obtained and partially described indicate the processes of self-organization in a complex nonequilibrium system called water and transforming weak impacts of various nature into active chemical compounds.

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**S7.522. Water relation in cell walls**

Meychik N.R.[1]*
[1]*Moscow State University;*
* meychik@mail.ru

The water relation in the cell wall (CW) is regulated through the swelling of their polymer matrix, which changes under the influence of the composition of the external environment, and is under cell metabolic control. The reason for the swelling of ion-exchange materials in an aqueous solution, including CW as a natural ion exchanger, is the presence of hydrophilic groups, the reason for insolubility is the existence of cross-links. The ability to swell is a property of a polymeric material (including the plant CW) determined by its structure and composition, and the swelling itself is not just a mechanical entry of water into voids and pores, which, in essence, are not present in the polymer, but is the result of intermolecular interaction, which is mainly due to the hydration of macromolecules, which indicates the colloid-chemical rather than physical nature of this process. In addition, the swelling coefficient of the CW (Kcw) is related to its osmotic pressure, and, consequently, to its water potential: the lower Kcw, the greater the osmotic pressure in the CW and the lower its water potential.

The value of the coefficient of swelling of cell walls depends on the plant specious, pH and ions concentration in the medium. Thus, the value of the swelling coefficient is lower in cereals and Chenopodium compared to legumes; for all plants, this indicator is lower in the acidic region compared to the neutral one. This means that the polymeric component of the root CW can decrease in volume (up to 10 times in some cases) with a decrease in pH in the medium or apoplast. Similar changes occur with an increase in the ionic strength of the solution. These data clearly show that the volume of the ion-exchange polymer component of the cell walls is not a constant value, but largely depends on the ionic conditions and pH in the environment and the apoplast.

It is important to note that for all plants, the swelling of cell walls isolated from dry and intact material is significantly different. This indicates that the water uptake by cell walls is provided not only by the difference in water potentials between the environment and the wall, but also by the conformation of polymers in cell walls.

In wheat roots, a decrease in the pH of the medium to 4 led to a decrease in the hydraulic conductivity of the cell walls. At the same time, it is known that the volume flow of water in a plant is changed in the same way as the hydraulic conductivity. Comparing these data with those obtained in the present study, it can be concluded that a change in the swelling of cell walls in response to changes in environmental conditions can lead to a change in the water flow through plant roots.

In addition, it can be said that there is a direct relationship between the biochemical composition of the polymer matrix of cell walls, their ionic environment, cell wall swelling, and water flow.
It is known that the lower the ionic strength of the soil solution, the greater the intensity of plant transpiration and, according to the results, the greater the swelling of the root CW. Moreover, it has been shown that in the absence of water deficit and at a high transpiration rate, a large gradient of water potential is formed in the soil-root-leaf-atmosphere continuum, the root resistance is low, the roots quickly absorb water from the soil, and the apoplastic pathway of water movement will dominate. These data also clearly demonstrate a direct relationship between root CW swelling and water flow and indicate an important physiological role of the polymer matrix in the regulation of not only ion transport, but also the water relation of plants.
However, the main factor that determines the ability to swell is the degree of crosslinking of polymer chains in the CW matrix. This parameter cannot be determined experimentally, but it is possible to estimate this indicator indirectly. Based on experimental and theoretical studies on the physicochemical properties of weakly cross-linked carboxyl-containing ion exchangers, it was found that the higher the degree of cross-linking of polymers, the lower the coefficient of swelling of the polymer material in water. In accordance with these data and the results of measuring the coefficient of swelling of cell walls in water, it can be concluded that in the series of legumes, cereals, chenopodium, the degree of polymer crosslinking in the CW of spinach and suaede is higher compared to other plants, because in the former, the coefficient of swelling in water is 2 and 4 times less than that of wheat and legumes, respectively. It can also be assumed that the degree of crosslinking of polymers in the cell wall of plant roots does not exceed 4%, since such values of this indicator are characteristic of synthetic carboxyl-containing ion exchangers with similar values of swelling coefficients in water.
Thus, there are differences in the structure of the polymer matrix of the cell walls of different plant species, which are due to different degrees of crosslinking of polymers in the CW matrix.

### S7.523. tPCS as a method for correcting cardiac arrhythmias in patients with myocardial infarction

Sorokina E.A.[1,3], Kade A.Kh.[1], Trofimenko A.I.[1,2,3]*
[1]*Kuban State Medical University, Krasnodar, Russia;*
[2]*Kuban State Technological University, Krasnodar, Russia;*
[3] *Scientific Research Institute – Ochapovsky Regional Clinical Hospital no. 1, Krasnodar, Russia;*
* artemtrofimenko@mail.ru

The elaboration of new non-drug methods for the prevention and treatment of cardiac arrhythmias (CA) developed after percutaneous transluminal coronary angioplasty (PTCA) with stenting during the treatment of myocardial infarction (MI) is an urgent task of modern cardiology.
The promising ways to solve the above problem is transcranial pulsed current electrotherapy (TES-therapy), since in a number of studies on models of myocardial ischemia, the method corrected nonspecific inflammation and oxidative stress, and prevented the negative effect of catecholamines on the heart.
TES-therapy is a method of non-invasive electrical stimulation with a bipolar pulsed current with a frequency of 77.5 Hz and a current flow density through the structures of the antinociceptive and stress-limiting system of the brainstem of 0.01-0.05 mA/cm2.
Objective: to study the effectiveness of TES-therapy in the correction of CA that developed after PTCA with stenting during the treatment of patients with myocardial infarction.
Materials and methods:
Inclusion criteria: men and women aged 35 to 75 years with Q-positive and Q-negative MI; PTCA with stenting; development of CA after PTCA with stenting; signing of voluntary informed consent to participate in the study.
Exclusion criteria: onset of an acute infectious disease during hospitalization; left ventricular ejection fraction (LVEF) $\leq 28\%$; coma; conducting systemic thrombolytic therapy; refusal to participate in the study at any stage.
Characteristics of patient groups: group 1 (n = 17, comparisons) – patients with MI after PTCA with stenting, standard treatment; group 2 (n = 21, main group) – patients with MI after PTCA with stenting, standard treatment and TES-therapy.
Control points of the study: 1st day - ECG, echocardiography, CPK, CPK-MB, Troponin I, potassium, β-endorphin; 5th day - the same, without echocardiography; 10th day - the same and ECG with the definition of harmony and quantum of the electromagnetic flow of the cardiocycle. PTCA was performed using Promus Premier stents (Boston Scientific, Ireland).
TES therapy was carried out using a two-program electrical stimulator "TRANSAIR-03" (Center for Transcranial Electrical Stimulation, Russia). The following electrical stimulation parameters were used: pulsed bipolar mode, fronto-mastoid electrode placement, current strength 2 mA, current frequency 77.5 Hz, session duration 45 min. In total, patients from the main group, starting from the 1st day of hospitalization, underwent 10 procedures, with a frequency of 1 session per day.
Results:
At the beginning of the study, the groups had an approximately equal number of patients with atrial fibrillation (AF) and ventricular extrasystole (VE) ($p = 0.7446$), on the 5th day - in the comparison group in 59% (n = 10) and in the main group, 5% (n = 1) had CA with a predominance of AF.
On the 10th day of the study, 24% (n = 4) of the comparison group had AF, while in the main group, sinus rhythm was restored in all patients.
During the systematic analysis of the ECG on the 10th day of the study, all patients revealed deviations from the normal values of harmony - 1.309 c.u. and the magnitude of the quantum of the electromagnetic flux of the cardiocycle - 0.246 s*mWb.
In patients of the main group, the studied parameters of the cardiocycle approached the optimal values, while the intergroup differences in harmony ($p = 0.002$) and the magnitude of the quantum of the electromagnetic flux of the cardiocycle ($p = 0.001$) were statistically significant.
At the beginning of the study, there were no statistically significant differences between the study groups in terms of CPK, CPK-MB, TP-I and β-endorphin ($p > 0.05$).
During the analysis of the concentration of potassium, when conducting both paired intergroup comparisons for the control points of the study, and in the dynamics within the studied groups, no statistically significant differences were found ($p > 0.05$).
At the same time, when conducting both paired intergroup comparisons on the 5th and 10th days of the study, and comparisons in dynamics within the studied groups, statistically significant differences ($p < 0.05$) were revealed in terms of CPK, CPK-MB and TP-I, indicating about a lower level of markers of myocardial alteration against the background of the use of TES-therapy. On the 10th day of the study, in all patients, the activity of CPK, CPK-MB almost returned to the range of normal values.
Against the background of the use of TES-therapy, the content of highly sensitive troponin-I is statistically significantly ($p = 0.0042$) lower by 109% than in the comparison group.
On the 5th day, against the background of the use of TES-therapy in group 2, the concentration of β-endorphin was higher by 38.3% than in the comparison group ($p < 0.05$).
On the 10th day in the main group, the serum concentration of β-endorphin was higher by 35.0% than in the comparison group ($p < 0.05$).

In the course of intragroup analysis, a statistically significant decrease in the concentration of β-endorphin by 42.4% ($p < 0.05$) was revealed in the comparison group ($p < 0.05$), while no statistically significant changes in this indicator were detected in the main group ($p > 0.05$).
Conclusion: The use of TES-therapy in patients with CA after PTCA with stenting due to MI, by the 10th day of the study, is accompanied by a tendency to regression of CA, normalization of the harmony and magnitude of the quantum of the electromagnetic flow of the cardiocycle, a drop in the level of highly sensitive troponin-I, which indicates in favor of the cardioprotective and antiarrhythmic potential of TES-therapy. The above positive changes are observed against the background of stabilization of the serum concentration of β-endorphin, which indicates the participation of the opioidergic system in the mechanism of the therapeutic effect of TES-therapy and is indirectly confirmed by the data of experimental studies on models of myocardial ischemia.

## S8. Environmental Biophysics

### S8.524. Measurement of radionuclide activity in soil samples of the city of Krasnodar

Filippov V.[1]*
[1]*Kuban State University;*
* slava.shor@mail.ru

Radioactive elements of natural origin are present everywhere in the human environment. In large volumes, artificial radionuclides are formed, mainly as a by-product at the enterprises of the defense industry and nuclear energy. Getting into the environment, they have an impact on living organisms, which is their danger [1].
At present, the release of technogenic radionuclides into the environment is strictly controlled at the enterprises of the nuclear industry, but the problem of ensuring environmental safety remains unresolved. In some cases, there is a leakage of radionuclides and their entry into the environment, involvement in the biological cycle with the ensuing consequences. This work is devoted to the study of the activity of radionuclides released into the environment and the assessment of the ecological state of the natural environment of the city of Krasnodar [2].
In the course of the study, soil samples of the city of Krasnodar were selected and studied at a depth of 0.5 m. Initial mechanical cleaning of the samples from stones and biological residues was carried out.
The activity measurement method is based on recording the hardware spectrum with a scintillation gamma radiation detector (MULTIRAD-gamma, Russia), followed by its processing using specialized software. Calculation of activity, specific activity and measurement uncertainty is carried out by the software on the basis of the measured spectrum and the mass of the countable sample determined by weighing.
Table 1 shows the results of assessing the activity of radionuclides of cesium-137, potassium-40, radium-226 and thorium-232 in soil samples of the city of Krasnodar for the purposes of background radioecological monitoring.
Table 1
The result of measuring the activity of radionuclides
Sample name
Value, units Conclusion Conclusion on the Value of a quantity Confidence interval boundaries
PROBA_3
137Cs, Bq/kg 4.3 ± 3.5 0.9 - 7.8
226Ra, Bq/kg 25.1 ± 7.2 17.9 - 32.3
232Th, Bq/kg 37.8 ± 8.5 29.3 - 46.3
40K, Bq/kg 600 ± 140 460 - 740
PROBA_10
137Cs, Bq/kg 3.6 0 - 3.6
226Ra, Bq/kg 20.8 ± 6 14.8 - 26.8
232Th, Bq/kg 26.6 ± 6.5 20 - 33.1
40K, Bq/kg 460 ± 110 350 - 570
Based on the obtained results, it can be concluded that the PROBA_10 sample ( 45.0223539°, 39.0308668°) has the lowest radionuclide activity, while the PROBA_3 sample ( 45.0502849°, 38.9190679°) showed the highest activity result. The table shows that the obtained data on the activity of radionuclides do not exceed the norms of the Decree of the Chief State Sanitary Doc-tor of the Russian Federation dated 07.07.2009 N 47 "On Approval of SanPiN 2.6.1.2523-09" (together with "NRB-99/2009. SanPiN 2.6.1.2523-09 . Radiation safety standards. Sanitary rules and regulations "(Registered in the Ministry of Justice of the Russian Federation on August 14, 2009 N 14534). This indicates a favorable radiation situation in the territory of the city of Krasnodar.
It should be noted that the radiation safety of the population is an important element of national security and implies the state of protection of the present and future generations from the harmful effects of radiation and its components. Any use of ionizing radiation sources in medicine, industry, and agriculture should be controlled and safe [3].
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### S8.525. Activation of bioelectrogenesis in soil microbial fuel cells with the introduction of model organic pollutants

Melkonyan K.K.[1,2]*, Volchenko N.N.[2], Gronina A.D.[2], Samkov A.A.[2], Khudokormov A.A.[2]
[1]*Kuban State Technological University;*
[2]*Kuban State University;*
* 010899karina@gmail.com

Bioelectrochemical systems involving microorganisms have been the object of active study in recent years. The principle of electron transfer under anaerobic (or microaerophilic conditions) from the electron transport chains of bacteria to an external acceptor is implemented in microbial fuel cells (MFCs). The final acceptor of negative charges in them is the bioanode. MFCs are biotechnological devices in which the energy of chemical bonds is converted into electric current due to the biochemical activity of bacterial cells. The voltage generated by such devices is small and most often does not exceed 1 V, which is enough to power low-power electronic devices. Biofuel cells are being actively studied for use in wastewater treatment systems, in situ bioremediation, as biosensors, etc. One of the still little studied areas is the use of the so-called soil (soil) elements to enhance the metabolism of soil organic matter. As well as the creation on this basis of bioelectrochemical systems for composting organic waste, aimed, among other things, at reducing the carbon footprint.
According to the UN analytical services, about a third of food is wasted in the world, food waste accounts for 8% of global greenhouse gas emissions. Several ways to reduce such emissions are being considered, for example, through the use of food residues as animal feed or recyclable materials, part is sent to biogas plants, etc.

We have studied the possibility of combining MFC and a system for composting organic residues, as well as their influence on electrogenesis. For this, a soil-type MFC was used. An electrode made of carbon fiber felt (anode) was placed at the bottom of a vertical plastic vessel. The middle layer was filled with 1 liter of soil mixed at a ratio of 10 to 1 with model food waste, including bakery and vegetable products. A cathode electrode was placed on the surface under aerobic conditions. As a control, an MFC of an identical design, but without a model pollutant, was used.

The initial voltage in both cells was 0.3 mV. In the first month of the experiment, the voltage in the MFC with organic residues was 20 mV, which was an order of magnitude lower than in the control variant. This is probably due to the rapid development of mold fungi in the biomass cell, assimilating the hydrocarbon substrate of the model food pollutant. They could also suppress the growth of bacteria involved in the processes of anodophilic electron transport.

Further, the bioelectrogenesis of the experimental MFC began to increase and reached 300 mV; this indicator, with some fluctuations, generally remained until the end of the experiment (90 days). A different picture was observed in the control cell, where the voltage dropped to 30 mV, which is probably due to the depletion of carbon sources. Measurements of the amount of carbon dioxide released from the soil during the period of activation of electrogenesis in the MFC was 1700 ppm, which is 1.5 times higher than in the control, which confirms the conclusion about the higher activity of microorganisms in the MFC with an organic pollutant.

In general, this experiment shows the possibility of using the technology of microbial fuel cells in soil bioelectrochemical systems and the possibility of using them to assess the biochemical activity of the soil.

**S8.526. Application of digital models of aquatic ecosystems for the sustainable operation of water bodies**

Ermachenko P.A.[1]*, Seredin D.S.[2], Belyaeva N.E.[3]
[1]*«Neo-Ecology» LLP, Almaty, Kazakhstan;*
[2]*«Progressive Aquaculture Center» LLC, Kamyshevakha, Russia;*
[3]*Biological Faculty, Moscow State University, Moscow, Russia;*
* neo-ecology@mail.ru

Degradation of aquatic ecosystems is taking place all over the world, which is associated with the uncontrolled development of toxic phytoplankton. Phytoplankton (phytocenosis) is a community of planktonic organisms that can carry out the process of photosynthesis and are the primary link in the trophic chain of an aquatic ecosystem. The state of phytocenosis characterizes the reservoir as an object of research [1]. As a rule, toxic phytoplankton in the bulk is represented by cyanobacteria. Cyanobacteria pose a threat to the health of aquatic ecosystems, since they emit many different toxins during the flowering of reservoirs [2]. As a result of the active development of cyanobacteria, water quality is rapidly deteriorating, fish and other aquatic organisms are massively dying, and the biodiversity of aquatic ecosystems is steadily decreasing. Research is dedicated to the development of mathematical models of aquatic ecosystems in order to find sustainable modes of operation of water bodies. We perform fitting of model parameters on the basis of experimental data obtained during the operation of water bodies and in laboratory model experiments. The structure of the mathematical models of aquatic ecosystems is based on the advection-diffusion-reaction problem. Also, the structure of mathematical models of aquatic ecosystems takes into account the data of the dynamics of the transformation of the energy of light quanta in the thylakoid membranes of phytoplankton by measurements of the kinetics of fluorescence induction of photosystem II [1].

When studying the nature of the stability of aquatic ecosystems, the results of simulation modeling. As a result, measures are proposed to restore the biodiversity of aquatic ecosystems.

One of the objects of simulation modeling are fish-breeding and biological ponds. Which are used for the disposal of wastewater with a high content of organic substances. The principle of functioning of these nature-like treatment facilities is based on the ability of aquatic ecosystems to self-purification. The mathematical models developed by us are based on the principle of an unbroken food chain from microorganisms to higher hydrobionts. At the same time, the rate of self-purification of aquatic ecosystems directly depends on the intensity of photosynthetic activity of phytoplankton [3-5].

In order to continuously monitor water bodies in the field, we measure chlorophyll fluorescence in living phytoplankton cells (in vivo) [6]. A kinetic model of primary photosynthesis processes is used to process the data obtained. We have tested the use of the isolated photosystem II model for assessing the state of aquatic ecosystems, using the example of measurements of fluorescence induction of phytoplankton samples from ponds in the Temernik River basin (Rostov-on-Don) [1].

The efficiency of the production of phytoplankton biomass in the reservoirs served by us averaged 0.20-0.50% of the spectrum-integral solar radiation coming to the water surface. Most of this energy remained in the ecosystem in the form of pure primary products.

The integrated approach proposed by us to the operation of water bodies has made it possible to reduce energy consumption for wastewater treatment by several times, reduce the carbon footprint during the cultivation of forage microalgae and provide conditions for the formation of stable hypertrophic aquatic ecosystems. In which fish regulate the concentration of suspended organic substances and the population of phyto-zooplankton. It has been proven that self-purification is most effective in ecosystems with more diverse trophic chains. Therefore, in order to restore the biodiversity of aquatic ecosystems, it is advisable to exploit reservoirs with the maximum bio-productivity for this climatic zone. At the same time, the stability of hypertrophic aquatic ecosystems should be maintained by catching excess biomass of hydrobionts in amounts equivalent to the intake of organic substances and biogenic elements (nitrogen and phosphorus) from the external environment into water bodies.

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### S8.527. Biological oxidation of high-energy compounds from industrial wastewater on example of nitrated cellulose

Saratovskikh E.A.[1]*, Avdeeva L.V.[1], Yarullin R.N.[2]
[1]*Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, RAS;*
[2]*Kazan (Volga region) Federal University;*
* easar@icp.ac.ru

The ideal economic activity of humanity should be based on the principle of natural ecosystems that optimally consume matter and energy, the waste of some organisms serves as a habitat for others, i.e. waste-free recycling technologies. Accordingly, the purification of chemical production effluents is possible only with the use of biological oxidation methods. In the middle climatic zone a high-energy compounds as nitrated cellulose (NC) practically does not decompose. The resistance of NC to biodegradation leads to the accumulation of waste in settling ponds, which has a negative impact on the environment and on human health. The search for effective microorganisms-destructors and the development of a method of biological oxidation of effluents produced by NC are an acute scientific and economic task.
The effluents of typical NC productions contain significant amounts of sulfates, therefore, sulfate-reducing bacteria are a promising model for studying the transformation of NC. They belong to the genera Desulfobacter, Desulfococcus, Desulfobacterium, Desulfosarcina, Archaeoglobus, and belong to chemoorganotrophic microorganisms. Some species of Desulfotomaculum are able to bring oxidation to CO2 and H2O. We performed studies on the oxidation of NC with a nitrogen content of 10.7 and 13.38 wt% using Desulfovibrio desulfuricans. Incubation of NC on bacteria was carried out for up to 65 days. The content of nitrate and nitrite groups in the solution was measured; changes in the molecular weight distribution, viscosity, heat release rate and heat of the thermal decomposition reaction and the elemental composition of the isolated and dried NC.
Microorganisms intended for the oxidation of effluents produced by NC should be able to metabolize lignin and its derivatives. The microorganism must grow rapidly, competitively prevail in the environment and degrade low- and high-molecular derivatives of lignin. White rot mushrooms meet these requirements. The oxidation of NC was carried out using a mycelial fungus of the genus Fusarium solani IFO 31093. It was shown that in the presence of F. solani, the N content was 10.61% (16 days) and 10.51% (38 days). During 65 days of incubation, the pH decreased from 7.15 to 6.53. The decomposition of NC was indicated by the appearance of nitrate and nitrite groups in the culture medium - 11.4 mcg/ml and 3.38 mcg/ml. After 5 days of F. solani incubation, the proportion of low molecular weight products was 15%, by 65 days the molecular weights of these fragments had significantly decreased, which is promising from the point of view of practical application.
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### S8.528. Biological rhythms - an algorithm for the stability of biological systems

Tuleukhanov S.T.[1]*, Shvetsova Y.V.[1], Kairat B.K.[1]
[1]*Al-Farabi Kazakh National University ;*
[2]*Al-Farabi Kazakh National University;*
* Elenna4444@mail.ru

A living system is an alloy of biological rhythms. Biological rhythms are found at all levels of organization of biosystems: from the molecular level to the organismic level. A wide range of biological levels refer to all biological processes and phenomena. The presence of biorhythms at all levels of organization of biosystems makes it possible to obtain information about the processes under study. All these biorhythms are interconnected both vertically and horizontally of living objects. These features of biorhythms allow biosystems to achieve a synergistic and matrix effect. Synergistic and matrix effects make it possible to maintain the stability of biosystems under the influence of various disturbing factors of both endogenous and exogenous origin. The dynamic nature of bioprocesses allows biosystems to be plastic and labile. The plasticity and lability of biosystems is developed during the evolutionary process and adaptation to dynamic environmental factors.
The state of systems, in which the parameters of biorhythms are preserved over time, is characterized by a stable stationary state, i.e. norm. At the same time, the body strives to work at the most favorable energy level. This property is of great importance for maintaining the stability of biosystems. If for some reason the biosystem deviates from the stationary state, then, due to the system's tendency to the minimum production of entropy, internal changes will occur in it, which will bring the biosystem closer to a stable stationary state, i.e. to self-stabilization.
The stability of the stationary state of organisms is maintained with the help of autoregulation mechanisms that have negative feedback, where there is a negative feedback, there is a rhythm, i.e. due to chronostructural parameters (acrophase, orthophase, mesor, amplitude, period), the stability of biosystems is maintained and ensured.
If the biosystem experiences a small external or internal impact, then the level of the stationary state is preserved. In the case of disturbances, the system passes from one level of the stationary state to another, more favorable under new conditions. And with a long-term perturbing effect, the biosystem passes into an unstable stationary state (pathology, etc.), which is characterized by the maximum rate of entropy increment, which will indicate a violation of the chronostructural parameters of biorhythms. And for an unstable stationary state, the presence of self-amplification mechanisms is characteristic, operating as a positive feedback. External or internal influences cause increasing changes in an unstable stationary system, as a result of which the system passes into a state of thermodynamic equilibrium, i.e. the destruction of biorhythms and the dominance of chaos.
Thus, a stable stationary state of the body is ensured by the preservation of biorhythms, and the instability of the state of biosystems indicates a violation of biological rhythms.

### S8.529. Bioluminescent Potential of Mesoscale Eddies of the World Ocean

Piontkovski S.A.[1]*, Melnik A.V.[2], Serikova I.M.[2], Minski I.A.[2], Juk V.F.[2]
[1]*Sevastopol State University;*
[2]*Institute of Biology of the Southern Seas RAS;*
* spiontkovski@mail.ru

Numerous research and reviews are dedicated to the mesoscale eddies of the ocean, with a characteristic diameter of 100-200 km and a life span ranging from weeks to months (Chelton et al., 2007, 2011; Korotaev, 2020, and etc.). These dynamic events gradually contribute to the spatio-temporal variability of the kinetic energy of geostrophic currents of the World Ocean. The spatial heterogeneity of plankton biomass and abundance distribution (including the bioluminescent fraction) is an ecological consequence of eddy energy dynamics (Piontkovski, 2005). The stimulated bioluminescence reflects the structural properties of plankton community (i.e. the bioluminescent potential of luminescent organisms), as well as its functional state (Tokarev, 2006, 2016).

The goal of our study was to elucidate characteristic properties of bioluminescent potential (BP) mesoscale variability of the upper 100 m layer in the regions with more or less pronounced eddy fields. In particular, a hypothesis about a provisional link of bioluminescent field mesoscale spatial heterogeneity with the eddy kinetic energy (EKE) was tested. Methods of EKE assessments were given elsewhere (Roach et al., 2018; Sharma et al., 1999, etc.).

The bathyphotometer "SALPA" of several modifications (Melnik et al, 2019) has been used over sampled regions. Night casts were analyzed in our work. A part of these casts was accompanied by phyto- and zooplankton sampling. Mesoscale eddies were identified by direct measurements of the speed and direction of geostrophic currents, as well as by remotely sensed sea surface anomalies (https://las.aviso.altimetry.fr).

Bathyphotometric measurements over oceanographic station grids and transects (including 5-20 consecutive casts per drift station) enabled us horizontal and vertical components of bioluminescent field spatial distribution to be assessed. The analysis of 24,000 bathy-photometric casts from 71 expeditions of USSR and Russian Academy of Sciences research vessels to the Atlantic Ocean, the Indian Ocean, and the Mediterranean Sea basin (1966-2017) was carried out.

The majority of BP maxima were associated with cyclonic eddy peripheries, with 3-4 fold differences between the eddy center and its periphery. The correlation of BP with net zooplankton biomass ($r = 0.7$, $p = 0.05$) in tropical waters of the Atlantic and Indian Oceans was noticed. This correlation has a seasonal pattern, in particular in the Indian Ocean, because of the changes observed in biomass taxonomic composition, due to monsoonal reversals of winds and currents. In open waters of the Black Sea, the correlation of BP with phytoplankton biomass has dominated.

The normalized variance of BP in the upper 50 m layer across 14 oceanographic grids and transects exhibited a correlation with EKE ($r = 0.8$, $p = 0.001$). The zooplankton biomass and EKE variations were in the range of one order of magnitude, while the BP varied in the range over two orders. The location of BP maxima over depth were higher in cyclonic eddies compared to that of adjacent background regions. The vertical scale of BP thin-layered heterogeneities was in a range of 1-7 m, in tropical and Black Sea regions. This range was assessed by spatial autocorrelation functions and was higher in cyclonic eddies compared to that of background regions.

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**S8.530. Bioluminescent method of air pollution assessment**

Melnikova A.A.[1]*, Rimatskaya N.V.[1], Kratasyuk V.A.[1]
[1]*Institute of Fundamental Biology and Biotechnology;*
* anastasiafb15@gmail.com

In recent years, the problem of atmospheric air pollution has become urgent, since most of the dangerous pollutants enter the natural environment through the atmosphere, which becomes an important input component of the geosystem from the point of view of technogenic effects[1]. Chemical methods of air analysis are actively used in Krasnoyarsk and other cities, but they do not allow analyzing the effect of pollution on living objects.

In this work, using the bioluminescent bioenzyme method, the state of the air environment was analyzed and the degree of its pollution was estimated.

Air sampling was carried out outdoors in the city using a PU-4E aspirator using Richter absorption vessels. The following were used as absorption media: distilled water; formaldehyde absorption medium[2]; absorption medium for nitrogen dioxide[3]. 30 air samples were taken. According to the results of spectrophotometric analysis, the content of formaldehyde and nitrogen dioxide in air samples was determined. Bioluminescent analysis of air samples was carried out using a soluble NAD(F) bioenzyme systemH:FMN oxidoreductase and luciferase.

A reaction mixture of the following composition was used:

- 350 µl 0.05 M potassium phosphate buffer (pH=6.9);
- 5 ml of crab solution; 50 ml of 0.0025% tetradecanal solution;
- 100 ml of 0.4 mM NADH solution;
- 10 ml of sample/control (for distilled water 50 ml);
- 10 ml of 0.5 mM FMN solution.

The toxicity of the test sample was assessed by the magnitude of the inhibition of the luminescence of the sample. The analyzed parameter is the value of the residual glow [1]. Spectrophotometric analysis of air samples showed that the concentration of formaldehyde was $0.016 \pm 0.002$ mg/m3, and nitrogen dioxide $0.063 \pm 0.014$mg/m3.

As a result of bioluminescence analysis, all the samples under study were contaminated, since the residual glow of the sample taken for the formaldehyde absorption solution was $134 \pm 4$, and for the absorption solution for nitrogen dioxide $77 \pm 7$.

Spectrophotometric analysis of samples, unlike biotesting, did not show exceeding the maximum permissible concentrations. This indicates that chemical analysis is not a full-fledged method of assessing the state of the air environment. An alternative integral method in such an analysis can be bioluminescent analysis using a soluble bioenzyme system of NAD(F)H:FMN oxidoreductase and luciferase.

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**S8.531. Biomonitoring of the Kalmius River surface water state using the fluorimetric method**

Chufitskiy S.V.[1]*, Bespalova S.V.[1]
[1]*Donetsk National University;*
* ChufitskyiSergey@yandex.ru

Introduction. The problem of saving and supplying the water resources in Donetsk region has been most acute in recent years. The need has significantly increased not only for searching the fresh water additional

sources, but also for saving the existing natural reserves. Surface natural water state assessment and water resources monitoring are a priority task in these conditions. At the same time, rapid methods that allow making an assessment of the water body condition within a short period of time are most in-demand. In this field, the most promising method is fluorimetry that implies using phytoplankton cells as a bioindicator.
Materials and techniques. 11 monitoring points were selected to monitor the state of the surface waters of the Kalmius riverbed and its two tributaries - the Bakhmutka and Durnaya rivers. A few monitoring points were located along the Nizhnekalmius reservoir. The points were also located in places of the potential pollution source wastewater ingress. All monitoring measurements were carried out in different seasons of the year (at least once in a single season) in order to determine the state of phytoplankton cells in various natural and climatic conditions.
The chlorophyll content in water samples was determined using a Phyto-PAM fluorimeter (Walz, Germany). The fluorescence induction curves were recorded using a fluorimeter FS-2. This device was developed employing the designs of a Special Engineering and Technology Bureau 'Turbulence' and the Department of Biophysics at Donetsk National University. The induction curves were processed using the pyPhotoSyn software [1], which allows calculating the parameters of the OJIP-test [2].
Results. In the winter, chlorophylls a and b were the main photopigments in water samples. The highest pigment concentrations were obtained for the Nizhnekalmius reservoir – about 6 mg/l. The accumulation of phytoplankton biomass in the reservoir is caused by hydro regime of the water body and is a peculiar feature regardless of the time of the year. During all the periods, in the Nizhnekalmius reservoir, high photopigment concentrations were observed compared with the other monitoring points. The Durnaya and Bakhmutka rivers were characterized by a low content of photopigments (for example, in the autumn period it was 3 times lower than in the riverbed) with a predominance of chlorophyll b. In the autumn period, the proportion of cyanobacteria photopigments in the reservoir waters increased significantly.
When analyzing chlorophyll fluorescence induction curves, 10 most exponential test-functions were determined. In the winter, chlorophyll fluorescence induction curves were not recorded due to low concentrations of phytoplankton cells in water samples.
Throughout a year, a decrease in photosynthetic activity of phytoplankton cells was observed in water samples from tributaries of the Kalmius River, which was expressed in the decline of quantum yield of fluorescence and overall photosynthetic index of photosystem II. In addition, during the summer period, low values of the minimum and maximum levels of fluorescence, quantum yield, and total photosynthetic index were observed near Donetsk Iron and Steel Works. A decrease in the quantum efficiency of electron transfer from the primary quinone and the probability of electron transfer from it was also observed. High photosynthetic activity of phytoplankton cells was revealed in the Nizhnekalmius reservoir. High values of the reservoir phytoplankton cell fluorescence were obtained, which is the evidence of phytoplankton biomass intensifying growth.
Conclusions. Separate monitoring points of the Nizhnekalmius reservoir and tributaries of the Kalmius River are primary exploration objects, since the photosynthetic pigment distribution, uncharacteristic for the most points, and the significant cyanobacteria pigment presence were obtained against the background of the total phytoplankton biomass increase in different seasons of the year. Based on the analysis of fluorescence induction curves, a decrease in photosynthetic activity of phytoplankton cells was observed in the Durnaya and Bakhmutka rivers, which was expressed in the decline in quantum yield of fluorescence and overall photosynthetic productivity index. All this indicates the contamination of the aforementioned tributaries of the Kalmius River.

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### S8.532. Biophysical indicators of deer skin derivatives from different habitats

Komarova S.A.[1]*, Oleshkevich A.A.[1]
[1]*Moscow State Academy of Veterinary Medicine and Biotechnology;*
* Black_panter27@mail.ru

The values of redox potentials of alkaline hydrolysates of hair from different types of deer (northern,spotted deer and red deer) were studied. Hair was taken from different parts of the body of healthy animals of different sex and age (males, pregnant and non-pregnant females, youngs). The sampling of material for the study was carried out in animals of different natural and climatic zones. In Russia, the reindeer lives in the north of the Russian-European plain, in the Ural mountains, in the Siberian taiga and in the Far East. It inhabits flat, mountainous regions, lives in the tundra, forest-tundra, coniferous forests. The Ussuri spotted deer is one of the rare animal species. In Russia, lives in the Primorsky Territory. The noble deer is represented mainly by the subspecies of the Altai deer (maral). It lives in the mountain forests of Altai, in the Sayans and in the Baikal region in Siberia.
The redox potential of hair was determined according to the method of the authors [1, 2]. 20-25 samples of deer skin derivatives from 8 localities were studied. Significant differences in the values of redox potentials in different animal species were revealed. However, the dependence of the redox potential on age, gender, area of residence and climatic conditions has not been established.
The redox potential of reindeer hair during incubation in the dark was in the range of 55-57 mV, and when irradiated with visible light, the range was 50-53 mV.
In the wool of sika deer, the redox potential during incubation in the dark was in the range of 48–51 mV; when irradiated with visible light, the range was 40–41 mV. The redox potential of red deer hair during incubation in the dark varied in the range of 60-64 mV, and when irradiated with visible light, the range was 56.5-58.5 mV.
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2. Laboratory examination technique for animal skin derivatives (hair, wool, fluff). A.A. Oleshkevich, S.A. Komarova, A.A. Guselnikova, E.I. Yarygina. RAD Conference Proceedings, vol. 4, pp. 95–100. DOI: 10.21175/RadProc.2020.20

### S8.533. Biophysical methods in oceanology

Pogosyan S.I.[1]*
[1]*Faculty of biology;*
* pogosyan@biophys.msu.ru

An urgent problem in oceanology is a more accurate and efficient determination of the primary production of phytoplankton in the water column of various water areas in connection with global climate change. Currently, the total production of the World Ocean is determined with an accuracy of ±40%, which cannot satisfy the scientific community and does not allow making informed decisions about economic activity.

Biophysical methods are capable of solving many urgent problems of ecology, including those mentioned above.
The report presents the integrating sphere cavity measurement method (ICAM) developed at the Department of Biophysics, which provides a high sensitivity for assessing the content of pigments of phytoplankton particles and dissolved organic matter without preliminary preparation of natural water samples. As an example of the use of ICAM, the results of measuring the absorption spectra of sea water samples from the Sea of Japan and the Black Sea are given. Employees of the Institute of Oceanology of the Russian Academy of Sciences named after P.P. Shirshov measured the content of pigments of phytoplankton particles and dissolved organic matter using the ICAM method in the Baltic, North, Barents, Kara Seas and in the Laptev Sea.
Methods for assessing the state of the photosynthetic apparatus of natural phytoplankton by the parameters of chlorophyll fluorescence are considered, which make it possible to determine the characteristics of the primary production of the studied area. In particular, a fundamentally new microfluorimeter is described, which makes it possible to assess the state of the photosynthetic apparatus of individual cells of planktonic algae by the parameters of chlorophyll fluorescence. The microfluorimeter makes it possible to evaluate the content of photosynthetic pigments in the cells of each species of algae in the phytoplankton community, to determine the efficiency of the primary processes of photosynthesis of each cell and the dependence of the electron flow through photosystem II on the light intensity (photosynthesis light curve), which is an indicator of cell production, and also to determine the level of non-photochemical suppression of the photosynthetic apparatus of the cell. The sensitivity limit of this microfluorimeter is 10-12 grams of chlorophyll in the object, which makes it possible to reliably measure the chlorophyll fluorescence parameters of single microphytoplankton cells.
Key words: primary production, chlorophyll, phytoplankton, absorption methods, microfluorometry.

### S8.534. Biophysical study of skin derivatives of reindeer from different zones of Yakutia

Oleshkevich A.A.[1]*, Komarova S.A.[1], Fedorov V.I.[2]
[1]*Moscow State Academy of Veterinary Medicine and Biotechnology;*
[2]*Federal State Budgetary Scientific Institution Yakutsk Research Institute of Agriculture named after V.I. M.G. Safronova;*
* kaffizmgavmib@mail.ru

Reindeer products are considered environmentally friendly. But the features of the physiological systems of reindeer in Yakutia have not been studied enough. Studies were carried out on the physiology of the reproduction of reindeer and the biophysical characteristics of skin derivatives depending on the natural and climatic habitat zone: mountain taiga, tundra and forest-tundra zones of Yakutia. Groups were formed according to the principle of physiological analogues: 350 animals from the mountain taiga zone, 380 animals from the tundra and forest-tundra zones.
Shot results. It has been established that domestic deer of the mountain taiga and tundra zones belong to the leptosomal type. Physiological indicators of growth and development of deer differ in different zones. To determine radioactive contamination, the presence of heavy metals (copper, lead, zinc) and to measure the redox potentials of alkaline hydrolysates of reindeer hair / wool from various northern zones, samples were taken from different animals (from males, females and cubs). As a result of studies of samples from physiological analogs, no significant differences were found in the thickness, strength, hair color, in the values of the redox potential of the hair of mountain taiga and tundra deer. The potential of the solution during incubation in the dark was in the range of 55-57 mV, when irradiated with visible light, the range was 50-53 mV. Then, upon incubation in the dark, it returned to the original dark values. In the wool of the cubs, the values of redox potentials did not differ from those of adults. A preliminary analysis of the presence of strontium-90 and cesium-137 in deer skin derivatives from different habitats did not reveal the presence of radioactive elements.

### S8.535. Changes in bioelectrogenesis of microbial fuel cells over time under the influence of some heavy metals

Gasyuk O.A.[1]*, Volchenko N.N.[1], Samkov A.A.[1], Khudokormov A.A.[1], Lazukin A.A.[1]
[1]*Kuban State University;*
* olgagasyuk2000@yandex.ru

The high anthropogenic load is currently forcing us to look for new and effective ways to clean up the environment. A large number of pollutants constantly enter the environment from various sources. Among the most dangerous pollutants are oil hydrocarbons, radioactive elements, heavy metals, surfactants, etc. One of the promising directions in environmental remediation is the use of biological agents (microorganisms, plants, fungi, and some animals). Bioremediation is the process by which harmful pollutants are converted by living organisms into nontoxic compounds.
Microbial fuel cells (MFC) are advanced bioengineering systems that can be used as an alternative energy source, also for monitoring and cleaning the environment, "Internet of things", etc. Due to the bioelectrochemical processes occurring in MFCs, they can be used in bioremediation processes. For the efficient use of microbial fuel cells in these environmental processes, it is necessary to find out how certain pollutants affect the performance of MFCs.
In this study, benthic-type microbial fuel cells with horizontal and vertical electrodes were constructed. Carbon fiber "Carbopon" and graphite rods were used as the basis for the electrodes. The bioremediation agent was Shewanella oneidensis MR-1 culture obtained from VKPM No. B-9861 and native soil microbiota, which was used as the MFC solid phase. The Shewanella oneidensis MR-1 strain was chosen for the study because it has electrogenic activity and is capable of reducing heavy metal cations to a less toxic state under anaerobic conditions, using them as electron acceptors. The electrogenic potential of the MFC under a load of 1 kOhm was recorded by an automatic voltmeter of the author's design Lazukin A.A. Soluble salts of nickel, copper, and lead were used as pollutants at a concentration of 7 MPC for each cation. The duration of the experiment was 3 months.
In the first 10 days of the experiment, the average value of the electrogenic potential in the MFC only with native microbiota and with horizontal electrodes was: in the presence of Pb2+ salts - 325.4 mV, Ni2+ - 368.1 mV, Cu2+ - 135.1 mV. In the presence of S. oneidensis MR-1, the average value of electrogenesis was: Pb2+ - 250.1 mV, Ni2+ - 380.8 mV, Cu2+- 413.2 mV. Thus, in MFCs with nickel, copper, and chevanella salts, the value of the electric potential is higher than in MFCs, in which only aboriginal microflora is present. In the MFC with vertical electrodes in the presence of S. oneidensis MR-1 for the first 10 days, the average value of bioelectrogenesis was: Pb2+ - 124.7 mV, Ni2+ - 65.1 mV, Cu2+ - 43.7 mV. As a result, vertical electrodes show a much lower potential value compared to horizontal electrodes. After three months of the experiment, the average value of the bioelectrogenesis of microbial fuel cells was analyzed again. As a result, it was found that in the MFC with horizontal electrodes and native microflora, the average value of the electric potential for 10 days was: Pb2+ - 28.8 mV, Ni2+- 37.7 mV, Cu2+ - 35.7 mV. In the MFC with horizontal electrodes and in the presence of Shevanella, the average voltage values for 10 days were: Pb2+ - 27.1 mV, Ni2+ - 31.1 mV, Cu2+ - 49.7 mV. In microbial fuel cells with vertical electrodes and S. oneidensis MR-1, the average value of bioelectrogenesis for 10 days was: Pb2+ - 5.1 mV, Ni2+ - 0.3 mV, Cu2+ - 0.1 mV.

Thus, after three months, there is a significant decrease in bioelectrogenesis, which is associated with the toxic effect of heavy metals on the MFC microbiota. Thus, in microbial fuel cells with horizontal electrodes and native microbiota, the voltage value decreased over three months: in the presence of Pb2+ - by 11.3 times, Ni2+ - by 9.8 times, Cu2+ - by 3.8 times. In this system, copper cations had the least detrimental effect on the operation of the MFC. In the MFC with horizontal electrodes in the presence of S. oneidensis MR-1, the value of bioelectrogenesis after three months was reduced: Pb2+ - 9.2 times, Ni2+ - 12.3 times, Cu2+ - 8.3 times. Here, a less toxic effect on bioelectrogenesis was produced similarly to copper salts. In MFCs with vertical electrodes and in the presence of the microorganism S. oneidensis MR-1, the magnitude of electrogenesis decreased over three months: Pb2+ - 24.5 times, Ni2+ - 217 times, Cu2+ - 437 times. As a result, lead cations have the least negative effect on MFC bioelectrogenesis.
Thus, the use of microbial fuel cells in the processes of bioremediation and environmental monitoring is possible, but these devices are not very stable and show different values of the electrogenic potential depending on the type of pollutant, its concentration, electrode type, microbiota composition, etc.

**S8.536. Changes in the state of the photosynthetic apparatus of the green algae Scenedesmus obliquis under separate and combined exposure to copper chloride (II) and dissolved organic matter**

Voronova E.N.[1]*, Drozdova O.Y.[2]

[1] *Lomonosov Moscow State University, Faculty of Biology;*
[2] *Lomonosov Moscow State University, Faculty of Geology;*
* vlena66@mail.ru

Pollution of the aquatic environment with heavy metals salts has a great impact on living organisms. Phytoplankton is the primary link in the system of food chains in the aquatic environment and determines the state of the whole aquatic ecosystem. Heavy metals affect many metabolic processes inside algae cells. One of the most sensitive to the impact of heavy metals processes is photosynthesis.
Dissolved organic matter (DOM) is present in all water reservoirs. Most of the DOM is humic acids. DOM plays an important role in protecting microalgae from heavy metal stress. In the presence of DOM, toxicity and bioavailability may change due to the formation of complexes with metals. The role of DOM in the biochemical behavior and toxicity of heavy metals to microalgae is very important, but complex and poorly explored.
Measurement of chlorophyll fluorescence parameters makes it possible to identify and determine at early stages the reasons for the decrease in the efficiency of photosynthesis by algae cells. The light absorption spectra of the suspension make it possible to control the growth rate of algae. In nature, organisms experience almost constant stress from various natural factors, therefore, to study the effect of toxicants on test objects, it is necessary to create an additional physiological load (increased light intensity, deficiency of mineral nutrition), not exceeding the tolerance limits of the test objects in terms of intensity.
This work considers the effect of Cu (II) ions at concentrations from 5*10-8 to 10-6 M and DOM at concentrations from 0.001 to 50 mgC/L on the photosynthetic apparatus of the green alga Scenedesmus obliquus. Measurements of chlorophyll fluorescence parameters and absorption spectra of chlorophyll in suspension were carried out for 72 hours from the moment of incubation of microalgae with a toxicant on a nitrogen-free medium under illumination (quantum flux density) of 80 μmol quanta m-2.s-1.
A day after the incubation of microalgae with Cu (II) salts at concentrations from 10-7 to 5*10-7 M, the values of fluorescence parameters at open and closed reaction centers increased slightly compared to the control. After 72 hours of cultivation of microalgae with Cu (II) salts in concentrations of 5*10-8 - 5*10-7 M, the values of fluorescence parameters, the value of non-photochemical quenching and the relative content of chlorophyll did not differ from the values in the control. With an increase in the concentration of Cu (II) salts to 10-6 M after 24 hours, the value of non-photochemical quenching multiplied by 4. After 72 hours of cultivation of algae with Cu (II) salts at a concentration of 10-6 M, the values of the parameters of fluorescence, non-photochemical quenching and optical density were 4 times lower compared to the same values in the untreated suspension of algae. The values of the variable fluorescence remained high in all experiments and were comparable with the controls. When cultivating algae with DOM at a concentration of >10 mgC/L, the values of the fluorescence parameters F0 and Fm decreased by 10–20%, and the value of non-photochemical quenching increased. Low concentrations of DOM <0.1 mgC/L had no effect on changes in chlorophyll fluorescence parameters. During the incubation of microalgae with DOM at a concentration of 0.25-1 mgC/l after 72 hours, the quantity of chlorophyll increased (values of optical density at D678) compared with the control. When cultivating algae with Cu (II) salts in the presence of DOM, the greatest decrease in the toxic effect was observed at a Cu (II) concentration of 10-7-5*10-7 M and a DOM concentration of 1 mgC/L. The ability of algae to grow in the presence of toxicants in the environment is due to physiological adaptation and selection of resistant individuals. DOM in concentrations close to natural can reduce the toxic effect of heavy metals.
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**S8.537. Cosmic physical factors and biorhythms in plants**

Kashulin P.A.[1]*, Kalacheva N.V.[1]
[1]*Polar-Alpine Botanical Garden-Institute of RAS ;*
* falconet1@yandex.ru

The peculiarity of long-term multi-diurnal time-course in nastic movement of indoor plant species Marantha leoconeura and Ctenanthe setosa were investigated under quiet“ the space weather” conditions and during high level solar activity. The Marantha genera species are susceptible to change in the atmospheric pressure, humidity change etc. and presumably to cosmic physical agents also. The plants were cultivated under lab controlled conditions and were underwent twice daily leaf-petiole angle change measurements at the noon and at the evening hours. To evaluate selectively the role of external factors of cosmic and geophysical provenance [1] in physiological reactions of the plants the unsusceptible to terrestrial environmental factors systems were used also. In parallel experiments the mechanical one namely coin toss events and electronic one systems generated random digits were used also. The ten random numbers with nine random digits were generated twice daily with portative generator “CITIZEN” SRP-285II. The deviation from most probable value for generation of either first 5 various digits were obtained in every experiment. The expected probability P for the generation of five consequent different digits expected to be as P = 0,3024 [2], i. e. one expects that every three of any ten numbers generated would include 5 first different digits under ordinary normal conditions. The solar activity was controlled in terms of 10,7 cm EM emission flux, and daily Wolf numbers, and Sunspot total square. The neutron monitor and external gamma flux data of the Polar Geophysical Institute of RAS were used. The spectral analysis of long-term leaf-petiole angle change measurements data have revealed the circaseptan and circasemiceptan cycles presence. Such cyles were inherent in periods with low solar activity days of the last main solar cycle minimum. The solar perturbation growth accompanied with CMEs in 2022 yr and in current year begining have caused the market disturbance of plant circadian rhythm. Simutaneously the series of uniform events in two output experiments distribution change were obtained. And also the fluctuations in expected three random numbers with different five first digits were registered which fluctuated either

down to zero or up to 7. The deviations were found for a number of auroral geophysial disturbances supplemented by fluctuations in outer gamma flux, last one in January 19, and Roybush fall in January 21 and February 8, respectively. The results obtained confirm the susceptibility of vascular flowering plants to geocosmical factors founded earlier [3]. Under quiet "space weather" conditions the sustained nastic movement circadian rhythms in plants and appearance of difference between diurnal and nocturnal experiment outputs in nonliving mechanical and electronic systems were observed. Which pointed out on plausible common causes of these differences with diurnal time course of radioactive isotope decay found in by S. Shnol' with colleagues [4] .

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### S8.538. Damaging effect of carbon nanoparticles on the cell membranes of peritoneal macrophages of mice

Shank M.[1,2]*, Jia S.[1], Turovetsky V.[2], Pirutin S.[1,2,3]
[1]*Shenzhen MSU-BIT University;*
[2]*Lomonosov Moscow State University;*
[3]*Institute of Theoretical and Experimental Biophysics, RAS;*
* mikhailshank@gmail.com

The growing population of the world requires more food and modern industrial and scientific breakthroughs. The development of the field of studying nanostructures makes it possible to study not only natural nanoparticles, but also to create artificial ones. The application of nanoparticles in various fields, for example, biology and medicine, is in the initial stage of research, and many in vivo studies are being conducted. Carbon nanoparticles are classified as a special type of newly developed quasi-spherical carbon-containing nanomaterials with sizes below the order of 10nm. However, the interaction of carbon nanoparticles with living cells can lead to adverse consequences, for example, such as oxidative stress. Thus, studies on living cells in vivo and in vitro become relevant. An important role in this kind of research is given to in vitro experiments on model systems based on cellular preparations. For such model preparations, it is reasonable to use macrophages – phagocytic cells capable of rapid functional response to various types of exposure.

In connection with the above, the purpose of this work was: to study the cytotoxic effect of carbon nanoparticles on cellular preparations based on peretoneal mouse macrophages.

The object of the study is the peritoneal macrophages of mongrel white male mice (hereinafter referred to as "macrophages" or "cells"). Macrophages were isolated by the standard method of cervical dislocation and injection of a 1.2 ml Hanks solution containing 10 mol/l HEPES (pH 7.2) into the abdominal cavity for one and a half minutes. Next, peritoneal fluid enriched with macrophages was extracted. After that, a liquid with macrophages of 30 μl was applied to the cover glasses, where macrophages were attached to the surface of the cover glass by 45-minute incubation in a moist chamber at a temperature of 20 ° C. After incubation, the cover glasses were washed with a drop of Hanks solution to remove non-attached cells, and placed in small Petri dishes (2 ml). After that, a 2ml Hanks solution with HEPES was added to the Petri dishes in which the glasses were placed. The cells were in this solution during the entire experiment.

The studies were carried out using microfluorimetric analysis of single cells using the method of cross-staining with fluorescent probes: fluoresceindiacetate (FDA) and ethidium bromide (BE) at a final concentration of 5mcg/ml. The incubation time of the cell preparations was 15 minutes in dark conditions, after which the cells were washed from the non-binding dyes and cells with a damaged membrane were counted. Cells with green fluorescence were considered whole, and those with bright orange or red with a damaged membrane because when the membrane was damaged, fluorescein flowed out of the cell, and BE penetrated through the defects formed in the membrane and bound to the DNA and RNA of the cells.

To analyze the detection of cells with a damaged plasma membrane, a luminescent microscope "LUMAM I3" was used, equipped with a halogen lamp to excite the fluorescence of drugs and a set of light filters.

Incubation of peritoneal macrophages with carbon nanoparticles was carried out in time up to 210 min and concentration intervals from 0.0009 to 0.0225 micrograms/ml at 20°C. As a result of experiments, it was revealed that significant statistically significant damage begins with 90 minutes of incubation at all these concentrations. Achieving maximum damaging effect by 210 minutes. An increase in the concentration of nanoparticles in the studied concentration range did not lead to a significant effect of increased damage.

The damaging effect of carbon nanoparticles on the plasma membranes of macrophages in the same concentrations at a temperature of 37°C and in a time interval of up to 60 minutes showed that already at the 15th minute of incubation of cells with nanoparticles there is a sharp increase in the content of cells with a damaged membrane. At the same time, an increase in concentrations leads to a more pronounced damage effect at the 15th minute of incubation and the absence of such severity at a later incubation period. The effect of damage to the plasma membranes of peritoneal macrophages is discussed from the point of view of increasing the generation of reactive oxygen species (ROS) and as a consequence of damage to the lipid bilayer of the plasma membrane due to the intensification of the process of lipid peroxidation (LPO).

Thus, the present study shows that carbon nanoparticles have a cytotoxic effect on peritoneal mouse macrophages, causing damage to the plasma membranes of cells. It was found that damage to plasma membranes depends on both the time and the concentration of nanoparticles, and significant damage occurs at all concentrations after 90 minutes of incubation at an incubation temperature of 37°C. An increase in the concentration of nanoparticles did not lead to a significant increase in damage, at least in the range of concentrations studied. The study also suggests that the damaging effect of carbon nanoparticles on the plasma membranes of macrophages may be associated with an increase in the generation of reactive oxygen species and increased lipid peroxidation. Overall, these results provide an important insight into the potential toxic effects of carbon nanoparticles on cells and highlight the need for further study of their biological effects.

### S8.539. Determination of seasonal variations of gas-air exchange in bee families depending on their physiological state using stable carbon isotopes (13C/12C)

Kuzmichev V.E.[1]*
[1]*Калужский государственный университет им. К.Э. Циолковского;*
* vekoff@yandex.ru

The aim of the work was to conduct a quantitative analysis of the gas-air environment in bee families depending on their physiological state and environmental factors based on quantitative mass spectrometric

measurements, including measurements of variations in the prevalence of stable carbon isotopes (13C/12C).
At the first stage of research, we have developed a fundamentally new effective technology for monitoring the physiological state of an integral bee community on external factors. Mass spectrometric analysis allows you to capture variations in the concentration of oxygen, nitrogen, carbon dioxide, argon and other gases in hundredths and thousandths of a percent.
Samples of the gas phase in bee hives were obtained by taking air in three repetitions from the middle part of the hive into ampoules with a volume of 10 ml. To do this, holes with a diameter of 5 mm were drilled in the necessary places of the walls of the hive, through which dropper tubes and temperature sensors were inserted into the hive. The sample was taken using a syringe and then moved into ampoules by displacing a saturated sodium chloride solution. In the experiment, 9 bee colonies of different strengths were used in different periods of the year. Preservation of the content of samples and their transportation to the place of analysis was carried out upside down in ampoules hermetically sealed with rubber stoppers with a gas-insulating water gate.
Gas samples were injected into the mass spectrometer through a thin needle connected to the sample preparation system of the mass spectrometer.
The composition of the gas phase was carried out by measuring the peak intensities of molecular ions in the air mass spectrum: nitrogen with m/z 28, oxygen with m/z 32, argon with m/z 40 and carbon dioxide with m/z 44. The composition of the air sampled near the tested hive served as a control. The measurement error of oxygen concentration did not exceed ± 1% (relative).
Quantitative dependences of changes in the concentration of oxygen and carbon dioxide in hives depending on the observation time during the day, outdoor temperature and temperature inside the hive were obtained. The concentration of carbon dioxide in the outdoor air (control) was 0.03...0.05%, the O2 content was 21.0...21.1%.
So, on 10.09.2013, at an outdoor temperature of +10°C ... +15°C, in families completing active brood cultivation, the mass fraction of CO2 ranged from 0.3% to 0.6%.
At the height of wintering on 17.01.2014 in hive No. 2 inside the hive at an outdoor temperature of about -10 ° C inside the wintering bee club, fluctuations in CO2 during the day from 0.16% to 0.35%, and oxygen - from 17.6% to 18.8% were noted.
A linear correlation of the temperature inside the hive during the day was noted from samples taken at intervals of 2 hours (in a row 29, 6;31,9;29,9;27,9;26,0;24,8;23,8;25,0;29,2° C) as with the CO2 content (0,594; 0,357; 0,299; 0,353; 0,462; 0,566; 0,499; 0.558; 0.312%, respectively), and O2 (18,67; 19,45; 19,37; 19,36; 19,06; 19,06; 18,90; 18,77; 19,37%, respectively). Calculations showed high reliability of differences in the Student (P<0.0001).
Thus, it is shown that quantitative indicators characterizing the respiratory activity of bee families vary depending on the time of day, ambient temperature, temperature inside the hive and the physiological potential of the bee family. The obtained absolute and relative regularities are in good agreement with the data of one of the largest specialists of the domestic beekeeping science, Eskov E.K. (1983, etc.) and his students, obtained by well-known chemical and physical methods. Which proves the viability of our proposed methodology.
For a more detailed study and regulation of the microclimate in a family of wintering bees, a model of a software and hardware complex based on the Mega 2560 R3 platform (ATmega2560-16AU CH340G) and the Ethernet Shield W5100 R3 network module (Vinogradsky et al.,2018) was developed and tested in the Laboratory of Genetics and Bioengineering of bees at Kaluga State University.
Mass spectrometry allows local sampling of the gas-air mixture, their accumulation and storage with subsequent analysis without loss of accuracy. Also of undoubted interest is the parallel analysis of various bee metabolites contained in the hive (uterine pheromones), for example, in order to express the prediction of swarming or to identify the causes of trouble in the family.
It is possible to analyze the content of gaseous waste products of pathogenic microflora for early diagnosis of infection of bee colonies. This method is also promising for detecting bee poisoning with pesticides.
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**S8.540. Diversity of Ice Nucleation Protein (inp) repeat number in Xanthomonas population in relation to their ice nucleation activity**

Tesic S.[1], Kyrova E.[2], Fokina E.[1], Kosenko A.[3], Ignatov A.[1]*
[1]*RUDN University;*
[2]*All-Russian Institute of Plant Protection;*
[3]*Biospark LLC;*
* an.ignatov@gmail.com

The model of genetic adaptation within phytopathogenic genus Xanthomonas (gamma-proteobacteria) was evaluated for ice nucleation protein (Inp). Ice formation is triggered by the Inp particles due to their specific surface properties, which reduce the energy barrier for freezing (Szyrmer & Zawadzki, 1997). Inp triggers ice formation at temperatures as high as -2°C (Morris et al., 2004). The central domain of Inp (1034–1567 aa) consists of tandem repeats with the 8, 16(17), or 48-aa sequences. NMR measurements were made for synthetic peptides corresponding to a section of the repetitive domains in Xanthomonas campestris Inp. Structure calculation reveals that the 17-residue peptide forms a circular loop (Kumaki et al. 2008). The relationship between the ice nuclei size of the Inp (the theoretical molecular weight of ice-binding sites) and the median freezing temperature was estimated as direct and positively correlated (Ling et al. 2018). The number of repeats was proportional to the ice active surface of Inp. We evaluated two factors: the molecular weight of 16 amino acid tandem repeats of Inp (Inp 16R) and the average number of monomeric subunits Inp 16R for 368 Xanthomonas genomes available in NCBI GenBank. Average, minimal, maximal number, and standard deviation of Inp 16Rs were calculated for comparison to taxonomic position of bacteria and inhabited area (climate zone). Strains of 15 Xanthomonas species and 15 strains without certain taxonomic assessment were analysed. As result of this study, we established a well-defined negative correlation between average temperatures in the pathogen distribution area and the number of Inp 16Rs. For example, subtropical pathogen or rice, X. oryzae, had only 12 16Rs, when pathogens, such as X. campestris, X. euroxanthea, X. hortorum, spread in temperate climate zone had from 48 to 84 repeats. The species of widest distribution, X. arboricola, pathogen of many cultivated trees and crop plants (Ignatov et al. 2015) had the broadest range of repeats number: from 3 to 74.
When the number of Inp 16R was reduced in a model experiment (Ling et al. 2018), the truncated version of the Inps retained a decreased ice nucleation activity, despite a more than fourfold reduction of the theoretical ice-binding surface compared to the intact version of the protein. Therefore, it can be assumed that all strains having Inp retain the ability to ice nucleation. Obtained results suggested that Xanthomonas population have genetic adaptation of size of the ice active surfaces proportional to temperatures across the pathogens distribution zones.
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**S8.541. Dynamics of intracellular oxidant-antioxidant relations in post-embryonic development**

İbragimova J.M.[1], Muxtarov M.M.[1], Bayramova S.D.[1]
[1]*Institute of Physiology. A.I.Karaev N.A.N.Azerbaijan, ;*
* Jaluzi2009@gmail.com

In recent decades, the density of electromagnetic fields in the environment has increased excessively, and as a logical consequence of this, the task of studying the biological impact of this EMP factor, which has a non-ionizing nature, has come to the fore. WHO indicates in its documents that the effects of exposure to electromagnetic radiation on individuals or on the human population are not yet clear, therefore, the principle of early warning must be actively pursued to ensure their safety. When studying the impact of non-ionizing electromagnetic radiation on the body, signs of its oxidative stress factor are revealed in limited periods of time. The impact of the electromagnetic web on the human field affects his thoughts, behavior, physiological functions and even vitality. There are enough facts indicating that prolonged exposure of living organisms to electromagnetic radiation (EMR) of the decimeter range (non-thermal intensity) changes are observed in particular (in the lens of the eye) and this is one of the reasons for the development of cataracts. Our work was aimed at studying the effect of oxidative stress induced by electromagnetic radiation with a frequency of 460 MHz on the dynamics of the intensity of lipid peroxidation in the lens of the eye during further development in newborn white rat. Materials and method. Newborn rat pups (aged 3-4 days) were irradiated in a metal chamber on the Volna-2 physiotherapy apparatus under the following conditions: output power 60 W, energy flux density 10 mW/cm2, for 20 minutes. Control newborn rat were subjected to "false" irradiation. At the end of the prescribed period (i.e., 20 and 30 days old), the rat pups were slaughtered and the concentration of MDA in the lens was determined. LPO levels were assessed by measuring the concentration of malondialdehyde. The significance of the results was calculated using Student's t-test. Research results. Our studies have shown that exposure to EMR of relatively low intensity on newborn rat pups affects the subsequent development, in any case, at the age of 20 and 30 days, the oxidative response to radiation of relatively high intensity weakens slightly, in other words, the redox status is maintained in the lens due to preirradiation. In terms of the intensity of lipid peroxidation, one can speak of a new oxidant-antioxidant state created under the influence of EMR in the body of a newborn in a later period of development, at least up to 30 days of age. As for the physical changes in the lens of the eye, here we can talk about biochemical reactions, the essence of which is reduced to the modification of redox reactions and the disruption of local redox homeostasis. In our previous studies, we studied various aspects of this phenomenon.

**S8.542. Eco-genetic plant stress tolerance as a strategy and tactic: super-molecular-proteomic design of the physicochemical nature of developmental biology**

Ivanova E.A.[1]*
[1]*Институт биологии;*
* fiona_belobor@mail.ru

Abstract. From the standpoint of eco-genetic adaptation of plants, from the position of interdisciplinary science - supramolecular physical chemistry, the dynamics of supramolecular topologically associated structures of the total chromatin matrix (TChrM) is considered: Np-nucleoplasm, ChrI-eu-, ChrII-heterochromatin and nuclear matrix. On the interface of which, the proteo-supermolecular reorganization of ensembles is presented: "linker", "core" histones and non-histones, the macrokinetics of which is important for understanding the features of biochemical processes in the genetic subsystems of a plant (root → mesocotyl → coleoptile) of the transition period from heterotrophic to autotrophic plant development. An algorithm for the features of the biological specificity of morphogenesis and structural stability of the genetic and proteomic basis of the TChrM model system, collection germs of wheat seeds, in the process of their organ-specific, coordinated-regular growth when switching development subprograms is shown where an experimental analysis of proteomic positioning in supermolecular assemblies was carried out: "linker", "core" and "non-histone" proteins in different genetic subsystems (mesocotyl → root → highly differentiated embryo), respectively: donor (spring) → transferred to winter (donor winter-phenotype) → transferred back to spring-phenotype. Based on the distribution of nucleosomal arginine-rich "core" histone (H3-H4)'' on the TChM interface: donor (spring) Np=ChrI (mesocotyl) → transferred to winter (donor winter-phenotype) Np˃ChrII≥NM (root)→transferred again into the spring phenotype Np˃ChrI˃NM˃ChrII) (highly differentiated embryo); possible switching of genetic subroutines of development in the genetic subsystems of the whole organism is assumed, which is carried out due to the combinatorial principle of proteomic ensembles, potential epigenetic networks of the "histone code", in the conditions of environmental ecosystems.
Key words: Proteomics, Interphase chromatin topology, Supramolecular biochemistry, Karyogenomics, wheat, signaling systems.

**S8.543. Ecological biophysics: food chains, agent-based programming and construction of Eltonian pyramids**

Savitsky M.A.[1], Suglobov A.S.[1], Kuznetsov A.V.[2,3]*
[1]*Center for Additional Education "Small Academy of Sciences", Sevastopol, 299055;*
[2]*Kovalevsky Institute of Biology of Southern Seas, RAS, Sevastopol, 299011;*
[3]*Sevastopol State University, Sevastopol, 299053;*
* andrei_kouznetsov@hotmail.com

Lotka-Volterra predator-prey models are used to study community ecology, but their ability to generate Eltonian pyramids versus field data has not been investigated in detail. Here, agent based modeling (ABM) was used instead of systems of ordinary differential equations (ODE). It was shown that the two-component producer-consumer system is unstable and the three-component system with two trophic levels is stable. Time sections in the course of program execution can generate both Eltonian pyramids and cascades. The simulation results are consistent with the data of field studies.
Introduction
Mathematical models such as exponential [Malthus, 1798] and logistic [Verhulst 1838; Pearl, Reed, 1920] growth equations, systems of linear

differential predator-prey equations [Lotka, 1925; Volterra, 1926] and matrix population models [Leslie, 1945, 1948] have been developed to describe competitive relationships in communities of organisms. Integral assessments are necessary for a general description of ecosystem functioning. A good example of such an approach is the ecological pyramid developed by Ch. Elton [Elton, 1927], which is a graphical representation of the relationship between producers and consumers at all levels. There are several types of ecological pyramids: species, numbers, biomass, energy, etc. The number of trophic levels is finite because of R. Lindemann's rule [Lindemann, 1942], according to which approximately 90% of matter and energy is lost at each stage of the food chain. This circumstance makes it possible to study the food chains of real communities.

A sequential filtration device Biber was created and tested to study marine plankton [Bazdyrev, 2021]. It has been discovered that the shape of Eltonian pyramids can be transformed into cascades [Ufimtseva and Kuznetsov, 2022]. It is assumed that the distortion of pyramids of species observed in individual samples occurs as a result of competitive relationships between organisms. This work is devoted to the study of plankton with sizes from 2 mm to 2 μm at a station in the area of Karavella beach at the Cape Fiolent near Sevastopol (Crimea, Black Sea) in the second half of summer 2022.

Material and Methods

The Biber-6 separator, 11 cm in diameter and 98 cm long, consisted of 5 sections inserted into each other, with nylon meshes between them. The diameter of holes of the inlet sieve was 2 mm, the subsequent ones were 300, 150 and 84 μm, the pore size of the last EN14683 fiber filter was 1-5 μm. Trawling was performed at a depth of 50 cm at a speed of ~5 km/h at a distance of 1500 m. Samples were washed from the filters and fixed with 2.5% glutaric aldehyde. Aliquots were examined under a ZEISS Stemi 305 binocular loupe at magnifications of 8x to 40x. The term "morphotype" was used, i.e., all objects with visually similar morphological features were referred to the same category.

The simulations were performed in the NetLogo environment [Wilensky, 1998, 1999; Liu, 2001]. The stability of producer-consumer systems with different numbers of consumers was investigated. In the simulation, the agents wandered randomly across the screen, where predators randomly encountered prey. Each step cost energy, so they had to feed. When the energy ran out, they died. In order for the population to continue to exist, each agent had a fixed probability of reproduction at the next time step. Varying parameters included: the initial population size of each agent, the amount of energy received by the predator for each victim eaten, and the probability of reproduction of the agents at the next time step. After the producers were eaten, they only grew after a certain time [Wilensky, Reisman, 1999].

Results

The two-component ABM model eventually proved to be unstable, leading to the death of all participants, in contrast to the analytical Lotka-Volterra model. The three-component system, with primary and secondary consumers, exhibited stability. Stability was maintained throughout the long computational experiment, despite significant fluctuations in the number of agents. This circumstance made it possible to compare the minimum model found with data from field studies.

Samples rinsed from filters in separate experiments were analyzed for morphotype richness separately for each size fraction. Numerical values obtained for time sections were used to make Eltonian pyramids, which visualize the number of morphotypes for successively decreasing pore size or numerical experiment data. Most of the results were visualised by Eltonian pyramids, a smaller portion of data represented distorted pyramids, i.e., cascades. However, combining the data by days resulted in a canonical ecological pyramid.

Analysis of the obtained size fractions of micro- and nano-plankton indicates in favor of a generalized two-link food chain: marine phytoplankton → copepods, where the latter feed on phytoplankton. It can be assumed that protoperidiniums eat Licmophora species, and copepods in turn eat protoperidiniums. Consequently, we can identify a longer food chain: Licmophora → Protoperidinium → Copepoda.

Discussion and Conclusion

Food web theory, in general, explains pyramids of numbers [Elton, 1927], consumer-resource oscillations [Lotka, 1925; Volterra, 1926], and cascades [Jonsson, 2017], yet remains a subject of debate. Recall that the energy paradigm [Lindeman, 1942] is based on the flow of energy through the food chain and explains how species are distributed by trophic levels or body size [Banse, Mosher, 1980; Sprules, Barth, 2015]. Energy arguments lead to pyramidal distributions, whereas dynamic models often do not [Jonsson, 2017]. We have explained the reasons for such disagreement using wet experimental data and agent-based programming.

## S8.544. Ecological niche as a hamiltonian determining the eigenvalues of the wave function of a living

Strigin M.B.[1]*
[1]*South Ural State University Department of Physics of Nanoscale Systems;*
* strigin1969@gmail.com

The idea of an ecological niche has been actively evolving over the past hundred years, starting with the works of Gause, Lack, Elton, which were continued by Hutchinson, Shelford, etc. This work hypothesizes that the definition of an ecological niche is an analogue of the definition of the Hamiltonian in quantum mechanics, which allows the use of the latter's tools. Then the kind of living thing (animals or plants) is the eigenvalue of some wave function and oscillates, like an electron in an atom, a phonon in a crystal, etc. In all cases, the eigenvalues are determined by the boundary conditions, or more simply, by some geometry of the potential well. The concept of the Hamiltonian in quantum mechanics is continuously becoming more complicated — the dimension of the phase space is growing.

In biology there is an idea of an ecological niche, within which there is a biological entity — a species of animals or plants. "Vasnetsov unequivocally recognized the "community member" or "animal" as the bearer of the niche, stipulating that the latter should be understood not as an individual, but as a species or genus" [1, p. 58]. J. E. Hutchinson constructed a model of hyper volume, where there is its own phase space, in which, along with spatial coordinates, others appear environmental factors.

Just as in quantum mechanics, where coordinates, pulses and spin parameters are independent, in the Hutchinson model it is assumed that the reaction to one factor does not depend on the influence of another factor and the factors are independent of each other. In his model, an n-dimensional cube is presented, where environmental factors lying in some ranges, called tolerance ranges by Shelford, are deposited on the axes. Like the phase space in quantum mechanics, the Hutchinson cube has two conjugate classes of environmental factors: fundamental, which are related to space, and realized, which are related to momentum, to the dynamics of the species, to predators and competitors.

This model can be described within the framework of the representation of the Schrodinger wave equation, where the Hamiltonian consists of several terms H_0+H_1+H_2. In terms of quantum mechanics, H_0 is determined by the nearest geometry, for example, an atom with an electron inside it, which can be called a fundamental niche (according to Hutchinson), defined by the geography of the niche: food resources, temperature and humidity aspects, etc. H_1 is a small correction, which is determined, for example, by the movement of an electron flying past — in terms of biology — a competitor, or by a predator atom flying past (since the first one can knock out the electron in question and take its place, and the second one can snatch an electron from the initial atom and assign it). H_1 can be called an implemented niche that modulates H_0. H_2 is a nonlinear part of the Hamiltonian that

is associated with self—action, in the case of the form, this part is determined by its cognitive capabilities. The key difference between this equation and the classical Lotka-Volterra equations is that it has moved into a complex plane and can take into account interference effects, the concept of which in macrophysics can be compared with the concept of synergy introduced by Haken.
Recall that the key constant defining the Schrodinger equation is Planck's constant, which characterizes a specific minimum phase cell of physical chaos. In our model, the evolution of a species is described by the semantic space of the Hutchinson cube and the strategies of the species (for example, its relations with competitors or with predators). Planck's constant must be replaced by a parameter denoting an individual who is a similar minimal chaos cell in the field of biological semantics. A specific individual of the species means the surrounding space, enriching it with possible strategies, adapting to it.
Many biological laws clearly demonstrate a quantum nature. For example, Gause's law that only one living species can live inside one ecological niche accurately reproduces Fermi statistics. Two species of squirrels can inhabit the same territory, but they are located at different levels of trees and slightly intersect with each other. At the same time, phytoplankton is subject to Bose statistics: "Hutchinson noted that such species diversity contradicts the principle of competitive exclusion of Gause and called this phenomenon the "phytoplankton paradox"" [2, p. 23]
You can imagine an ecological niche in the form of a potential pit, inside which the species oscillates, adjusting to its shape. But if the niche is transformed significantly, when the restriction constraining the view inside it is significantly reduced, the synthesis stage occurs, and the view occupies a fundamentally new niche. Such transformations can be considered the Darwinian way of evolution.
The researchers note that it is possible for the species to "leak" beyond the ecological niche, by analogy with the tunnel junction in quantum mechanics. Such an evolution can be called Lamarckian. "Tunneling" through the barrier and occupying a new ecological niche is possible at the expense of internal energy resources. For example, David Lack found [3] that the increase in beak size in Galapagos finches is more of a social nature, rather than a response to changes in the food chain.
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### S8.545. Ecosystem monitoring vs mathematical modeling. Convergence

Medvinsky A.B.[1]*, Adamovich B.V.[1,2], Minaev I.S.[1], Minaev N.S.[1], Nurieva N.I.[1], Rusakov A.V.[1], Tikhonov D.A.[1,3]
[1]*Institute of Theoretical and Experimental Biophysics of RAN;*
[2]*Belarusian State University;*
[3]*Institute of Mathematical Problems of Biology, Keldysh Institute of Applied Mathematics;*
* alexander_medvinsky@yahoo.com

The problem of the correspondence of the data obtained during the monitoring of natural ecosystems and the results of mathematical modeling aimed at identifying the mechanisms that determine the dynamics of population abundance observed during monitoring is considered. An approach is proposed in which monitoring data is directly incorporated into mathematical models. This approach makes it possible to analyze the dynamics of biotic factors that were not measured during monitoring, and to assess the relationship of this dynamics with variations of ecologically significant abiotic factors.

### S8.546. Estimation of ion-induced polysaccharide hydrogels sorption capability based on elemental analysis data

Zueva O.S.[1]*, Khair T.[1], Yanushevskaya Ya.S.[1]
[1]*Kazan State Power Engineering University;*
* ostefzueva@mail.ru

Environmental recovery polluted by smoke emissions, industrial and domestic effluents requires the creation of innovative technologies for water, air and wastewater treatment. Recently, much attention has been paid to enhancement of highly efficient adsorbents, including composite materials based on natural biopolymers [1]. Natural polysaccharides contain carboxyl, hydroxyl and other active functional groups that can react with heavy metals through ion exchange, thereby adsorbing them from wastewater [1]. The development of technologies for ionotropic gels formation and microspheres or microfibroids preparation from these gels [2, 3] has created many possibilities for modifying their structure and properties by changing the conditions and components during their formation.
To prepare innovative materials, solutions of natural polyelectrolytes are crosslinked with divalent metal cations. This work is devoted to studying of various crosslinking ions influence on the morphology and elemental composition of alginate hydrogel microspheres obtained using alkaline earth metal divalent cations Ba2+, Ca2+, Sr2+. The study of elemental composition of metal-alginate microsphere walls after ion-induced gelation makes it possible to investigate the alginate chains association and to evaluate the sorption abilities of the resulting hydrogels. The main component of hydrogels are linear alginic acid molecules built from the residues of β-D-mannuronic (M units) and α-L-guluronic acids (G units) in the pyranose form and linked by 1→4 bonds. The chemical formula of alginic acid (C6H8O6)n reflects the composition of both M units and G units, what is more the number of units M>G. However, the spatial structures formed by the MM, MG, GG blocks differ quite strongly. When divalent metal salts are added to sodium alginate solutions, pairwise joining of blocks of neighboring alginate chains of hydrogels takes place. Crosslinking occurs due to formation of metal polyelectrolyte complexes resulting from electrostatic interactions between negatively charged carboxyl groups of polysaccharide molecules and positively charged metal cations. To describe the mechanism of alginate chains crosslinking with divalent Ca2+ ions and their further association into flat sheets, the previously proposed egg-box model was used, which was repeatedly improved. It turned out that alginate chains crosslinking with alkaline earth metal ions takes place in several stages. The first stage is formation of single crosslinks between biopolymers. The diaxial bond in a homopolymeric chain of guluronates determines a curved fiber structure forming cavities in each GG block. Metal cations prefer to be located within these negative cavities, ensuring their crosslinking. Since MM and MG blocks do not form such cavities, Ca2+ ions prefer to bind to GG blocks. Therefore, when alginate chains are connected into dimers, some of the cells remain unoccupied.
The methods of electron microscopy and energy-dispersive X-ray spectroscopy were used for the study. It was shown that elemental composition of hydrogels in the form of freezing dried microspheres give information on the structure of junction zones in the polysaccharide hydrogel network, on the degree of filling of egg-box cells by cations, the type and magnitude of interaction of cations with alginate chains, most preferred types of alginate egg-box cells for these cations binding and suggest the nature of alginate dimers binding in junction zones. It was found that in the case of alkaline earth metals in metal-alginate

hydrogels the number of cations of various Me2+ metals per C12 block, which theoretically should be equal to 1 for completely filled cells, is less than this number and is 0.3 for calcium, 0.6 for barium and 0.65-0.7 for strontium. This fact points to the electrostatic binding of alkaline earth cations with alginate chains. A consequence of relatively weak electrostatic interaction is a different degree of complexation of these cations with alginate blocks of various types (GG, MM, GM), which manifests itself in the presence of a certain number of unoccupied sites in the connected alginate dimers structure. The using of combinatorial analysis made it possible to calculate the probabilities of various types cells formation from alginate blocks, which appear under alginate chains association with a ratio of units M/G = 1.5 during gelation. It was shown that some of them cannot be occupied by alkaline earth metal cations. The presence of free cells in the formed structures explains the absorption possibilities of heavy metal cations from the environment, which additionally contributes to the strengthening of hydrogel structure [3]. It was concluded that calcium alginate can be used to obtain the most effective materials in terms of sorption capability for their use in environmental technologies.

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### S8.547. Fluorimetric devices for diagnosing the functional state of photosynthetic organisms in the natural environment

Konyukhov I.V.[1]*, Pogosyan S.I.[1]
[1]*M.V. Lomonosov MSU, Faculty of Biology, Biophysics department;*
* vanka.kon@gmail.com

Variable chlorophyll fluorescence detection is an important non-invasive method for studying photosynthetic reactions in living organisms. Today, serious laboratory experimental work with photosynthetic organisms (plants, unicellular and multicellular algae, lichens and cyanobacteria) relies on fluorimeters with the widest functionality known as PAM-fluorometers (Walz company, Germany) where the researcher by means of several independent light sources can exert various kinds of treatment on the electron transfer between photosystem 2 and photosystem 1. The most famous feature of the PAM device is the presence of weak measuring light modulated in the range of several kilohertz (tenths and hundredths of μmol•m-2•s-1). On the one hand, such a beam guarantees a negligible intrinsic effect on photosynthetic electron transport, on the processes of photosynthesis regulation, and on the quantum yield (intensity) of chlorophyll fluorescence. But on the other hand, the low intensity of the measuring beam significantly limits the performance of the device when it comes to measure fluorescence in natural conditions - in a measuring chamber open to direct sunlight or at low chlorophyll content typical for natural waters.

The report presents a complex of fluorometric devices designed at the Department of Biophysics and built on a slightly different technology for controlling a fluorescence excitation source known as "fast-repetition-rate" (FRR). Using a single powerful light source, the FRR protocol allows to measure the fluorescence induction and relaxation curves at a high signal-to-noise ratio even in the presence of an intense and noisy background natural irradiance and to determine the photochemical quantum yield of PS2 (Fv/Fm ratio). FRR fluorometers can also pre-adapt an object to actinic light and measure light slopes of non-photochemical quenching (NPQ) and relative electron transport rate (rETR) whose characteristics depend highly on temperature, on the plant spesies or phytoplankton taxonomic composition, and other conditions. The ease of use of the instruments as well as fluorescence measurements accuracy in natural conditions are also significantly increased due to specialized software developed at the Department of Biophysics.

The set of devices includes:

- A portable fluorometer «Smart 8» with a darkened measuring chamber and a 50 ml cuvette for water samples with the temperature control and additional registration of the delayed fluorescence induction curve on phytoplankton samples;
- A portable device «Smart 4» which has no measuring chamber - for fluorescence signals recording from objects under natural irradiation and from microalgae suspensions in transparent flasks or photobioreactors;
- «Tubby FRR» portable device with 4 ml vial for reach phytoplankton samples and algae cultures (chlorophyll contents 5 μg/l of higher) and for quantification of chlorophyll a content in acetone extracts;
- All-weather laser fluorometer with long-wavelength excitation (650nm) for year-round registration of fluorescence on young shoots (branches) of woody plants;
- A microscope with FRR excitation protocol to measure fluorescence transients arising from individual phytoplankton cells.

The features of each instrument are demonstrated in examples of in situ research performed on seawater samples, lichens and leaves during outdoor observations, as well as on microalgae used in bioassay experiments and photobioreactors.

### S8.548. Impact of extreme physical factors during stratospheric flight on model biological objects as part of "Cubesat" microsatellites

Volchenko N.N.[1]*, Khudyakova Y.E.[1], Novomlinova O.S.[1], Reut E.S.[1], Egupets L.V.[1], Krylova A.K.[1], Palagutina E.E.[1], Kritskaya A.A.[1], Popko K.S.[2]
[1]*Kuban State University ;*
[2]*Center "Perspektiva";*
* volchenko.n@mail.ru

One of the promising areas of modern biophysical research may be experiments on the «Cubesat» microsatellites. These devices are launched into Earth orbit as secondary payloads on a launch vehicle. Cubesats are capable of performing most of the functions of a classical artificial Earth satellite, with adjustments for their minimal size and the absence of orbit correction systems. A significant number of physical experiments in the field of radiation, radio transparency of the atmosphere, optical observations, etc., are currently being carried out on the basis of such devices. However, biological experiments are still poorly represented, which is due to natural limitations on the size of biosystems that do not allow miniaturization to the same degree as physical ones.

Our goal was to design microorganisms-based biological experiments that would be possible to subsequently implement it in microsatellites raised into the stratosphere under conditions of extreme physical environment. A stratospheric launch is simpler and more technically accessible than a space launch, but still allows the biological samples to experience conditions close to those in near space: low temperatures, minimal atmospheric pressure, high levels of radiation, including ultraviolet, and so on. One of the key problems is the weight and size limitations of cubesats, which are containers up to $10 \times 10 \times 30$ cm in size. The biological payload must be placed in a module with a volume of no more than 1000 cm3.

The microsatellites were designed and manufactured by the employees and students of the space team «Perspektiva» (Kurganinsk, Krasnodar Region). Biomodules for various organisms were developed by the employees and students of the club «Matrix» and space team «Stantciya» (Krasnodar). The microbial fuel cell with a data recording

system was developed and manufactured by D.A. Barybin and A.S. Prutskiy. Microbiological experiments were developed and implemented by the students and employees of the Faculty of Biology of KubSU, mentioned in the list of authors of this work. The launch into the stratosphere was carried out on a balloon by the «Perspektiva» and «ToSky» (Tomsk) teams. During the flight, an altitude of 24.6 km was reached,with the minimum air temperature of -42..-55 °C and the radiation level of about 300 μR / h. The flight duration was 86 minutes. After reaching the maximum height and rupturing the shell of the ball, the return of the microsatellites was carried out by a parachute.

To assess the influence of these extreme physical factors, 4 versions of biological experiments to be placed in three microsatellites were developed and implemented.

The purpose of the experiment with a microbial fuel cell (MFC) was to study bioelectrogenesis under extreme conditions. The dynamics of electrical voltage was used as an indicator of the anaerobic respiratory activity of bacteria. A monoculture of an electrogenic strain of the Shewanella genus and anodophilic microbial associations of silty communities were studied as samples. It was shown that bacteria can create a potential of more than 300 mV with a non-critical reversible decrease during the flight.

The experiment with microalgae Chlorella vulgaris was aimed at studying the growth dynamics of photosynthetic organisms in laboratory conditions after extreme impacts of stratospheric flight. The evaluation of the growth of the Chlorella suspension was carried out by the photometric method in Prat's media. It was shown that all algae samples retained their viability, and a similar level of growth was observed in Chlorella, which also was in the biomodule with and without thermostating.

An experiment was carried out with the growth of bacteria of several strains on a dense agar media in Petri dishes without thermostating and sealing. It was shown that the microorganisms retained their viability, which depended on the pigmentation of their colonies. Cultures with yellow pigments grew better than the strains of other colors; bacteria with pink pigment showed partial color loss and poor growth. This may probably be due to the protective properties of carotenoids, which may neutralize free radicals formed when exposed to radiation.

A similar experiment was conducted on an agarized medium with the «Escherichia coli/bacteriophage» system. The fact of penetration of the genetic material of viruses into the bacterial cells was registered visually by the diameter of the lysis zone. It was shown that both phage and E. coli remained viable, although the lytic ability of the virus was significantly lower than in the control group.

Based on the results of the biological experiments, we can conclude that the microorganisms we studied can be used as model objects for studying extreme physical factors in the upper atmosphere, and in the future, near space. Further development of equipment for biomodules of cubesat microsatellites as a platform for biological research is expedient.

### S8.549. Influence of hypomagnetic conditions, dissolved oxygen and water salinity on reproduction and morphometric characteristics of Daphnia magna Straus

Sizova A.A.[1]*, Sizov D.A.[1], Krylov V.V.[1]
[1]*Papanin Institute for Biology of Inland Waters Russian Academy of Sciences;*
* batrakova_a@mail.ru

In recent years, manifestations of global climate change have become more noticeable. The consequences of environmental transformation for freshwater ecosystems probably will lead to an increase in water salinity and a decrease in dissolved oxygen levels. In addition, there is a possibility that a reduction of the geomagnetic field intensity may occur due to the inversion of the Earth's magnetic poles. Changes in salinity, the amount of dissolved oxygen in water reservoirs, and magnetic conditions may significantly affect the life and reproduction of aquatic organisms.

The crustacean Daphnia magna Straus is one of the convenient objects for studying the effects of these factors. In the scientific literature, there is information on the influence of salinity, the amount of oxygen dissolved in water, and magnetic fields but not its combinations on these crustaceans. However, we did not find publications on the combined effect of hypomagnetic conditions and factors accompanying global climate changes on animals.

We carried out experiments to study the combined and separate effects of an increase in the salinity of the aquatic environment and hypomagnetic field, as well as the effects of a decrease in the dissolved oxygen content and hypomagnetic field on the morphometric and production parameters of D. magna.

In the experiments, we used water with a salinity of 0.5, 1.5, and 3 g/l, which corresponds to the currently found salinity in the water reservoirs of the Yaroslavl region and the probable increase in this parameter due to climatic changes. Waters with an oxygen level of 2, 5, and 8 mg/l were used for the second series of experiments. The last value corresponds to the oxygen level in the water reservoirs of the Yaroslavl region; the first and second simulate a probable decrease of this parameter due to climate changes. The reduction of dissolved oxygen level was achieved by bubbling water with liquid nitrogen. We used an unmodified geomagnetic field (51.7 μT) and hypomagnetic conditions ($0 \pm 200$ nT), which simulates changes in the process of reversal of the Earth's magnetic poles in our experiments.

Newborns no older than 24 hours were randomly selected from the synchronized culture of D. magna. The crustaceans were placed in containers filled with water of different salinity or oxygen level, one individual in each container. A part of the containers was placed in the center of Helmholtz coils that generated a hypomagnetic field. Another part remained in the geomagnetic field.

Hypomagnetic field and different salinity. The effects of an increase in the size of juveniles and the number of individuals in the first brood were revealed in daphnids kept under a hypomagnetic field compared to females exposed under control conditions. The salinity also affected the size and the number of offspring produced in the first brood. It was expressed in an increase of these parameters in the crustaceans maintained in water with a salinity of 3 g/l. The interaction of factors did not affect the size and the number of offspring produced in the first brood. These results are in agreement with the literature data.

The time of the first brood appearance depended on the salinity. In less degree, this indicator was influenced by magnetic conditions and the interaction of factors. The analysis of variance showed a significant effect of the magnetic environment on the period between broods. It was associated with an increase of this indicator in daphnia in the geomagnetic field in comparison with crustaceans kept in hypomagnetic conditions.

Despite good production under hypomagnetic conditions in the first brood, the dynamics of the produced offspring in the following broods indicated an adverse effect of the hypomagnetic field. At any salinity starting from the fourth brood, daphnia kept in the geomagnetic field reproduced more offspring compared to the crustaceans maintained in a hypomagnetic field. A decrease in the size parameters of females under hypomagnetic conditions was found. The strongest effect was observed at the salinity of 3 g/l.

A trend to increase of caudal spine length with an increase in the salt concentration in water and a slight enlargement of this morphological trait under hypomagnetic conditions were observed. We did not find information about the effect of salinity on caudal spine length in the literature.

Hypomagnetic field and hypoxia. It is possible to distinguish a group of effects caused by a change in the oxygen level that includes a decrease in the number of offspring produced in 3-6 broods, a reduction in the size parameters of adults, and an increase in the length of the caudal spine in daphnids kept under hypoxia.

The hypomagnetic field led to an increase in the sizes of the produced offspring. At the same time, the females' length under hypomagnetic

conditions was significantly lower than that in the geomagnetic field. On the contrary, the caudal spine length was increased in the hypomagnetic field. In addition, we observed an increase in the length of the caudal spine in D. magna maintained in water with low oxygen levels. The oxygen level and magnetic conditions had a noticeable effect on the reproduction and morphometric parameters of D. magna. The salinity varies significantly inside the habitat of this species and had a lesser effect compared to the lowering in the geomagnetic field induction. Paleomagnetic data indicate that the geomagnetic field has not experienced reversals and sudden weakening over the past 42,000 years. It is quite rational that changes in such a stable factor were unexpected by the organism and, therefore, led to more noticeable effects in our experiments. The obtained results indicate that the environmental changes, which may occur due to global climatic and geophysical processes, significantly affect the freshwater crustaceans D. magna.
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**S8.550. Monitoring studies of fluorescent parameters and biochemical components of Hedysarum daghestanicum L**

Pinyaskina E.V.[1]*
[1]*Pry-Caspian Institute of Biological Resources, DFRC of RAS, Makhachkala, Russia;*
* elpin1@yandex.ru

Plant growth is controlled by a variety of physiological, biochemical and molecular processes, and photosynthesis is a key process that contributes significantly to plant growth and development. Measurement and analysis of chlorophyll a fluorescence parameters allows studying the reactions of the photosynthetic apparatus under stress conditions and assessing the adaptive capabilities of plants. The aim of the study was a comprehensive study of the biochemical composition and fluorescent parameters of the Hedysarum L. Daghestanicum, a narrow-local endemic of Dagestan, growing on arid areas of stony slopes from 320 to 1800 m above sea level. m. with a low level of moisture and a high level of insolation. When monitoring Hedysarum daghestanicum, fluorescent parameters were studied both during dark adaptation (to determine the potential of the plant) and under natural conditions measured in the light. The maximum quantum yield of photosynthesis (Fv/Fm) is very high and approaches the theoretically possible. The relative rate of noncyclic electron transport along the ETR in plants is high, which makes it possible to maintain photosynthesis at the required level. The loss of excess energy in the form of maximum fluorescence is not large and amounts to 15%, while the main part of the absorbed light goes to photochemistry with great speed. According to the data obtained, the potential level of energy realization in photochemistry is about 80%, controlled losses (Y(NPQ)) - 20%, uncontrolled Y(NO) - 12%. A high level of Y(NPQ) means that the excess energy flows are well regulated (due to the work of ΔpH and zeaxanthin-dependent mechanisms) and the excess excitation energy is safely dissipated at the antenna level.
The qualitative and quantitative analysis of the photosynthetic pigments of the Dagestan Hedysarum showed that the content of Chl a, is 64% (of the total), the ratio of Chl a/b≈3.22, and Chl (a+b)/Car ≈5.5, which are characteristic of photophilous plants. Amino acid analysis revealed 13 amino acids, 7 of which have antioxidant properties, moreover, their content in the underground part is many times higher than in the aboveground parts. The content of proline in the roots was 33% of the total content of amino acids, and in the aerial parts 85%. In addition, secondary metabolites of flavone and isoflavone nature (caffeic acid and luteolin-7-O-glucoside and lutein, genistein and formononetin) necessary for plants to overcome stress limitations when adapting to a changing environment have been identified.
The conducted comprehensive studies have shown the high adaptability of the Dagestan kopek to abiotic stress factors. The mesophyte, growing in conditions of strong insolation and lack of moisture, has a complex of specific anatomical, morphological, physiological adaptations that are aimed at minimizing the amplitude of changes in the physical parameters of the environment that prevent the destruction of cells or their organelles.

**S8.551. On the circulation of higher animals under the physical conditions of external environment**

Bratsun D.A.[1]*, Kostarev K.V.[1]
[1]*Perm National Research Polytechnic University;*
* dmitribratsun@rambler.ru

Collective behavior in complex biological systems can act as a mechanism for conservation of energy and play a key role in the survival of a group of organisms [1]. One well-known example is bioconvection, which is observed in liquid solutions with aerobic microorganisms and a free surface. The instinctive movement of bacteria along the oxygen gradient leads to a second-order phase transition that triggers a macroscopic ordered circulation of fluid and bacteria. The phenomenon of bioconvection is interpreted within the framework of Prigogine's synergetic paradigm and has the same nature as the Rayleigh-Benard instability, i.e., can be explained within the physical concept. Similar phenomena in higher animals are much more difficult to differentiate from social behavior in a group, so they are poorly understood. For example, complex oscillatory movements are observed in birds, fish, and deer. But a simple explanation of these phenomena based on physical mechanisms is no longer applicable here, since the main trigger for such behavior is social adaptation. In this paper, we point out one important exception, when representatives of higher animals exhibit collective behavior that is even more similar to the phenomenon of classical thermal convection in a fluid than microbial bioconvection. This is the adaptive behavior of emperor penguins (lat. Aptenodytes forsteri) during wintering in Antarctica. Until recently, the features of the life cycle of this species have been studied poorly because of the difficult access to their habitat. In addition, the most interesting things happen in winter, when conditions in the habitat of emperor penguins become some of the harshest on the planet. Nevertheless, it was established experimentally in [2] that hundreds of birds are involved in collective behavior, and a comfortable temperature is established eventually inside a dense flock. In that work, a concept was also proposed that puts a correspondence between rearrangements in a flock of penguins and first-order phase transitions. In this work, we study the effect of a sudden liquefaction of a dense flock of penguins, in which there is a macroscopic circulation of birds from the edge of the group to its center and back. For the first time, we propose a mathematical model of the phenomenon, which is based on the hypothesis that the effective buoyancy force in a flock is generated by a temperature gradient (analogous to thermal convection). The model was developed both within the framework of the microscopic theory and within the framework of the continuum approach. In the first case, individuals in the model are represented as a set of discrete bodies interacting with each other under the effective potential, the form of which depends both on physical effects and socio-physical processes in the flock. The control parameters of the problem are the ambient temperature and wind speed. We found that a discrete model with individual dynamics of elements reproduces most of the phenomena observed in a flock of emperor penguins observed in natural conditions. For example, demonstrate a phase transition to vortex motion with a decrease in the ambient temperature, as well as an asymmetry of the final pattern under a side wind. It was also found that the liquefaction of a dense flock occurs first locally, while most of the animals do not circulate at the beginning. This behavior looks like the phenomena in

a granular medium, in which different aggregate states of the medium can also coexist.
In the continuum approximation, we propose a mathematical model of the phenomenon, which is reduced to the equation of a self-gravitating porous disk saturated with an incompressible fluid that generates heat. We derive the governing equations in the Darcy-Boussinesq approximation and write a nonlinear boundary value problem. An exact solution to the linearized problem for infinitesimal perturbations of the base state is derived, and the critical values of the control parameter for the onset of bioconvection are calculated. For finite-amplitude perturbations, the boundary value problem is solved using a finite-difference method. We show that there is an axial symmetry, the most dangerous is the four-vortex motion. If wind breaks the symmetry of the problem, the two-vortex circulation of penguins becomes the most dangerous.
Thus, we can interpret the observed phenomenon as a second-order phase transition similar to the mechanism of thermal convection, in which the desire of penguins to move towards the temperature maximum plays the key role of an effective buoyancy force. The authors of this work are not aware of another example of macroscopic circulation caused by physical processes in media of higher animals. The involvement of penguins in the collective process, which proceeds under the control of external physical phenomena, can be explained by too harsh conditions for the existence of the flock during polar wintering. Any deviation of individuals from the rigid program of behavior is punishable by death.
The theoretical results of the study of the microscopic and continuum models are compared. We also compare our theoretical findings with the results of recent experimental observations of emperor penguins in the Antarctic.
This study was financially supported by the Ministry of Science and Higher Education of the Russian Federation (grant no. FSNM-2020-0026).
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### S8.552. Peculiarities of adaptational changes in blood of yakut ground squirrels in the autumn

Teplova P.O.[1]*, Komelina N.P.[1], Lizorkina K.I.[1], Zakharova N.M.[1]
[1]*Institute of Cell Biophysics of RAS, Pushchino, Russia;*
* p.o.teplova@gmail.com

Hibernation of ground squirrels is a unique natural model for studying adaptive mechanisms of organism to conditions of critically low temperatures for survival of humans and other warm-blooded animals. When studying hibernation, most attention is focused on comparing the summer and winter periods corresponding to the maximum and minimum activity of hibernating animals, while ethological, morphological and physiological changes in preparation for hibernation occur as early as in the autumn time. Unfortunately, the data concerning the autumn period of preparation of hibernators for hibernation are extremely scarce in the literature. Meanwhile, we obtained convincing results concerning the changes occurring in Yakut ground squirrels during the autumn pre-hibernation period: in the functional activity of the brain, in the monoaminergic and opioid systems of the brain, in the heart, and in the skeletal muscles [1-4]. The present work continues the cycle of studies of the autumn preparation to the winter hibernation. The cardiovascular system plays a key role in maintaining body homeostasis; therefore, it is extremely important to understand the peculiarities of hematological rearrangements during the seasonal adaptations. The aim of the work is to reveal the peculiarities of adaptation changes in white blood cells of the Yakut long-tailed ground squirrels during the autumn period. The ground squirrels Urocitellus undulatus of both sexes (n = 50), were taken for the experiment during summer (June-July, 38 °C, n = 25) and autumn activity (October, 37-38 °C, n = 25). All animal procedures were performed in accordance with Directive 2010/63/EU and were approved by the Biosafety and Ethics Commission of the ICB RAS. Hematological examination of whole blood was performed on a BC-2800Vet multiparameter veterinary analyzer (Mindray, China). Statistical evaluation was performed using the Whitney-Mann criterion for independent samples with GraphPad Prism software (v. 9.4.1). The total number of leukocytes was found to increase 1.2 times in autumn. At the same time the count of granulocytes increased by 1.8 times, lymphocyte count decreased by one third, and monocytes did not change. Thus, there is a shift to the left in the leukogram from the agranulocytic fraction to the granulocytic one. This phenomenon may indicate the release of neutrophilic granulocytes from the bone marrow into the circulating blood. The mammalian bone marrow is known to contain a reserve of granulocytes many times greater than their level circulating in the bloodstream, which is triggered, for example, in stressful situations. Increased granulocyte levels may also be associated with a process opposite to leukocyte extravasation - penetration of leukocytes outside the blood vessels (so called adherent pool) and temporary "storage" in secondary lymphoid organs (spleen and lymph nodes). The developing lymphocytopenia can be explained by the gradual involution of the thymus, which seems to start already in the autumn period in preparation for hibernation. [5]. A significant increase in the total number of leukocytes in autumn has also been recently found in heterothermic bats [6]. Significant increases in thrombocytes and thrombocrit by a factor of 1.4 were also detected in active autumn ground squirrels. At the same time, platelet volume distribution width (PDW) remains unchanged, which indicates the general preservation of the percentage of mature and young platelet fractions, and is further confirmed by the unchanged MPV parameter - the mean platelet volume. A possible mechanism of platelet release into the bloodstream is related to their ability for reversible adhesion, migration and accumulation on the walls of blood vessels, as well as in various organs (spleen, lungs, etc.). Presumably, leukocytosis may be one of the reasons for the increase in thrombocrit. The reason for the changes observed in the blood of hibernating animals during torpor is associated mainly with the consequences of hypothermia experienced by animals [7]. Meanwhile, we can conclude that there are extremely important, non-temperature-related adaptive changes in "white blood" of ground squirrels during the pre-hibernation euthermal autumn period.
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**S8.553. Phytoplankton growth rate oscillations are phase-synchronized with temperature variations in a lake ecosystem**

Nurieva N.I.[1]*, Adamovich B.V.[1,2], Medvinsky A.B.[1]
[1] *Institute of Theoretical and Experimental Biophysics of RAS;*
[2]*Belarusian State University;*
* nailya.nurieva@mail.ru

To describe the dynamics of populations in a predator-prey system, differential and difference equations have been used. They describe the prey growth, independent of the density of the predator, and the prey decrease as a result of the predation. At the same time, the growth of the predator population is due to the transformation of the prey biomass into the predator biomass.
In our work, phytoplankton and zooplankton of the Naroch Lakes (Belarus) is considered as the predator-prey system. The novelty of our approach is a result of the fact that our model operates directly with the time series obtained during environmental monitoring. In the framework of this approach the growth rate and predator functional response are presented not analytically, but in the form of time series. We show here that the phytoplankton growth rate is phase-synchronized with temperature, whereas the oscillations of phytoplankton biomass is not synchronized with temperature. Thus, the effect of temperature on phytoplankton is disguised as a result of zooplankton predation.

**S8.554. Proximal sensing of plant condition in the field: spectral detection vs. machine learning**

Solovchenko A.E.[1]*, Shurygin B.M.[1]
[1]*Lomonosov Moscow State University;*
* solovchenkoae@my.msu.ru

Quantitative objective assessment of assorted plant traits is central place to modern methods of diagnosing the condition of natural and artificial plant communities (agroecosystems). This approach, termed "phenotyping", is also used in accelerated breeding of cultivated plants to increase their productivity and stress resilience [1-3]. Plant phenotyping methods are also integral to advanced precision farming practices. The need for rapid screening of plants in vast territories as well as for evaluation of large batches of ancestral forms and hybrids in experimental plots, fields, and orchards fueled the creation of automated, non-invasive express methods of high-performance plant phenotyping [2]. Initially, phenotyping was frequently based on remote sensing of vegetation by the sensors on satellite and airborne platforms from a relatively far distance—from hundreds of kilometers to hundreds of meters. Currently, we see a boom of plant phenotyping techniques carried out at relatively small distances—from a few centimeters to several meters, collectively called "proximal sensing".
Traditionally, registration and analysis of light reflected by plants (analysis of reflection spectra) [3-7] were used for remote and proximal sensing. Depending on the spectral resolution (the number of available spectral channels), multi- and hyperspectral approaches are distinguished. The analysis of hyperspectral images based on spatially resolved plant reflection data provides a huge amount of structural, biochemical and phenological information about wild and cultivated plants [3,5,7]. The advent of inexpensive hyperspectrometers has made this method affordable for many scientists and practitioners (agronomists and plant breeders). Along with the analysis of reflected signals, functional diagnostics of plants and communities based on visualization of amplitude-kinetic parameters of chlorophyll a fluorescence induced by sunlight (solar-induced fluorescence, SIF) is rapidly gaining popularity. Recently, the possibilities of proximal sensing of plants have expanded dramatically by relying on the novel mathematical methods for image analysis via artificial neural networks—machine learning (ML). Moreover, ML-image analysis is increasingly being put forward as self-sufficient method for proximal sensing of plants. At the same time, these approaches often overlook the physiological, biochemical and phenological processes of the studied object (plant). Overall, a balanced approach combining spectral analysis of the reflected signal or fluorescence emission with morphological analysis of plant structures by ML method seems to be more effective.
Extracting information from hyperspectral images, as well as optimizing ML algorithms for their analysis, is still a challenge. This report compares the advantages and limitations of the above-mentioned proximal sensing methods and suggests a strategy for choosing the optimal approach. The use of spectral indices, including new approaches based on remotely measured absorption coefficients, is given as an example. The extraction of quantitative information about the development of plants (aging, maturation, phenophase progression), their biochemical composition (the content of primary and secondary carotenoids, anthocyanins, and chlorophylls) from hyperspectral images obtained under environmental conditions in the field and under controlled conditions in the laboratory is considered. Based on own and published data, we conclude that the "synthetic" approach enriches the proximal sensing and plant phenotyping with more diverse and hence more valuable information about the physiological condition, stress acclimation, and development of plants.
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**S8.555. Study of sorption capabilities of strontium-alginate hydrogels, including reinforced by carbon nanotubes, based on elemental analysis and electron microscopy data**

Zueva O.S.[1]*, Khair T.[1], Shaidullin A.I.[1]
[1]*Kazan State Power Engineering University;*
* ostefzueva@mail.ru

The development of innovative technologies for purification of various aqueous media requires highly efficient adsorbents capable of binding

hazardous compounds, including heavy metal ions. Solving similar problems is also important for biomedical applications. Hydrogels prepared from natural biopolymers, in particular, based on divalent metal alginates, can be used as such adsorbents. One of the most promising materials for environmental and biomedical applications is strontium alginate. Natural strontium occurs as a mixture of four stable isotopes. It is included in the composition of microorganisms, plants and animals and is a low-toxic element, which in its properties is an analogue of calcium. When drops of sodium alginate enters a solution of a concentrated salt of strontium chloride, neighboring polymer chains are connected due to the occurrence of ionic bonds and complex formation between strontium ions and carboxyl groups of alginate chains, leading to appearance of gel microspheres. Such a hydrogel, as well as materials obtained on the basis of freeze-dried gel microspheres, have good sorption properties. The purpose of this study was to study the sorption capabilities of strontium alginate hydrogels, including those in the presence of carbon nanotubes added to hydrogels to enhance their mechanical strength [1, 2], according to elemental analysis and electron microscopy.

When alkaline earth metal cations, in particular strontium, are added, linear alginate chains are joined in pairs and flat junction zones are formed. The structure of these zones, according to the "egg-box" model, consists of cells of various types. Alginate is a natural copolymer having an irregular block structure consisting of units of different uronic acids: M (mannuronic) and G (guluronic), and usually the number of M units prevail over the number of G units. The spatial structures formed by MM, MG, GG blocks, and even more so by cells of pairwise connected blocks in each dimer, differ quite strongly, despite the fact that the chemical formula of alginic acid for M and G units is the same. For a block of any two units, it can be written as (C12H14O12Na2) n. After crosslinking with strontium ions, this chemical formula is converted to the form (C12H14O12SrX)n. The symbol X denotes the number of divalent metal ions per block of two C12 monomeric units. The limiting theoretical value of this number of ions, corresponding to completely filled cells of the egg-box, is 1. The value of X observed in the experiment turned out to be less than one, which indicates that alkaline earth strontium does not fill all the cells of the egg-box. Part of the cells remains vacant and can be occupied by adsorbed heavy metal ions [3].

Elemental analysis data for freeze-dried gel microspheres showed that the average block occupation number of the “egg-box” cells is approximately equal to X = 0.64, i.e. 36% of the cells can additionally adsorb heavy metal ions due to coordination bond. However, according to the same data, in addition to chemical bonds with alginates, there are opportunities for physical adsorption of certain associates, which can be retained near alginate chains due to weaker (mainly van der Waals) interactions. In particular, in the case under consideration, additional physical adsorption of SrCl2 was observed, corresponding to 1.42 units for each block of C12, indicating good sorption capabilities of strontium alginate.

A study of carbon nanotube-reinforced gel microspheres showed a significant difference in results. In particular, the average block occupation number of the “egg-box” cells increased to X = 0.81, which indicates the appearance of a more ordered structure from alginate chains, leading to a decrease in the possible adsorption of heavy metal ions due to coordination bond with alginate. However, the number of places of additional physical adsorption of SrCl2 increased and became equal to 1.97 units for each C12 block. Thus, the addition of carbon nanotubes to the hydrogel structure changes its sorption capabilities, leading, first of all, to an increase in physically adsorbed molecules.

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### S8.556. Study of the action of herbicide Roundup (glyphosate) on horseradish peroxidase

Avdeeva L.[2], Vahterova Y.[1]*, Saratovskikh E.[2]
[1]*Lomonosov Moscow State University;*
[2]*Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, RAS , Chernogolovka, Russia;*
* vahterova_yana@mail.ru

The effect of the herbicide Roundup (Rp) or Glyphosate (Gl) on horseradish peroxidase (HRP) was studied. Rp is the most common drug in terms of production and use in agricultural practice in the world. However, Rp has a toxic effect on living organisms of all trophic levels; causes many pathologies in people: disorders in the functioning of the liver, kidneys, oncological diseases. In March 2015, the WHO International Agency for Research on Cancer published a conclusion that Gl is a “possible human carcinogen” (hazard category “2A”). In conclusion, data are presented on an increased level of development of non-Hodgkin’s lymphoma in those working with Gl [1]; on the development of cancer in laboratory rats and mice [2,3]; about the ability of GL to damage DNA and cause chromosome aberrations in human and animal cells cultivated in vitro; and also lead to an increase in the frequency of chromosomal damage (micronuclei) in blood cells [3].

It is believed that Rp inhibits 5-enoylpyruvyl-shikimate-3-phosphate synthase [4]. However, it was previously shown that Rp inhibits the activity of the enzyme NADH oxidoreductase, which catalyzes many redox reactions [5]. Naturally, the question arose about the ability of the red-ox system of a plant or animal organism to oxidize and neutralize this chemical toxic compound. The ability of environmental objects to self-purify, i.e. decomposition of pollutants, is largely determined by the occurrence of enzymatic red-ox processes in the cells of plants and microorganisms. Therefore, we studied the effect of Rp specifically on the oxidative enzyme.

The choice of the enzyme was due to the fact that peroxidases belong to the enzymes of the main antioxidant system that destroys hydrogen peroxide and oxidizes a wide range of substrates. Typically, peroxidases function in the presence of hydrogen peroxide.

The herbicide Rp (or Gl) has many commercial names, but the active ingredient is the chemical compound N-(phosphonomethyl)-glycine (N-PMG). In this regard, we carried out work with the active substance N-PMG, which was isolated from the commercial drug Rp by double recrystallization from water.

Hydroquinone (HC) was chosen as a substrate, which belongs to the group of rapidly oxidized PO substrates. The kinetics of HC oxidation by horseradish peroxidase in the presence of Rp was studied in this work. HC oxidation was recorded on a PerkinElmer UV-VIS Spectrometer "Lambda EZ 210", manufactured in the USA to reduce absorption at a wavelength of λ = 290 nm. Experimental conditions: HC (0.05–0.4 10-3 M), H2O2 (0.64 10-3 M), horseradish peroxidase (15 10-9 M), 0.05 M Na-acetate buffer, pH 5.4, T = 250C.

In a preliminary study, the stability of both HC and Rp under the experimental conditions and the absence of interaction between them was established.

In the course of the study, the following kinetic parameters of PO in the HC oxidation reaction were determined: the Michaelis constant Km

(according to HC) for system I (HC + H2O2 + PO) and for system II in the presence of Rp (HC + H2O2 + PO + Rp) is 0.168 μM; Vmax for system I is 4.31 μM/s and in the presence of Rp for system II 3.44 μM/s.
The type of inhibition and the inhibition constant were determined in double reciprocal Lineweaver-Burk coordinates. It has been shown that Rp exhibits a non-competitive type of inhibition with respect to the oxidizing enzyme PO.
It has been shown for the first time that N-PMG inhibits the enzymatic activity of peroxidase from horseradish roots. This result is a serious addition to the available literature data on the targets targeted by the roundup.
The results obtained prove that N-PMG blocks the functioning of the antioxidant system, which in warm-blooded organisms can cause the accumulation of free radicals and oncological degeneration of tissues and organs.
Acknowledgments
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### S8.557. Study of the effect of carbon nanoparticles on green microalgae Scenedesmus quadricauda

Todorenko D.A.[1]*, Gvozdev D.A.[1], Tsoraev G.V.[1], Baizhumanov A.A.[1], Lukashev E.P.[1], Matorin D.N.[1]
[1]*M.V. Lomonosov Moscow State University, Faculty of Biology, Department of Biophysics;*
* dariatodor@mail.ru

Carbon nanomaterials have been attracting the attention of researchers since the 1980s due to their unique properties and the possibility of application in various fields. Usually, carbon nanomaterials are understood as nanodiamonds, fullerenes and carbon nanotubes, however, at present, the term has expanded significantly due to the emergence of various types of carbon nanoparticles (CNPs), which were originally described as luminescent fragments of carbon nanotubes. CNPs are divided into carbon nanodots (cNDs), which have an amorphous structure and do not exhibit quantum size properties, spherical carbon quantum dots (cQDs) and graphene quantum dots (GQD). The widespread production and use of CNPs in various fields, as well as the formation of CNPs as by-products of anthropogenic activities, inevitably leads to environmental pollution. At the same time, their impact on living organisms has not yet been sufficiently studied. In this work, we studied the effect of CNPs synthesized via electrochemical (GQD), hydrothermal (hND), and microwave methods (mND) on the green microalgae Scenedesmus quadricauda (Turp.) Breb. In the presence of CNPs, a decrease in the growth rate of S. quadricauda was observed. Inhibition of cell growth in most cases had a dose-dependent effect, which made it possible to establish a half-maximal effective concentration (EC50) for 72 hours for GQD and hND – 3.63 ± 0.8 μM (or 140 ± 30 mg/L) and 1.76 ± 0.21 μM (or 80 ± 10 mg/L), respectively. Unlike GQD and hND, carbon nanoparticles obtained by the microwave method (mND) did not have pronounced dose-dependent effects; therefore, it was not possible to determine the EC50 for this type of CNPs. We have shown that the synthesized CNPs are able to be adsorbed on the algae cell wall due to the large number of functional groups on the surface of the nanoparticles. We did not reveal any specific toxicity of the obtained CNPs, which could be associated with oxidative stress. The photosynthetic activity of microalgae, determined by the maximum quantum yield (Fv/Fm), also did not change significantly. The data obtained indicate that the synthesized CNPs do not affect the photochemistry of PSII in the S. quadricauda culture. We believe that the main factor that reduces the growth rate of microalgae in suspension is the limitation of the available light flux due to light absorption by nanoparticles (shading effect). The data obtained can be used to evaluate and predict the interaction of carbon nanoparticles with microalgae cells, which will reduce the adverse impact on aquatic ecosystems in the future.
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### S8.558. The effects of ligands on the adsorption of copper ions by the cell wall of vetch (Vicia sativa L.) plants

Nikushin O.V.[1]*, Meychik N.R.[1], Nikolaeva Yu.I.[1], Kushunina M.A.[1]
[1]*Lomonosov Moscow State University;*
* nikushin.94@mail.ru

Russian regions with developed industry and agricultural production are in danger of agroecosystem pollution. The usual and most dangerous pollutants of ecosystems are heavy metals (HM). The toxic effect of HMs on plants is primarily due to the disruption of ion homeostasis, water balance, the functioning of enzyme systems and the cytoskeleton, which leads to multiple metabolic disorders.
Because of its lifestyle, to survive on soil with high metal concentrations, plants have to develop several specialized resistance mechanisms. Intracellular defence mechanisms are widely studied and covered in a sufficient number of reviews [2,5]. At the same time, much less attention is given to the extracellular mechanisms of plant defence against HMs. The cell wall is the first structure of the plant cell interacting with the environment. In this regard, several authors believe that cell wall plays key roles in protecting plants from HMs, due to their high cation exchange capacity [1,3].
Another mechanism of plant defence against HMs is the release of root exudates containing ligands, which can chelate metals in soil solution, such as citrate, malate, and some amino acids [4,6]. Although there are numerous studies on the effect of organic ligands on the accumulation and availability of HMs, the subtle mechanisms of these phenomena have not yet been established.
In this regard, the purpose of this work is to study the effect of ligands (histidine and glutamine) on the adsorption of copper ions by the cell walls of plants of vetch (Vicia sativa L.).
In this work, a comparative study of the absorption of copper ions by the roots of intact plants and cell walls (CW) isolated from roots and shoots at different concentrations of histidine (His) or glutamine (Gln) in the presence of 10 and 100 μM copper in the solution was carried out. In experiments with His, concentrations of 0.5 mM and 1 mM were used; glutamine Gln concentrations of 1 mM and 5 mM were used. According to the results, with an increase in the concentration of Cu2+ in the growing solution, its content in the roots and shoots also increased. His and Gln decreased the intake of metal into the plant. 1

mM His, in the presence of 100 μM copper, significantly reduces the supply of metal to the root by 4.3 times. 5 mM Gln in the presence of 100 μM of the metal decreased by 86.6% to the variant without the ligand.
In the case of isolated cell walls, a similar tendency was observed to increase metal sorption as the concentration of copper in the medium increased. Under conditions of 10 μM copper, His did not affect the adsorption of copper by root CW. The addition of 5 mM Gln, on the contrary, increased copper adsorption by 13% relative to the ligand-free variant. In the presence of 100 mM copper in the solution, the addition of amino acids reduced adsorption by 16% in the variants with Gln and by 25% in the variants with 1 mM His, 0.5 mM His had no effect. In the case of the cell wall of the shoots, the ligands did not affect the adsorption of copper ions per gram dry weight of the shoot.
When treated with solutions of CuCl2 at a concentration of 10 μM and in the absence of ligands, the root CW adsorbs 2 times more metal ions than is absorbed by the transpiring plant. As the concentration of ligands in the solution increases, the difference between the copper content in the root and the adsorption of isolated root CW increases. At the maximum concentration of His and Gln, the adsorption of CW is 4.4 and 13.4 times higher than the internal concentration of copper in the roots of experimental plants per gram of root dry weight.
Thus, the studied ligands reduce the uptake of copper by intact plants. At the same time, it does not affect the adsorption of copper ions by isolated root CW, which, in turn, exceeds by several times the endogenous concentration of the metal in the experimental roots. The obtained results give grounds to believe that: 1) CW is the main site of HM deposition in the plant; 2) the studied ligands limit the intake of copper ions by intact plants without affecting the adsorption capacity of cell walls.
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### S8.559. The model of the phytoplankton population functioning in the arctic regional seas in the summer period

Fursova P.V.[1]*, Riznichenko G.Yu.[1], Konyukhov I.V.[1], Pogosyan S.I.[1]
[1]*Lomonosov Moscow State University;*
* fursova@biophys.msu.ru

Sunlight and the availability of mineral nutrition are the main factors that ensure the existence of phytoplankton communities. Their impact primarily affects photosynthetic activity, which can be assessed using fluorescent methods. By measuring the parameters of algal chlorophyll fluorescence, one can determine the potential efficiency of photosynthesis, find out its dependence on different light intensities, detect a deficiency of mineral nutrients, establish the degree of algae adaptation to light intensity in the phytoplankton habitat. According to the expeditions data in the basins of the Kara Sea and the Laptev Sea, in situ measurements of the chlorophyll content by the fluorimetric method demonstrate extremely diverse depth distributions [1]. The maximum can be near the surface and at depths below the jump in water density, or in the upper and lower layers simultaneously. At the same time, some cells from samples of the upper horizon demonstrate adaptation to dark conditions, and from samples of the lower horizon, to high illumination. It should be noted that diatoms make up a significant part of the phytoplankton biomass in the Arctic seas in summer, they are not capable of active movement. The water column consists of two practically non-mixing layers of water - the upper desalinated, deficient in nutrients, and the lower, more saline and enriched in nutrients. The upper layer of water is sufficiently well lit, while in the lower layer the light intensity is too low for photosynthesis. A natural question arises: how are diatom populations maintained in a stationary state in such a system? In mathematical models, the vertical movement of phytoplankton in the absence of directional transport can be described, for example, by turbulent diffusion [2, 3]. However, under conditions of stratification of the aquatic environment, this process cannot explain the observed distributions. In the present work, the following hypothesis is suggested [4]. In the illuminated layer of water, the cell accumulates biomass due to photosynthesis and increases its density by maintaining the volume due to the presence of solid silicon valves. As density increases, the cell gradually sinks until its density equals that of more saline and denser water. In this layer, rich in minerals, the cell replenishes its intracellular reserves. Once in the absence of light, the cell begins to spend the accumulated carbohydrates on various metabolic processes, including respiration. The released carbon dioxide is retained around the cell in the resulting mucus sac. At the same time, the specific gravity of this "formation" (cell + "bag") gradually decreases. Upon reaching the critical density value, the cell floats to the surface, and the gas bubble collapses. Based on the proposed hypothesis, an agent-based model of the phytoplankton population dynamics was built in the NetLogo environment (http://ccl.northwestern.edu/netlogo/). Cells-agents are divided into two groups - upper and lower. For each cell increase/decrease of its density; storage/expense minerals; division; death; transition from one group to another; moving are available. Cells of the upper group photosynthesize increasing density, while consuming intracellular minerals. The cells of the lower group replenish the intracellular content of minerals and carry out the main metabolism, their density decreases. The medium is divided into 2 layers: in the upper layer, the nutrient content is assumed to be zero, the light intensity decreases with depth in accordance with the exponential law. In the lower layer, there is no illumination, nutrients are evenly distributed. It is assumed that all "resources" are in abundance, their content does not change when consumed by agent cells.
The behavior of model solutions was analyzed. The key parameters were the level of near-surface illumination and the distance by which cells are displaced in a random direction at each moment of time. By changing these parameters, one can obtain different distributions of cells—numbers in the upper and lower layers—typical for expeditionary observations. With small displacements of cells, the population demonstrates a periodic change in abundance. The constant predominance of cells in one of the layers or the successive change in dominance is determined by the given illumination. Moving cells over long distances smooth out population fluctuations. The model experiment demonstrates a decrease in the total number of cells compared to a similar experiment in the absence

of significant cell movements, since the "availability" of sunlight is reduced. Under conditions of higher surface illumination, the total number of cells moving over long distances becomes higher, since they are less susceptible to photodegradation. The dynamics of the numbers in each of the layers also changes: at a normal level of illumination, the maxima of the numbers of the upper and lower cells replace each other, which is typical for cells that move weakly in low light conditions. Further studies will be aimed at refining the parameters of the model and a more detailed comparison of the results with experimental data.

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## S9. Medical biophysics. Neurobiophysics

### S9.560. 2-Ethyl-6-methyl-3-hydroxypyridine N-acetylcysteinate prevents stress-indused mitochondrial dysfunction

Zhigacheva I.V.[1]*, Rusina I.F.[2], Krikunova N.I.[1], Mil E.M.[1]
[1]*Emanuel Institute of Biochemical Physics of RAS;*
[2]*N.N. Semenov Institute of Chemical Physics of RAS, Moscow, Russia;*
* zhigacheva@mail.ru

Various stresses lead to disruption of the bioenergetic functions of mitochondria and excessive generation of ROS, which underlie the development of pathological processes [1]. Since mitochondria are the main source of ROS under stress conditions, it is possible that the main mechanism of action of adaptogen drugs is to reduce the excessive generation of ROS by these organelles. This function can be performed by antioxidants, in particular 3-hydroxypyridine derivatives, which have a wide spectrum of biological activity [2].They are heterocyclic analogs of aromatic phenols and, therefore, exhibit antioxidant and antiradical properties [3]. In our work, we used 2-ethyl-6-methyl-3-hydroxypyridine N-acetylcysteinate, which is a derivative of 3-hydroxypyridine and acetylcysteine, as an object of study. The aim of our study was to study the antiradical properties and biological activity of this drug. 2-ethyl-6-methyl-3-hydroxypyridine N-acetylcysteinate in the concentration range of 10-6-10-11M and 10-13M prevented LPO activation in the membranes of mouse liver mitochondria in the model system of mitochondrial "aging". At the same time, the chemiluminescent method showed high values of the antiradical activity of this drug, which could indicate the presence of adaptogenic properties in 2-ethyl-6-methyl-3-hydroxypyridine N-acetylcysteinate. The study of these properties was carried out on models of acute hypobaric hypoxia (AHH) or acute alcohol poisoning (AAP). At the same time, the influence of these effects on the functional state of mouse liver mitochondria was studied. AHH and AAP caused the activation of lipid peroxidation in mitochondrial membranes, which was accompanied by changes in the content of fatty acids (FA) having 18, 20 and 22 carbon atoms and swelling of mitochondria. The content of linoleic acid, one of the main fatty acids that make up cardiolipin, in the total lipid fraction of mitochondrial membranes decreased by 6%. Pool 20:3ω6 and 20:5ω3 decreased by 18% and 32% respectively. Considering that eicosanoids are signaling molecules and have a wide range of biological functions [4], a decrease in the content of these FAs, possibly, as well as a decrease in the content of linoleic acid, affected the body's resistance to stress. A 5-day administration of the drug at a dose of 10-6 M/kg before stress prevented the activation of lipid peroxidation, changes in the FA composition of membranes, and swelling of mitochondria.

Changes in the functional state of mitochondria affected the body's resistance to stress factors. The injection of 10-6M drug to mice for 5 days increased the lifespan and survival of mice under conditions of various types of hypoxia by 57-92% and 15-40%, respectively. At the same time, the lifespan and survival of mice under conditions of acute alcohol poisoning increased by 3.9 and 12 times respectively.

The adaptogenic properties of the drug, apparently, are due to its high antiradical and antioxidant properties.

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### S9.561. A new approach to evaluate the effective intracellular concentration of bioactive molecules on the example of modular nanotransporters capable of interacting with the Nrf2 system of cells

Khramtsov Y.V.[1]*, Bunin E.S.[1,2], Alieva R.T.[1,2], Slastnikova T.A.[1], Sobolev A.S.[1,2]
[1]*Institute of Gene Biology RAS;*
[2]*Faculty of Biology, Moscow State University;*
* ykhram2000@mail.ru

When studying intracellular interactions of bioactive molecules with target proteins, the problem of determining the concentrations of these molecules in a cell often arises. Of particular interest in this case is the concentration of only those molecules that are able to interact with the target protein, i.e., their effective concentration. In the cytoplasm, it can differ significantly from the average concentration, for example, due to the fact that the bioactive molecule and the target protein can be spatially separated, for example, in different cellular compartments. To determine the effective concentration, we developed an approach based on the processing of data from cellular thermal shift assay using a simple equilibrium mathematical model of interaction between molecules. This approach was tested on the example of protein constructs capable of interacting with the Keap1 protein, thereby leading to the release of the transcription factor Nrf2 from the complex with Keap1.

Our laboratory is actively developing polypeptide modular delivery systems for bioactive molecules that can bind to a selected receptor on the surface of target cells, internalize into cells by endocytosis, and exit endosomes. In this work, an antibody-like molecule, the R1 monobody, is delivered to the Keap1 protein. For binding to cells and subsequent internalization, another antibody-like molecule, affibody to the epidermal growth factor receptor (EGFR), was used. The translocation domain of diphtheria toxin (DTox) was responsible

for the exit from endosomes. The E.coli hemoglobin-like protein connected all the modules together and gave the construct the necessary solubility. Such a modular nanotransporter is further designated as MNT, while the similar one lacking Keap1 monobody is designated as MNTc.

Internalization of MNT labeled with a fluorescent dye was studied using flow cytometry on mouse liver cells (alpha mouse liver 12, AML12). Just after 15 minutes incubation of AML12 cells with MNT, the fluorescence of the cells significantly increased compared to the control without the addition of MNT. It has been shown that internalization is mainly provided by the interaction of MNT with EGFR, rather than by nonspecific binding to cells.

The ability of DTox within the MNT to cause pH-dependent damage to the integrity of lipid membranes was studied by the release of the fluorescent dye calcein from phosphatidylcholine liposomes loaded with calcein at a fluorescence self-quenching concentration. The obtained results indicate that DTox retains its activity in the obtained MNT, which is evident from the presence of a peak of membranolytic activity at a pH value of 5.5.

The ability of R1 monobody in MNT to interact with Keap1 in solution was tested using the thermophoresis method. It was shown that the dissociation constant of the MNT complex with Keap1 is $8 \pm 3$ nM, i.e., this interaction is highly affine. Given that the affinity of the free monobody for Keap1 is much higher than that of the monobody in MNT, cleavage of this monobody in endosomes will facilitate its interaction with Keap1 in cells.

Evidence for the interaction of MNT with Keap1 in cells was obtained using the Förster resonant energy transfer method using fluorescence lifetime microscopy. The essence of this method is that the lifetime of the donor is significantly reduced if the acceptor molecule is located at a distance of no more than 10 nm. AML12 cells were transformed with Keap1 protein fused to green fluorescent protein (eGFP), while MNT and MNTc were labeled with the fluorescent dye AlexaFluor568. After 1 hour incubation of cells with MNT, but not MNTc, a significant decrease in the average lifetime of eGFP fluorescence is observed.

The interaction of MNT with Keap1 and the subsequent displacement of Nrf2 from the complex with Keap1 can be quantitatively described using cellular thermal shift assay. This method allows one to determine the so-called melting curve of the selected protein, which depends on the formation of its complex with another molecule. Thus, using Western blot, for example, with antibodies to Nrf2, it is possible to estimate the free Nrf2 fraction following different times of incubation of cells with MNT. Using this fraction and a simple equilibrium model of the competitive interaction of MNT with the Nrf2 system, one can estimate the concentration of MNT in the cell cytoplasm. Using our approach, we estimated the Nrf2, Keap1, and MNT cytoplasmic concentrations in several cell lines, and these results are similar to data obtained by Western blot with antibodies to the selected protein and the corresponding calibration dependences.

To increase its efficiency endosomal protease cathepsin B cleavage site was introduced into MNT between monobody and the rest of MNT molecule. The use of our proposed approach for such a modified MNT showed a significant increase in its efficiency, both due to the fact that the free monobody has a higher affinity for Keap1 compared to the monobody within MNT, and due to an increase in the proportion of monobody released from endosomes.

Thus, we have proposed a new approach for assessing the effective intracellular concentration of a bioactive molecule capable of interacting with a selected target protein. This approach was used to estimate the concentration of modular nanotransporters capable of interacting with the Keap1 protein and leading to the displacement of the transcription factor Nrf2 from the complex with Keap1.

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## S9.562. A new technology for assessing the metabolic response of gliomas to chemotherapy using time-resolved fluorescence microscopy

Sachkova D.A.[1,2]*, Shirmanova M.V.[2], Druzhkova I.N.[2], Shcheslavskiy V.I.[2], Mozherov A.M.[2], Yashin K.S.[2], Yuzhakova D.V.[2]
[1]*National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
[2]*Privolzhsky Research Medical University of the Ministry of Health of the Russian Federation, Nizhny Novgorod, Russia;*
* sachkova.collins@gmail.com

Introduction. The central problem in the treatment of glioma is the low effectiveness of standard chemotherapy regimens that do not take into account the individual characteristics of the patient's tumor. A developing direction in solving this problem is the development of a personalized approach based on the use of patient-specific models and evaluation of the effectiveness of therapy using a promising method of fluorescence time-resolved microscopy (FLIM) of metabolic coenzymes. FLIM is based on the detection of fluorescence lifetime of molecules, and differs from standard assessment methods in that it does not require the use of dyes, destruction of cells and tissues, and allows you to obtain data in real time.

The purpose of the work. Development of a new technology for assessing the metabolic response of gliomas to chemotherapy using time-resolved fluorescence microscopy.

Materials and methods. The studies were provided on cell cultures obtained from postoperative material of patients diagnosed with high-grade astrocytoma (Grade II-IV). Short-term cultures were isolated by mechanical disaggregation and cultivation of small tumor fragments. Chemotherapy was performed with Temodal (Orion Pharma, Finland). Visualization of autofluorescence of the metabolic coenzyme nicotinamide dinucleotide (phosphate) NAD(P)H was carried out using a confocal microscope LSM 880 (Carl Zeiss, Germany) with a FLIM application based on a time-correlated count of single photons TCSPC (Becker & Hickl, Germany) (excitation 375 nm, registration 435 – 485 nm).

Results. A library of primary cultures of patients' gliomas was recruited. A technology was developed to assess the metabolic response of glioma cultures after exposure to chemotherapy. The technology includes optimized conditions for isolation and cultivation of short-term cultures, selected doses of a chemotherapy drug for exposure to cultures and parameters of NAD(P)H visualization using the FLIM method.

It has been shown that the use of the FLIM method makes it possible to identify glioma cultures with a metabolic response developing as a result of the action of chemotherapy. An increase in the relative amplitude of the protein-bound NAD(P)H form a2% was demonstrated in the average lifetime of fluorescence in tumor cells after incubation with Temodal for 72 hours, which correlates with a decrease in viability after chemotherapy according to the results of the MTT test. The presence of such changes may be associated with a metabolic shift towards oxidative phosphorylation and a decrease in the proliferative activity of tumor cells as a result of an effectively selected therapy regimen. In the event that there are no changes in the parameters of the lifetime NAD(P)H, or a decrease in the relative amplitude a2 is recorded, this correlates with the absence of a cell response to chemotherapy.

Conclusions. The innovative FLIM imaging method is able to detect metabolic changes in glioma cultures of patients after chemotherapy based on the parameters of the fluorescence lifetime of NAD(P)H. The developed technology for assessing the metabolic response of tumor cells to treatment can serve as an effective approach for the selection of personalized therapy. The work was carried out with the financial support of the Russian Science Foundation grant (project No. 21-75-00098).

**S9.563. A spatio-temporal map of 5-HT4R expression in the mouse brain at various developmental stages**

Mitroshina E.M.[1]*, Perenkov A.D.[1], Vedunova M.V.[1]
[1]*Lobachevsky State University of Nizhny Novgorod;*
* helenmitroshina@gmail.com

Depression is one of the major concerns in Public Health in Russia and around the world. The serotonin system modulates the acute stress response, and has been implicated in both the etiology of depression and anxiety as well as the response to treatment. Several serotonin receptors, including 5-HT1A and 5-HT2 have been proposed to modulate morphogenic events elicited by serotonin. These findings are in agreement with observations of 5-HT involvement in certain aspects of neural development, such as neurite outgrowth, regulation of somatic morphology, growth cone motility, and control of dendritic spine shape, in addition to its classical role as a neurotransmitter.
However, little is known about the role of other types of serotonin receptors in the development of depressive disorders, in particular 5-HT4R receptors. Much attention has recently focused on the 5-HT4R's positive effects on learning and memory, since deficits in these cognitive functions are often associated with anxiety, depression as well as neurodegenerative diseases, such as Alzheimer's disease.
We have created a spatio-temporal map of 5-HT4R receptors expression in the brain of C57BL/6 mice. The level of 5-HT4R expression was studied in the cortex, cerebellum, and hippocampus at various developmental stages (P5, P15, P30, 6 months, 12 months, 18 months, and 22-24 months was assessed by RT-PCR.
It has been shown that the level of 5-HT4R expression in the cerebral cortex increases from P5 to 1 year, as in the cerebellum, and at 18 months it begins to irreversibly decrease. In the hippocampus, an irreversible decrease in 5-HT4R expression occurs starting at 6 months of age. Comparison of the 5-HT4R expression level in different parts of the brain showed that for the neonatal period (P5) the maximum level of 5-HT4R expression was found in the cerebellum (3.1 times higher than in the cortex). During the period of fertility (from P15 to 6 months), the highest level of 5-HT4R expression was in the hippocampus. At the age of 12 months, 5-HT4R is expressed at the same level in all studied brain regions. In elderly animals aged 18 months or older, 5-HT4R expression in the cerebellum is significantly reduced compared to the cortex and hippocampus.
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**S9.564. A study of the peripheral part of the occurrence of pain during migraine in conditions of a chronically increased level of homocysteine in rats**

Ermakova E.V.[1]*, Koroleva K.S.[1], Kabirova A.A.[1], Sitdikova G.F.[1]
[1]*Kazan Federal University;*
* EliVErmakova@kpfu.ru

Migraine is a common disease that causes chronic headaches. This disorder affects up to 10–15% of the population, but its neurochemical mechanisms are not well-understood [1]. Several clinical studies have shown the connection between high concentrations of the sulfur-containing amino acid homocysteine (Hcy) in plasma (hyperhomocysteinemia, HHcy) and the frequency of migraine attacks. However, the mechanism and targets of Hcy effect in the trigeminal system remain unexplored, such as changes in the excitability of its peripheral part in conditions of the increased levels of this amino acid.
It was suggested that Hcy could change the number or activity of glutamate receptors in trigeminal ganglion (TG) neurons. Glutamate is the most common excitatory neurotransmitter in the nervous system. It provides a transmission from primary afferents to second-order nociceptive neurons in the brainstem. However, its role in the peripheral mechanisms of migraine during the generation of nociceptive signals in the meninges also remains unclear. Glutamate excites neurons through ionotropic AMPA, NMDA, or kainate receptors. Among these types, NMDA receptors (NMDAR) have the highest affinity for glutamate and relatively low desensitization, which makes them the most suitable candidates for participating in the effects of glutamate in the meninges. Our study aimed to analyze the excitability of the peripheral part of rat trigeminal nerve as well as the excitability of isolated TG-neurons under conditions of prenatal HHcy modelling. Besides, current work aimed to test the role of NMDAR in the activation of the peripheral endings of the rat trigeminal nerve.
The study involved two groups of animals: the control group and the group with prenatal HHCy, which was modelled as described previously [2]. Action potentials (AP) generated in the peripheral endings of the trigeminal nerve were recorded using an extracellular glass electrode. To do this half-skull preparation of P30-40 rats was used. Half of the cranium was cleared of peripheral tissues, while the hard shell of the brain with blood vessels and nerve fibres was left intact. Substances were applied to the divergence of the medial meningeal artery. Registration of evoked Ca2+ responses was performed using a primary culture of neurons isolated from the trigeminal ganglion of P10-14 rats; cells were visualized using the Fluo4 AM marker with the following quantitative analysis [3]. The patch-clamp technique was used to measure the membrane potential of TG-neurons, which allowed us to evaluate changes in the electrical properties of the membrane under conditions of prenatal HHcy. Application of the NMDAR agonist (100 μM) on TG neurons was performed using a rapid perfusion system (application time 20 s). A magnesium-free external solution supplemented with the co-agonist glycine (30 μM) was used in all experiments with the NMDAR agonist.
It was shown that the threshold concentration of KCl, which caused an increase in the frequency of trigeminal nerve spiking, differs in the control and HHcy groups. In control threshold concentration was 25 mM while in HHCy group it was 5 mM. An analysis of the passive and active electrical properties of the membrane of TG-neurons showed that the input resistance, the resting membrane potential, the AP amplitude, and the AP generation threshold did not differ significantly in control and prenatal HHCy groups, however, the mean rheobase for neurons of animals with prenatal HHcy was significantly lower than the control values. It was also shown that 30.3% of neurons in the control group and 23% of neurons in the HHCy group generated AP series in response to the increase in the strength of the injected current up to two rheobases. The average number of APs per 1 s increased than twice for neurons in HHcy group under conditions of such stimulation compared with control. During the extracellular recording of APs in trigeminal nerve frequency of spikes was almost doubled by applying an NMDAR agonist. In control animals NMDA caused a transient increase in Ca2+-levels in 14.3% of TG neurons (n=101/706), which also indicated a functional expression of NMDA receptors [4]. In rats of the HHcy group, NMDA-induced Ca2+ transients were observed only in 6.19% of TG neurons (n=31/501), which may be associated with chronic oxidative stress. At the same time, the average amplitude, area, and FWHM of responses to NMDA in the HHcy group did not change significantly compared with the control parameters.
In summary under conditions of prenatal HHcy occurs a higher excitability of trigeminal nerve afferents and increased excitability of isolated TG-neurons. It can be assumed that an increase in the frequency of migraine attacks associated with a high level of Hcy in blood may be explained by the sensitization of the trigeminal system which provides innervation to the dura mater. Activation of the dura mater is a trigger of nociceptive impulses.
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### S9.565. Aberrant oscillations and motor dysfunction in the rat model of Pakinson's disease: new therapeutic strategies and goals

Brazhnik E.S.[1], Novikov N.I.[1]*
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
* nikolay_novikov@hotmail.com

Parkinson's disease (PD) - a progressive neurodegenerative disease caused by a selective loss of dopaminergic innervation of the basal ganglia (BG), resulting in impaired motor function. In PD, activity in neural motor networks is characterized by the appearance of sustained 30-Hz beta-oscillations, which are thought to impair movement processing and lead to motor symptoms (1-4). Levodopa eliminates these oscillations, which is accompanied by a temporary restoration of motor function. However, with long-term treatment with levodopa, most patients experience serious side effects - levodopa-induced dyskinesia (LID) with the characteristic appearance of pathological 100-Hz gamma-oscillations in the thalamus and motor cortex (5-6). The mechanisms underlying pathological synchronization in BG-thalamocortical neurocircuits and their contribution to the process of occurrence of the PD motor symptoms and levodopa-induced dyskinesia are not fully understood. Identification of the critical components of the brain neural networks involved in this process can become the basis for the development of new methods of treating patients with PD.
We investigated the effects of reversible suppression of the activity of the nuclei of the motor thalamus (VM and Pf) and GPe (the strategic nucleus in BG; 7, 8) by local administration of muscimol, a GABA-A receptor agonist, or increasing their activity with picrotoxin, a GABA-A-receptors antagonist, on the severity of aberrant rhythms and the ability to restore normal locomotion in rats with experimental parkinsonism. We have shown that, upon modulation of the neuronal activity of the motor thalamus and GPe (activation or inhibition), the power of 30-Hz beta-oscillations in the BG-thalamocortical circuits significantly decreased, while its effect on locomotion was not unidirectional. It should be noted that upon inhibition the activity of thalamic Pf and GPe, the motor deficit was eliminated, while after inhibition of thalamic VM the motor deficit increased. Conversely, an increase in the activity of VM and GPe with picrotoxin was accompanied by the restoration of normal locomotion, but the introduction of picrotoxin into Pf increased the motor deficit. In rats treated with high doses of levodopa, leading to the development of LID, infusion of muscimol into GPe and VM abolished 100-Hz gamma-oscillations in the thalamocortical neural network. However, only inhibition of GPe activity prevented or significantly reduced the incidence of levodopa-induced dyskinesia.
The obtained results indicate that the presence of aberrant oscillations in motor neurocircuits can not be fully considered as the cause of motor symptoms in PD, but to some extend this could be the manifestation of the imbalance in brain activity. It is important that GPe, as a critical component of BG, can be considered as the main target for therapeutic intervention in order to normalize motor activity in PD and, possibly, prevent LID. Note that the use of modern chemogenetic methods will allow the development of new approaches to the elimination of movement disorders in Parkinson's disease (9).
Abbreviations: PD - Parkinson's disease; GPe - external segment of globus pallidus nucleus; BG - basal ganglia; Pf - parafascicular nucleus of the thalamus; MCx - motor cortex; VM - ventromedial nucleus of the thalamus; LID - levodopa-induced dyskinesia.
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### S9.566. Accessing and quantification of marker transport in a hydrogel phantom mimicking the brain's parenchyma

Vanina A.S.[1]*, Sychev A.V.[1], Postnikov E.B.[1], Lavrova A.I.[2], Grekhnyova E.V.[1], Kudryavtseva T.N.[1], Gavrilov P.V.[2], Andropova P.L.[3]
[1]*Kursk State University;*
[2]*Saint-Petersburg State Research Institute of Phthisiopulmonology;*
[3]*N. P. Bechtereva Institute of the Human Brain of the Russian Academy of Sciences;*
* vanina.nast.05@gmail.com

Among modern problems of brain physiology, special attention is paid to transport processes in its extracellular space, which are crucial for understanding neuroglial metabolism, addressing drug delivery, and preventing neurodegenerative diseases [1].
One of the still unresolved questions is the mechanism of solute transport through the parenchyma. There are debates about the existence of either directional fluxes or diffusive transport. Since direct recording such processes in vivo is difficult, there is demand for developing phantoms with properties mimicking the brain parenchyma as physical models for testing the feasibility of different approaches to describing the transport of substances.
This work deals with accessing and quantification of transfer processes in a new phantom tissue [2] based on collagen hydrogel imitating the structure and consistency of the brain parenchyma. Electron scanning

microscopy and computed tomography revealed the existence of network-like and microporous structures corresponding to the characteristics of the brain's extracellular space. Taking these data into account, the transport of molecular markers or nanoparticles in the synthesized hydrogel can be expected as following the same diffusion pattern as in an inhomogeneous environment with traps and barriers.
Two approaches were used: 1) a spatiotemporal fixation of the two-dimensional projection of low-molecular fluorescent markers' spread, and 2) the three-dimensional spread's picture of the contrast agent used in clinical computed tomography. The first resulted in the determination of an effective macroscopic diffusion coefficient, which is shown as biologically relevant. The second revealed a complex process combining the rapid water spread in this structure after injection, followed by a slow diffusion process influenced by the microstructure of the medium. The correspondence of the obtained regularities to the biophysical models of these processes is discussed.
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**S9.567. Acoustic structure of communication calls of the house mouse (Mus musculus): analysis and fundamental features**

Lupanova A.S.[1]*, Egorova M.A.[1]
[1]*Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences;*
* sozdanie21@gmail.com

Acoustic communication in animals refers to a complex type of behavior, where the meaning of a signal is determined by the properties of its structural elements (Hauser et al., 2002; Pinker, 2004). All structural elements of the signal can be divided into two groups. The main, so called spectral-temporal characteristics, include duration, intervals between sounds, repetition frequency of sounds, fundamental frequency at the beginning and end of the signal, its maximum and minimum values, and frequency modulation. The second group of characteristics includes non-linear phenomena such as biphonations, subharmonics, deterministic chaos, sidebands and frequency jumps (Wilden et al., 1998).
In early studies of animal calls, when describing their acoustic structure, attention was paid mainly to the call duration, fundamental frequency, the number of harmonics, and the number of calls in a series (Konstantinov and Movchan, 1985). Later the role of nonlinear phenomena in encoding information about the species, gender, individual characteristics and the current emotional state of the animal has become known (Ehret, 2006; Volodin, 2007).
The recognition of a communication signal and an adequate behavioral response to it are of great importance for social animals (Kanwal, Rauschecker, 2007). The house mouse , leading a social lifestyle and having a well–developed acoustic communication, is a traditional object of laboratory studies of acoustic behavior and neurophysiological mechanisms of sounds coding by the auditory centers of the brain. Despite numerous studies of the vocalizations of these animals, the data on their vocalization repertoire are very fragmentary (Portfors, 2007; Hammerschmidt et al, 2009) and do not provide a complete picture of the acoustic characteristics of calls and the role of acoustic behavior in the survival of individuals. Thus, insufficient knowledge of the acoustic structure of calls and their semantic meaning, as well as the almost complete absence of information on the formation of vocalizations in the mice ontogenesis determined the purpose of our study.
We did an audio-video recording of the acoustic behavior of mice when simulating elements of various types of behavior. Experiments were made on 33 mature mice - hybrids of CBA and C57BL\6 lines (19 males and 14 females) and 92 pups.
The recorded vocalizations reflected the entire range of behavioral activity of mice - their social, reproductive and individual behavior. Spectral-temporal analysis of early ontogenesis calls (ultrasound (US) pups calls and wriggling calls), calls of a subordinate male during agonistic behavior, defensive calls of males and US vocalizations of a male during sexual behavior, US calls of females during social contacts and search for litters were performed. All calls studied covered frequencies from 1 to 90 kHz and occupied two non-overlapping frequency ranges – sound and ultrasonic. US vocalizations in the repertoire of mice were mostly represented by a series of tonal signals with a small amount of non-linear phenomena. Thus, the presence of broadband noise was noted in 18% of the US pups calls, 16% US calls of males during sexual behavior and in 4% of the US vocalizations of females when searching for litters. Low-frequency vocalizations, on the contrary, were distinguished by a well-pronounced noise component: in 60% of the calls of the subordinate male and 75% of the wriggling calls and the female defensive calls. A similar data were also obtained for such non-linear phenomena as discontinuities in the spectrum (in 14% of pups US calls, 16% of the low-frequency calls of males) and subharmonics. The subharmonics were not encountered in the structure of US vocalizations. Vocalizations of the sound range had well-pronounced frequency modulation and subharmonics in at least 40% of the signals. An analysis of ontogenetic changes in the structure of pups calls (from 2 to 14 days of life) also showed differences in US and sound vocalizations in the presence and severity of non-linear phenomena. US calls in 14-day-old pups had less noise inserts and frequency jumps than in pups on the second day of life. And on the contrary, with the maturation of the mice, more discontinuities in the spectrum and subharmonics were noted in the low-frequency wriggling call.
Thus, the study of the acoustic structure of vocalizations made it possible to identify their fundamental characteristics. The most stable of them for all recorded signals were the frequency range of the calls, its duration, the frequency of the fundamental tone, the number of harmonics, the presence of subharmonics and frequency modulation in the structure of low-frequency vocalizations. It is likely that low-frequency calls with a large number of stable parameters play a special role in encoding information about the individual and his emotional state.
The analysis of ontogenetic changes in the structure of mouse pups calls showed a change in the characteristics of signals with the maturation of animals due to the physiological development of the vocal apparatus and an increase in social interactions.
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**S9.568. Activity of glutamate receptors in hippocampal neurons of rats with prenatal hyperhomocysteinemia**

Kurmashova E.[1], Gataulina E.[1], Yakovlev A.[1]*
[1]*Kazan Federal University;*
* alv.yakovlev@gmail.com

Homocysteine, a sulfur-containing amino acid formed from methionine, is a risk factor for the development of a number of pathologies. An increase in the level of homocysteine (hyperhomocysteinemia, hHCY) during pregnancy leads to various complications of pregnancy, fetal hypoxia and, as a result, the development of early and delayed postnatal pathologies. One of the mechanisms of the neurotoxic action of homocysteine is the activation of ionotropic and metabotropic glutamate receptors in cells, the stimulation of which leads to the development of neuronal hyperexcitability, and prolonged exposure to high doses in the prenatal period can lead to changes in the expression, regulation, or activity of ionotropic glutamate receptors. The aim of the study was to analyze the role of glutamate channels in the development of prenatal hHCY in rat hippocampal neurons in the first week of postnatal development. The experiments were carried out on rat horizontal slices of rat pups in the first week after birth. For chronic model of prenatal hHCY we used high methionine diet during the pregnancy of female rats. The synaptic activity and the ratio of AMPA/NMDA receptors of pyramidal neurons of the hippocampus was carried out using patch clamp registration: AMPA-receptor-mediated excitatory postsynaptic currents (AMPA-EPSC) were recorded at a fixation potential of -70 mV, and NMDA-receptor-mediated excitatory postsynaptic currents (NMDA-EPSC) at +40 mV, in the presence of the AMPA receptor blocker - CNQX (10 μM). The activity of postsynaptic NMDA or AMPA receptors was recorded using focal application of NMDA (50 μM, 50 μM glycine, 10 μM CNQX) or glutamate (1 mM, 40 μM d-APV) at a distance of 150–200 μm from the soma of neurons. To avoid receptor desensitization, agonists were applied at 5 min intervals. All experiments on registration of excitatory postsynaptic currents in hippocampal CA3 synapses were performed in the presence of a GABA(A)-receptor blocker, bicuculline (10 μM). Under control conditions, in the first week of postnatal brain development, the amplitude and decay time of spontaneous NMDA-EPSC in hippocampal CA3 neurons were 27±1 pA and 76±13 ms and (n=20), respectively, and the NMDA-EPSC frequency was 0.2±0.1 Hz (n =20). In slices of the hippocampus from rats with prenatal hHCY, it was observed the decreasing of NMDA-EPSC amplitude and an increasing frequency, without changing the decay time. Focal application of the agonist NMDA induced an NMDA-mediated postsynaptic current in hippocampal neurons with an amplitude of 0.9±0.1 nA (n=26), while under conditions of prenatal hHCY, the amplitude of postsynaptic NMDA response increased up to 1.6±0.2 nA (n=39 , $p<0.05$). An analysis of the amplitude-temporal characteristics of AMPA-EPSC indicated rising of amplitude and frequency of spontaneous excitatory currents. In hippocampal CA3 neurons of rats with prenatal hHCY it was observed a significant increasing in both the amplitude and frequency of AMPA-EPSC, without a significant change in the decay time. The analysis of the distribution of AMPA-EPSC amplitudes in hippocampal neurons showed an increase in the number of high-amplitude events in rats with prenatal hHCY. The focal application of glutamate caused an AMPA-mediated postsynaptic current in hippocampal neurons with an amplitude of 0.4±0.02 nA (n=55), and under conditions of prenatal hHCY, an increase in the amplitude of responses of postsynaptic AMPA receptors up to 0.6±0.03 nA was observed (n=29 , $p<0.05$). Thus, the data obtained indicate that a chronic high concentration of homocysteine during the pre and postnatal period of rat brain development causes a change in the activity of NMDA and AMPA-receptors in hippocampal neurons. It can be assumed that under conditions of prenatal hHCY in neurons there is a change in the subunit composition or the number of receptors on the postsynaptic membrane, which underlies the increased sensitivity of the hippocampus of rats with prenatal hHCY to the generation of epileptiform activity. This research was funded by RSF No.20-15-00100.

**S9.569. Adaptogenic properties of antioxidants**

Zhigacheva I.V.*, Kricunova N.I.
[1]*N.M. Emanuel Institute of Biochemical Physics of RAS, Moscow, Russia;*
* zhigacheva@mail.ru

Mitochondria occupy key positions in energy, redox and metabolic processes in the cell. Nevertheless, under stress conditions, these organelles are one of the main sources of reactive oxygen species (ROS) [1]. Excessive generation of ROS leads to peroxidation of membrane lipids, primarily cardiolipin, and swelling of mitochondria. The consequence of "peroxide" swelling of mitochondria (or the formation of large pores in the outer membrane) is the release of apoptogenic proteins from the intermembrane space into the cytoplasm and activation of the mitochondrial pathway of apoptosis. It can be assumed that drugs that reduce excessive generation of ROS by mitochondria will increase the body's resistance to stress factors. This role is primarily claimed by antioxidants. The object of the study was selected antioxidants from the class of spatially hindered phenols: potassium phenosan (potassium 3,5-Di-tert-butyl-4-hydroxyphenyl propionate ) and sodium anphen (sodium (1-carboxy)-1- (N-methylamide) - 2-(3,5-di-tert-butyl-4-hydroxyphenyl-propionate), antioxidant - a derivative of 3-hydroxypyridine, which is a heterocyclic analogue of aromatic phenols: 2-ethyl-6-methyl-3-hydroxypyridinium hydroxy-butane-dioate (ethoxidol) and an antioxidant of natural origin - polyphenol resveratrol. The aim of the study was to study the effects of the studied antioxidants on the functional state of rat liver mitochondria under stress. Since mitochondria are the main source of ROS under stress conditions, it was necessary to develop a model simulating stress, i.e. find the conditions under which ROS production by mitochondria will increase, and, consequently, LPO will be activated [2]. We solved this problem by developing a model of "aging" (incubation of mitochondria in a hypotonic medium at room temperature). "Aging" caused an increase in the generation of ROS, which was reflected in an increase in the fluorescence intensity of the final products of lipid peroxidation (Schiff bases) by 3 times. The introduction of drugs into the incubation medium of mitochondria led to a decrease in the fluorescence intensity of LPO products, and this decrease depended on the concentration of the drug in the incubation medium. Sodium anphen at concentrations of 10-9-10-11, 10-13 and 10-14M reduced the fluorescence intensity of LPO products to the control level. The most effective concentrations for potassium phenosan were 10-7-10-8 and 10-13 and 10-14M, and for ethoxidol - 10-5M. As for resveratrol, effective concentrations for it were 10-6-10-14M. At higher concentrations, it exhibited prooxidant properties. The decrease in the fluorescence intensity of LPO products by the drugs probably indicated that they have anti-stress properties, the presence of which was tested in the model of acute hypobaric hypoxia (AHH). The choice of the AHH model is due to the activation of lipid peroxidation (LPO) and mitochondrial dysfunction under these conditions [3, 4]. In the experiment, drugs were used in doses that effectively reduce the intensity of LPO in a model experiment: sodium anphen 10-9 mol/kg, potassium phenosan - 10-8 mol/kg, ethoxydol - 2 × 10-5 mol/kg, resveratrol 10-5 mol/kg.

AHH led to the activation of LPO in mitochondrial membranes: the fluorescence intensity of LPO products increased by 2-4 times. Administration of sodium anphen and potassium phenosan to rats 45 minutes before exposure prevented the activation of LPO. Injection of 2×10-5

mol/kg of ethoxydol for 7 days or 10-5 mol/kg of resveratrol for 5 days before stress exposure also led to a decrease in the intensity of lipid peroxidation to the control level. Changes in the physicochemical properties of mitochondrial membranes caused by peroxidation of membrane lipids could affect lipid-protein interactions and, consequently, the.activity of mitochondrial respiratory chain enzymes.

Indeed, AHH resulted in a 27% decrease in the maximum oxidation rates of NAD-dependent substrates and a 38% decrease in the efficiency of oxidative phosphorylation. Sodium anphen and potassium phenozan administered to animals 45 minutes before exposure prevented changes in the functional characteristics of liver mitochondria. The same result was achieved by 7-day administration of ethoxidol or a 5-day administration of resveratrol.

By preventing the activation of LPO under stress and thereby preventing changes in the functional characteristics of mitochondria, the drugs also affected physiological parameters: the life expectancy of animals with the introduction of antioxidants before stress exposure under conditions of various types of hypoxia increased by 1.8-3 times, and the survival rate increased by 1.8-3 times. 10-40%.

Based on these data, it can be concluded that the studied antioxidants can be used as adaptogens. Drugs that prevent the activation of lipid peroxidation probably ensure the effective operation of mitochondrial electron transport chains by preventing a decrease in the activity of NAD-dependent dehydrogenases.

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**S9.570. Adhesion is a target of anticancer therapy**

Bocharova O.A.[1]*, Karpova R.V.[1], Bocharov E.V.[1], Kucheryanu V.G.[2]
[1]*N.N. Blokhin National Medical Research Center of Oncology of the Ministry of Health of Russia, Moscow, Russia;*
[2]*Institute of General Pathology and Pathophysiology of RAS, Moscow, Russia;*
* imufarm@rambler.ru

Violation of cell differentiation with loss of control over proliferative processes is considered to be an inseparable feature of tumor cells. Close attention deserves the fact that tumor cells lose their interaction with each other, with immune effectors, and, as a result, with the whole organism. We can consider the violation of adhesive interactions as the key mechanism of neoplasia [1].

The beginning of our research series devoted to this problem was the study of determining the cohesive forces between cells in tissue (liver and lung) by the Coman method in early postnatal ontogenesis in 7 different inbred lines of mice - CBA, C3HA, C3H, Balb/c, CC57Br, A, C57Bl. Thus, a previously unknown phenomenon of an increase in cohesive forces during intercellular interaction in epithelial tissues in the early postnatal period was established, which leads to the completion of the tissue homeostatic system formation and ensures an increase in its resistance, including to the processes of blastomogenesis [2,3,4].

It was also found that extracts of adaptogens (Panax ginseng, Rhodiola rosea) increase the cohesive forces between cells in tissues (liver and lung) predisposed to tumor growth. At the same time, the administration of an extract of Rhodiola rosea to high-cancer mice-males CBA in the preventive and therapeutic modes increased the cohesive force of target tissue (liver) cells, had an immuno-, hormone-modulating and anti-stress effect, as well as reduced the frequency of hepatocarcinomas in the second half of ontogenesis [5]. It has also been demonstrated in the oncology clinic that the use of Rhodiola rosea extract enhances the cohesive force between bladder urothelial cells (homotypic histospecific adhesion), exerting an immunomodulatory effect while increasing the expression of beta2-leukocyte integrins (heterotypic immunoadhesion), reducing the incidence of bladder cancer recurrence in patients [6].

It should be noted that, of course, pure biologically active compounds were isolated from adaptogen plants (Panax ginseng, Rhodiola rosea, Eleutherococcus senticosus, etc.). However, their use is significantly limited for many reasons, including low bioavailability, rapid excretion from the body and high toxicity. Therefore, the use of extracts remains preferable. In turn, the use of extracts of individual plants is accompanied by increasing tolerance, that is, the loss of action on the body. We tried to solve this problem when developing a complex preparation.

We have developed a multiphytoadaptogen (MPhA) consisting of forty plant extracts components including adaptogens (Panax ginseng, Rhodiola rosea, Eleutherococcus senticosus, etc.) that is effective for preventive oncology and age-related pathologies and does not cause the tolerance in patients. However, in clinical studies we were unable to demonstrate in details the correction of adhesive mechanisms which probably play a key role in the antitumor activity of MPhA. We decided to investigate the role of immunoadhesive interactions between tumor cells and immune effectors in the control of tumor formation, life expectancy and quality of life in a large experiment on high-cancer mice-males CBA (about 1000) genetically predisposed to hepatocarcinomas.

In the experiment the medication was administered in a preventive and therapeutic modes. It was shown that the increased expression of beta-2 leukocyte integrins is significant for the activation of antitumor cytotoxicity of T-lymphocytes and life expectancy [7]. Taking into account the information of the international database Cortelis Drug Discovery Intelligens (2022), it became known that synthetic activators of antitumor T-cell immunity and infiltration of tumors by cytotoxic T-lymphocytes, «first-in-class», are in phase 1 clinical trials. Among them are the LFA-1 adhesion molecule agonist (7HP349, Hills Pharma, USA), as well as the Mac-1 adhesion molecule agonist (ADH-503, Leukadherin1-cholin, GossamerBio, USA). This emphasizes the relevance and timeliness of our research.

The implementation of the adhesive concept creates a certain perspective for improving the effectiveness of diagnosis methods, prevention and treatment, which can be a step towards solving the problem of malignant neoplasms.

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**S9.571. Age-dependent changes in the calcium activity of astrocytes in vitro**

Mitroshina E.M.[1]*, Mishchenko T.A.[1], Yarkov R.S.[1], Krivonosov M.I.[1], Vedunova M.V.[1]
[1]*Lobachevsky State University of Nizhny Novgorod;*
* helenmitroshina@gmail.com

Recent studies have shown the unique role of astrocytes in the functioning of neural networks in the brain. Astrocytes can model brain activity and slow down or accelerate neurodegeneration processes by performing an exceptional role in maintaining neuronal homeostasis. In the age aspect, the response of brain cells to hypoxia, one of the main pathogenetic factors of ischemic brain damage, is of particular importance [1]. In this regard, the study of age-dependent changes in astrocytes is of exceptional importance.

The study of age-dependent functional reorganization of calcium activity was carried out on primary astrocyte cultures and dissociated hippocampal primary cultures using a D-galactose induced aging model (D-galactose was administered from 3 to 14 days of cultivation). Modeling of hypoxia in vitro was performed on 14 DIV by replacing the culture medium with a low oxygen medium. Visualization of calcium activity was performed on 21 DIV. A laser scanning microscope LSM 800 (Zeiss, Germany) was used for imaging studies of the functional calcium activity of nerve cells. The technique allowed us to visualize the functional architecture of the neuronal network of the culture at the cellular level. Oregon Green 488 BAPTA-1 AM (Invitrogen, USA) was used as a calcium sensor.

It was shown that during the pharmacological modeling of aging using D-galactose, changes in the main parameters of calcium activity of astrocyte cultures are expressed in a decrease in the number of functionally significant connections formed by the cell ("Control" 5.8±0.85; "D-galactose" 3.27±0,32). However, the correlation level of calcium activity, as well as the signal propagation rate, do not change. Modeling of hypoxia led to a decrease in connectivity indicators in both the experimental and control groups.

The study of the reorganization of the network activity of neuron-glial primary hippocampal cultures showed that modeling of accelerated pharmacological aging leads to a pronounced inhibition of calcium activity and disruption of neuron-glial network connectivity. More pronounced destruction of neural network are observed when modeling hypoxia in the group with D-galactose.

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**S9.572. Age-related changes in the permeability of the blood-brain barrier in rats undergoing prenatal hyperhomocysteinemia**

Detterer A.S.[1]*, Yakovlev A.V.[1]
[1]*Kazan Federal University;*
* anna.detterer@gmail.com

The blood-brain barrier (BBB) is a structure that provides selective permeability between blood components and the brain parenchyma. Homocysteine is a sulfur-containing amino acid involved in the methionine cycle. An increased level of homocysteine in the blood (hyperhomocysteinemia, HHC) causes a violation of the structure of the BBB, through increased production of reactive oxygen species and activation of matrix metalloproteinases, which play an important role in the destruction of the protein matrix and lead to increased vascular permeability. The purpose of the study was to analyze age-related changes in the BBB permeability of rats under conditions of chronic action of homocysteine and its derivatives.

The following groups of animals were used in the experiments: the control group, animals with prenatal HHC and with injection of homocysteine-thiolactone. To create a chronic model of prenatal HHC, a dietary methionine load (7.7 g/kg feed) was used throughout the pregnancy of female rats. The experiments were carried out on offspring at the age of 1.5 and 6 months after birth. To assess the permeability of the BBB, extravasation of albumin in the brain tissue was determined using the dye Evans Blue (EB, 2ml/kg). Evans Blue's passage through the BBB was assessed 60 minutes after its intravenous injection. Determination of the dye concentration in the animal cerebellum homogenate was carried out spectrophotometrically (620 nm) using a tablet ELISA reader Multiscan FS (Thermo scientific, USA) using calibration curves. To analyze the effect of homocysteine derivatives, homocysteine-thiolactone (10 mg/kg) was injected subcutaneously 60 minutes before dye injection.

The experimental data showed that there was no release of the dye from the cerebral vessels after intravenous injection of EB under control conditions. The EB concentration in the cerebellum cell homogenate of the control group of animals at the age of 1.5 months was $0.1 \pm 0.04$ μg/mg brain tissue ($N = 11$). Preliminary homocysteine-thiolactone injection caused an increase in BBB permeability and the EB concentration was $0.4 \pm 0.2$ μg/mg brain tissue ($N = 9$; $p < 0.05$). Under conditions of prenatal HHC and in the brain tissues of the offspring, BBB permeability was impaired, and the EB concentration in the cerebellar tissues was $0.6 \pm 0.09$ μg/mg brain tissue ($N = 8$; $p < 0.05$). The BBB permeability index was 16±2% (N=8) in rats with prenatal hyperhomocysteinemia and did not exceed 1% in the control group. At the same time, in rats of the experimental group at the age of 6 months, restoration of BBB permeability was observed and the concentration of EB in the cerebellum homogenate was 0.14±0.01 μg/mg brain tissue (N=6, $p > 0.05$) relative to the control.

Thus, the obtained data indicate, that homocysteine and its derivatives have a negative effect on the permeability of the blood-brain barrier in rats in the prenatal period of development. It is possible that the toxic effect of homocysteine is associated with a violation of the structure of the endothelium of the brain vessels, dysfunction of astrocytes and neurons.

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**S9.573. An ex vivo study of the effects of lactoferrin and vitamin D3 on the markers of halogenative stress and NETosis in the blood of healthy subjects and type 2 diabetes mellitus patients during induced activation of neutrophils**

Ivanov V.A.[1], Galkina N.V.[1], Maximov D.I.[1], Gusev S.A.[1], Kostevich V.A.[1,2], Gorbunov N.P.[1,2], Sokolov A.V.[1,2], Ostrovsky E.M.[1], Panasenko O.M.[1,3]*
[1]*Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia;*
[2]*Institute of Experimental Medicine, St. Petersburg, Russia;*
[3]*Pirogov Russian National Research Medical University, Moscow, Russia;*
* o-panas@mail.ru

Type 2 diabetes mellitus (T2DM) is a serious metabolic disease. Lack or inadequate antidiabetic therapy combined with poor diet and other

factors leads to hyperglycemia in which blood glucose levels can reach 10-12 mM. T2DM and accompanying hyperglycemia cause a variety of complications reducing quality of life, such as diabetic retinopathy, polyneuropathy, or diabetic foot disease. The major cellular component of the immune response are neutrophils which are essential part of innate immunity having broad range of protective and signaling functions. Neutrophils migrate to sites of inflammation, where they undergo activation resulting in degranulation or NETosis, both leading to exocytosis of the contents of cytoplasmic granules. As a result, various biologically active molecules, in particular myeloperoxidase (MPO), appear in the extracellular milieu. MPO catalyzes the formation of reactive halogen species (RHS) which exert antimicrobial actions, but at the same time their excessive production leads to halogenative stress, an imbalance between RHS and the body's ability to neutralize their excess which causes damage to molecules, cells and tissues of the body [1]. Halogenative stress contributes to the development of cardiovascular, metabolic, neurodegenerative and other diseases. Thus, searching for means and approaches to regulate MPO blood level and to prevent halogenative stress is of great importance. Lactoferrin (LF) and vitamin D3 could be one of such therapeutics. LF is abundant in all secretory fluids, and it is also present in neutrophil specific granules. LF displays immunomodulatory, antimicrobial, antioxidant, and other biologically important functions [2]. Vitamin D3 and its metabolites have anti-inflammatory and antioxidant properties as well as prevent NETosis [3].

The aim of the work was to study the effects of LF and vitamin D3 on the content of MPO and the markers of halogenative stress and NETosis in blood of healthy individuals and T2DM patients during induced activation of neutrophils in ex vivo experiments.

Materials and Methods. The study involved 15 people of different sex, aged 45 to 86 years, among whom there were 8 healthy volunteers and 7 T2DM patients with persistent hyperglycemia (blood glucose level of > 7 mM, glycated hemoglobin level of 7-9%). Venous blood was collected in EDTA-containing vacuum tubes. LF and vitamin D3 were added to the blood to a concentration of 1 mg/ml and 5 ng/ml, respectively. Following incubation for 2 h at 37°C, neutrophils were activated by adding phorbol-12-myristate-13-acetate (PMA, 0.16 μM) and incubated for another 0.5 h. After incubation, MPO, halogenative stress markers (chlorinated albumin (HSA-Cl), ceruloplasmin (CP-Cl), and low-density lipoproteins (LDL-Cl)) as well as DNA-MPO complex, a marker of NETosis, were determined in plasma by ELISA method [4].

Results. The ELISA study showed that addition of the neutrophil activator PMA to the blood of both healthy subjects and T2DM patients resulted in a significant increase in MPO, the markers of halogenative stress (HSA-Cl, CP-Cl and LDL-C) and the NETosis marker DNA-MPO. Preincubation of blood with LF significantly reduced all parameters under study by 40-90% compared to control. Incubation of blood with vitamin D3 significantly decreased DNA-MPO. A tendency to decrease MPO was also observed. The results indicate that LF and vitamin D3 can reduce neutrophil secretion of MPO and prevent NETosis. In addition, due to its effect on activated neutrophils, LF impedes halogenative stress.

Our results suggest that LF is able to translocate into the plasma membrane, making it difficult for neutrophil extracellular traps, which include MPO, to be released from the cell. This should decrease MPO concentration in plasma and, thereby, lead to a decrease in the markers of halogenative stress. The observed decrease in LDL-Cl levels can be explained by the ability of LF to compete with MPO for binding to the LDL surface. Due to displacing the functioning MPO from LDL-MPO complex, LF prevents LDL modification caused by MPO-produced RHS [5]. Vitamin D3 was able to reduce NETosis markers, which is in agreement with our previous data [3].

The results obtained extend the possibility of using LF and vitamin D3 as a basis for creating new tools and approaches aimed at the regulation and correction of neutrophil and MPO functions to prevent halogenative stress, which should have a positive effect on wound healing and the course of chronic inflammatory diseases.

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## S9.574. Analysis of homocysteine-thiolactone induced epilepsy in the hippocampus of rats of different ages

Gerasimova E.V.[2], Yakovlev A.[1], Enikeev D.[3], Bogatenkov G.[1], Sitdikova G.[1]*

[1]*Kazan Federal University;*

[2]*Sirius University of Science and Technology, Sochi, Russia;*

[3]*I.P. Pavlov Institute of Physiology, Russian Academy of Sciences;*

* sitdikovaguzel@gmail.com

Background. Epilepsy is a chronic neurological disease characterized by recurrent seizures resulting from excessive neuronal discharges and accompanied by a variety of motor, sensory, autonomic and psychiatric disorders.

One of the etiological factors in the development of epilepsy can be an accumulation of sulfur-containing amino acid - homocysteine. Small increases in plasma homocysteine levels correlate with age-related cognitive impairment, neurodegenerative and cerebrovascular diseases, and the development of epilepsy [1, 2]. One of the mechanisms of the neurotoxic effect of homocysteine is the activation of ionotropic and metabotropic glutamate receptors in neurons, which excessive stimulation causes hyperexcitability [3,4,5].

Study objective: to investigate the effect of the homocysteine metabolite homocysteine-thiolactone (Hcy-thiolactone) on electrical activity in the rat hippocampus.

Materials and Methods. Experiments were carried out on Wistar rats of two ages p6-8 and p35-90. A 16-channel electrode was placed in the hippocampus, and electrical activity was recorded in the CA1 area. Hcy-thiolactone solution or 2 μl of artificial cerebrospinal fluid (ACSF) was injected into the CA1 area using a microinjector at the rate of 200 nl/s. The doses of Hcy-thiolactone were 0.24*10-6 mg, 0.24*10-3 mg, 0.03 mg, 0.06 mg or 0.12 mg. The total spectral power of local field potentials (LFP) and the frequency of multiple action potentials (MUA) were estimated using MatLab software package.

Results. Low electrical activity was observed in the CA1 zone of the hippocampus in control group (p6-8 animals), including rare low-frequency waves in the theta range and MUA bursts lasting from 0.5 to 3 s. In p35-95 animals, high electrical activity corresponding to the age of the animals was observed. Administration of ACSF (2 µl) or Hcy-thiolactone at low doses (0.24*10-6 mg, 0.24*10-3 mg) into the hippocampus did not result in changes in electrical activity in both groups. Hcy-thiolactone in doses of 0.03-0.12 mg induced the development of epileptiform activity with increased spectral power of LFP in alpha and theta ranges. In 3 of 4 animals (p6-8 group) Hcy-thiolactone (0.03 mg) administration resulted in rapid (within the first 1-2 minutes) development of epileptiform activity: the spectral power of LFP increased up to 1331±662% ($p<0.05$, n=3) and the frequency of MUA increased up to 1155±598% ($p<0.05$, n=3). In the p35-90 group, only 2 of 4 animals showed an increase in MUA frequency up to 212±82% 10 minutes after application of Hcy-thiolactone, which reached 343±61% by 30-40 minutes ($p<0.05$, n=2). Administration of Hcy-thiolactone at a dose of 0.06 mg in the p6-8 group (n=4) caused epileptiform activity in all animals: power spectrum of LPT and frequency of MUA increased up to 463±156% and 259±45% ($p<0.05$, n=4), respectively. In the p35-90 group (n=4), Hcy-thiolactone (0.06 mg) induced epileptiform activity in 3 of 4 animals: LPT spectral power increased to 454±32% ($p<0.05$, n=3) and MUA frequency increased to 396±126% ($p<0.05$, n=2). Hcy-thiolactone at a dose of 0.12 mg caused epileptiform activity in all animals in both groups, and there was an increase in spectral power of LFP and frequency of MUA in the p6-8 group to 1205±596% and 956±203%, and in the p35-90 group to 856±143% and 296±98% ($p<0.05$), respectively.

Latency period of epileptiform activity in p6-8 animals was significantly shorter than in p35-90 group at doses of 0.06 and 0.12 mg, and was 3.23±0.29 min and 2.01±0.12 min, respectively. In p35-90 group, the latency period was 6.2±1.8 min after administration of Hcy-thiolactone (0.06 mg) and 4.92±1.61 min for Hcy-thiolactone (0.12 mg) ($p<0.05$). Thus, Hcy-thiolactone, when injected into hippocampus, dose-dependently enhanced the electrical activity and induced epileptiform activity starting from 0.03 mg in p6-8 and 0.06 mg in p35-90 rats. The obtained data indicate a higher sensitivity to Hcy-thiolactone of animals in the first postnatal week of development and experimentally confirm the high risk of epilepsy in children with high levels of homocysteine in the prenatal or early postnatal periods. This work was supported by the Russian Science Foundation (Project No. 20-15-00100)

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**S9.575. Analysis of the activity of hippocampal neural networks in vivo in a freely behaving mouse**

Gerasimov E.I.[1]*, Mitenev A.V.[1], Pchitskaya E.I.[1], Erofeev A.I.[1], Chukanov V.S.[1], Vlasova O.L.[1], Bezprozvanny И.Б.[1,2]
[1]*Лаборатория молекулярной нейродегенерации;*
[2]*Отделение физиологии ;*
* evgeniigerasimov1997@gmail.com

Visualization of the neuronal activity of a certain area of the brain of a freely behaving animal in vivo allows researchers to obtain an extensive array of information about the patterns of neuronal activity, changes in their excitability and the relationship between each other [1]. A method that allows recording the activity of individual groups of neurons in a mouse in free movement is a miniature fluorescence microscopy – a miniscope [2]. With a microscope weight of 3 grams, the miniscope has a resolution sufficient to visualize individual neurons, which makes it possible to detect changes in the work of neurons using the expression of genetically encoded calcium indicators in them – GCaMP6f.

In this study, the injection of the AAV-Syn-GCaMP6f virus was performed in the hippocampus (AP -2.1, ML +2.1, DV -1.8) of 5-month-old B6SJL mice, and after 3 weeks a GRIN-lens was fixed over the area of interest, which is the optical path for the miniscope. Next, a baseplate was implanted, on which a miniscope was subsequently fixed to visualize the calcium dynamics of hippocampal neurons. Accommodation to a miniature fluorescent Miniscope v3 was carried out under experimental conditions of the "open field" test for 3 days. To move from qualitative analysis of the data on neuronal activity obtained by the miniscope, a software package in Python was developed, which allows researchers to quantify the data by calculating statistical data on the neural network, in this study - the hippocampus. At the initial stage, the active state of the neuron is determined, the search for gaps when the intracellular calcium concentration increases, which leads to an increase in the intensity of the calcium indicator GCaMP6f. The active state of a neuron is determined based on the derivatives of input signals after primary processing by means of the Minian software product. A neuron is in an active state if the derivative of its signal exceeds the threshold value, which is calculated using this formula: $\boldsymbol{threshold = median(x) + mad(x)}$, where $x$ are the values of the derivative of the signal, *mad* is the average absolute deviation. Next, statistics describing the neural network are calculated: the activity level of the neural network is the number of active neurons for a certain period of time; calculation of pairwise correlations between neurons with calculation of the Pearson coefficient; burst activity of neurons - the number of "cell activations" over a set period of time. According to the obtained distributions of correlation values, connectivity is calculated – the proportion of “connected” neurons depending on the threshold level. A module for estimating the dependence of the correlation coefficient between neurons on the distance between them is also implemented.

At the next step, the shuffling module was created. This module is necessary to determine the regularity of the statistics obtained (they have a biological/ physiological nature) or they are random variables. When comparing the values of statistics obtained from the initial data, as well as shuffled, there is a decrease in the average value of each of the presented statistics for each of the days of registration. This is probably due to the fact that when shuffling data, information about the connections between neurons is lost, which determines the initial values of statistics. Based on these data, it can be assumed that the recorded change in intracellular calcium concentration, which correlates with changes in the excitability of neurons, and statistics calculated based on the analysis of calcium signals, are biologically significant, determining the functioning of the hippocampal neural network.

This approach to studying the work of neural networks of the brain, in particular the hippocampus, will allow to identify and determine

violations in their work in pathological conditions of the brain in neurological and neurodegenerative diseases, including Alzheimer's disease.
The work was supported by the strategic academic leadership program "Priority 2030" of the Russian Federation.
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### S9.576. Anxiety level and permeability of the blood-brain barrier in mice in the simulation of irritable bowel syndrome

Sorokina D.[1]*, Yakovleva O.[1], Yakovlev A.[1], Mitrukhina O.[1], Sitdikova G.[1]
[1]*Kazan Federal University;*
* dinagabita@mail.ru

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized not only by visceral pain, changes in bowel and intestinal microflora, and food digestion, but also by cognitive impairment, allodynia, depression, and increased anxiety (Arslanova et al, 2021). In most cases, the symptoms of IBS are not associated with organic causes and are largely dependent on stress and anxiety, suggesting that the connection between the brain and the gut is involved in this disease. One of the criteria for diagnosing IBS is the development of visceral hypersensitivity (Agafonova et al., 2018). But the exact pathogenesis of visceral hypersensitivity is not yet fully understood. In our work, using behavioral tests, we analyzed the anxiety-phobic state, development of visceral hypersensitivity in mice with a chronic post-inflammatory model of IBS, and also assessed the content of malonic dialdehyde in the brain and permeability of the blood-brain barrier. Methodology. Chronic model of post-inflammatory IBS is an experimental model of neonatal sensitization induced by rectal administration of a dilute acetic acid solution in mice. Presumably, it causes non-transmural inflammation characterized by increased infiltration of neutrophils into the intestinal tissue, as well as massive necrosis of the mucosal and submucosal layers. Animals at the age of P10 (P – day after birth) were randomly divided into two groups: control (n = 25) and experimental (n = 25) with the IBS model. Mice of the control group were injected with NaCl 0.9% (0.5 ml daily for P10-P15 and 1 ml daily for P16-P21). To create an experimental model of IBS, mice of P10 were injected with a solution of acetic acid during 10 days (0.5 ml daily for P10-P15 and 1 ml daily for P16-P21). The body weight of mice in all groups was measured simultaneously at the age of P11, P21, P26 and P40. Anxiety was assessed using the classic behavioral tests "Open Field", "Dark Light Box", "Integral Index of Anxiety (IIA)". Colonic visceral hypersensitivity was assessed by measuring the intensity threshold of the abdominal flexion reflex required to induce a behavioral response during colorectal distension with characteristic elevation of the animal's posterior part of the body and clearly visible abdominal contraction. The level of oxidative stress was determined by the content of malonic dialdehyde in the brain tissues using of spectrophotometric method according to Ohkawa et al., 1979. The permeability of the blood-brain barrier in mice was monitored using the Evans Blue dye according to the standard method (modified by Wick et al., 2018) in hippocampus and cerebellum. Statistical assessment of the differences between the compared samples was evaluated for a 5% significance level. The normality of the sampling distribution was determined using the Fisher F-test and the Shapiro-Wilk test using the OriginPro 8.5 program (OriginLab Corporation, Northampton, MA, USA). The significance of differences was calculated using the Mann-Whitney test for non-parametric samples. Results and discussion. During the formation of the IBS model, animal mortality was 8%. Survival rate in control group was 100%. The introduction of acetic acid to mice was also accompanied by a low increase in body weight of mice in this group compared to the control group. Anxiety analysis showed an increase in IIA in mice of both groups immediately after the end of IBS modeling (1.23±0.28 points). But 2 weeks after the simulation, in the control group, IIA decreased to 0.47±0.08 points, in the experimental group, IIA remained at a high level of 1.38±0.21 (n=25; $p<0.05$). Analysis of the anxiety-phobic state based on the time spent in the light chamber, the time of exit from the center of the open field, the number of acts of grooming and defecation showed a significant increase in anxiety in mice of the experimental group (n=25; $p<0.05$). It was found that in response to colon distension with volumes of 0.35 and 0.5 ml, the values of the abdominal flexion reflex in the experimental group (2.26±0.14 and 3.38±0.18) were significantly higher than in the control group (1.83±0.18 and 2.9±0.14), respectively, (n=15; $p<0.05$), indicating the appearance of visceral hypersensitivity in models of post-inflammatory IBS. We found an increase in the content of malonic dialdehyde to 0.23±0.01 mg/g in the brain tissues of mice of the experimental group in relation to the control 0.20±0.008 mg/g (n=20; $p<0.05$). Analysis of the permeability of the blood-brain barrier did not reveal the dye outside the cerebral vessels under control conditions. In the model of chronic post-inflammatory IBS, a 3-fold increase in the concentration of Evans Blue in the tissues of the cerebellum and hippocampus (n=7; $p<0.05$) was observed, which indicated a significant violation of the permeability of the blood-brain barrier. Conclusion. Thus, in this work, we have shown an increase in anxiety in mice in a chronic model of post-inflammatory IBS, which may be associated with the appearance of visceral hypersensitivity, increased oxidative stress, and blood-brain barrier permeability.
The work was supported by the Russian Science Foundation No. 22-25-20045.
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### S9.577. Application of the method of correlation ratios for comparison of EEG of healthy subjects and neuromental profile patients

Sobolev M.E.[1]*, Gorelik A.L.[2], Vlasova O.L.[1]
[1]*Peter the Great St. Petersburg Polytechnic University;*
[2]*V. M. Bekhterev national medical research center for psychiatry and neurology;*
* m.e.sobolev@mail.ru

Electroencephalography (EEG) is a long-known and widely used non-invasive method for studying the functional state of the brain in scientific research and clinical practice. EEG allows you to observe various bioelectrical processes in dynamics, as well as to see the response to various stimuli. One of the main characteristics of electroencephalograms is the amplitude and frequency of the measured bioelectric signal. At present, the development of quantitative analysis and processing of electroencephalograms requires the application of appropriate mathematical approaches and methods, since the possibilities of visual analysis of the EEG, according to many experts, are practically exhausted. One of those who suggested the diagnostic significance of the distortion of the correlation between the quantitative characteristics of the EEG in patients with various neuropsychiatric diseases were Naryshkin A.G. with colleagues [1]. To progress this idea, we have developed a physical and mathematical model that adequately describes the

amplitude-frequency characteristics of human electroencephalograms [2]. In addition, other authors proposed the method of correlation ratios [3]. This method is based on the non-linearity of the interaction between EEG electrodes, compared to coherence coefficients using a linear approach. The method of correlation ratio allows you to establish not only the fact of interaction between channels, but also the direction of the connection.
Results. Using the method of correlation ratio in our work, we compared the EEG in a state of calm wakefulness with eyes closed in healthy subjects with patients with depression and subjects with paranoid schizophrenia. Significant differences were obtained in the spatial organization of the EEG between groups of subjects in the frequency ranges: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz). The obtained differences in the correlation ratio between healthy patients and subjects with depression and schizophrenia were similar in the parietotemporal channels. However, there were also enough differences in other channels between healthy subjects and subjects with depression and schizophrenia. From this it follows that using the method of correlation ratios, it is possible to determine the differences in interchannel interaction that are characteristic of a particular neuropsychiatric disease. In addition, the direction of the connection between the channels was established.
Conclusion. Using the method of correlation ratio, a comparison was carried out in the interchannel interaction of human electroencephalograms. Significant differences were found between healthy subjects and neuromental patients.
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### S9.578. Application of the pseudo triglyceride approach in the synthesis of new lipophilic derivatives of 3`-azido-3`-deoxythymidine and 2`,3`-dideoxy-3`-thiacytidine

Darnotuk E.S.[1]*, Vojtik K.V.[1], Shastina N.S.[1]
[1]*MIREA – Russian Technological University;*
* Mslizirichi@yandex.ru

Currently, highly active antiretroviral therapy (HAART) is used for the treatment of HIV infection, which is based on the use of classical drugs based on nucleoside reverse transcriptase inhibitors (NRTI), which are highly effective in suppressing HIV replication and preventing further progression of the disease. It is known that the main disadvantages of most of the drugs used are high toxicity and low bioavailability [1-2]. All this negatively affects during the prolonged use of drugs, leading to serious side effects and the emergence of resistant viral strains. Thus, the decrease in the effectiveness of anti HIV therapy makes it necessary and important to modify reverse transcriptase inhibitors, which will eliminate most of the existing shortcomings of these drugs.
Therefore, it is extremely relevant to use a multi-purpose prodrug approach that allows the development of drugs with improved characteristics, which will minimize the above disadvantages. The prodrug approach, which includes the use of substances of a lipid nature, in particular 1,3-diacylglycerols, contributes to the fact that the nucleoside drug will be included in lipid metabolism and thereby directionally reach the lymphatic system (HIV reservoir), absorbed together with fats in the small intestine, thus bypassing primary degradation in the liver [3].
Therefore, the purpose of this work is to obtain and study the properties of conjugates of 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxy-3'-thiacytidine (3TC) and derivatives of 1,3-dipalmitoylglycerol modified by phosphorus center with L-alanine esters.
Glycerolipid derivatives of 3'-azido-3'-deoxythymidine and 2',3'-dideoxy-3'-thiacytidine were obtained by known methods [4], in which the binding of molecular fragments was carried out using functional phosphorus bonds. It is known that phosphoramidate derivatives of nucleoside preparations are of greater interest, since many of them exhibit high antiviral activity and increased resistance to chemical and enzymatic hydrolysis. In order to increase the stability of the resulting AZT and 3TC conjugates to the action of hydrolysis in various biological media, as well as to modulate the inhibitory activity of such compounds, their phosphoramidate derivatives with amino acid esters were developed [5].
We have conducted studies the kinetics hydrolysis of synthesized compounds in various buffer solutions that simulate the pH of physiological media. We have established that the hydrolysis half-time for these molecules exceeded 20 h. When investigating the enzymatic hydrolysis of these prodrugs by the porcine pancreatic lipase in vitro, the hydrolysis half-time was 30 s. This can mimic the metabolic pathways of natural glycerolipid compounds.
The study of the cytotoxicity of the compounds on the MT-4 cell line showed that most of the conjugates have of low toxicity (CC50 >100 μM). The results of determining the antiviral activity of a compounds in HIV-1 infected MT-4 cells showed that among of AZT conjugates, the derivative containing the L-alanine ethyl ester residue (EC50 0.012 μM) turned out to be the most active. And among the 3TC derivatives, the compound containing L-alanine tert-butyl ester (EC50 25.46 μM) showed the highest inhibitory activity.
The study of the biological activity of the obtained glycerolipid derivatives of AZT and 3TC showed that some of the compounds have advantages (low toxicity, high level of anti-HIV activity) compared to the original nucleosides, which makes it possible to use these prodrugs to expand the arsenal of drugs included in antiretroviral therapy regimens.
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### S9.579. Applying of transcranial pulsed current stimulation in the combined treatment of osteoarthritis

Onishchuk V.V.[1], Kade A.Kh.[1], Trofimenko A.I.[1,2,3]*
[1]*Kuban State Medical University, Krasnodar, Russia;*
[2]*Scientific Research Institute – Ochapovsky Regional Clinical Hospital no. 1, Krasnodar, Russia;*
[3]*Kuban State Technological University, Krasnodar, Russia;*
* artemtrofimenko@mail.ru

Osteoarthritis (OA) is a chronic disease predominantly affecting large joints, its structural characteristics are cartilage degradation, subchondral bone remodeling, osteophyte formation, changes in the synovial

membrane and joint capsule, which leads to a number of typical clinical symptoms, such as pain in joints, stiffness and a significant decrease in their mobility, the course of the disease is accompanied by a decrease in the productivity and quality of life.
Modern conservative therapy for OA includes medication - non-steroidal anti-inflammatory drugs, chondroprotectors and intra-articular injections of hyaluronic acid, glucocorticoids and synthetic viscoprostheses, as well as non-drug interventions - lifestyle modification, weight loss, exercise, orthoses and physiotherapy.
Pharmacotherapy often provides only limited pain relief and is associated with the risk of serious side effects, and therefore the development of conservative non-pharmacological approaches to the treatment is relevant.
It has been shown that pain in OA is associated with the reorganization of the somatosensory and motor cortex, as well as the restructuring of its neuroplasticity. The above disorders can be modified by the central effects of transcranial electrostimulation.
Due to the impact on the structures of the antinociceptive system of the brainstem, TES-therapy is of particular interest among the methods of transcranial pulsed current stimulation.
TES-therapy is a method of non-invasive electrical stimulation with a bipolar pulsed current with a frequency of 77.5 Hz and a current flow density through the structures of the antinociceptive system of the brainstem of 0.01-0.05 mA/cm2.
Objective: to study the effectiveness of TES-therapy in the framework of conservative treatment of patients with OA of large joints.
Materials and methods:
Inclusion criteria: men and women aged 35 to 60; verified diagnosis of OA large joints (knee and hip) II and III stage (according to ICD-10); signing of voluntary informed consent to participate in the study.
Criteria for exclusion and non-inclusion: decompensated somatic pathology; endocrine pathology; oncopathology; autoimmune diseases; drug addiction and/or alcoholism; viral hepatitis, HIV, tuberculosis; other diseases and conditions - epilepsy, taking immunosuppressive therapy, immobilization, intra-articular administration of glucocorticoids, peptic ulcer of the stomach and duodenum; the presence of contraindications for TES-therapy; development of acute infectious diseases during the study period; refusal to participate in the study.
Characteristics of patient groups: group 1 (n = 30, control) - "conditionally healthy" patients; group 2 (n = 30, comparisons) – patients with OA of large joints who received only standard treatment; group 3 (n = 30, main group) – the same, TES-therapy together with standard treatment.
At the control points of the study (before the start of treatment; after 2 weeks and 6 weeks), the intensity of the pain syndrome was assessed using the visual analogue scale of pain (VAS), as well as the determination of β-endorphin, MCP-1 in the blood serum.
TES-therapy was carried out using a TRANSAIR-03 electrical stimulator (Centre for Transcranial Electrical Stimulation, Russia). The following electrical stimulation parameters were used: pulsed bipolar mode, fronto-mastoid electrode placement, current strength 2 mA, current frequency 77.5 Hz, session duration 45 min. In total, patients from the main group, starting from the first visit to the doctor, underwent 10 procedures, with a frequency of 1 session per day; then 8 sessions, 2 procedures per week.
Results: Before the start, in groups No. 2 and No. 3 the concentration of β-endorphin was 63.4 and 65.3% lower ($p<0.05$), while the content of MCP-1 was 93.0 and 85, 2% higher ($p<0.05$) than in "conditionally healthy". There were no statistically significant differences ($p>0.05$) between groups 2 and 3 in terms of VAS scores.
Two weeks after the start of treatment, the concentration of β-endorphin in patients of group No. 2 was 51.5% and 73.5% lower ($p<0.05$) than in "conditionally healthy" and in group No. 3. The concentration of MCP-1 in groups No. 2 and No. 3 remained 81.3 and 65.7% higher ($p<0.05$) than in "conditionally healthy". In group No. 2, the intensity of the pain syndrome was 18.2% higher ($p<0.05$) than in group No. 3.
Six weeks after the start of treatment, in group No. 2, the concentration of β-endorphin remained 46.7% and 43.3% lower ($p<0.05$) than in "conditionally healthy" and in group No. 3. Also, in group No. 3, the level of MCP-1 is statistically significantly ($p<0.05$) lower by 49.0% than in group No. 2 and higher by 43.6% relative to group No. 1. In group No. 2, the intensity of pain syndrome was 50.0% higher ($p<0.05$) than in group No. 3.
When intragroup analysis of the concentration of β-endorphin in dynamics according to the control points of the study: in group No. 2, a trend towards an increase in the indicator by 19.7% ($p > 0.05$) was revealed, and in group No. 3, a statistically significant increase by 89.9% ($p < 0.05$). Analysis of the dynamics of MCP-1 concentration showed: in group No. 2, a decrease in the indicator by 6.1% ($p > 0.05$) and in group No. 3, a statistically significant decrease by 37.3% ($p < 0.05$). When analyzing the VAS score in group No. 2, its decrease by 26.5% ($p < 0.05$) was revealed; in group No. 3, a decrease in the intensity of pain syndrome by 56.5% was also observed ($p < 0.05$).
Conclusion: It has been shown that patients with OA of large joints are characterized by severe hypoendorphinemia and a persistent increase in the serum MCP-1. Against the background of TES-therapy, a statistically significant increase in the concentration of β-endorphin and a decrease in MCP-1 were observed, which was accompanied by a pronounced regression in the intensity of the pain syndrome.

### S9.580. Aspects of assessing the metrological characteristics of biophysical methods in clinical practice

Semenova E.[1]*
[1]*Academician I.P. Pavlov First St. Petersburg State Medical University;*
* katemelnikova@mail.ru

Of the medical sciences, laboratory medicine is the most receptive to the introduction of achievements in fundamental sciences. Laboratory methods are responsible for forming ideas about sanogenetic systems, and the individual clinical picture of the disease consists of the combination of pathogenesis and sanogenesis. The assessment of the metrological properties of biophysical methods in the formation of diagnostic informativity of laboratory technologies is based on the application of various methods of medical informatics. Algorithms of information support for clinical decisions allow available data to highlight the minimum set of features that provide the best results. The results of highly sensitive, but non-specific biophysical technologies gain significant diagnostic efficiency when using modern medical informatics methods and become a tool in evidence-based medicine (V.S. Duke et al., 2018).
However, biophysical technologies are practically not represented in the "Register normative and reference information of the healthcare system»" document, in particularly the “Federal Handbook of Laboratory Research. Handbook of laboratory tests” (current version 3.36 dated 07/29/2022). The advantage of biophysical methods is that they do not affect the native properties of biomaterials and intermolecular compounds. Unlike, for example, biochemical studies, which usually register the products of chemical reactions. The purpose of biochemical testing is to measure the concentration of a particular analyte in a biological sample, which is thereby destroyed and acts as an enviroment for chemical reactions. The result obtained in vitro is approximated by the content of this component in biological medium in vivo (Y.A. Mitin et al., 2021; Landa, S et al. 2022).
The possibilities of the integral biophysical method of "dynamic light scattering" - a method of laser correlation spectroscopy, which allows to estimate the subfractional composition of biological fluids by particle size in a wide range of values from units to thousands of nanometers, have been studied. A method unspecific in terms of biological fluid composition, but unique in terms of the ability to study polydisperse biological media without disturbing their native properties.

The method of examining the spectral properties of biological substances using infrared radiation is increasing in application. This method is based on recording vibrational spectra of molecules, which provide detailed information about the state of molecules or functional groups in molecules or functional groups in a test specimen.
An important advantage of infrared spectroscopy is the ability to monitor the intermolecular interactions of proteins. In addition, this method is highly sensitive, easy to use and measure, and it is also possible to use small amounts of sample. Fourier infrared spectroscopy does not require optical transparency of the sample, which allows the analysis of the protein spectrum to be carried out in suspension, in an aggregated state and also as a part of large membrane fragments.
It is relevant to study the peculiarities of the protein composition of crystallization centers. A special pathochemical process is a disturbance of colloidal stability in biological media, primarily urine, leading to pathological crystallization of mineral components and the development of urolithiasis. Since the transition from ash to gel state is a biophysical phase phenomenon , but dependent on the composition of the biological medium, the study of causal relationships and biochemical composition and biophysical properties remains a relevant problem both in basic research and as an important part of applied research to improve the diagnosis of various diseases.

### S9.581. Assessment of connectivity in the cortico-hippocampal brain network in epilepsy model rats

Grishchenko A.A.[1,2]*, Suleymanova E.M.[3], Vinogradova L.V.[3], Sysoev I.V.[1,2]
[1]*Kotel'nikov institute of radio engineering and electronics of RAS;*
[2]*Saratov State University;*
[3]*Institute of Higher Nervous Activity and Neurophysiology of RAS;*
* vili_von@mail.ru

Temporal lobe epilepsy is the most severe form of epilepsy. During secondary generalization, this local epileptic activity can spread along synaptic connections widely beyond limbic structures[1]. In experimental animals, spontaneous limbic seizures develop after epileptic status induced by pharmacological [2] or electrical stimulation [3]. It was previously found that limbic seizures can also develop in non-epileptic rats in response to administration of an endocannabinoid CB1 receptor antagonist [4]. In between seizures, the animal models develop interictal adhesions visible on an electroencephalogram [5]. We have previously studied connectivity in the cerebral cortex of rat models during spike-wave discharges [6, 7], while interictal dynamics have not been studied. The aim of this work is to compare interictal activity with discharges, as well as to search for connectivity during such activity. In this work we analyzed two-hour EEG recordings from 11 mice, the EEG was recorded from the left and right cerebral hemispheres. Then we used the Granger causality method to find connectivity [8]. The idea of the Granger causality method is to build predictive models. When checking the possible impact of system Y on system X, first an empirical predictive model is built for system X using data from its own time series, and then using the time series of system Y . If the data from the time series of the system Y can significantly reduce the prediction error of the future of the system X, the conclusion about the influence of Y on X is made. Based on the connectivity analysis, we can conclude that there is connectivity in the brain during interictal activity, with the left hippocamp being most actively involved.
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### S9.582. Assessment of elastic modulus in lymph nodes using compression optical coherence elastography

Gubarkova E.V.[1], Vorontsov D.A.[2], Hramushina I.A.[3], Bederina E.L.[1], Sovetsky A.A.[4], Plekhanov A.A.[1]*, Zaitsev V.Y.[4], Sirotkina M.A.[1], Gamayunov S.V.[2], Vorontsov A.Y.[2], Krivorotko P.V.[5], Gladkova N.D.[1]
[1]*Privolzhsky Research Medical University;*
[2]*Nizhny Novgorod Regional Oncologic Hospital;*
[3]*Lobachevsky State University of Nizhny Novgorod;*
[4]*Institute of Applied Physics of the RAS;*
[5]*N.N. Petrov National Medicine Research Center of oncology;*
* strike_gor@mail.ru

Assessment of the lymph nodes (LNs) condition is an important factor for determining the stage of the disease and choosing the optimal treatment tactics for breast cancer. Traditional dissection of the LNs often reveals that only a small proportion of the LNs are metastatic, and this fact must be weighed against the potential complications of dissection, such as lymphedema. The gold standard for assessing the state of the LN remains postoperative histopathological analysis, which can be implemented only if the LN is removed, and is not achievable with intraoperative diagnosis without lymphadenectomy. The radioisotope method and a number of new technologies, such as fluorescent lymphography, ultrasound with contrast-enhancement, etc., are used as methods for intraoperative marking of signal LNs [1]. Limitations of these methods are the administration of exogenous agents and the potential for false negative results. There remains a need to develop and apply new intraoperative, high-resolution and highly sensitive methods capable of determining the necessity of LN removal, which will reliably evaluate the extent of the tumor process in regional lymphatic collectors without lymphadenectomy. In this context, a promising method is Optical Coherence Tomography (OCT) with its new elastographic modality - OCT elastography (OCE), which demonstrates a high contrast of morphological structures of the LN and is able to quantify their elastic properties, which change significantly with metastatic lesions of the LN. Recently, our studies showed the potential of using OCE with an assessment of the elastic modulus for the differential diagnostics of breast cancer subtypes and asssesment of breast cancer surgical margins [2].The aim of this study is to determine the stiffness values

of various morphological structural components of intact LNs and LNs with metastatic lesions in breast cancer using the compression OCE method.

The OCE study was performed on 19 postoperative samples of LN from 17 patients (aged from 42 to 72 years) with breast cancer after axillary lymphadenectomy. All samples were divided into three groups according to the results of pathomorphological examination: intact (n=5), LNs with reactive inflammatory changes (n=8) and metastatic (n=6) LNs. The study used a high-speed spectral multimodal OCT facility (IAP RAS, Russia) with a central wavelength of 1310 nm, radiation power of 20 mW, lateral resolution of ~ 20 μm, axial resolution of ~ 15 μm, scanning depth of ~ 1.7 mm, and 20,000 A-scans/sec scanning rate. The system is capable to perform phase-sensitive compression OCE to visualize tissue elastic properties. This method for mapping tissue deformation is based on the vector approach for estimating the interframe variation of the phase gradient of the OCT signal [3]. The use of a calibration silicone layer with a known stiffness on the surface of the tissue under study makes it possible to calculate the absolute values of the tissue stiffness - Young's modulus of elasticity (measured in kPa). OCE images are obtained in real time with a spatial resolution of 40-50 μm enabling 2D lateral scanning within a range of 4,0 × 1,25 mm. For the construction and analysis of OCE images, a standardized level of pressure on the tissue is selected, which allows not only qualitative comparison, but also quantitative assessment of stiffness in various morphological structures under various conditions.

As a result of this study, characteristic ranges of stiffness values were established for various morphological structural components of unchanged LNs and LNs with metastatic lesions. In the case of intact LNs on the OCE image, the adipose tissue is characterized by the lowest stiffness values (50-150 kPa); the fibrous capsule and trabeculae between the follicles are identified by high stiffness values (400-800 kPa); in the cortex the follicles are presented as oval-shaped areas with medium stiffness values (150-400 kPa). In addition, OCE makes it possible to differentiate LN with reactive changes, which are characterized by the growth of lymphatic tissue and disruption of the normal structure of the LNs. On OCE images, reactive LNs are visualized as homogeneous areas with average stiffness values (200-400 kPa). At the same time, a clear boundary between the capsule, trabeculae, and cortex is lost on OCE images. In case of metastatic lesion of the LNs, confirmed by pathomorphological examination, the normal structure of the cortex of the LNs is disturbed on the OCE images, the identification of the capsule and follicles is impossible, there is a predominance of areas with the highest stiffness values (more than 800 kPa), morphologically corresponding to accumulations of tumor cells.

Compression OCE based on the assessment of stiffness values, allows not only to determine the status of the LN, but also to statistically significantly differentiate the main microstructural components of the LN, such as the connective tissue capsule, cortex with follicles, trabeculae, adipose tissue, and areas of accumulation of tumor cells. Compression OCE is a new highly effective method for intraoperative evaluation of the LNs condition in situ. Further research is needed to develop an algorithm for introducing this technique into clinical practice.

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## S9.583. Axotomy-induced changes in the beta-amyloid precursor protein, ADAM10, BACE1, presenilin 1 and nicastrin expression in the rats spinal ganglia

Dzreyan V.A.[1]*, Kalyuzhnaya Yu.N.[1], Batalshchikova S.A.[1]
[1]*Southern Federal University;*
* dzreyan2016@mail.ru

Brain and spinal cord injuries are one of the leading causes of death and disability. The APP protein has been intensively studied since the 1980s due to its central role in the development of Alzheimer's disease (AD). It is involved in the development, differentiation, and function of neurons, neurite growth, synapse and long-term memory formation, brain integrity maintenance, and neuronal response to damage. However, the specific biochemical and physiological functions of APP and its proteolytic products are still unknown. The accumulation of APP in damaged neurons after peripheral nerve transection indicates its important role in after axotomy -induced pathological processes in the nerve tissue.

Work objective. In this study, we investigated the expression and localization of APP in rat dorsal root ganglia (DRG) of the rat spinal cord after sciatic nerve transaction (SNT). We studied the expression and localization of α-secretase ADAM10, β-secretase BACE1, and components of the γ-secretase complex presenilin 1 and nicastrin involved in APP proteolysis in rat DRG in the early stages after axotomy.

Materials and Methods. The model of peripheral nerve injury was rat dorsal root ganglion (DRG) after SNT. Symmetrical ganglia from the contralateral side of the same animal were used as a control. Immunoblotting was used to estimate the expression and intracellular distribution of N- and C-terminal fragments of APP and secretases involved in APP proteolysis in rat DRG cells after axonal injury. 4, 24 hours or 7 days after the SNT, decapitation and isolation of the 4th and 5th DRGs were performed, followed by sample preparation and immunoblotting. A more detailed picture of the intracellular distribution of the APP is provided by the electron immunohistochemistry data.

Results. According to the immunoblotting data, SNT stimulates the accumulation of the C-terminal APP fragment in the axotomized ganglia. After 4 hours the level of C-APP in the cytoplasmic fraction is low. However, after 24 hours and 7 days, it significantly increased in the cytoplasmic fraction relative to control ganglia. The level of N-APP was also low in the cytoplasmic fraction at 4 hours but not at 24 hours and 7 days after SNT. At 24 hours and 7 days after axonal injury of DRG neurons by sciatic nerve transaction, the level of N-APP in the tissue significantly increased from the control ganglia. α-secretase, ADAM10 is mainly present in the cytoplasmic fraction of the DRG cells. A sufficiently high level of protein is noticed in as well as contralateral ganglia and axotomized DRG at 4 and 24 hours after SNT. However, the level of ADAM10 decreased 2-fold in the cytoplasmic fraction relative to nonaxotomized ganglia 7 days after SNT. Thus, axotomy caused a significant decrease in ADAM10 in the cytoplasmic fraction 7 day after SNT compared to the protein level at 4 and 24 hours after axotomy. Electron microscopy shows that the C- and N-terminal fragments of APP were associated with the plasma membranes of the processes of nerve cells. Our attention was drawn to the pronounced clustering of APP and ADAM10 but not BACE1. This is unexpected since BACE1 and presenilin1 (PS1), the catalytic unit of γ-secretase, are localized mainly in detergent resistant membranes (DRM) or lipid rafts, while ADAM10 is localized mainly in non-lipid raft domains. However, no significant difference was found at different times after SNT for the expression of BACE1 (the expression of BACE1 in DRG relatively low) and components of the γ-secretase complex presenilin 1 and nicastrin.

Here, we showed that C- and N-terminal fragments of APP are found only in cytoplasmic fraction but not in nuclear fraction DRG. SNT caused the growth of both C- and N-terminal regions of APP already

after 24 h. A high level of C- and N-APP (that could be predominantly a product of α-secretase (sAPP activity) persisted at cytoplasmic fraction of axotomized neuron the 7th day after SNT. Axotomy caused a significant decrease in ADAM10 in the cytoplasmic fraction 7 day after SNT affecting the balance between amyloidogenic and non-amyloidogenic pathways.
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## S9.584. BIOAVAILABILITY OF NEW NANOEMULSIONS MODIFIED WITH CURCUMIN AND CERIUM DIOXIDE NANOPARTICLES

Shirokikh A.D.[1], Zamyatina E.A.[2], Anikina V.A.[2], Mishchenko E.V.[1], Koroleva M.Y.[1], Popova N.R.[2]*
[1]*Mendeleev University of Chemical Technology ;*
[2]*Institute of the theoretical and experimantal biophysics of RAS;*
* nellipopovaran@gmail.com

Increasing the bioavailability of a number of active compounds of organic and inorganic nature is an urgent task of modern biomedicine. It is known that some compounds promising for biomedical applications have poor solubility in water, which potentially limits their use. One of these compounds is curcumin, which has anti-inflammatory, antioxidant, antimicrobial, wound-healing, anti-cancer activities. In addition, it has been shown that under certain conditions curcumin can exhibit cytotoxicity in relation to normal cells, inducing oxidative stress. However, it was found that when the curcumin complex was obtained with nanocrystalline cerium dioxide, the latter leveled the toxicity of curcumin in relation to normal cells, due to the inactivation of reactive oxygen species, but retained its therapeutic effect on cancer cells. Nanocrystalline cerium dioxide has unique physicochemical properties and is an inorganic antioxidant that can perform the function of some oxidoreductases: catalase, superoxide dismutase, oxidase and phosphatase. It is known that nanocerium is able to enhance the antibacterial, antioxidant and wound healing properties of nanoemulgel with curcumin during wound healing. Thus, one of the important tasks is to develop effective forms of curcumin and its complexes in order to increase their bioavailability and therapeutic effect for medical use. To achieve this task, nanoemulsions (NE) can be used, used as carrier systems for encapsulating active components, increasing their bioavailability without losing their activity. In this work, new nanoemulsions based on hydrocarbon oil modified with curcumin and cerium dioxide nanoparticles were obtained, its properties and toxic effects in in vitro and in vivo systems were investigated.
In the synthesis of NE, a mixture of nonionic surfactants - Tween 60 and Span 60 - was used to stabilize them. Droplet sizes of the dispersed phase of nanoemulsions and CeO2 particles were analyzed by dynamic light scattering (Zeta SizerNano, Malvern). The average diameter of the nanoemulsion droplets was 60±10 nm. When incorporating 4 wt.% curcumin the size of the primary droplets remained unchanged, however, the presence of aggregates 210 ±30 nm was observed. CeO2 particles (20 ± 5 nm) included in the composition of nanoemulsions both without and with curcumin led to the formation of larger aggregates - 1500 ± 200 nm, while the average diameter of the primary droplets also remained unchanged. However, a wider size distribution was observed in nanoemulsions with curcumin. Further, a comprehensive study of the toxicity of 4 types of nanoemulsions obtained (1 - NE; 2 - NE+CNP; 3 - NE+curcumin; 4 - NE+curcumin+CNP) in vitro on mouse embryonic fibroblasts (MEF) by determining the dehydrogenase activity by MTT and the ratio of the number of living and dead cells (staining with fluorescent dyes Hoechst 33342 and propidium iodide). It was found that the metabolic activity of mouse embryonic fibroblasts (MEF) during incubation with all the studied NE at a concentration of 1% from 24 to 72 hours significantly decreased by 40-90% relative to the control group. The metabolic activity of MEF during incubation with NE at a concentration of 0.0001% from 24 to 72 hours does not significantly differ from the control. In the NE+CNP group, by 72h, the metabolic activity of MEF increases by 30% relative to the control, whereas in the NE+curcumin group, by 72h, there is a decrease in metabolic activity by 15% relative to the control. This effect may be due to the fact that curcumin could partially segregate on the surface of oil droplets in NE.
Acute toxicity of all obtained nanoemulsions was investigated in vivo. The experiments were carried out using 8-9 weeks of white mongrel male SHK mice (30-35 g). The animals were kept in accordance with Directive 2010/63/EC of the European Parliament and of the Council of the European Union on the protection of animals. It was found that after a single intraperitoneal injection of NE at a dose of 860 mg / kg in all groups of experimental animals, animal death was not observed during 14 days of the experiment. The dispersion medium was a saline solution (NaCl solution with a concentration of 0.9 wt.%. In addition, the toxic effect of NE was assessed by the general condition of the animals and their survival. The counting of the surviving and dead animals was carried out within 14 days after the introduction of NE, followed by observation of the surviving animals for two weeks after intraperitoneal administration. The animals were under continuous observation for the first 6 hours. During the entire observation period, the animals felt normal. During 14 days of observation of animals, no noticeable deviations in motor activity were detected; the presence of seizures; coordination of movements, skin condition, hair and color of visible mucous membranes; water and food intake; body weight. Thus, the nanoemulsions obtained in this study do not show toxicity to mouse embryonic fibroblasts in vitro and after a single intraperitoneal administration to mice in vivo. The data obtained demonstrate the possibility of using nanoemulsions containing curcumin and cerium dioxide nanoparticles in advanced biomedical applications.
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## S9.585. Biochemical in vitro diagnostics and targeted drug delivery in vivo based on magnetic nanoparticles

Nikitin P.I.[1]*, Bragina V.A.[1], Orlov A.V.[1], Skirda A.M.[1], Gorshkov B.G.[1], Nikitin M.P.[2]
[1]*Prokhorov General Physics of the Russian Academy of Sciences;*
[2]*Moscow Institute of Physics and Technology (State University);*
* gpiru@mail.ru

Magnetic nanoparticles (MPs) based on iron oxides possess a wide range of unique properties and are promising for many biophysical and medical applications. 5 g of iron is present in the human body in various forms and are vital for organism's functioning. Iron nanooxides feature low toxicity and are approved in many countries for intravenous injecting to humans.
As a metrological tool of developments in biophysics, the authors introduced a principle of highly sensitive quantification of MPs in opaque 3D objects using nonlinear remagnetization. Based on the principle and synthesized MPs, new methods have been developed for:
1) non-invasive "liquid biopsy", express biochemical diagnostics and food safety control;
2) effective delivery of drugs to cancer tumors and metastases in the animals;
3) a comprehensive study of the long-term biodegradation of MPs and their toxicity in vivo, as well as the identification of degradation products in a living organism.
Based on the use of MPs as labels for immune and DNA reactions, a variety of express methods were developed for measuring the concentrations of small molecules, protein markers of diseases, and extracellular vesicles (EVs) in human biological fluids. The EVs, being

carriers of protein and DNA fragments of cancer cells, are promising for non-invasive "liquid biopsy" [1]. The limit of EV detection in clinical samples of patients with breast cancer was 3.7·10e5 pcs/μl. That is 1-2 orders of magnitude better than that of the most sensitive commercial tests. The limit of detection of prostate-specific antigen (PSA) in blood serum of 19 pg/ml was obtained at a dynamic range > 3.5 orders. That is promising for diagnosis of both prostate cancer and its relapses after radical prostatectomy using trace PSA amounts [2]. The limit for cardiac troponin I detection was 0.08 ng/ml at a dynamic range > 3 orders, which is important for early diagnosis of myocardial infarction [3]. Another method enables rapid (20 min) measuring of small molecules concentrations, in particular ochratoxin A in foods from 11 pg/ml within a dynamic range of 5 orders. That is on the level of reference methods, which are labor- and time-consuming [4].

With regard to therapeutic tasks, we note that most drug nanocarriers become ineffective in vivo due to rapid elimination from the bloodstream by the mononuclear phagocytes (MPS). We have developed a breakthrough technology in nanomedicine, which enables significant (32-fold) increase of circulation time in the blood of almost any nanodrugs. That increases their therapeutic efficacy [5]. It was demonstrated that this technology called "MPS-cytoblockade", led to a significant increase in the delivery of chemotherapy drugs to five types of tumors of various nature: from melanoma to breast cancer, including two types of human tumors inoculated into mice. In particular, it was shown that it increased the efficiency of "active" magnetic delivery of chemotherapy drugs to cancerous tumors in animals by a factor of 23. This produced a significant suppression of tumors while minimizing side effects. Besides, it was shown that targeting polymeric and magnetic nanoparticles with lectins to the glycosylation profile of cancer cells, followed by a photodynamic therapy, represents a promising strategy for the treatment of aggressive tumors [6].

An important aspect for creating drug delivery agents is their rapid biodegradation after performing therapeutic tasks. In the experiments with animals in vivo, a comprehensive study of the complete life cycle of MPs was carried out for the first time, starting from their introduction into the bloodstream of animals and up to biodegradation in a living organism [7, 8]. Dependences were established for long-term (1 year) biodegradation of 17 types of nanoparticles upon injected dose, hydrodynamic size, ζ-potential, type of surface coating and structure of nanoparticles. In particular, it has been shown that coating the particles by a 39-nm layer of polystyrene prolongates the MP degradation from 40 days to 1 year. The dynamics of decay of the contrasting properties for MRI, as well as for detection by non-linear remagnetization have been established. It has been found that MP biodegradation enhances expression of genes of iron-associated proteins, increases the number of erythrocytes and the level of hemoglobin in the blood, as well as the absence of significant toxicity of the MPs. The results are important for employment of different types of nanoparticles in clinical practice.

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## S9.586. Biomimetic carriers of bioactive substances sensitive to external physical influences

Potapenkov K.V.[1,2]*, Grigoryan I.V.[1,4], Spiridonov V.V.[3], Yaroslavov A.A.[3], Taranov I.V.[4], Cherepenin V.A.[4], Gulyaev Yu.V.[4], Khomutov G.B.[1,4]
[1]*M. V. Lomonosov Moscow State University, Faculty of Physics;*
[2]*MIREA — Russian Technological University;*
[3]*M. V. Lomonosov Moscow State University, Faculty of Chemistry;*
[4]*Institute of Radio-engineering and Electronics;*
* potapenkov.kirill@physics.msu.ru

An important and actual area of research in biophysics and a number of related fields of science, is solving a problem of a radical increase in the effectiveness of drug therapy for oncological and a number of other diseases is creation of biocompatible means of encapsulation, targeted delivery and controlled release of biologically active substances (including drugs) directly to target areas of the body. Despite the ever-increasing number of scientific groups around the world actively working in this direction, a search of effective approaches of solving this problem is far from over.

We have developed new biocompatible colloidal carriers for the controlled delivery of biologically active substances in aqueous mediums including biological fluids. They were based on biomimetic vesicles - phosphatidylcholine liposomes functionalized with inorganic nanoparticles. For functionalization of liposomes, magnetite nanoparticles or gold nanoparticles were used, which ensured the susceptibility of drug carriers to external controlling physical influences. Functional inorganic nanoparticles were localized directly in the hydrophobic region of the bilayer lipid membrane. The ability to use this key feature of the described carrier vesicles was provided by preliminary hydrophobization of nanoparticles.

The resulting nanocomposite carrier vesicles were loaded with model substances such as an anticancer antibiotic doxorubicin and a fluorescent dye carboxyfluorescein. A possibility to release these encapsulated model substances from vesicles can be quantified by using their fluorescence, which has a characteristic effect of concentration quenching. We studied an effect of external pulsed electric fields on nanocomposite vesicles with hydrophobized gold nanoparticles in the membrane loaded with doxorubicin. For this purpose, solution samples containing vesicles were subjected to electric field pulses with a strength of about 2.25*106 V/m and a duration of about 10 ns. Before and after exposure, the fluorescence intensity of doxorubicin was measured, and based on a relative change in fluorescence intensity, a degree of destruction of liposomes and changes in their penetration into encapsulated molecules were estimated. The experimental data obtained in this way indicates a release of encapsulated doxorubicin from nanocomposite vesicles as a result of pulsed electrical action. Transmission electron microscopy was used as an additional method confirming the destruction of vesicles in solution.

The effect of destruction of nanocomposite vesicles under influence of external electric field pulses is explained by a significant increase in an electric field strength near nanoparticles conducting gold as a result of their polarization in an external electric field. This can lead to local breakdown of liposomal membranes near the particles and destruction of the liposome with a release of encapsulated substances.

Magnetite has semiconducting properties, which ensures that vesicles functionalized with magnetite nanoparticles are susceptible to external electric fields, as is the case with gold nanoparticles. However, we are mostly interested in an effect of external fields'influence on vesicles with magnetite nanoparticles localized directly in the membrane.

Liposome membranes with magnetite nanoparticles localized in the hydrophobic region can be considered as a magnetoelastic.
We studied an effect of external magnetic fields on liposomes containing hydrophobized magnetite nanoparticles in their membranes; carboxyfluorescein dye was used as a model substance loaded inside. The solution containing nanocomposite vesicles was kept in a constant magnetic field of 1.9 kOe for an hour. Before and after exposure a fluorescence intensity of the sample was measured.
A change in the fluorescence intensity of carboxyfluorescein indicates the release of a dye from the carrier vesicles into the solution under the influence of an external magnetic field. As in the case of electric field experiments, the vesicles exposed to the magnetic field were further characterized by TEM. Analysis of micrographs obtained by this method indicates a change in the shape of liposomes from quasi-spherical to ellipsoidal.
Theoretical calculations based on the analogy with an electrostatic model, as well as the numerical solution of the Laplace equation for a spherical ferrofluid layer in an external magnetic field, indicate that the shape of an ellipsoid extended along the direction of the external magnetic field strength is the most energetically favorable. Nanocomposite magnetic liposomes change their shape from spherical to ellipsoidal under the influence of an external magnetic field, the membranes of such vesicles are deformed, which leads to an increase in their permeability to dye molecules.
The effects we have discovered give us a possibility of creating new biomimetic biocompatible colloidal systems for encapsulating drugs that have a capabilities of controlled non-thermal release of encapsulated substances by using external physical influences.
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**S9.587. Biophysical investigations nerve cell functions**

Maksimov G.V.[1]*
[1]*Biology faculty of Moscow university ;*
* gmaksimov@mail.ru

Using the modern optical methods (Raman spectroscopy, laser interference microscopy, confocal microscopy), molecular and cellular changes during the functioning of the nerve cell were studied. It was found that when a series of action potentials or receptor activation is commanded, not only the membrane potential changes, but the viscosity of the plasma membrane, as well as the state of the mitochondria and the cytoskeleton of the cell.

**S9.588. Biophysical basis of epileptic activity: the hypothesis of membrane contamination**

Hernandez-Caceres J.[1]*, Dzhimak S.S.[2,3], Drobotenko M.I.[3], Nechipurenko Y.D.[4,5]
[1]*Cuban Center for Neurosciences;*
[2]*Southern Scientific Centre of the RAS;*
[3]*Kuban State University;*
[4]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences;*
[5]*Sevastopol State University;*
* jose.caceres@cneuro.edu.cu

The commonly accepted electrophysiological hallmark of epileptic activity is the paroxysmal depolarization shift (PDS). More than fifteen years ago it was hypothesized that epilepsy is associated with membrane pollution. This hypothesis includes the following assumptions: 1) PDS arises as result of the transition of pacemaker potentials into hypertrophied long-term high-amplitude depolarizations; 2) disruption of the order of the lipid bilayer (for example, as a result of the inclusion of amphiphilic substances) causes the transformation of pacemaker potentials into PDS [1].
Many well-known facts about epilepsy can be explained in the framework of this hypothesis, such as the fact that refractory epilepsy can be treated with a ketogenic diet [2] and vagus nerve stimulation [3], the antiepileptic effects of valproate, cholesterol and other membrane fluid stabilizers.
Additional studies are needed to clarify points 1 and 2. Previously, it was shown that by changing the parameters of the pacemaker potential model, one can trace the transition from the pacemaker potential to paroxysmal depolarization [2]. But the question of determining the channels involved in the generation of the pacemaker potential remains open. To model the described processes, both experimentally and mathematically [4], a new biophysical model is needed. We develop approaches to such a model in [5].
Indirect evidence supports the idea that bilayer contamination with amphiphilic substances can enhance epileptogenicity, but there is no clear demonstration that membrane pollution promotes transformation the pacemaker potential into paroxysmal depolarization [6].
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**S9.589. Biophysical regularities of nitrogen monoxide and copper content changes in rat brain during simulated cerebral ischemia**

Gainutdinov Kh.L.[1,2]*, Andrianov V.V.[2,1], Yafarova G.G.[2], Bazan L.V.[1], Bogodvid T.K.[2,3], Iyudin V.S.[1], Filipovich T.A.[4], Shanko Yu.G.[4], Tokalchik T.P.[4], Kulchisky V.A.[4]
[1]*Zavoisky Physical-Technical Institute of the Federal Scientific Center of Russian Academy of Sciences, Kazan;*
[2]*Kazan Federal University, Kazan;*
[3]*Volga Region State University of Physical Culture, Sport and Tourism, Kazan;*
[4]*Brain Center, Institute of Physiology, National Academy of Sciences, Minsk, Belarus;*
* kh_gainutdinov@mail.ru

Nitric oxide (NO) is one of the key signaling molecules that regulate the body's physiological functions, including the nervous system,

both normal and pathological [1]. Studies of the role of NO in the vital activity of organisms began shortly after the discovery of the regulatory role of NO in normal vascular tone as a mediator of vasodilation [2,3,4]. Through synthases, the level of NO is controlled in neurons, neuroglia and microglia of the brain in normal and pathological conditions by enzymatic and non-enzymatic reactions. Under physiological conditions, the function of NO is consistent and in coordination with many other regulatory systems in the nervous tissue. Of great interest is the involvement of NO in the underlying mechanisms of the development of various pathological conditions in the body [5]. It was found that in some pathological processes NO plays both a protective role and a destructive one, which is governed by many factors [6]. The development of pathological processes in the brain (hypoxia and ischemia)is associated with an increase in the activity of the regulatory systems of the brain (including the NO system) This is naturally accompanied by an increase in oxygen consumption (which exacerbates hypoxia) and an increase in underoxidized products in brain tissue [4,7].

Although, the role of the NO system in these conditions is systematically across the globe, there are still many ambiguities in this fundamental and applied problem. One of the reasons for such a pessimistic situation is the technical complexity of regulating the NO level since NO is formed during rapid chemical reactions involving a wide range of molecules and intermediaries, including metals, thiols, free radicals, amino acids, calcium, and oxygen. It is relevant to study the biophysical patterns of changes in the NO content during ischemic processes in the brain. Therefore, the matter of using modern methods for detecting and quantifying the NO content in the tissues of living organisms in normal and experimental models of pathologies became relevant. One of the most effective methods for identification and quantifying NO in biological tissues is the electron paramagnetic resonance (EPR) method [8]. The authors attempted to detail some biophysical patterns of nitrogen monoxide formation in cerebral ischemia. This method is based on a technique developed by prof. Vanin et al that depends on the reaction of a radical (in this case, NO) with a spin trap. . As a result of the reaction, an adduct with a characteristic EPR spectrum is formed. The authors applied the Fe2+ complex with diethyldithiocarbamate (DETC) to capture NO and form a stable triple complex (DETC)2-Fe2+-NO in animal tissues. These complexes are characterized by an easily recognizable EPR spectrum with a g-factor value of g=2.035 – 2.040 and a triplet hyperfine structure [8,9]. The method has a sensitivity of 0.04–0.4 nM, allows direct measurements, and is highly sensitive due to the use of spin traps [8]. The work aimed o study the effects of experimental ischemic brain damage on the intensity of NO production and copper content (as an indicator of superoxide dismutase) in the hippocampus of rats using EPR spectroscopy with spin trap technique. The results show a significant decrease in NO content in the hippocampus 1 day after modeling ischemia by carotid artery ligation. When modeling ischemia with simultaneous intranasal administration of mesenchymal stem cells (MSCs), no significant difference in NO content was found between ischemic and control rats. After 2 days, the NO content in the hippocampus of ischemic rats was restored. In the hippocampus of rats in which ischemia was modeled with simultaneous intranasal administration of MSCs, no significant difference in NO content relative to ischemic rats was found after 2 days. The copper content in the rat hippocampus decreased unreliably 1 day after modeling ischemia caused by carotid artery ligation and there was a unreliably tendency to increase 2 days later. All in all, NO measurements indicate a tendency to restore the level of NO characteristic to the intact animals. So was found a tendency to increase the effectiveness of the antioxidant system both 1 and 2 days after ischemia.

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## S9.590. Brownian-yet-not-Gaussian diffusion in brain's parenchyma: experimental evidence and mathematical modeling

Postnikov E.B.[1]*, Lavrova A.I.[2,3], Postnov D.E.[4]
[1]*Kursk State University;*
[2]*Saint-Petersburg State University;*
[3] *Saint-Petersburg State Research Institute of Phthisiopulmonology;*
[4]*Saratov State University;*
* postnicov@gmail.com

Brownian-yet-not-Gaussian (BnG) diffusion discovered about ten years ago [1] in some complex biophysical media is characterized by the nontrivial combination features: when one explores the mead-squared displacement of random walkers, it follows the usual linear time dependence but the spatial profile of their probability density function does not correspond to the normal distribution.

Among the different physical origins of such a process, it has been shown [2] that the BnG can emerge as a consequence of the quenched disorder when a marker diffuses in a random medium with a local correlation of inhomogeneities. Such a structure is typical for the extracellular space in the brain's parenchyma where local gaps uniformly filled by the interstitial fluid form a complex random porous structure at a large scale. The targeted search for features typical for the BnG already confirms their existence [3].

Thus, this work explores the data obtained using the MRI mapping of the Gd-based contrast agent concentrations in rat's brain in vivo. It is demonstrated that fitting the dynamic sequence of radial cross-sections of the concentration's spatiotemporal distribution follows the Laplacian probability. density function at intermediate time scales. Further, it is followed by its transition to the Gaussian one at large time scales (but supplied with narrowing localised central peak in the point of injection) that is in line with the theory of BnG homogenization process.

In addition to the direct processing of the biophysical experimental data, the modelling approach based on the master equation will be presented and discussed with a special focus on the perspectives for distinguishing between interpretations of underlying stochastic processes. In addition, outlooks for the usage of such models for quantification of the brain extracellular space's structure and topology from results of macroscopic dynamical pictures of the solute transport in the parenchyma will be discussed.

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### S9.591. Cellular disorders in fibroblasts carrying mutations associated with Parkinson's disease

Kritskaya K.A.[1], Fedotova E.I.[1,2]*, Berezhnov A.V.[1,2]
[1]*Institute of Cell Biophisics RAS;*
[2]*Orel State University named after I.S. Turgenev;*
* delf-fenka@rambler.ru

Parkinson's disease (PD) is a progressive neurodegenerative disease in which the death of dopaminergic neurons in the substantia nigra occurs. The literature describes hereditary forms of PD, which are characterized by mutations in a number of genes encoding the alpha-synuclein protein (mutations A30P, E46K, A53T and multiplications of the SNCA gene locus) and proteins associated with mitochondrial functions (mutations in PINK1, LRRK2, double mutation PINK1/Parkin). Many studies have been aimed at elucidating the molecular mechanisms of PD, among which the accumulation of alpha-synuclein aggregates, mitochondrial disorders, apoptotic and non-apoptotic programmed cell death, autophagy disorders, endoplasmic reticulum stress, and calcium homeostasis disorders have been identified.

It is believed that mitochondrial dysfunction plays a key role in the pathogenesis of PD. In this case, the operation of complex I of the electron transport chain is disrupted, reactive oxygen species (ROS) production is enhanced, mitochondrial DNA disorders are accumulated, and the activity of the mitochondrial network is changed. The current hypothesis is that moderate activation of processes associated with mitochondrial network remodeling (fusion, fission and mitophagy) may help prevent neurons' death during Parkinson's disease and return to normal activity of cell.

In this work, the features of hereditary cellular models of PD were studied - cultures of human fibroblasts with mutations in PINK1 and LRRK2, leading to the development of this pathology, and differences between the studied cell cultures and control ones were revealed.

Using fluorescence and confocal microscopy, it was shown that, the intracellular pH level is lower in cells with the PINK mutation than in the control fibroblast culture, that may be associated with an altered level of mitophagy/autophagy.

We have studied the level of mito/autophagy. It was shown that the degree of colocalization of mitochondria and lysosomes in fibroblasts with mutations did not differ significantly from control cells. Analysis of changes in the mRNA expression level of auto/mitophagy genes in model cells revealed an increase in the expression of genes encoding Nix and Fundc1 proteins involved in the alternative (non-canonical) receptor-mediated mitophagy pathway, which indicates compensation for functions affected by mutations.

The morphology and dynamics of the mitochondrial reticulum (network) in cell models of PD were evaluated and a decrease in the average length of mitochondria was found both in the mitochondrial networks and in individual mitochondria in cells with the PINK mutation compared with the control. The data were confirmed by analysis of the expression of genes associated with mitochondrial dynamics, which resulted in an increase in the mRNA level of Fis1 responsible for mitochondrial division.

The differences in the bioenergetic state of cells, namely in the levels of mitochondrial potential, NADH and reduced glutathione, between the control and cellular models of PD were revealed. In cells with mutations, a decrease in mitochondrial potential and the level of reduced glutathione were observed compared to control fibroblasts.

It was determined that the rate of ROS production in mitochondria is increased in cells with the PINK1 mutation relative to control cells. At the same time, the mutant cells were characterized by a reduced calcium buffer capacity of mitochondria.

The viability of cells in fibroblast cultures was assessed: the number of dead cells did not differ significantly in the control culture and in mutant cells. The study of apoptosis genes revealed an increase in the expression of few genes. However, mutant cells were more sensitive to stress induced by the addition of exogenous hydrogen peroxide.

Thus, it was shown in the work that in cells with the PINK1 mutation, the pH level is lower than in the control fibroblast culture. Despite the fact that basal mitophagy is not increased in mutant cells, evaluation of the morphology of the mitochondrial network in PINK1 cells showed that the length of mitochondria in mitochondrial networks and individual mitochondria is impaired. Also, in these cells, the rate of mitochondrial ROS production was increased, bioenergetics disorders were revealed, and the calcium buffer capacity of mitochondria was reduced. At the same time, the viability of cells in mutant cultures does not differ from that of control fibroblasts.

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### S9.592. Changes in mitochondrial redox state and lipid-protein composition of cells in tumoral and peritumoral regions under high-and low-grade gliomas

Morozova K.I.[1]*, Popov A.v.[2], Denisov P.A.[2], Evgeniya E.U.[1], Medyanik I.A.[3], Yashin K.S.[3], Brazhe A.R.[1,2], Shestopalova M.S.[2], Zalygin A.V.[2], Oleinikov V.A.[2], Semyanov A.V.[2], Brazhe N.A.[1]
[1]*Lomonosov Moscow State University;*
[2]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS;*
[3]*Privolzhskiy Research Medical University;*
* xenia.i.morozova@gmail.com

Gliomas are the most common brain tumors in adult patients, which are classified into four grades according to their malignancy with high-grade gliomas carrying the worst prognosis for the patient. In the present study we applied Raman microspectroscopy with laser excitations at 532 and 633 nm to assess the mitochondria redox state and the lipid-protein composition of cytoplasm of cells in the tumoral and peritumoral regions. The studied preparations were 200-250 μm brain slices prepared from the patient brain tissue removed during the surgery. All studies were carried out with the permission of the Ethical Committee of the Privolzhskiy Research Medical University on the basis of informed consent of patients in accordance with the necessary ethical conventions. We demonstrated that Raman spectroscopy allows to discriminate between low-and high-grade gliomas and to distinguish peritumoral regions from the tumoral area and normal cortex (access tissue). Our results indicate the increase in the relative amount of protein in tumor cells under both low- and high-grade gliomas. Using the specified Raman peaks, we demonstrated that the relative amount of reduced cytochromes b, c and a in the respiratory chain (electron transport chain, ETC) was increased in cells of the peritumoral area under low-grade gliomas and decreased in cells of peritumoral area under high-grade gliomas. The decrease in the relative amount of reduced cytochromes indicates fewer electrons in the ETC and may suggest faster electron transfer due to the formation of the supercomplexes consisting of complexes III and IV in cells of peritumoral area under high-grade gliomas. The redox state of the mitochondrial ETC also changed in high-grade gliomas compared to the low-grade gliomas. The assembled dataset of Raman spectra acquired from different regions of patients tissue samples and the described metabolic characteristics was used for the development of the AI tools for the diagnostics of brain tumor grades and regions. Overall, Raman microspectroscopy allowes us to reveal the metabolic features specific for the peritumoral regions under gliomas of different grades.

### S9.593. Changes in phase and correlation relationships between oscillations of cardiovascular parameters during heating in patients with type 2 diabetes mellitus

Tikhonova I.V.[1], Tankanag A.V.[1], Guseva I.E.[2], Grinevich A.A.[1]*
[1]*Institute of Cell Biophysics of RAS;*
[2]*Hospital of Pushchino Scientific Centre of Russian Academy of Sciences;*
* grin_aa@mail.ru

Type 2 diabetes mellitus (T2DM) is a socially significant chronic metabolic disease characterized by insulin resistance, hyperglycemia, and beta-cell secretory dysfunction. It leads to pathological changes in cardiovascular system (CVS), the development of angiopathies, and as a consequence, peripheral blood circulation disorders of extremities. The search for new noninvasive methods of an assessment of peripheral blood flow is an actual task both for understanding the mechanisms of pathological changes and detection of these changes at early stages.
It is considered that oscillations in CVS reflect the activity of different regulatory mechanisms providing its normal functioning and adaptation to different influences. Oscillations are divided into non-overlapping functional frequency intervals. For heart rate variability (HRV) these are high frequency interval (HF, 0.15-0.4 Hz), reflecting parasympathetic regulation; low frequency interval (LF, 0.04-0.15 Hz), reflecting sympathetic and parasympathetic regulation; and very low frequencies (less than 0.04 Hz). For skin blood flow, these are cardiac (C, 0.6-2 Hz), respiratory (R, 0.145-0.6 Hz), myogenic (M, 0.056-0.145 Hz), neurogenic (N, 0.021-0.056 Hz), and endothelial (E, 0.005-0.021 Hz) intervals. Oscillations in E, N, M intervals characterize low-frequency local regulation, and ones in R and C intervals characterize high-frequency central regulation. The concept of network physiology assumes the presence of relationships between different regulatory mechanisms and, consequently, between oscillatory processes in CVS, which is proven experimentally. We assumed that changes in these relationships may reflect pathological changes in CVS and changes caused by different influences. The aim of the study was to assess and analyze changes in the relationships between different oscillations forming HRV and dynamics of skin blood flow of extremities, at rest and under local heating in T2DM patients.
Healthy volunteers (control) and T2DM patients participated in the study. The control group included volunteers close in age to the patients with normal blood pressure, lipid profile, glucose level, glycated hemoglobin, and other biochemical parameters. Exclusion criteria were smoking, taking any medication, caffeine or alcohol 12 hours before measurement. Volunteers gave written consent. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association (2013) and was approved by the Local Ethics Committee (№2 dated 10.04.2014).
Measurements were performed after a 15-min adaptation in the supine position at ambient temperature 23 ± 1°C during 35 min, of which: 15 min at rest (32°C), 20 min at local heating (38°C). We recorded simultaneously 4 signals: 1) electrocardiogram to determine HRV; 2) pneumogram; 3) dynamics of skin blood flow by laser Doppler flowmetry (LDF) from two skin areas - the outer surface of the right forearm (LDFfr) and the back of the right foot (LDFft), using LDF-probes with heaters. Signal analysis (15 min of rest and the last 15 min of heating) was performed in the Matlab software. Relationships between regulatory processes were assessed by calculating group phase coherence between all pairs of signals, which was averaged over functional frequency intervals (external couplings) and Spearman correlation between spectral components for each signal (internal couplings). The discriminative power of the strength and number of couplings was determined by ROC analysis.
The following results were obtained when comparing patients with controls. The strength of external couplings was lower in LF and higher in HF intervals both between HRV and LDFfr, and between HRV and LDFft at rest and during heating. Between extremities, the strength of external couplings was lower in M interval and higher in C interval at rest and during heating, but appeared differently in R interval: it was lower at rest and higher during heating. Between respiration and other signals, the strength of external couplings was higher at rest and during local heating, with especially strong differences during heating on the foot. Estimation of internal couplings showed that the number of significant couplings was higher at rest for HRV and LDF on extremities, and during the heat test only for LDF on extremities, especially for LDFft. The strength of significant internal couplings was lower for HRV at rest and for HRV and LDFfr during heating, but higher for LDFft at rest and during heating. ROC analysis revealed that parameters of internal couplings were the most effective predictors for distinguishing groups. At the same time, the number of such predictors decreased under local heating. The obtained results showed that local heating followed by analysis of relationships in CVS can be a good test for detecting early changes and the degree of cardiovascular disorders in T2DM.
This study was supported by the Russian Science Foundation [grant number 22-15-00215].

### S9.594. Changes in the structural characteristics of blood cells in patients with COVID-19 and associated cardiovascular diseases

Chernov Ya.V.[1], Shirokova O.M.[1]*, Arkhipova E.V.[1], Kovylin R.S.[1,2], Makarova E.V.[1,3], Lyubavina N.A.[1], Mukhina I.V.[1,4], Galova E.A.[1]
[1]*Privolzhsky Medical Research University;*
[2]*G. A. Razuvaev Institute of Organometallic Chemistry of the Russian Academy of Sciences, 49 Tropinina St., 603950 Nizhny Novgorod, Russia;*
[3] *Nizhny Novgorod Research Institute of Hygiene and Occupational Pathology of Rospotrebnadzor, Nizhny Novgorod, Russian Federation;*
[4]*National Research Lobachevsky State University of Nizhny Novgorod., 603022, Nizhny Novgorod, Gagarin Ave., 23;*
* shirokovaom@gmail.com

Pathophysiological changes in blood cells in case of COVID-19 and concomitant cardiovascular diseases have not been studied in sufficient detail. There is increasing evidence that the biochemical and biophysical properties of erythrocytes can actively contribute to hypercoagulation in various clinical conditions (Soma P. et al., 2022). However, practically there are no studies of ultrastructure of blood cells. The aim of the work was to determine the ultrastructural features of blood cells of patients with COVID-19 during the course of the confirmed community-acquired bilateral viral pneumonia with cardiovascular pathology and without concomitant cardiovascular diseases using scanning and transmission electron microscopy.
For electron microscopy, blood samples were taken from male patients in order to exclude the influence of female sex hormones on the studied parameters. In the main group there were 8 male patients over the age of 18 with a diagnosis of a new coronavirus infection caused by COVID–19, confirmed, community-acquired bilateral viral pneumonia: 5 of them with cardiovascular pathology (hypertensive heart disease and coronary heart disease) and 3 people without concomitant cardiovascular diseases. The control group consisted of 8 patients, comparable in demographic indicators and concomitant pathology, selected according to the "head-to-head" principle, who were not ill with the new coronavirus infection COVID-19 and were not vaccinated against SARS-Cov-2, who had negative values of blood analysis by enzyme immunoassay (ELISA) for the content of anti-SARS antibodies-Cov-2 IgG. Scanning and transmission electron microscopy was carried out on microscopes Regulus SU8100 (Hitachi) and MERLIN VP (Zeiss), Morgagni 268D (FEI). For scanning electron microscopy a whole blood smear was evaluated without carbon deposition. For transmission

electron microscopy cells were fractionated to obtain three fractions - erythrocytes, platelets and leukocytes.
A study of blood smears revealed a strong aggregation of erythrocytes of patients with COVID-19. The cells were glued together and with platelets. Platelets had an activated form, increasing the aggregation of red blood cells. In addition, the properties of the membrane changed significantly in the erythrocytes of patients with COVID-19, regardless of the concomitant diagnosis, that particles of various genesis settled on the surface (for example, salt buffer, cell residues, platelets, etc.). Statistically significant differences in the ultrastructural organization of erythrocytes between groups with cardiovascular pathology and without concomitant cardiovascular vascular diseases were not detected.
Thus, additional studies are needed to determine the causes of the increased aggregation capacity of the erythrocyte cell membrane in SARS-Cov-2.
Primary analysis of blood smears by scanning electron microscopy was carried out using the equipment of the center for collective use "Analytical Center of the IOMC RAS" with the financial support of the grant "Ensuring the development of the material and technical infrastructure of the centers for collective use of scientific equipment" (Unique identifier RF-2296.61321X0017, Agreement number 075-15-2021-670). Ultrastructural studies on transmission electron microscopy and morphometry were performed at the Correlation Microscopy Center of PIMU, within the framework of Strategic Academic Leadership Program "Priority-2030".

### S9.595. Characteristics and interpretation of EMR signals in SC tissue 7 days after traumatic injury

Yurtaeva S.V.[1]*, Yafarova G.G.[1,2], Yatsyk I.V.[1], Gainutdinov Kh.L.[1,2]
[1]*E.K. Zavoisky Physical Technical Institute of the FRC of RAS;*
[2]*Institute of Fundamental Medicine and Biology, Kazan Federal University;*
* svetlana.vish@rambler.ru

Recently the study of the molecular mechanisms of damage of neural tissues and the search for the ways to restore resulting disorders after it, have been very intensive. Severe spinal injury, complicated by spinal cord (SC) damage remains one of the urgent medical and social problems, because it leads to severe disability of the patients. The current lack of effective methods of treatment and rehabilitation for this pathology encourages the intensive study of the molecular mechanisms of neural tissue injury. Iron is one of the metabolites actively involved in the development of post-traumatic conditions in the case of damage of the neural tissue.
It is known that the injury of SC is accompanied by the death of cells and bleeding, which result in increase of free iron pool. It is believed that an increased amount of iron can initiate the secondary tissue damage by growing free-radical processes. In this case, the increase of iron biomineralization process in SC tissue is possible. That results in formation of crystalline iron oxides. The EPR method allows us to detect such crystals.
To date, it is known about the observation of Electron Magnetic Resonance signals (EMR), depending on orientation of magnetic field in injured neural tissues. These signals were registered in injured sciatic nerve of frog [1] and in injured cat spinal cord [2]. In the first study, which discovered the anisotropic signal in the neural tissue, its ferromagnetic origin was suggested, but the sources of the signals and their characteristics were not established. The defining the nature of the EMR signals can give an additional information about molecular processes developing after neural tissue injury, and can contribute to the development of new methods to correct the emerging metabolic shifts.
In this study the method of EPR spectroscopy to investigate iron biomineralization in spinal cord tissues in conditions of injury was used. The nature of signals emerging in injured rat SC 7 days after trauma was investigated. A quantitative comparison of signals in injured and healthy tissues was carried out.
The tissues of control rats (n = 3) and rats with an experimental model of the spinal cord injury (SCI) (n = 6) were studied. SCI modeling was made according to Allen method [3]. In SC tissues two types of EMR signals corresponding to crystalline iron oxides formed as a result of biomineralization were detected. Their temperature and angular behavior have been studied. The first type, characterized by the orientation dependence of Hres in magnetic field, is attributed to nanocrystalline magnetite. The second type, characterized by superparamagnetic temperature behavior, is attributed to the crystalline core of ferritin, ferryhydrite. The first type signal prevailed in injured tissues, the second - in control and adjacent sections to injured SC. The integral intensities of EMR signals in the tissues were evaluated. An evident increase in EMR signal was found directly in the area of injury compared to adjacent SC sections, as well as compared to the similar uninjured SC tissues of control rats, on average twice as much and more, indicating the accumulation of crystalline iron in injured tissue. In the rat, with the maximum effect, the signal in the injury area 10 times increased. The estimated increase in amount of crystalline iron oxides in the injured SC may be due to the phenomenon of "iron homeostatic response" [4]. The signals found in this study in injured SC tissues and adjacent tissues (above and below the injury site) are due to iron oxides, magnetite and ferritin. Different types of angular anisotropy of Hres were found, demonstrating varied distribution of accumulating crystalline particles (in the form of films, 3D structures, dispersed nanosized grains).
It should be noted that according to the literature [5], the period of 7 days after the injury is characterized by intensive processes of axon demyelination and an increase of macrophages that can hadle the iron by means of ferritin and protect cells from death [6].
The study of such signals is of importance, since their amplitude may correlate with the intensity of demyelination pathological process in the nerve fibers, and the evaluation of the intensity of the signals in the blood allows to control the intensity of these processes during the treatment of spinal injuries. The effect we detected is consistent with the mechanisms of the development of tissue molecular processes at spinal cord injury in the literature. At the same time, the use of the EPR method allows to move towards a semiquantitative assessment of the observed phenomenon.
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### S9.596. Chemiluminescent analysis of the antioxidant capacity of aqueous extracts from yerba mate (Ilex paraguariensis)

Teselkin Yu.O.[1]*, Babenkova I.V.[1], Osipov A.N.[1]
[1]*Pirogov Russian National Research Medical University;*
* teselkin-box@mail.ru

It is known that aqueous extracts from the Paraguayan holly (Ilex paraguariensis) leaves and stems treated according to a traditional technology and named «yerba mate» or «mate» possess a wide spectrum of biological

activity such as antidyslipidemic, antidiabetic, anti-inflammatory, cardioprotective, antibacterial, antitumor, antioxidant, and other properties. That served a justification for application of yerba mate in pharmaceutic and food industry, and cosmetology in some countries [1]. It is believed that the antioxidant capacity (AOC) of aqueous extracts from mate is due to biologically active substances (BASs) of a polyphenolic nature, such as caffeic acid, chlorogenic acid, rutin, quercetin, kaempferol. A comprehensive study of the AOC of aqueous extracts from mate using various radical-generating model systems will make it possible to understand the mechanisms of the antioxidant action of mate in vivo. The great opportunities for studying the antioxidant properties of the BASs of plant origin are provided by the method of chemiluminescence (CL), which has a high sensitivity and allows for kinetic measurements.

The purpose of this work is to study the AOC of aqueous extracts from mate using the kinetic CL.

Mate of the Amanda trademark of the Desplada category (La Cachuera S.A., Argentina) was the object of this study. Aqueous extracts from mate were prepared with bidistilled water as described in [2]. Single-layer liposomes from egg phospholipids were formed in 50 mM Tris-HCl buffer containing 100 mM KCl, pH 7,4.

The AOC of aqueous extracts from mate was studied by recording the CL kinetics in two model systems: 1) luminol oxidation induced by 2,2'-azobis(2-amidinopropane)dihydrochloride (ABAP-luminol system) [3]; 2) lipid peroxidation of liposome suspension induced by Fe(II) ions [4].

The AOC of the blood plasma of volunteers was determined using the ABAP-luminol system [2, 3]. Eight apparently healthy male volunteers aged 34–50 years were divided into two groups of 4 people each. The first group had a tea drink made from 4 g of mate on an empty stomach, the second group had a tea drink made from 8 g of mate.

It was found that the addition of the aqueous extract from mate into the ABAP-luminol system was accompanied by the appearance of a CL latent period, the duration of which was directly proportional to the amount of the added sample. The appearance of a latent period is caused by the fact that BASs in mate scavenge the water-soluble radicals-initiators of luminol oxidation, formed in the system. The AOC value of the aqueous extract from mate, presented in the form of trolox equivalent, was on average 1,31 mmol/g of dry plant raw material. We have studied under the same conditions the AOC of quercetin, rutin, chlorogenic and caffeic acids found in aqueous extracts of mate. The addition of these BASs to the ABAP-luminol system also led to the appearance of the CL latent period. The AOC of quercetin (in trolox equivalent) did not differ from the AOC of rutin and was 1,2–1,3 higher than that of chlorogenic and caffeic acids ($p < 0,05$).

The addition of aqueous extract from mate to a suspension of liposomes resulted in a dose-dependent rise in the time period within which the «slow flash» of CL reached its maximum value and a decrease in its intensity. This indicates a decrease in the oxidation rate of Fe(II) ions and a decrease in the rate of lipid radical formation, respectively. Similar results were obtained after addition of the classical radical inhibitors butylhydroxytoluene and trolox to liposomes. However, unlike radical inhibitors, a further increase in the concentration of aqueous extract from mate in the liposomal suspension (more than 2,5 μg of dry plant raw material/ml) was accompanied by a gradual decrease in the time period within which the «slow flash» of CL reached its maximum value, which was typical of the action of iron-chelating agents (EDTA and deferoxamine) and caused by a decreased time of Fe(II) ions oxidation to «the critical concentration» [4]. It can be supposed that BASs in the composition of aqueous extract from mate exhibit both radical-scavenging activity and iron-binding ability. The effects of quercetin, rutin, chlorogenic acid, and caffeic acid on Fe(II)-induced liposomal CL were studied. It has been established that quercetin in a liposome-based model system acted as radical inhibitor. A mixed type of action, radical-scavenging and iron-chelating, was observed in rutin, caffeic and chlorogenic acids.

We studied the AOC change of the blood plasma of healthy volunteers after a single consumption of tea prepared from 4 and 8 g of mate. In the first case, after 1 h, there was a tendency to an increase in this indicator as compared to the initial value: the AOC increase was 7,4%. In the second case, after 1 h, the blood plasma AOC increased by 14,5% ($p < 0,05$), and after 2 h the increase was 8,9% ($p < 0,05$).

Thus, the AOC of aqueous extracts from yerba mate is due to radical-scavenging and iron-chelating properties, which probably determine the main mechanisms of the antioxidant action of yerba mate in vivo.

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### S9.597. Chloroamine derivatives of analogues of adenosine - covalent inhibitors of platelet functions

Murina M.A.[1]*, Roshchupkin D.I.[2], Sergienko V.I.[1]
[1]*Federal Research And Clinical Center Of Physical-Chemical Medicine, Moscow, Russia;*
[2]*Russian National Research Medical University named after N.I. Pirogov, Moscow, Russia ;*
* marina_murina@mail.ru

Now, high efficacy of covalent antiplatalet agents (platelet functions inhibitors) for thrombosis prevention has been convincingly proved. Such antiplatalet agents suppress platelet functions by a chemical modification of molecular targets. Acetylsalicylic acid (aspirin) reacting with prostaglandin H2-syntase and thienopyridines whose metabolits adduct to ADP-receptor as a result of the reaction with sulfhydryl group are widely used. We found [1] that taurine and amino acid chloramines have the ability to irreversibly suppress platelet activity. N-acyl and N-alkyl derivatives of taurine chloramine have been developed, which have two important properties: increased stability and chemoselectivity [2,3].

This work is aimed at the development of an antithrombotic substance from among the chloramine derivatives of adenosine analogs (CDA). We believed that the effectiveness of the antiaggregant action of the chloraminic compound would increase if at first there is binding specifically to the platelet membrane and then undergoes irreversible modification of the target. In this regard, CDA attracts attention, since platelets have specific receptors on the outer surface for a number of structural analogues of adenosine [4]. A procedure was developed for the preparation of the interested chloraminic compounds in the reaction of sodium hypochlorite with a solution of the starting compounds [5]. Further, the reactive properties of new chloramines, which are important for the manifestation of their antiaggregant activity, were studied. The rate constants of the reactions of the studied CDA with sulfur-containing compounds (methionine, cysteine, acetylcysteine, reduced and oxidized glutathione, albumin, fibrinogen) were determined. It was found that CDA exhibit increased reactivity with respect to the sulfhydryl atomic group. It has been established that chloramines of adenosine analogues exhibit specific pharmacological activity as antiplatelet agents in three cell systems. They effectively inhibit the aggregation of isolated platelets, platelet-rich plasma and whole blood when activated by collagen or ADP. The studied chloramines in micromolar concentrations cause inhibition of platelet functions, not only inhibiting their aggregation, but also suppressing the reaction of ejection of the contents of dense granules, as well as inducing dissociation of aggregates of platelets.

Obviously, when CDA is introduced into whole blood, they will act not only on platelets, but also on other blood cells. To determine the sensitivity of erythrocytes and leukocytes to the action of chloramines, the rate of hemolysis of erythrocytes and the change in the rate of formation of reactive oxygen species in a suspension of neutrophils were determined. Experiments with a dilute suspension of erythrocytes showed that hemolysis (observed after 24 hours) occurs at a high concentration of chloramines: there are approximately 10^11 molecules per erythrocyte. In whole blood, strong inhibition of platelet aggregation is achieved when this ratio is about 3 orders of magnitude lower. The studied chloramines do not cause significant suppression of luminol-dependent chemiluminescence in a suspension of neutrophils activated by phorbol-12-myristate-13-acetate (PMA). Thus, at a CDA concentration of 100 μM, the luminescence intensity decreases by approximately only 15%. In the presence of CDA, there is also no increase in chemiluminescence in the system neutrophils - luminol without stimulation of PMA cells, i.e. chloramines themselves do not activate cells. Thus, chloramines in the blood act selectively on platelets: at the level of significant inhibition of platelet aggregation activity, there is no change in the properties of erythrocytes and leukocytes.

In this work, the modification of proteins of the blood plasma coagulation system under the influence of CDA was studied. Coagulation was initiated in three ways: contact, administration of thromboplastin (tissue factor) and administration of thrombin. It turned out that at concentrations at which inhibition of platelet aggregation occurs, the studied compounds do not affect the blood coagulation system, i.e. are not anticoagulants.

The antiplatelet property of chloramine analogs of adenosine is probably due to their ability to modify the sulfhydryl group of plasma membrane receptors. In active concentrations, analogues of chloramine adenosine do not affect other blood cells.

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**S9.598. Coding of biologically significant time intervals of sound sequences by neurons of the higher auditory centers in anesthetized and awake mice**

Egorova M.A.[1]*

[1]*Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences ;*

* ema6913@yandex.ru

Establishing the mechanisms of the communication calls processing in the auditory system of humans and animals is one of the main and difficult to solve neurophysiological tasks attracting the attention of the leading hearing researchers. Despite the efforts made in this area, the main principles of the temporal processing of communication calls in the brain auditory centers, and the specialization of neurons in this processing are to be studied.

The vast majority of biologically significant acoustic signals, including human speech, consist of sequentially generated sound events (Bregman, 1990; Gaub, Ehret, 2005). It is evident that the temporal structure of these sounds including the intervals between sound events, are of great value for their recognition. Post-stimulus neural adaptation, one of the most vivid forms of the brain plasticity directly related to sensory information processing, has been proposed as a possible mechanism providing the ability of the auditory system to group sound sequences into a single auditory event, separate them in time, and recognize them as biologically significant (Ulanovsky, 2004; Bibikov, 2010).

The presented work shows the participation of neuronal adaptation in the temporal processing of biologically significant acoustic signals by the example of sequences of the communication calls of mice – the wriggling calls of mouse pups.

We carried out extracellular recording of the impulse activity of the auditory midbrain (central nucleus of the Inferior colliculus) neurons as well as primary auditory cortex neurons (primary and anterior auditory fields) in female house mice, F1 hybrids of the outbred strains CBA and C57BL/6 aged 8–15 weeks. The experiments were performed both under general anesthesia, supported by injections of ketamine (ketavet, 35 mg/kg) and xylazine (rompun, 0.1 mg/kg), and in awake animals. The stimuli were series of four tonal signals whose frequency corresponded to the characteristic frequency of the neuron, and intensity was 40 dB above the response threshold, which corresponded to the optimal response regions of most neurons. The duration of each stimulus was 100 ms, including the rise and fall time (5 ms each). The intervals between the tonal components of the same series were equal; in different series, they varied from 0 to 1000 ms. The responses of the single neurons to the tone series were recorded at inter-tone intervals of 0, 2, 4, 10, 20, 50, 100, 200, 500, 700 and 1000 ms. Each series was presented 20 times at 2-s intervals. The parameters of tone series and inter-tone intervals were set based on the results of a psycho-physical study of the relationship between sound production and perception of the sequences of mouse pups wriggling calls (Ehret, Riecke, 2002; Gaub, Ehret, 2005). It was shown that mouse pups produce series of wriggling calls each consisting of 2–5 signals, but the pup's mothers perceive the call as a meaningful for releasing instinctive maternal behavior if series consist of 4 calls with the inter-call intervals of 100–400 ms.

We studied the temporal dynamics of post-stimulus adaptation of single neurons, i.e. the number of spikes in responses to each of the four tones in the sequence on the inter-tone interval in the sequence of wriggling call models (response-recovery curves).

Two-thirds of neurons in the auditory midbrain center and all neurons of the primary auditory cortex demonstrated an adaptation effect in responses to a series of wriggling call models. The effect was ontained in both awake and anesthetized mice. At short inter-tone intervals (0 – 50 ms in the auditory midbrain, 0 – 100 ms in the auditory cortex), neurons either responded only to the first tone of the four-tone series or responses evoked by the second, third and fourth tones were considerably reduced. An increase in inter-tone intervals in a series led to a gradual recovery of responses to the second, third and fourth tones up to the full recovery of the response. In different neurons, response recovery began with different inter-tone intervals (4 – 50 ms in the auditory midbrain, 10–200 ms in the auditory cortex). Full recovery of responses to the 2nd– 4th signals of the series, when they did not differ from the response to the first tone, occurred at the inter-tone intervals from 200 to 1000 ms. Statistical evaluation of the temporal dynamics of adaptation over all recorded units showed that at inter-tone intervals of 0–200 ms responses to the first tone of the series significantly exceeded those to the 2nd, 3rd and 4th tones (ANOVA on ranks, Dunn's test, $p < 0.01$). Responses to the 2nd–4th tones in the series did not differ.

Since the inter-tone interval of 500 ms, the responses to all tones of the series did not differ significantly. So, within the temporal window of 0 – 500 ms the phenomenon of adaptation of the neuronal responses to repetitive sounds was observed. Thus, the time domain of 100–400 ms intervals in wriggling call series, which is important for triggering maternal behavior, corresponds to the time domain of the release from adaptation to their models. It can be assumed that the variation of adaptation time scales of individual neurons serves as the basis for the formation of optimal time windows in processing of the sound events grouping and separation relevant for the perception of animal communication signals and human speech.

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### S9.599. Combination of methods of lifetime calcium imaging and electrophysiological recording of mouse hippocampal neuronal activity

Vinokurov E.[1]*, Erofeev A.[1], Vlasova O.[1], Bezprozvanny I.[1,2]
[1]*Peter the Great St. Petersburg Polytechnic University, Laboratory of Molecular Neurodegeneration;*
[2]*University of Texas Southwestern Medical Center, Dallas, TX, USA;*
* eg.vinokurov@yandex.ru

The study of fundamental and applied bases of brain functioning is among the priority areas of modern science. In vivo studies provide data on the lifetime activity of the neural network, which is a set of neurons and other cell types interconnected and performing specific physiological functions. Neural networks provide complex and highly organized work of the brain, and its activity affects both mental and physical aspects of human life. That said, important results obtained in brain research are currently incomplete, as they have been obtained in vitro or ex vivo, so in vivo brain research is an important task of modern neurobiology.

In this project, we have designed and fabricated a microelectrode that will allow us to combine methods of lifetime calcium imaging using a miniature fluorescence microscope (miniscope) and electrophysiological registration.

In vivo visualization of neuronal activity can be performed using special sensors that fluoresce when the concentration of various ions, such as calcium, changes. The family of genetically encoded calcium indicators GCaMP (which is a hybrid of green fluorescent protein (GFP), calmodulin (CaM) and M13 (myosin light chain kinase peptide sequence)) is widely presented in scientific practice and often used in studies of neuronal activity. Calcium signaling links membrane excitability and cellular biological functions and plays an important role in imaging neuronal activity [1,2]. Calcium imaging technique provides an opportunity to understand functional relationships by recording large populations of neurons [3,4].

The technology of lifetime calcium imaging using a single-photon miniature fluorescence microscope (miniscope) is an important modern tool for studying neuronal networks in various brain regions. The use of the miniscope makes it possible to record neuronal activity on freely moving laboratory animals, in contrast to the traditionally used two-photon imaging.

However, the achievable temporal resolution is limited by the slow (millisecond) kinetics of Ca2+ binding, because genetically encoded calmodulin-based Ca2+ fluorescent indicators always exhibit an extended fluorescence attenuation time. In other words, registration of high-frequency action potentials may be limited by the dynamics of the calcium sensor. Therefore, calcium imaging in vivo does not reflect the entire activity of neuronal networks, which negatively affects the understanding of the mechanisms underlying memory formation, learning, sleep, social behavior, feeding, as well as the processes underlying their disruption [5,6]. One possible way to solve this problem is to combine electrophysiological registration and optical imaging. For this purpose, in this work we designed and manufactured a microelectrode adapted to the size of the gradient lens and combined with it.

This microelectrode is a three-layer rectangular structure of polyimide film and thermoformed gold contact/conducting tracks. On one side of the film are 12 open conductive contacts for recording local field potentials, and on the other side a similar number of conductive tracks for connecting a connector that transmits data to the processing board. The developed microelectrode will allow combining the methods of in vivo calcium imaging using a miniscope and electrophysiological registration. This approach will enable a more detailed study of the activity of neural networks, as well as mapping the brains of freely moving laboratory animals with high spatial and temporal resolution, which will expand the possibilities of research in the field of neurobiology.

The aim of our future work will be to analyze the activity of hippocampal neurons based on miniature fluorescence microscopy data and parallel recording of the local field potential due to the developed microelectrode in wild-type laboratory mice and with an Alzheimer disease model during the evaluation of conditionally-reflexive freezing. This study will reveal abnormalities in Alzheimer's disease at the neural network level and, as a consequence, suggest potentially new therapies or mechanisms of pathology associated with progressive memory loss in Alzheimer's disease.

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### S9.600. Comparison of the properties of the neurons in brainstem and cerebral cortex levels of the auditory pathway

Bibikov N.G.[1,2]*
[1]*Институт проблем передачи информации им. акад А.А. Харкевича РАН;*
[2]*A.A. Kharkevich Institute for Information Transmission Problems of the RAS, Moscow, Russia;*
* nbibikov1@yandex.ru

For many years, I have carried out electrophysiological studies of neurons in the brainstem nucley of the auditory pathway of various vertebrates, and in recent years, thanks to the use of a unique setup developed by I.N. Pigarev, together with this tragically deceased researcher, I managed to study the properties of neurons in the auditory cortex of an awake and sleeping cat. This allows, based on our own experience, to compare some properties of neurons located in the brainstem and cortical levels of the auditory pathway. Neurons localized in the nuclei

of the direct auditory pathway of the brainstem can differ sharply from each other in their internal properties. Many of them are characterized by a long and sharp decrease in excitability after spike generation, which is due to the functioning of specific ion channels, and which phenomenologically manifests itself as a generalized refractoriness. In some other specialized neurons (for example, the octopus cells of the ventral cochlear nucleus), an extremely low input impedance causes effective differentiation of the input action. At the same time, in other neurons (for example, in many cells of the dorsal cochlear nucleus and the inferior colliculus), the time constant of the membrane are extremely large that, under the action of a fixed synaptic input, the response increases in time over tens of milliseconds. The architecture of synaptic inputs to each specific neuron is just as diverse. There are regions in which the vast majority of cells receive one or only a few powerful inputs from the underlying sections of the auditory pathway (spherical cells of the anterior cochlear nucleus, almost all neurons of the nucleus of the trapezoid body). Other, often closely spaced, cells have a highly branched dendritic structure with many synapses. It is natural to assume that all these features of the structure of individual stages of the auditory pathway provide the implementation of specialized operations that serve to describe in detail all the features of a one-dimensional time function that describes changes in pressure at the entrance to the inner ear after preliminary frequency and amplitude filtering in the inner ear. As a result, a detailed analysis of a relatively high-frequency sound stimulus can be carried out using relatively inertial elements, which are brain neurons. It seems that the main function of these stages of sound processing is to extract the temporal features of the perceived signal, which will be further used to identify signals and make appropriate decisions. Note that in the neural networks of the auditory pathway, the frequency features of the signal are also represented as temporal relationships of the firing rate in different channels. In this case, it is usually possible to simulate the reaction of a neuron in the brainstem sections of the auditory pathway to a new signal, knowing its responses to simple tonal stimuli. On the other hand, the cells of the cortical sections of the auditory analyzer surprise with the homogeneity of their internal properties. Among the hundreds of cells examined, I have never observed a long-term decrease in excitability caused by spike generation. Many models of cortical cells simply ignore the concept of refractoriness, limiting themselves to a brief reset of the membrane potential to zero. In almost all neurons, after a post-spike pause lasting only 1–2 ms, a period of sharply increased excitability is observed, which causes the appearance of burst activity and partially fractal properties of the temporal point process of firing. According to these parameters, not only the cells of the auditory cortex are similar to each other, but also neurons located in completely different cortical zones. However, if in terms of their internal properties the cortical neurons appear to be rather simple and homogeneous, this cannot be said about their reactions to sound stimuli. Recent work carried out on waking subjects in the absence of any anesthesia has convincingly shown the extreme complexity of the behavior of cortical cells under the action of real sounds. In fact, some authors have come to the conclusion that it is difficult or even impossible to predict the reaction of the studied neuron to a new signal, even if its responses to sounds of various frequencies have been studied in detail. It is clear that this is determined by the complexity of the architecture of a huge number of input synaptic structures. Such features of the behavior of cortical neurons force us to reconsider the fundamental approach to understanding the operation of the auditory analyzer. It can be assumed that cortical cells, initially homogeneous in their internal properties, undergo learning in the process of ontogeny and further development using some feedback algorithms, which themselves can be quite simple. Given that each cortical neuron has several thousand synaptic inputs, a significant number of which are plastic, such a multilayer deep neural network can perform efficient analysis of audio signals in real time. At the same time, an analogy arises with deep neural networks, which by now have brilliantly shown themselves in the tasks of recognizing even continuous speech. It is possible that the above considerations also apply to other sensory systems that have representation in the cerebral cortex.

### S9.601. Complex of low-molecular collagen peptides and glycosaminoglycans to treat and prevent musculoskeletal system diseases

Nikolaeva T.I.[1]*, Laurinavichus K.S.[2], Molchanov M.V.[1], Kuznetsova S.M.[1], Emelyanenko V.I.[1], Shekhovtsov P.V.[1]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
[2]*Institute of biochemistry and physiology of microorganisms of the RAS;*
* tomivnik@yandex.ru

Diseases of the human musculoskeletal system represent both medical and biological problem. In such the diseases the structure of connective tissues is destroyed, their main components are collagen fibrils and proteoglycans. To solve this problem glycosaminoglycans (chondroprotectors) are used in the Russian Federation and abroad. However, chondroprotectors are not restore the structure of the tissues completely. Another attempt is the usage of collagen hydrolysates in a number of countries [1].

One of the promising solutions of this problem is the production of the nutraceuticals based on collagen hydrolyzate containing low molecular weight peptides in combination with glycosaminoglycans. Low molecular weight peptides can perform not only structural, but also regulatory function. Collagen di- and tripeptides regulate the functions of the neuroendocrine and immune systems [2].

The missing concentrations of collagen and proteoglycans can be replenished with the substances obtained from the connective tissues of farm animals. We have developed nature-like technology for obtaining the complex of collagen peptides and glycosaminoglycans that includes the stages of raw material homogenization and enzymatic hydrolysis. The availability of enzymes for collagen molecules packed in fibrils is facilitated for particles having minimal size. Such particles were obtained in the high-pressure homogenizer “Donor-3” under the conditions of elevated temperatures and pressures. We investigated the enzymatic hydrolysis of cartilage tissue biopolymers depending on pH, temperature, enzyme concentration and duration of the process. The effect of enzyme preparations based on papain of Russian manufacturers of caripazim, which are produced by CJSC Vifitech and LLC MedFlorina, in the town of Obolensk, Moscow Region, was tested.

Comparative analysis of the physicochemical properties of homogenates obtained depending on the homogenization conditions was carried. At the temperatures of about 70ºC and 80ºC samples with different physicochemical properties were obtained. More homogeneous sample of hyaline cartilage was obtained at the temperature of 80ºC. More complete denaturation of collagen also occurs at this temperature of 80ºC. The particle sizes in the homogenate at 80ºC are 1,5 times smaller than at 70ºC. Collagen molecules in the homogenate at 80ºC are more accessible for active enzymes. Thus, we have determined the optimal temperature for cartilage homogenization, which is 80ºC.

In the study of enzymatic hydrolysis, the degree of hydrolysis was higher in hydrolysates obtained under the action of karipazim made at LLC "Medflorina". After the influence of caripazim on the homogenate at the temperature of 50°C, the enzyme concentration of 5%, the peptide profile was located in the range from 240 to 780 Da. With the increase of temperature up to 55°C, and a concentration of caripazim up to 10%, lower molecular weight peptides are formed in the range from 240 to 620 Da. Using the NMR method, spectra were obtained, in which peptides and hyaluronic acid were identified. The molecular weight distribution of glycosaminoglycan oligosaccharides ranges from 240 to 720 Da. Thus, we obtained the complex of low molecular weight peptides of collagen and glycosaminoglycans. If we compare with imported analogues the molecular weights of collagen hydrolysates

obtained by us, it should be noted that we have created samples that are not inferior to Flexinovo (Poland) and have advantages over BioCell Collagen II (USA).

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**S9.602. Conformational changes in p53, acetylated at lysine 320, promote its transition into the cytoplasm and protects neurons in the perifocal area of the photothrombotic stroke from apoptosis**

Demyanenko S.V.[1,2]*, Bachurin S.S.[2], Guzenko V.V.[1]
[1]*Southern Federal University;*
[2]*Rostov State Medical University;*
* demyanenkosvetlana@gmail.com

Acetylation of lysine residues of histone and non-histone proteins regulates the most im-portant functions of cells, including apoptosis. Protein p53 is the key regulator of apoptosis. On the model of photothrombotic stroke (PTS) in rats (rose bengal 20 mg/kg i.v., diode laser 532 nm, 60 mW/cm2, Ø3 mm, 30 min) and in mice (rose bengal 150 mg/kg i.p., diode laser 532 nm, 0.2 W/cm2, Ø1 mm, 15 min) we have shown that ischemia causes an increase in p53 acetylation level in lysines at position 320 (acp53K320) and 373 (acp53K373). On the first day after PTS, acp53K320 level decreases almost threefold in the nuclear fraction and increases in the cytoplasmic fraction of penumbra tissue. Such a tendency persists up to 7 days after PTS. Furthermore, total content of acp53K320 in neurons of the perifocal area of the photothrombotic stroke (PTS) increases within these time intervals. Acp53K373 has predominantly nuclear localization in neurons. A significant increase of acp53K373 occurs on the first day after PTS.

Molecular dynamic simulation was performed in the Gromacs 2022.3 software [http://dx.doi.org/10.1016/j.softx.2015.06.001]. It has shown that conformational stability of the (ALA 237 - ASP 354) p53 fragment changes upon acetylation of lysine 320. The main difference between the conformations p53 fragment, acetylated and unacetylated at lysine 320, is the compactness. Apparently, the acetyl fragment in this site allows the formation of a hydrophobic domain that directs the compaction of the free peptide chains. On the contrary, the K320-unacetylated fragment shows a chaotic position of the free peptide chains relative to the structured domain with alpha helices. Probably, the more compact conformation of p53, which it acquires upon K320 acety-lation after PTS, allows the protein to move between the nucleus and cytoplasm in penumbra neurons. In contrast, acetylation of p53 at lysine 373 does not induce any significant changes in the conformational preferences of the p53 C-terminal fragment (SER 367 - LYS 386), which could be due to its role in the protein, i.e. binding to regulatory sites of enzymes or other service proteins.

The analysis using co-immunoprecipitation and Duolink PLA (proximity ligation assay) methods showed that acetyltransferases PCAF and, to a lesser extent, p300, but not HAT1, acety-late p53 in the cells of the perifocal region after PTS. The administration of the PCAF inhibitor plumbagin (1 mg/kg, i.p., 3 days after PTS, once a day) reduced p53 acetylation at lysine 320, but not acetylation at lysine 373, and increased the level of apoptosis in the cells of the perifocal region of PTI. Moreover, the effect was more expressed when combined with the administration of p53 inhibitor pifithrin-α (8 mg/kg, i.p., 3 days after PTS, once a day), capable of reducing the level of p53 in the cytoplasm. On the contrary, the administration of the p300 inhibitor embeline (0.6 mg/kg, i.p., 3 days after PTS, once a day) reduced the level of p53 acetylation at lysine 373, as well as apoptosis in the cells of the perifocal region 3 days after PTS. The co-administration of embeline and a p53 inhibitor pifithrin-μ (8 mg/kg, i.p., 3 days after PTS, once a day), capable of reducing the level of p53 in neuronal nuclei, enhanced the antiapoptotic effect of the p300 inhibitor.

Thus, the acetylation of p53 at lysine 320 changes the protein's conformation and promotes its accumulation in the cytoplasm of neurons in the perifocal area after PTS. The acetylation of p53 at lysine 320 is more preferably than the acetylation at lysine 373, and, probably, promotes the survival and repair of penumbra neurons after stroke. The strategies, aimed to increase the acetylation of p53 at K320 by increasing the activity of PCAF will be promising for neuroprotective therapy of stroke.

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**S9.603. Delivery of biologically active substances into compartments of a living cell: achievements, problems and prospects**

Sobolev A.S.[1,2]*
[1]*Faculty of Biology, Lomonosov Moscow State University;*
[2]*Institute of Gene Biology, Russian Academy of Sciences;*
* alsobolev@yandex.ru

The report summarizes the results of the last 4 years on the creation of polypeptide means for delivery of various bioactive substances to specified compartments of target cells - modular nanotransporters (MNT). The principle underlying the design of MNT is the use of natural cellular transport processes. The main groups of bioactive substances being delivered are 1) antibody mimetics (monobodies, nanobodies, etc.) targeting specific intracellular molecules, and 2) cytotoxic agents (radionuclides emitting short range particles). The results of experiments on blocking transcription factors and/or their inhibitors (e.g., Nrf2, Keap1, c-Myc) or viral proteins (e.g., nucleocapsid N-protein of the SARS-CoV-2 virus) in a living cell using specific antibody mimetics delivered to cells with the help of MNT are presented [1-4]. Also described [5] are the results of the use of MNT with antibody mimetics for recognition of target cells, delivering the radionuclide In-111, the Auger electron emitter, into the nuclei of these cells. Such MNT variants can already be used as experimental tools for studying the functions of living cells. The task waiting to be solved is the development of an MNT variant for systemic administration.

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**S9.604. Detection of radiation-induced changes of optical properties of white matter of the brain using optical coherence tomography**

Achkasova K.A.[1]*, Moiseev A.A.[2], Bogomolova A.Yu.[1], Gladkova N.D.[1]
[1]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia;*
[2]*Institute of Applied Physics of RAS, Nizhny Novgorod, Russia;*
* achkasova.k@bk.ru

Introduction. Radiation therapy is an actively used method of treatment of malignant neoplasms of the brain. In order to increase the effectiveness of treatment, it is necessary to irradiate both the tumor itself and the adjacent nerve tissues. Thus, radiotherapy can slow down tumor growth, but leads to pathological changes in the surrounding brain tissues, in particular, in the white matter. Unfortunately, the occurrence of structural damage to the white matter tissue is not always detected at early stages of development, which emphasizes the importance of finding new ways to detect them. The present study is aimed

at investigating the possibility of using optical coherence tomography (OCT) to detect radiation-induced changes in the white matter of the brain.
Materials and methods. The study was carried out on ex vivo rat brain samples divided into a control group and an X-ray exposed group. At 7 time points (2-14 weeks) after a single irradiation of the right hemisphere at a dose of 15 Gy, an OCT study of the frontal sections of the brain of laboratory animals was performed. The subsequent processing of the OCT data included the calculation of the attenuation coefficient in co-polarization Att(co), as well as the construction of color-coded maps. The study of the structural characteristics of the samples was carried out by staining histological sections with hematoxylin-eosin, as well as by immunohistochemical studies using antibodies to myelin basic protein (MBP). The corpus callosum was chosen as the region of interest for detailed study.
Results. Histological analysis of the studied samples made it possible to register the appearance of structural changes in the tissue of the corpus callosum at 2, 6 and 12 weeks after irradiation, which corresponds to periods of acute and early delayed injuries. At these time points, edema of the corpus callosum was identified, characterized by an increase in the spaces between the myelinated fibers. At the same time, 2 weeks after irradiation, edema was detected only in the region of the irradiated hemisphere, while at stages 6 and 12 weeks it was recorded in both hemispheres, which indicates the spread of the process along the myelinated fibers. An analysis of the OCT data made it possible to identify the corresponding changes in the values of the attenuation coefficient. Thus, at 2, 6, and 12 weeks, statistically significant decreases in the Att(co) value in the area of the corpus callosum in the irradiated hemisphere were found ($p<0.0086$) compared with the control group. In addition, at 6 and 12 weeks after exposure, a decrease in the attenuation coefficient was also noted in the region of the contralateral hemisphere ($p<0.0346$). Thus, the registered changes in the OCT signal confirm the detected structural damage.
Conclusion. In the present work, acute and early delayed radiation-induced changes in the white matter tissue were recorded, the reversibility of which is confirmed by their subsequent disappearance. These changes are characterized by a decrease in the scattering properties of the tissue, which can be detected using OCT.
The study was financially supported by Russian Science Foundation, project No23-25-00118.

**S9.605. Determination of the relative biological efficiency of a pencil scanning beam of protons with an energy of 90-150 MeV under irradiation of mice before and at the Bragg peak**

Strelnikova N.S.[1]*, Balakin V.E.[1], Rozanova O.M.[2], Smirnova E.N.[2], Belyakova T.A.[1], Shemyakov A.E.[1,2], Smirnov A.V.[1]
[1]*Physical Technical Center, Lebedev Physical Institute of RAS, Protvino, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*

* strelnikova.ns@lebedev.ru
Introduction. Currently, protons are most often used in radiation therapy with accelerated charged particles. The value of the relative biological effectiveness (RBE) of protons practically does not differ from that of photons, and in clinical practice, when planning exposure, a coefficient equal to 1.1 is used. In recent years, the fixed RBE value has been questioned from the standpoint of safety assessment, because if the dose in the tumor is too low, the risk of recurrence increases, and if it is too high, the probability of side effects increases. In recent years, the fixed RBE value has been questioned from the standpoint of safety assessment and efficacy of treatment, because if the dose in the tumor is too low, the risk of recurrence increases, and if it is too high, the probability of side effects increases. The RBE value depends on many factors, both of the biological nature (species, sex, and age of the animal; cultured mammalian cell lines; damage detection method, oxygen concentration in tissues; cell cycle stage; and conditions of in vitro cultivation and in vivo metabolism) and the physical and technical characteristics of irradiation (method of delivery and dose calculation, dose per fraction, linear energy transfer (LET) of particles (exposure before or at Bragg peak), composition and uniformity of the ion beam, and secondary radiation).
Purpose. Determination of the value of RBE of a pencil scanning proton beam depending on the linear energy transfer of particles after total irradiation of mice at doses of 6.5–8.5 Gy using the 30-day survival test.
Materials and methods. The experiments were carried out in accordance with international requirements on 2-month-old male mice of the SHK colony, which were kept under standard vivarium conditions (ITEB RAS). A total of 120 mice were used in the work. Each of the groups was divided into subgroups ($n = 10–15$) for exposure to different types of radiation and doses. For individual experimental points, two or three independent experiments were performed. Animals were irradiated totally in individual well ventilated containers.
Animals were irradiated with protons at the proton therapy complex Prometheus of the PTC LPI RAS (Protvino) with a thin scanning beam from one direction in a pulsed mode (pulse duration 200 ms, 1 pulse per 2 s) and a beam sigma of 2.8–3.6 mm in two ranges of the Bragg curve with doses of 6.5–8.5 Gy. Under irradiation before the Bragg peak with protons with a particle energy of 150 MeV, the LET, which was calculated using the planning program, was $0.7\pm0.04$ keV/μm. At the Bragg peak, the particle energy at the accelerator output was 91–123 MeV, with an average LET value of $2.5\pm0.7$ keV/μm. Dose control was performed with a PTW UNIDOS dosimeter (Germany) and a dosimetric film (EBT3, United States), absorbed dose error of ~ 5%. To determine the RBE coefficient, the control groups of mice were irradiated with hard X-ray radiation on an RUT device (200 kV, 2 keV/μm, 1 Gy/min; Shared-Use Equipment Center "Ionizing Radiation Sources Sector", Institute of Cell Biophysics, Pushchino). Then, within 30 days after the radiation exposure, the number of dead animals was counted daily, and the mice were weighed twice a week. The survival curves were obtained, according to which the dynamics of death and the average life span (AL) of the mice that died from irradiation were estimated.
Results and discussion. It was shown that survival of mice after irradiation with protons before and at the Bragg peak within the dose range studied, depended on the dose. The dynamics of death of animals under irradiation at a dose of 6.5 Gy practically did not differ, and by the 30th day the survival rate at the Bragg peak was 70%, and before the peak – 80%. A significant difference in the dynamics of death was observed only at 7.5 Gy: at the Bragg peak, the majority of animals died from days 10 to 13, and before the peak, death took place evenly throughout all 30 days. In all groups, regardless of the dose, the maximum weight loss of mice up to 25–30% was observed on days 12–14 after proton irradiation. The AL of dead animals was as follows: 1) at a dose of 6.5 Gy: at the Bragg peak – $18\pm4$ days, before the peak – $14\pm4$ days; 2) at a dose of 7.5 Gy: at the Bragg peak – $14\pm7$ days, before the peak – $17\pm9$ days; 3) at a dose of 8.5 Gy: at the Bragg peak – $12 \pm 4$ days, before the peak – $14 \pm 5$ days.
To determine the LD50/30 dose value, at which 50% of animals survive for 1 month and which is the basic radiobiological characteristic of radiation, probit analysis was used as a method for transforming mortality curves, on the basis of which LD50/30 values were calculated: for protons before the Bragg peak it was equal – 7.6 Gy, at the Bragg peak – 6.9 Gy, and for X-Ray – 5.5 Gy. According to the criterion of LD50/30 values, the RBE values before and at the Bragg peak were counted, which were 0.72 and 0.80, respectively; no statistically significant difference between the values was found. The obtained values are consistent with the results of a number of studies on the RBE determination by the acute skin reactions of mice irradiated at the hind leg

with a proton beam at the Bragg peak and with hard X-ray radiation, where the RBE value is in the range of 0.85–0.97. Experiments on mammalian cultures and tissues on assessing the short-term effects of protons at similar doses showed that, in the low LET range of 0.3-10 keV/μm, the RBE of protons was below or close to 1, that is, in the case of 2–3-fold increase in LET, no increase in RBE was observed, in contrast to other accelerated particles that are used in radiotherapy, the LET of which is much higher.

The data obtained will allow a wider use of the capabilities of the proton therapy complex Prometheus for the development of advanced therapy schemes, the search for new radioprotectors, as well as the clarification of radiation risks from galactic cosmic rays during long-term flights.

**S9.606. Development of a new class of theranostics for the purposes of hadron therapy**

Romanov M.V.[1]*, Shemyakov A.E.[1,2], Popov A.L.[1]
[1]*ITEB RAS;*
[2]*PTC LPI;*
* rmvya@yandex.ru

Radiosensitizers based on nanoparticles are considered as one of the most promising classes of theranostic agents. One of the most promising materials is gadolinium-doped cerium oxide (Ce0.9Gd0.1O2-x). Previously, we have shown that Ce0.9Gd0.1O2-x nanoparticles have radiosensitizing properties when exposed to X-rays, inducing the generation of hydroxyl radicals and hydrogen peroxide, and can also act as an effective MRI contrast agent [1]. Thus, this composition of the nanomaterial provides high catalytic activity under ionising radiation and the ability to visualise its localisation by MRI.

The cytotoxicity of this nanocomposite was evaluated on cultures of healthy (fibroblast line NCTC L929) and transformed (melanoma line B-16) cells. We found that dextran-stabilized Ce0.9Gd0.1O2-x nanoparticles at concentrations of 500, 250, 125, and 62.5 μg/ml did not cause cell death of both types of cell cultures after 24, 48, and 72 hours of co-incubation, but contributed to a decrease of their mitochondrial membrane potential in a dose-dependent manner. The results of the MTT test for both types of cells showed their viability in the region of 100% of the control, and statistical analysis did not reveal significant deviations for all concentrations studied. For the NCTC L929 line, the ratio of dead cells to their total number did not exceed 2%, and for the B-16 line it did not exceed 5% among all concentrations. On the first 2 days, nanoparticles at concentrations of 500, 250, and 125 μg/ml led to a decrease in the mitochondrial potential in NCTC L929 cells; on the third day, only the two highest concentrations led to this result. On the first day, Ce0.9Gd0.1O2-x nanoparticles led to a decrease in the mitochondrial potential of melanoma cells at all concentrations, and on the next 2 days, all concentrations except 62.5 μg/ml led to this effect. We have developed the design of the experiment with a culture of B-16 mouse melanoma cells to identify the radiosensitizing properties of various nanomaterials using the Prometheus proton therapeutic complex (JSC Protom) (doses of irradiation and positioning of the irradiated object were selected, as well as the conditions for the preparation and cultivation of cell culture). In the future, it is planned to evaluate the radiosensitizing properties of Ce0.9Gd0.1O2-x nanoparticles upon irradiation with a proton beam of cell cultures in the Bragg peak.

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**S9.607. Development of a radionuclide 223-radium delivery system based on amorphous calcium carbonate nanoparticles**

Akhmetova D.R.[1,2]*, Karpov T.E.[1,2], Antuganov D.O.[2], Sysoev D.S.[2], Timin A.S.[1,2]
[1]*Peter the Great St.Petersburg Polytechnic University;*
[2]*Russian Scientific Center of Radiology and Surgical Technologies named after Academician A.M. Granov;*
* ahmetova.darya1999@yandex.ru

Inorganic particles based on calcium carbonate have proven to be promising agents for the efficient delivery of biologically active compounds and radioisotopes due to their physical and chemical properties: hardness, large surface area, characteristic pH sensitivity and controlled biodegradation [1].

In biomedicine, amorphous structures of calcium carbonate are of interest, characterized by a porous structure, a spherical shape, and a high efficiency of loading therapeutic agents [2]. Therefore, a desirable direction is the synthesis of nanosized amorphous calcium carbonate and its use as a delivery system for therapeutic radioisotopes for the treatment of malignant tumors.

In this study, we used nanosized supports based on amorphous calcium carbonate obtained by coprecipitation of Na2CO3 and CaCl2 salts in the presence of polyacrylic acid (PAA) as a particle size stabilizer. This technique makes it possible to obtain a stable system of nanoparticles with a narrow average size (80–120 nm). Nanoparticles have high rates of biocompatibility and aggregative stability, which has been proven by several in vitro studies.

To use the nanoparticle system to deliver therapeutic radioisotopes for the treatment of malignant tumors, it is necessary to develop an effective radiolabeling protocol. In this work, a number of methods for incorporating the 223Ra isotope into carriers were tested:

(1) Isotope incorporation at the stage of salt coprecipitation – stepwise mixing of PAA+ CaCl2/RaCl2+ Na2CO3 solutions to form RaCO3 and incorporate it into the nanoparticles.

(2) Inclusion at the stage of "post-synthesis" by the method of physical adsorption - incubation of ready-made carriers in a solution of RaCl2.

(3) Incorporation of the isotope at the stage of salt coprecipitation followed by modification of the particle surface with biocompatible polymers (bovine serum albumin and tannic acid) by layer-by-layer deposition.

(4) Incorporation at the “post-synthesis” stage in the presence of Ba+ and SO4+ ions as catalysts for the Na+/Ra2+ ion exchange reaction and subsequent incorporation of the isotope into the nanoparticles.

To verify the effectiveness of the radiolabeling technique, parameters such as the efficiency of loading activity into particles and the radiochemical stability of the finished drug in physiological fluids were studied.

To measure the percentage loading of the radioisotope into the nanoparticles, after completion of the radiolabeling protocol, the particles were centrifuged at 14,000 rpm and the supernatant was removed from the sample. The activity was measured with a Triathler scintillation gamma camera (HIDEX, Finland). The percentage of isotope loading is calculated by comparing the initial activity with the nanoparticles remaining in the precipitate.

The radiochemical stability of the finished drug is measured by incubating the obtained nanoparticles with the included isotope in physiological saline and human blood serum with the measurement of activity in the particles and the incubated liquid for 1, 3, 7 days.

The results of these studies radiolabeling technique (4) showed high activity loading efficiency (≈70%) and radiochemical stability (>90% on day 7), which corresponds to generally accepted criteria for quality control of radiopharmaceuticals.

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**S9.608. Development of an integrated information system to study genetic regulators and physiological mechanisms of individual resistance to the formation of acquired neurodegenerative disorders**

Stepanov S.V.[3], Naydenov I.E.[1], Korchagina V.M.[1], Kololeeva V.C.[1,4], Glytov I.V.[1,5], Osypov A.A.[1,2,3]*
[1]*ITEB RAS;*
[2]*IHNA&NPh RAS;*
[3]*PushGU;*
[4]*IBH MPGU;*
[5]*RSMU;*
* aosypov@gmail.com

Neurodegenerative diseases are one of the most common causes of disability and death, and most of the strategies developed for their treatment are palliative and ineffective, despite years of efforts by medical and biologists. Studies have identified many risk factors and individual elements of pathological processes, but there is still no general understanding of the fundamental mechanisms of neurodegeneration and virtually no work devoted to an integrated analysis of data obtained by different methods in different research areas.
As part of scientific work on the study of genetic regulators and physiological mechanisms of individual resistance to the formation of acquired neurodegenerative disorders, we are developing an integrated information system for collection, storage, analysis and presentation of all types of data relevant to the issue under study: taxonomic, genetic, chemical, biochemical, physiological, electrophysiological, histological, behavioral and others. The system contains a constantly replenished set of tools for comprehensive data analysis, including bioinformatics and artificial intelligence methods, in order to reveal mechanisms of neurodegenerative pathology formation and search for methods of its prevention and treatment.
Hippocampus is a comprehensive information system consisting of a database and a set of tools that allow performing various types of analysis in manual, automatic or semi-automatic modes. The database is of mixed relational-file type and contains information on all types of data relevant to the question under study: taxonomic, genetic, morphological, physiological, including electrophysiological, chemical and biochemical. Data sources are reference international databases, data obtained from mass medical sources (results of experiments and analyses of patients), literature data and data obtained from direct experiments of our laboratory and its colleagues.
All objects of the information system allow complex cross-correlation structural and functional analysis due to the use of universal identifiers and can be grouped into the following sections: Bibliographic, Protocol Section, Taxonomic, Intraorganismal Localization Section, Chemical, Genetic, Electrophysiological, Behavioral, Histo-Morphological, Biochemical, Metabolic Pathways Section, Mathematical Modeling Section.
The presented information system can serve as a platform for research in the declared field, as well as a template for development of similar systems for similar tasks, and even a basis for creation of a unified complex of research and development support in the considered field of scientific and practical activity.

**S9.609. Development of combination therapy based on radionuclide, photothermal and chemotherapy using gold nanorods for the treatment of melanoma**

Peltek O.[1]*, Ageev E.[1], Postovalova A.[1], Rogova A.[1], Timin A.S.[1], Zyuzin M.V.[1]
[1]*ITMO University;*
* peltek.oleksii@gmail.com

Cancer is the leading cause of death globally, with 3 million new cases and 1.7 million deaths recorded annually.1 Traditional cancer treatments, including surgery, chemotherapy, and radiotherapy, are not always effective in completely eliminating solid tumors and their metastases, and can result in undesired side effects on healthy organs. Chemotherapy drugs, for example, lack targeting ability and can harm healthy cells. Thus, there has been a shift towards using a combination of cancer therapies, as the combination of different treatment modalities can improve efficiency and achieve a synergistic effect.
Recent advancements in nanostructured material design and application have boosted progress in combined cancer therapy. One such material is Au NPs, which can convert light into heat for locally induced hyperthermia which can be used for photothermal therapy (PTT).2 Currently there are numeral clinical trials of PTT that show promising results. PTT offers various advantages such as selective spatial and temporal targeting and increased immunogenicity, but it still has certain limitations.3 Combining PTT with other cancer therapies, such as radionuclide therapy and chemotherapy, which have different mechanisms of action, can overcome these limitations.4
In this study, we investigate the synergistic effect of treating melanoma tumors with radio-, photothermal- and chemo- combined therapy using gold nanorods (Au NRs). For this, Au NRs with the maximum plasmon band at 1160 nm, which enables an effective light conversion5 and subsequent heating under near infrared (NIR) light, were radiolabeled with the therapeutic isotope rhenium-188 (188Re). For the maximization of melanoma treatment efficiency, third therapy, namely, chemotherapy, was employed by introduction of a clinically relevant cytostatic drug (Paclitaxel, PTX).
The development of multimodal nanocarriers with combined chemo-, radionuclide and photothermal therapy properties against melanoma tumor growth was achieved. The photothermal efficient Au NRs were synthesized and radiolabeled with therapeutic isotope 188Re for internal radionuclide therapy, with a 96% radiolabeling efficiency and high in vitro stability. Intratumoral injection showed steady distribution of 188Re-Au NRs throughout the tumor without significant leakage of isotopes. The combination of radionuclide therapy with 188Re-Au NRs, photothermal therapy with Au NRs, and chemotherapy with PTX resulted in synergistic inhibition of tumor growth in B16-F10 melanoma tumor-bearing mice. The combined therapy using 188Re-Au NRs showed high therapeutic efficacy against B16-F10 melanoma tumor without significant side effects on healthy tissues. The favorable properties of Au NRs suggest great potential for combined radio-, photothermal and chemotherapy in future clinical melanoma tumor treatment.
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**S9.610. Development of the specialized platform for bioprinting tissue-engineered equivalents of eye structures and components of organ-on-chip systems for application in experimental ophthalmology**

Kravchenko S.V.[1]*, Myasnikova V.V.[1,2], Sakhnov S.N.[1,2]
[1]*Krasnodar branch of S.N. Fedorov National Medical Research Center "MNTK "Eye Microsurgery", Krasnodar, Russia;*
[2]*Kuban State Medical University, Krasnodar, Russia;*
* ksv.1991@yandex.ru

The studying of the physiology and biophysics of visual analyzer and testing new approaches in the treatment of its diseases needs advanced methods. The tissue equivalents of various structures of the eye, as well as microphysiological systems of an organ-on-a-chip are important methods for it. An example of the using of tissue-engineered equivalents in ophthalmology is tissue-engineered corneal equivalents, including innervated ones, which allow studying the interaction of corneal nerves and stroma, assessing the mechanical and optical properties of tissue-engineered corneas and the prospects for their using for keratoplasty [1]. The organ-on-a-chip technology makes it possible to model the cornea and the eye surface together with its auxiliary apparatus with high accuracy, which is important for studyingof the pathogenesis of dry eye disease (biophysical processes that depend on the evaporation of tear and its osmolarity) and the search for effective methods its therapy. It is also possible to model the retina and its hematoretinal barriers, which allows reproducing age-related macular degeneration, diabetic macular edema, diabetic retinopathy, and glaucoma for screening neuroprotective and antiglaucoma drugs [2]. In both cases, it is possible to use bio- and 3D-bioprinting [3, 4].
The aim of this work was to develop a specialized platform for bioprinting of tissue-engineered equivalents of eye structures and components of organ-on-a-chip systems for application in experimental ophthalmology.
The designed device has a moving table (moves along the Y axis) and an extruder holder (moves along the X and Z axes). The extruder holder allows installing various types of extruders and printheads. The printing field size (30*35*20 mm) is optimized for most eyeball structures and organ-on-chip systems, which, unlike many open source systems, which using ordinary 3D printing platforms. It allows optimizing the overall device's size, for placement it in a laminar flow cabinet, and reduces vibrations, increasing the accuracy and quality of printing. The system supports two concepts: 3D printing, which involves the creation of 3D volumetric objects, and bioprinting on a plane, for example, printing cells on the surface of an adhesive material in one layer. The current version of the platform will use a print head developed for it with a syringe-type extruder, which allows 2D and 3D printing with various gels containing cells or without them, as well as dosing and accurately applying a medium with a suspension of various types of cells on adhesive surfaces and distribute them among the chambers of the organ-on-chip microfluidic system.

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**S9.611. Developmental exposure to endocrine disrupting chemicals affects secretory machinery of catecholamine-producing cells**

Obernikhin S.S.[1]*, Yaglova N.V.[1], Timokhina E.P.[1], Yaglov V.V.[1], Nazimova S.V.[1]
[1]*Avtsyn's Research Institute of Human Morphology of Federal State Budgetary Scientific Institution «Petrovsky National Research Centre of Surgery»;*
* ober@mail.ru

The secretory machinery of catecholamine-producing cells includes formation of secretory granules, intracellular transport, deposition, and excretion of products by both exocytosis and molecular secretion. Endocrine disruption of secretory process in chromaffin cells is poorly studied. Dichlorodiphenyltrichloroethane (DDT) is known as the most wide-spread environmental contaminant with long period of half-life and a potent endocrine disrupter affecting sex steroid, mineralocorticoid, glucocorticoid and thyroid hormone production [1,2]. Exposure of growing organism to endocrine disruptor DDT has been shown to significantly decrease production of adrenaline and norepinephrine by adrenal chromaffin cells in rats [3]. The present study is aimed at evaluation of secretory process in adrenal chromaffin cells in postnatal period in rats exposed to low doses of DDT during prenatal and postnatal development. mechanisms of these disorders have been studied.
Female Wistar rats received solution of o,p-DDT 20μg/l ("Sigma-Aldrich", USA) ad libitum instead of tap water since mating, during pregnancy and lactation (n=10) and other female rats received the same solution of DDT only during lactation (n=10). After weaning the progeny of the rat dams received the same solution of o,p-DDT during postnatal development. The main experimental group included male rats (n=20) exposed to low doses of o,p-DDT prenatally and postnatally. A group of male rats (n=20) exposed to DDT only during postnatal development, was included in the experiment to differentiate effects of prenatal exposure. The male offspring (n=22) of intact rat dams was used as a control. Half of the control and exposed rats were sacrificed in pubertal and postpubertal periods. Light and transmission electron microscopy and morphometry were used for adrenal medulla examination. Tyrosine hydroxylase production was detected by immunohistochemistry. Plasma epinephrine levels was quantified by enzyme-linked immunosorbent assay.
In pubertal rats exposed to DDT from the conception, a significant decrease in number of mitochondria and secretory granules and smaller sizes of chromaffin cells and nuclei were revealed as well as downregulation of tyrosine hydroxylase expression and decrease in epinephrine production. The findings indicate a multiple mechanism of reduced epinephrine production. After puberty, intact rats showed a physiological decrease in the production of catecholamines by the adrenal glands, despite an increase in the size of the adrenal medulla and a high level of tyrosine hydroxylase. The study revealed an age-dependent decrease in the number of mitochondria in chromaffin cells, which ensure the release of secretory granules. In rats developmentally exposed to DDT, slowdown of the medulla growth and a decrease in the synthesis of tyrosine hydroxylase were observed. Electron microscopy revealed a smaller number of mitochondria along with destructive changes. Rats exposed to DDT postnatally did not have medulla growth retardation and, however, experienced an earlier decrease in the number of mitochondria, leading to accumulation of secretory granules in the cytoplasm and reduced release of adrenaline into the bloodstream. In the

cytoplasm, signs of hydration of secretory granules and dissolution of their contents were observed.
Thus, along with the dysmorphogenetic mechanism, the endocrine disruptor DDT changed the secretory machinery in chromaffin cells, negatively affecting the rearrangement of the mitochondrial apparatus and granule exocytosis, which led to the activation of molecular secretion as a compensatory mechanism in response to a decrease in catecholamine production. Evaluation of different regimens of exposure showed that impaired secretory machinery of adrenal chromaffin cells was evoked by postnatal exposure to DDT.
The research was funded by the Ministry of Science and Higher Education of the Russian Federation, reg. number FGFZ-2022-0035.
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### S9.612. Differences in spleen morphodynamics in rats developmentally exposed to endocrine disruptor DDT

Gagulaeva B.B.[1], Yaglova N.V.[1]*, Timokhina E.P.[1], Obernikhin S.S.[1], Yaglov V.V.[1], Nazimova S.V.[1]
[1]*Avtsyn's Research Institute of Human Morphology of Federal State Budgetary Scientific Institution «Petrovsky National Research Centre of Surgery»;*
* yaglova@mail.ru

Morphodynamic processes in organism reflect realization of genetic program and may be affected by plenty of internal and external factors. Among external factors endocrine disruptors are of great concern as universal pollutants [1]. Endocrine disruptors are mainly anthropogenic chemicals that disrupt endocrine system functioning and the regulation of homeostasis by endogenous hormones [2]. Endocrine disruptors are of great concern due to ability to penetrate placental barrier [1,2]. The ability of endocrine disruptors to provoke changes in morphodynamic processes remains an open question. The aim of this study was to determine the main parameters of morphodynamics in rats exposed to low doses of the wide-spread endocrine disruptor dichlorodiphenyltrichlorotane (DDT) during prenatal and postnatal development. Female Wistar rats received solution of o,p-DDT 20μg/l ("Sigma-Aldrich", USA) ad libitum instead of tap water since mating, during pregnancy and lactation (n=5). After weaning the progeny of the rat dams received the same solution of o,p-DDT during postnatal development. The main experimental group included male rats (n=22) exposed to low doses of o,p-DDT prenatally and postnatally and male progeny of intact dams (n=23) were examined. The rats were sacrificed at the age of 1 (newborns) and 7 (suckling period) days, 6 (puberty) and 10 (adult) weeks. Body and spleen masses were measured. Histology and computer histomorphometry of spleen sections were evaluated. The data were statistically processed.
No significant differences in organ mass were found in newborn rats. In the suckling and pubertal period, the dynamics of the organ mass was similar to the control, that is, it increased with age, especially before puberty. After puberty an increase in organ mass was insignificant in the control rats, and in rats that developed under low-dose exposure to DDT, a significant increase in organ mass was revealed after sexual maturation. The relative mass of the organ also significantly exceeded the values of the control group. Evaluation of the morphodynamics of the spleen parenchyma found that development of the white pulp in the control rats differed from the development of the organ as a whole. In newborn rats, the white pulp was absent, and its growth began later. A similar pattern was observed in animals developmentally exposed to DDT. Assessment of the white pulp parameters showed that the age-related morphodynamics of lymphoid formations was the same in the compared groups at early stages of postnatal development.
The study showed that in rats that exposed to DDT during ontogeny, morphodynamic processes in the spleen differs from the control with age. After pubertal period the increase in the organ mass, that is, its growth, does not stop, but, on the contrary, was activated. Postpubertal growth of the spleen was provided by active development of lymphoid tissue as well as red pulp. Thus, the low-dose exposure to the endocrine disruptor DDT disturbs morphodynamic processes in the spleen.
This work was supported by the Russian Science Foundation (Grant No: 23-25-00012).
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### S9.613. Does enrichment of organism with deuterium influence thyroid function?

Yaglova N.V.[1]*, Obernikhin S.S.[1], Timokhina E.P.[1], Yaglov V.V.[1], Nazimova S.V.[1]
[1]*Avtsyn's Research Institute of Human Morphology of Federal State Budgetary Scientific Institution «Petrovsky National Research Centre of Surgery»;*
* yaglova@mail.ru

Deuterium, a stable isotope of hydrogen is abundant in living organism. Scientific investigations have shown that deuterium plays an important role since it participates in control of cell proliferation, apoptosis, and senescence [1-3]. Impact of deuterium content in function of cells and inner organs, especially endocrine glands, is poorly studied. Our previous investigations revealed that short-term reduction in deuterium supply evoked rapid response of the thyroid gland [4].
The investigation was aimed at evaluation of changes in thyroid hormones production and pituitary regulation of thyroid function in response to pronounced and prolonged deuteration of organism. The experiment was performed on adult male Wistar rats weighed 200-220gg (n=20). The rats were housed in local vivarium at room temperature and given a pelleted standard chow. Consumption of deuterium-enriched water with [D]=500000 ppm (manufactured by S.-Peterburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Center "Kurchatov Institute", Russia) instead of tap water for 21 days was used as a method of reduction of deuterium body content. The control rats (n=10) consumed distilled water with deuterium content [D]=146ppm, identical to tap water. Total and free fractions of thyroid hormones andthyroid stimulating hormone were quantified in serum by enzyme-linked immunosorbent assay.
The control rats demonstrated an increase in the body mass by the 21st day of the experiment. The rats that consumed deuterium-enriched water showed significantly smaller growth, and their mass on the

21st day of the experiment was significantly less than in the control group. After 24h of exposure enhanced total thyroxine production and regular decrease in thyroid stimulating hormone secretion were registered. Subsequently, the production of thyroid hormones continued to increase, but by the 7th day it returned to normal parameters. However, thyroid stimulating hormone levels remained low. On the 14th day, a significant decrease in the concentration of all thyroid hormones and thyroid stimulating hormone was revealed. By the 21st day, the production of thyroid hormones increased to control values, and the feedback principle was restored, which was manifested by a decrease in thyroid stimulating hormone secretion in response to an increase in the production of thyroid hormones.

The revealed alterations indicate that thyroid gland is sensitive to shifts in hydrogen isotope balance. The thyroid gland demonstrated higher sensitivity to deuterium/protium disbalanse than the hypothalamic-pituitary complex. An increase in the deuterium content in the systemic circulation exerted a stimulating effect on secretory processes in the thyroid gland, and then led to a decrease in thyroid stimulating hormone secretion by the pituitary gland and the development of transient hypothyroidism. Restoration of hormone production and pituitary control of thyroid activity indicated that deuteration does not inhibit hormone synthesis in the thyroid gland. Determination of the free fractions of thyroid hormones in the exposed rats showed that their concentrations did not undergo significant changes, in contrast to the control group, that is, the level of the unbound hormones became more stable with an increase in deuterium body content. It indicates that shifts in deuterium/protium balance do not significantly affect either production of plasma binding proteins or their binding capacity.

Thus, thyroid cells demonstrated higher sensitivity to changes in deuterium body content and responded by increase of hormone secretion to higher deuterium content unlike pituitary thyrotropes, which on the contrary, responded later by reduction of hormone production during deuteration of the organism. Consequently, the intensity of secretory processes in the thyroid gland depends to some extent on the deuterium gradient in the blood and cells and in the cytosol and organelles.

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## S9.614. E2F1 Deacetylation by HDAC1 after Peripheral Nerve Transection

Dzreyan V.A.[1]*, Guzenko V.V.[1], Kalyuzhnaya Yu.N.[1], Romanchenko E.D.[1]
[1]*Southern Federal University;*
* dzreyan2016@mail.ru

Not only histones, but also non-histone proteins such as chaperones, signaling proteins, and transcription factors are subjected to posttranslational acetylation/deacetylation by histone acetyltransferases (HAT) and histone deacetylases (HDAC). The activity of non-histone proteins, protein-protein interactions, and their cellular localization is regulated via acetylation/deacetylation, which determines cell growth, differentiation, migration, and survival both in norm and pathology.

Peripheral nerve injury is one of the common causes of disability and mortality and, as a consequence, one of the current problems of public health not only in Russia, but all over the world. The situation is worsened by the lack of effective neuroprotective agents, especially for the first hours after injury. According to recent studies, the transcription factor E2F1 is one of the nonhistone substrates of some HDACs. It is known to regulate the expression of pro-apoptotic proteins such as caspases 3, 7, 8, and 9, SMAC/DIABLO, Apaf-1, p53, p73, and Bcl-2 family proteins and thereby promote apoptosis. However, there are very few studies on acetylation/deacetylation of nonhistone proteins, including E2F1, in neurodegeneration, and there are almost no studies on these processes in peripheral nerve injuries.

Work objective. To study the processes of acetylation and deacetylation of the E2F1 transcription factor in mammalian PNS neurons and glial cells in different periods after peripheral nerve axotomy.

Materials and Methods. The model of peripheral nerve injury was axotomized rat dorsal root ganglion (DRG) obtained by sciatic nerve transection. The experiments were performed on adult male rats (Wistar, wild-type), weighing 200-250 g. Sciatic nerve transection and DRG isolation were performed according to the standard protocol described by Savastano et al. Quantitative PCR was performed using qPCRmix-HS SYBR. The primers were synthesized at Eurogen, CJSC. Immunoblotting and double immunofluorescence microscopy were used to estimate the expression and intracellular distribution of acetylated forms of E2F1 in rat dorsal ganglia cells. Immunoblotting was performed by semi-dry transfer using the Trans blot Turbo system 1, 4, 24 hours, or 7 days after axotomy, combining 4th and 5th DRGs from three rats. To confirm the interprotein interaction between HDAC1/acetylated E2F1(K120), co-immunoprecipitation was performed with a kit from the Sileks company using magnetic particles with G protein according to the manufacturer's recommendations. The histone deacetylase activity of HDAC1 towards E2F1 was determined in the obtained immunoprecipitate. The study was performed using a commercial solid-phase enzyme immunoassay kit for HDAC1. Visualization of protein-protein interactions was performed using Duolink® PLA (proximity ligation assay) technique. The nonselective HDAC class I inhibitor sodium valproate (i.p. 300 mg/kg) was used to study the mechanisms of involvement of the studied proteins, acetylation, and deacetylation in ganglion cell death, and the possibility of neuroprotection after the neurotrauma (sciatic nerve transection). Statistical analysis was performed using ANOVA.

Results. In the axotomized DRGs, the earliest and most specific changes were observed in HDAC1 histone deacetylases, whose expression increased as early as in one hour after the sciatic nerve transection. The expression of the E2F1 transcription factor in axotomized ganglion neurons increased in four hours. Acetylated E2F1 (K120) levels decrease in the cytoplasm of ganglion neurons in 24 hours after axotomy. The neurotrauma induces the translocation of HDAC1 and E2F1 from the nucleus to the cytoplasm in the first 24 hours after the injury. Sciatic nerve axotomy is associated with increased expression and activity of HDAC1 in axotomized rat ganglia, resulting in a decrease of acetylated E2F1 (K120) in the neuronal cytoplasm. HDAC inhibitor administration for seven days after sciatic nerve transection decreases the level of E2F1 in the cytoplasm but increases it in the nucleus, indicating a redistribution of the transcription factor between the nucleus and the cytoplasm, which is also indicated by the colocalization coefficient with the nuclear marker. Thus, administration of the HDAC class I inhibitor sodium valproate withdraws the axotomy-induced translocation of E2F1 from the nucleus to the cytoplasm and increases the level of E2F1 acetylation at K120 in ganglion neurons, protecting their cells from apoptosis. This suggests that, firstly, the level of protein acetylation depends on the deacetylase activity of HDAC1, and secondly, it affects the intracellular localization of the protein.

Summarizing all the data:
1. Protein-protein interactions of the E2F1 transcription factor with HDAC1 were studied.
2. After identifying the "substrate-enzyme" protein pair, the deacetylase activity of HDAC1 towards E2F1 was determined.
3. This allowed us to establish the dependence between acetylation of the regulatory protein and its activity towards downstream targets, as well as apoptosis of ganglion cells after axonal damage using a HDAC class I inhibitor.
The obtained data will enable new ways of viewing the functions of histone acetyltransferases and histone deacetylases, which may be more extensive than the transcription regulation. This knowledge will underlie a theoretical framework that can be further used to develop novel selective HDAC inhibitors as potential neuroprotectors that protect neurons and glial cells in the early periods after axonal stress.
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**S9.615. Effect of local heating on cardiovascular oscillatory processes in type 2 diabetes mellitus**

Tikhonova I.V.[1], Grinevich A.A.[1], Guseva I.E.[2], Tankanag A.V.[1]*
[1]*Institute of Cell Biophysics RAS;*
[2]*Hospital of Pushchino Scientific Centre of Russian Academy of Sciences;*
* tav@icb.psn.ru

The concept of network physiology considers the body as an integrated network of organs and systems connected at different spatial and temporal scales to coordinate their functionality. The harmonious interaction of regulatory mechanisms ensures the normal activities of individual organs and systems, as well as the whole organism. Heart rate variability (HRV) analysis is one of the widely used non-invasive methods to assess contribution of sympathetic and parasympathetic components of autonomic nervous system in regulation of cardiac function. The functioning of large central and small peripheral vessels is regulated by different physiological factors that determine peripheral hemodynamics. There are various non-invasive methods for monitoring peripheral microvasculature, among which laser Doppler flowmetry (LDF) and photoplethysmography (PPG) are the most accessible and popular. The use of these methods in combination with local heating is one of the common methods to study microvascular functional state and detection of associated complications in various pathologies, including type 2 diabetes mellitus (T2DM). It is known that in T2DM thermoregulation is impaired, which may lead to decreased skin vasodilation of extremities due to the development of neurovascular dysfunction. Analysis of changes in mechanisms of HRV and skin hemodynamic regulation in response to local heating in T2DM patients may be an indicator of early microvascular dysfunction in noninvasive diagnosis. The aim of the study was to assess changes in spectral components and phase relationships between HRV, tissue blood volume dynamics and skin blood flow oscillations in upper and lower extremities of healthy volunteers and T2DM patients in response to local heating.
Twenty-two T2DM patients (5 males and 17 females) and twenty-two healthy volunteers (8 males and 14 females) participated in the study. Measurement procedures were conducted in a quiet room at 23 ± 1°C after a 15-minute adaptation period. All subjects were in supine position during registration. Five 35-minute signals were recorded simultaneously for each subject - electrocardiogram (ECG), tissue blood volume dynamics of the right index finger pad (PPGfg) and the right second toe pad (PPGtoe), as well as skin blood flow dynamics from the outer surface of the right forearm near the wrist joint (LDFfm) and of the right foot between the heads of the 1st and 2nd metatarsal bones (LDFft). The local heating test was performed by heating both skin sites from 32 to 38 0C in the area where LDF probes were placed. For each participant, two 15-minute fragments of the entire 35 min signals analyzed: the initial 15 minutes without heating (rest) and the last 15 minutes of probe (heating). Amplitude-frequency spectra were analyzed using adaptive wavelet transform, as well as phase interactions between pairs of analyzed signals were assessed by the value of wavelet phase coherence (WPC) function. Statistical significance of obtained WPC values was estimated by surrogate method, the number of which was equal to 100. Statistical analysis was performed using Wilcoxon tests for independent samples, significance differences were considered reliable at $p < 0.05$.
The amplitudes of HRV ultra-low (< 0.04 Hz) and low (0.04 - 0.15 Hz) frequency components were significantly lower in patients both at rest and during local heating compared to controls. Amplitudes of respiratory oscillations (~ 0.3 Hz) of forearm blood flow (as measured by LDF) were higher at rest and during heating in patients versus healthy subjects. In comparison with controls the amplitudes of myogenic (~0.1 Hz) oscillations of foot blood flow were lower at rest and the amplitudes of cardiac (~1 Hz) oscillations - under local heating. No significant differences in spectral components of finger and toe tissue blood volume oscillations (as measured by PPG) were found between patients and healthy participants neither at rest nor under local heating. In patients WPC values for myogenic (~0.1 Hz) oscillations of LDFfm - LDFft and PPGfg - PPGtoe pairs were lower as compared to controls. There were no significant differences between patients and controls for these signal pairs under local heating. In addition, no significant changes were found between WPC values characterizing the phase relationship between HRV and skin blood flow oscillations of both extremities neither at rest nor under local heating. For phase interactions between HRV and toe tissue blood volume dynamics a significant decrease of patient WPC values at ~ 0.1 and ~ 0.3 Hz both at rest and during local heating was revealed, while for finger tissue volume no differences were detected. The results obtained can provide the basis for development of methods for early noninvasive diagnosis of microvascular disorders and ways of their therapeutic correction in various socially significant diseases, including T2DM.
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**S9.616. Effect of methylglyoxal-modified human serum albumin on myeloperoxidase activity and neutrophil function**

Panasenko O.M.[1,2]*, Ivanov V.A.[1], Mikhalchik E.V.[1], Gorudko I.V.[3], Grigorieva D.V.[3], Basyreva L.Yu.[1], Gusev S.A.[1], Kostevich V.A.[1,4], Gorbunov N.P.[1,4], Sokolov A.V.[1,4]
[1]*Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia;*
[2]*Pirogov Russian National Research Medical University, Moscow, Russia;*
[3]*Belarusian State University, Minsk, Belarus;*
[4]*Institute of Experimental Medicine, St. Petersburg, Russia;*
* o-panas@mail.ru

Methylglyoxal (MG) is a highly reactive α-ketoaldehyde generated in human body as a by-product of a number of metabolic pathways and due to non-enzymatic glucose oxidation in hyperglycemia and diabetes mellitus [1]. Myeloperoxidase (MPO; EC 1.11.2.2) stored in neutrophil azurophilic granules catalyzes generation of reactive halogen species which is a key factor of neutrophil antibacterial activity [2]. It has been shown that exposure of neutrophils to advanced glycation end products (AGEs) stimulates cellular expression of MPO [3]. The previously discovered paradoxical growth in phagocytic capacity and in MPO expression together with increase in intracellular bacterial persistence in AGEs-stimulated neutrophils made us hypothesize that AGEs could inhibit MPO enzymatic activity and hence, bactericidal function of neutrophils [4]. Our aim was to elucidate if modification of human serum albumin (HSA) under hyperglycemia-like conditions

affects enzymatic activity of MPO, its release from neutrophils by degranulation and NETosis, generation of reactive oxygen (ROS) and halogen species (RHS) by neutrophils, which are key factors of neutrophil bactericidal activity.
Methods. Peroxidase activity of MPO was registered by oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and its chlorinating activity – by decolorization of Celestine blue B dye. Binding of HSA-MG to MPO was studied by disk-electrophoresis and also by solid phase immunoassay using monoclonal antibodies to MPO. ROS and RHS generation were detected by lucigenin and luminol chemiluminescence (CL), respectively. Neutrophil degranulation was assessed by flow cytofluorometry using the fluorescent labeled antibodies to the marker proteins CD63 from azurophilic granule and CD11b from peroxidase-negative specific/gelatinase granules. NETosis was assayed by quantifying of NETs in blood smears colored by Romanovsky.
Results. It was shown that HSA-MG binds to MPO giving a stable complex (Kd = 1.1 nM) which is consistent with the data on competition of HSA-MG with monoclonal antibodies to MPO. HSA-MG non-competitively inhibited peroxidase and chlorinating activity of MPO. It also induced degranulation of peroxidase-negative granules with no impact on exocytosis of the content of azurophilic granules, including MPO. Moreover, HSA-MG enhanced lucigenin CL of neutrophils in itself or under their stimulation with phorbol 12-myristate 13-acetate (PMA) suggesting effects on priming and activation of NADPH-oxidase mediated ROS production. At the same time, HSA-MG did not influence luminol CL of neutrophils which depends mainly on MPO-mediated RHS production. HSA-MG did not induce NETosis and did not affect NETosis stimulated with PMA.
Conclusions. Thus, HSA modified under hyperglycemia-like conditions, stimulated NADPH oxidase in neutrophils but did not activate their functions dependent on azurophil granule degranulation and MPO secretion, inhibiting its enzymatic activity. This phenomenon could underlie downregulation of bactericidal activity of MPO and neutrophil, and hence, of innate immunity, giving rise to wound healing impairment and susceptibility to infection in patients with hyperglycemia.
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### S9.617. Effect of nitric oxide and hydrogen sulfide donors on the degree of mast cell degranulation in the dura mater of the rat brain

koroleva K.S.[1]*, Nurmieva D.A.[1], Petrova K.A.[1], Sitdikova G.F.[1]
[1]*Institute of Fundamental Medicine and Biology;*
* k.s.koroleva@yandex.ru

Introduction. Migraine is a neurological disease characterized by severe headaches and difficult to treat. The pathophysiology of migraine involves the trigeminovascular system, as well as mast cells, which, localized in the dura mater near the blood vessels and afferents of the trigeminal nerve, form a neuroimmune synapse. In this structure, active substances secreted by mast cells can activate nearby nociceptive fibers, and compounds released from active fibers, in turn, can lead to their degranulation.
To study migraine, a model was developed using nitroglycerin, an NO donor, that provokes the onset of migraine pain. However, the literature mainly considers the vascular role of nitric oxide in the pathogenesis of migraine and there is insufficient data on the effect of NO on the state of mast cells of the meninges of the brain. At the same time, there is evidence of the participation of another gaseous mediator, such as hydrogen sulfide (H2S), in the process of nociceptive signal formation during a migraine attack. It is known that endogenous hydrogen sulfide can have a pro-nociceptive effect in the trigeminal afferents due to the activation of TRP receptors. At the same time, it was shown that the donor of hydrogen sulfide, sodium hydrosulfide (NaHS), reduces the activity of P2X3 receptors in the trigeminal nerve endings and in the soma of the trigeminal ganglion neuron. Whereas the effect of H2S on the state of mast cells of the rat brain membrane is not considered.
Aim of the study. Effect of NO and H2S donors on the degree of mast cell degranulation in the dura mater of the rat brain.
Materials and methods. To study the effect of gas mediators on the state of mast cells, intact brain membranes were incubated in a solution containing the test substance. To study mast cell degranulation, we used a histological method with staining of the dura mater of the rat brain with toluidine blue. Shooting of finished preparations was carried out using a PC at a x20 increase. The degree of mast cell degranulation was assessed visually, calculations were made as a percentage of the total number of cells.
Results. The results of the experiments showed that incubation in the H2S donor - NaHS - did not lead to a change in the morphology of mast cells, and the number of degranulated cells did not exceed the control values (26.4±4.1%; n=4). We have shown that pre-incubation in NaHS for 10 min followed by the addition of ATP (100 μM) to the incubation solution for 20 min also did not cause an increase in the number of degranulated mast cells (28.4±2.9%; n=4). Whereas incubation of membranes in ATP caused a significant increase in the number of degranulated cells (49.4±3.02%; n=4; $p<0.05$) compared with the control group (22.4±1.8%; n=4; $p<0.05$). In addition, it was shown that incubation with the addition of the P2X7 receptor agonist BzATP led to an increase in the number of degranulated cells up to 43.5±4.2% (n=4, $p<0.05$;) and this effect decreased against the background of preincubation in the presence of NaHS.
Thus, at the level of mast cells, the modulating effect of H2S on the pronociceptive action of ATP in the trigeminal nerve can also be manifested by reducing the activity of P2X7 receptors and preventing ATP-induced degranulation of rat brain mast cells.
Whereas incubation of the drug in a solution containing an NO donor (sodium nitroprusside, SNP 200 μM) did not lead to degranulation of mast cells in the meninges of the rat brain, which is consistent with previous studies where exogenous NO donors did not change the state of mast cells under ex vivo conditions. Apparently, degranulation is caused by systemic administration of an NO donor, due to the release of pro-inflammatory compounds from endothelial cells of vessels and nerve endings.
Conclusions. NO donor (SNP) and H2S do not cause degranulation of mast cells in the meninges. However, the H2S donor, NaHS, prevents mast cell degranulation under the action of ATP, which may be due to a decrease in the activity of P2X7 receptors.
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**S9.618. Effect of xylazine-zoletyl anesthesia on the radiosensitivity of mice exposed to radiations with different linear energy transfer values**

Belyakova T.A.[1]*, Balakin V.E.[1], Rozanova O.M.[2], Smirnova E.N.[2], Strelnikova N.S.[1]
[1]*Physical Technical Center, Lebedev Physical Institute of RAS, Protvino, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* belyakovata@lebedev.ru

Introduction. Recently, in connection with the use of accelerated charged particles for cancer therapy, which requires high-precision control of patient positioning, it becomes necessary to use anesthesia, especially for children and the elderly. Anesthesia affects many physiological parameters that influence both the outcome of treatment and the radiosensitivity of the organism. The observed effects depend on the composition of the anesthetic mixture, concentration, mode of administration, species and age of animals, the criterion for registration of lesions, and the range of doses for total or local irradiation. It is also relevant to search for new experimental animal models for studying the effect of charged particle sources, which require convenient and safe methods of animal immobilization for precise positioning of the target in certain coordinates when irradiated with protons or carbon ions, as well as adjusting the target irradiation taking into account movements, related to breath.
Purpose. Study of the effect of anesthesia on the radiosensitivity of mice by a 30-day survival test with total irradiation of mice with beams of protons and carbon ions in vivo before and at the Bragg peak, as well as with X-rays.
Materials and methods. The experiments were carried on 2-month-old male mice of the SHK colony. A total of 405 mice were used in the work. Animals were divided into two groups for irradiation with and without anesthesia. Each of the groups was divided into subgroups (n=10–15) for exposure to different types of radiation and doses. For individual experimental points, two or three independent experiments were performed. Animals were irradiated totally in individual well ventilated containers. Mice were anesthetized 10 min before irradiation with a combination of selective drugs Xylazine - 0.7 mg/kg and Zoletil 100 - 3.4 mg/kg. The depth of anesthesia was assessed visually by the absence of uncoordinated movements in the mouse and a negative response to a painful stimulus (pinching the tail with tweezers). The selected scheme of anesthesia ensured the immobilization of mice during their laying and irradiation, as well as 100% recovery of mice from anesthesia without the administration of additional antagonistic drugs and the absence of animal death. Animals were irradiated with protons at the proton therapy complex at the PTC LPI RAS (Protvino) with a pencil scanning beam at an interval of doses of 6.5–8.5 Gy before the Bragg peak (LET=0.7 ± 0.04 keV/μm) and at the Bragg peak (LET=2.5 ± 0.7 keV/μm), and carbon ions with an energy of 450 MeV/nucleon at a dose of 6.5 Gy before the Bragg peak (LET=15 keV/μm) and at the Bragg peak (LET=39 keV/μm) using the U-70 Carbon Beam Radiobiological Stand of the National Research Center Kurchatov Institute–IHEP (Protvino). As a positive control, mice were irradiated with X-ray radiation in the dose range of 6.0–8.5 Gy using a RUT device (LET=2 keV/μm) at the Shared Core Facilities of the Pushchino Scientific Center for Biological Research.
Results and discussion. Irradiation of mice with X-rays in the presence of anesthesia resulted in a significant increase in the 30-day survival ($p \leq 0.01$) at all doses. The average life span (AL) of dead mice did not depend on the use of anesthesia in the studied dose range.
Anesthesia had no effect on the dynamics of mortality of mice, the course of radiation sickness and the AL of dead animals after irradiated with protons at the Bragg peak at doses of 6.5 and 7.5 Gy, as well as before the Bragg peak at all doses, but after irradiation at the Bragg peak with a dose of 8.5 Gy, a significant increase in survival was observed: by day 30, as many as 43% of animals survived compared to the unanaesthetized mice, where 7% survived.
The dose modification factor (DMF) was calculated under the action of anesthesia for X-rays and protons. For X-rays, DMF=1.13, which indicates a weak protective effect of anesthesia in the dose range studied. For protons, DMF=0.94 at the Bragg peak and DMF=0.95 before the peak; that is, no effect of anesthesia on the radiosensitivity of mice was detected.
The effect of anesthesia on the 30-day survival of mice irradiated with carbon ions at a dose of 6.5 Gy was significant, both in the position before the peak and at the Bragg peak. After irradiation of mice without anesthesia, 100% death was observed at the Bragg peak by day 7, and when anesthesia was used, 30% of the animals survived to 30 days, the main mortality was observed within 10 days. When mice were irradiated with carbon ions before the Bragg peak, the 30-day survival rate in the experimental groups drastically differed: 30% without anesthesia and 76% with anesthesia. The AL of the dead animals did not depend on the use of anesthesia.
Thus, when mice were irradiated with X-Ray, the effect of xylazine-zoletyl anesthesia on the radiosensitivity of mice was insignificant and did not depend on the dose. The presence of anesthesia during proton irradiation both at the Bragg peak and before the peak at doses of 6.5 Gy and 7.5 Gy did not affect the radiosensitivity of animals, but when mice were irradiated at the Bragg peak at a dose of 8.5 Gy, survival increased by 1.7 times. This effect was even more pronounced for carbon ions, which have a higher LET compared to X-Ray and protons, and it increased by a factor of 3.3 after mice were irradiated at the Bragg peak, at the maximum LET, and when irradiated to the peak, the effect of anesthesia decreased to 1.2. The results show that the classical ideas are not sufficient to explain the effects observed when using this anesthesia, which has a hypoxic effect. The data obtained are primarily of practical importance in the introduction of new methods of radiotherapy in veterinary medicine, the development of adequate models for biomedicine, preclinical trials of new radiation sources, as well as studies of the mechanisms of the combined action of pharmaceuticals and radiation.

**S9.619. Effects of NO donors, substrates and synthesis inhibitors on the nociceptive activity of the trigeminal nerve**

Ananev A.S.[1]*, Buglinina A.D.[1], Svitko S.O.[1], Koroleva K.S.[1], Sitdikova G.F.[1]
[1]*Kazan Federal University;*
* anton990124@mail.ru

Migraine is one of the most common diseases that lead to temporary disability and a significant decrease in patients' quality of life. The main complaint in this case is a headache. It is necessary to study the cause of pain to develop an effective treatment for this problem. Generation and conduction of nociceptive information from the periphery to CNS is associated with the activation of trigeminal nerve afferents. It is possible to use various substances in experimental models of migraine. In particular, it is common to use nitroglycerin as a donor of nitric oxide (NO). It is known that the introduction of nitroglycerin leads to migraine-like headaches. However, studies with this substance focus on vascular reactions, but neuronal mechanisms are not well understood. Because of that, the purpose of this work was to study the effect of NO on the nociceptive activity of the trigemino-vascular system.
Preparation of dura mater was used to record the nociceptive activity of the trigeminal nerve. Preparation is isolated rat skull with sagittal dissection without brain. The intact dura mater contains a branch of the trigeminal nerve (nervus spinosus) that innervates the middle meningeal artery. Under visual control, the nervus spinosus was partially extracted and sucked into a recording electrode attached to a manipulator. Recording of action potentials (AP) was carried out using the DAM 80 amplifier (World Precision Instruments, Sarasota, FL,

USA). Initially, the basic electrical activity of the trigeminal nerve was recorded. Then, studied substances were applied in the area of divergence of the middle meningeal artery. Electrical activity was recorded for 20-40 minutes, after which washing was carried out.
In the course of the work carried out, the following data were obtained. Donors of NO, Sodium nitroprusside (SNP) ($p = 0.04$; $n = 4$) and SNAP ($p = 0.02$) at a concentration of 200 μM caused a double increase in the frequency of PD. Light-inactivated SNP at the same concentration did not alter trigeminal nerve activity ($p = 0.62$; $n = 4$). However, the use of nitroglycerin at a concentration of 10, 100 and 200 μM in an acute application did not significantly affect the electrical activity of the trigeminal nerve. Based on this, the most effective in acute experiments is the use of SNP or SNAP.
L-arginine as substrate of NO sythase was used to analyze the role of endogenous NO in the regulation of trigeminal nerve activity at a concentration of 200 μM ($p = 0.04$; $n = 4$). Its application caused more than twice increase of AP frequency. The L-NAME NO synthase blocker (100 μM) did not change the base frequency of AP ($p = 0.72$; $n = 4$). L-arginine used with L-NAME did not cause an increase in the frequency of AP ($p = 0.18$; $n = 4$). The data obtained suggest that NO is formed endogenously in the innervation region of the trigeminal nerve and increases the excitability of its afferents. During inhibition of endogenous synthesis of NO, we observed a decrease in the intensity of the pro-nociceptive action of ATP and capsaicin, and also, the decrease of receptors sensitivity to low concentrations of serotonin.
Nucleotides such as cGMP and cAMP are involved in modulating the nociceptive signal by activating protein kinases, which in turn phosphorelate membrane channels and receptors. Using cAMP (8-Br-cAMP) ($p = 0.013$, $n = 5$) and cGMP (8-Br-cGMP) ($p = 0.01$; $n = 5$), an increase in nociceptive activity in the afferents of the trigeminal nerve was shown to double.
An ODQ inhibitor at a concentration of 10 μM was used to identify the role of soluble guanylate cyclase in the effects of NO. Incubation of the drug in ODQ did not lead to changes in the frequency of AP ($p = 0.87$; $n = 4$). The subsequent addition of SNP did not cause a significant change in the frequency of AP ($p = 0.62$; $n = 4$). In contrast, the use of an adenylate cyclase inhibitor, MDL 12330A (5 μM), did not prevent an increase in frequency after SNP application ($p = 0.02$; $n = 5$). The findings suggest a leading role of Guanylate cyclase in the effects of SNP.
The results obtained in the course of the work demonstrate that nitric oxide has a nociceptive effect, as it causes activation of the sensory fibers of the trigeminal nerve. The main mechanism of action of NO is related to the activation of soluble guanylate cyclase.
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**S9.620. Effects of mucin on activation of neutrophils by montmorillonite microparticles in vitro**

Mikhalchik E.V.[1], Firova R.K.[1], Klinov D.V.[1,2], Kraevsky S.V.[1,3], Morozova O.V.[1,2,4], Obraztsova E.A.[1,5], Filatova L.Yu.[6], Balabushevich N.G.[6], Panasenko O.M.[1]*
[1]*Department of Biophysics, Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, 119435, Moscow, Russia;*
[2]*Laboratory of Biomaterials, Sirius University of Science and Technology, 354340 Sochi, Russia;*
[3]*V.N. Orekhovich Institute of Biomedical Chemistry, 119121, Moscow, Russia;*
[4]*N. F. Gamaleya National Research Center of Epidemiology and Microbiology, 123098 Moscow, Russia;*
[5]*Moscow Institute of Physics and Technology, 141701, Dolgoprudny, Moscow Region, Russia;*
[6]*Faculty of Chemistry, Lomonosov Moscow State University, 119991 Moscow, Russia;*
* o-panas@mail.ru

Montmorillonite (MM) is a porous natural aluminomagnesium silicate clay of a subclass of smectite with high adsorption capacity which contributes to its efficacy as enterosorbent and promising component for drug delivery systems, also due to its biocompatibility exceeding that of other nanomaterials [1]. In the intestine it can function as probiotic-protecting agent with potential protective effect for mucosa [2].
In conditions of intestinal inflammation, the local effects of MM could depend on interaction between mineral particles and neutrophils infiltrating gastrointestinal wall. Earlier it was shown that MM induces fast lysis of neutrophils in absence of proteins of blood serum, presumably, via electrostatic interactions between particles surface and phospholipids of cell membrane accompanied by activation of neutrophil respiratory burst [3]. Serum proteins adsorbed onto the particles prevent neutrophil lysis and activation but there is no data on effects of mucin, the major component of mucosa. Earlier we have shown that mucin acquired proinflammatory properties towards neutrophils when adsorbed onto microparticles of calcium carbonate [4] or phosphate [5]. Our aim was to study effects of mucin on activation of neutrophils by MM microparticles in vitro.
Methods: evaluation of mucin adsorption by decrease of its concentration in solution after incubation with MM and its visualization using scanning electron microscopy (SEM); analysis of activation of respiratory burst of neutrophils isolated from normal human blood as superoxide radical generation measured by lucigenin chemiluminescence (Luc-CL); analysis of cytotoxicity of MM by direct cell count using Goryaev chamber; evaluation of surface charge using zeta-sizer.
Results. MM microparticles with surface charge of -30±3 mV stimulated generation of superoxide radical by neutrophils with lysis of 50-70% cells; Luc-CL value correlated with concentration of undestroyed cells in the probes. Fast sorption of 90% of lucigenin added into the probes (52 ± 7 nmole/mg MM) was registered, with no direct impact on CL values. Incubation of MM in mucin solution resulted in binding of glycoprotein which varied from 2 to 17 μg/mg MM. According to SEM images, there were particles not coated with mucin or with partial mucin coating or totally coated with mucin. Binding of mucin to MM did not influence subsequent sorption of lucigenin by the particles, and only at maximal adsorption of mucin the partial decrease in cell lysis (by 23 ± 13%) was detected in the probes after CL measurement. Like with untreated MM particles, Luc-CL was proportional to whole cells number, so no specific enhancement of neutrophil activation was induced by adsorbed onto MM mucin, unlike our results obtained earlier with mucin-coated calcium carbonate or phosphate microparticles [4, 5]. Dependence of effects of adsorbed mucin on the nature of mineral particles obviously originates from different mechanisms of their interaction with neutrophil membrane.
Conclusion. Mucin coating of MM partially prevents neutrophil lysis, with no enhancement of generation of superoxide radical by the cells. The absence of proinflammatory effects of mucin adsorbed onto MM microparticles is important for safe peroral application of these particles.
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**S9.621. Effects of the NO synthesis inhibitor L-name on the formation of long-term contextual memory in the terrestrial snail**

Deryabina I.B.[1]*, Andrianov V.V.[1], Bogodvid T.K.[1,2], Muranova L.N.[1], Habib Z.C.[1], Gainutdinov K.L.[1]
[1]*Kazan (Privolzhsky) Federal University;*
[2]*Physical Culture, Sports and Tourism;*
* IBDeryabina@kpfu.ru

Nitric oxide (NO) is one of the most important mediators, playing vital roles in the functioning of various body systems. Ample evidence for the participation of NO in the processes associated with plasticity has been established [1, 2]. In molluscs, nitric oxide plays the role of an intercellular messenger and signaling molecule. All experiments were carried out on the terrestrial snail Helix lucorum. The animals were trained to acquire a contextually conditioned reflex (CCR) according to the contextual paradigm "on the ball" - in a situation where the shell of the animal was rigidly attached to a tripod in a fixed position, although at the same time, they retained freedom of movement on the surface of a ball floating in the water. The training consisted in presenting an unconditioned stimulus (electric stimulation) while the snail was in this particular context - on the ball. The training was carried out according to the following protocol: presentation of 5 electrical stimulations per day for 5 days by manually making contact between two metal electrodes and the back and front dorsal parts of the snail leg. Electrical stimulation parameters: rectangular current pulses with a frequency of 50 Hz, current intensity 1-2 mA, duration of stimulation - 1 s. The interval between stimuli was 15-20 minutes [3, 4]. The intensity of the stimulation current was selected to be sufficient to trigger a defensive reaction associated with retraction of the head and anterior part of the body and did not exceed 2 mA. During the training procedure (5 days), the snails did not receive food [5]. Testing was carried out before the start of the training and on the following days after training. To do this, the amplitude of retraction of the ommatophores was measured in response to tactile stimulation, which was a tangential movement of brush hair over the skin of the dorsal side of the front of the leg at a standard speed. The CCR was considered formed if the reaction on the ball significantly exceeded that on a flat surface. The results were statistically processed and presented as mean ± SEM. The significance of differences was assessed using Student's t-test and Mann-Whitney U-test. The SigmaStat32 program was used. Statistical significance was estimated at $p<0.05$. The following day after the confirming test for the development of the CCR, the animals were divided into four groups. Animals of the first group were injected with the NO synthase blocker L-NAME 30 min before the reminder procedure, followed by an anisomycin (AN) injection. A reminder consisted of placing the animals on the ball for 20-30 minutes, the same environment where they acquire the CCR, but without the presentation of tactile and electrical stimuli. The animals in the second were injected with the NO synthase inhibitor L-NAME 30 min before the reminder procedure. The third group underwent a reminder procedure followed by an AN injection. The fourth group was a control group, in which the animals were injected with saline solution (SS) after the reminder procedure. The results obtained show that the injection of SS after the procedure of the contextual reminder does not lead to a change in the amplitude of the defensive reaction to tactile stimulation when tested on the ball. On the other hand, injections of AN after the reminder led to complete forgetting of the formed memory. Administration of the NO-synthase inhibitor L-NAME 30 min before the reminder procedure, followed by an injection of AN, did not lead to a decrease in the amplitude of the defensive reaction to tactile stimulation when tested on the ball. The snails that were injected with L-NAME 30 min before the reminder procedure without inhibiting protein synthesis showed a gradual decrease in the amplitude of the defensive response from 77.9% to 48.2%. in the context "on the ball". The values of tests on the plane in all experimental groups did not differ significantly. These results indicate the involvement of NO in learning and memory formation. Nonetheless, it is possible that inhibiting NO synthase prevents the initiation of the reconsolidation process.

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**S9.622. Efficacy of photodynamic therapy against uropathogenic bacteria**

Elagin V.V.[1]*, Ignatova N.I.[1], Budruev I.A.[2], Antonyan A.E.[1], Bureev P.A.[2], Streltsova O.S.[1], Kamensky V.A.[1,3]
[1]*Privolzhsky Research Medical University;*
[2]*Lobachevsky State University of Nizhny Novgorod;*
[3]*Institute of Applied Physics of the Russian Academy of Sciences;*
* elagin.vadim@gmail.com

The frequent, prolonged, and uncontrolled use of antibiotics in the treatment of infections has resulted in an increasing number of bacterial strains resistant to a wide range of antibiotics. The antibiotic resistant bacteria such as Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species are of great significance in the case of infected urinary stones. During lithotripsy pathogens may diffuse in organ cavity and induce postoperative complications, such as pyelonephritis, systemic inflammatory reaction syndrome, and urosepsis. Antimicrobial photodynamic therapy is considered to be an alternative to the antibiotic treatment of localized infectious processes. The purpose of the study was to evaluate the antibacterial efficacy of photodynamic inactivation against human antibiotic-resistant bacterial uropathogens. Uropathological microorganisms were isolated from renal calculi preliminary selected based on their X-ray density. The disk diffusion susceptibility method was used according to the approved standard. The accumulation of photosensitizer, Fotoditazin, by microorganisms was studied. The overnight culture of microorganisms were diluted to 2.5x108 CFU/mL in a phosphate buffer saline. The photosensitizer was added to the bacteria with subsequent incubation for 15 min in the dark at room temperature. For laser irradiation 100 ul of the bacteria suspension were transferred into a 96-well plate. The fiber-coupled diode laser with a wavelength of 662 nm was used to illuminate samples. Laser irradiation was performed for 9 minutes and various output power.
It was found that 78.7±5.2 % of renal calculi were contaminated. Testing the sensitivity of the isolated strains to 10 antibiotics of different mechanisms of action showed that the studied strains have a high antibiotic resistance. Staphylococcus aureus and Enterococcus faecalis strains were shown to be resistant to all the tested drugs (0 sensitive

out of 10). It was found that Escherichia coli was susceptible to nitrofural (1 sensitive out of 10) and Proteus mirabilis had intermediate sensitivity to ofloxacin and nitrofural (2 intermediate out of 10). The interaction between photosensitizer and urapothogenic microorganisms was analyzed. Since the samples were washed from free molecules of photosensitizer, the fluorescence was only detected from the photosensitizer that had penetrated into cells and/or was bound with cell wall. The photosensitizer accumulation was estimated to be dependent on both incubation time and concentration. The fluorescence intensity was found to be higher for Gram-negative strains than for the Gram-positive ones regardless of the photosensitizer concentration. The strains of E. faecalis and S. aureus demonstrated the enhancement of the fluorescence intensity in a time-dependent manner with the maximal value at 60 min. E. coli and P. mirabilis had the maximal value of fluorescence intensity after 30 min and that significantly decreased by 60 min. The optimal incubation time was found to be 30 minutes. However, this technique is planning to use during laser lithotripsy for sanitation, where the time is a limiting factor; therefore, 15 min of incubation was chosen. The concentration of the photosensitizer was selected to be 50 μg/mL. After 15 minutes of incubation in the dark followed by 15 minutes of manipulation (dilution, inoculation) at ambient light, no colony of S. aureus and E. faecalis was detected on the plates. The treatment of either Gram-positive or Gram-negative bacteria by laser light only did not induce significant reduction of CFUs. The survival rate of P. mirabilis for photodynamic inactivation was power-dependent. The number of viable bacteria was decreasing from 65% to 10% with an increase in power from 50 mW to 150 mW. The maximal bactericidal effect was reached at 150 mW.

Next, the antimicrobial photodynamic therapy was adapted for Gram-negative species. The efficacy of aPDT of E. coli washed from an unbound photosensitizer under continuous wave irradiation was only 5%. Tween 80 provided an insignificant enhancement of aPDT efficacy of up to 9%. The efficacy of aPDT of E. coli incubated with photosensitizer and Triton X-100 achieved 52.5%. It was found that washing of the extracellular photosensitizer led to loss of the aPDT efficacy. K. pneumoniae was not sensitive to aPDT without extracellular photosensitizer, while the efficacy of aPDT with the photosensitizer was 89%. E. coli had low sensitivity to aPDT without extracellular photosensitizer as well as with it. The high sensitivity of P. aeruginosa to aPDT with extracellular photosensitizer significantly reduced after washing of the photosensitizer. The efficacy of aPDT of P. mirabilis did not change after washing of the extracellular photosensitizer. It was demonstrated that the aPDT efficacy depended on laser power in all studied species, excluded K. pneumoniae. The efficacy of K. pneumoniae treatment did not exceed 93%. The irradiation of other bacteria species with a power of 450 mW provided an aPDT efficacy of 99.99%. To test the efficacy of the developed aPDT technique, urine cultures of the patients were incubated with a photosensitizer and Triton X-100 for 15 minutes in the dark. Then, the unwashed samples were illuminated by a continuous wave laser at 450 mW of output power. The efficacy of the aPDT of infected urine cultures was not less than 99.996%.

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### S9.623. Elevated aggregation of RBC and platelets and its correction in blood samples of patients suffering from arterial hypertension

Lugovtsov A.E.[1]*, Ermolinskiy P.B.[1], Semenov A.N.[1], Priezzhev A.V.[1]
[1]*Lomonosov Moscow State University;*
* anlug1@gmail.com

Aggregation of RBCs and platelets is one of key factors, which determines the blood flow and thereby affects the blood rheology and microcirculation. Alterations in these properties lead to changing the blood viscosity and, as a consequence, to changes in blood flow through vessels and capillaries. This can lead to significant impairment of blood function. Arterial hypertension (AH), also known as high blood pressure, is a long¬term medical condition in which the blood pressure in the arteries is persistently elevated. AH can lead to severe alterations of vitally important systems of the human organism including the cardiovascular system and resulting in damage to blood vessels and capillaries, impairment of blood hemorheology.

Fibrinogen-induced red blood cells (RBC) aggregation was assumed to be caused by nonspecific binding of fibrinogen molecules to cell membranes and further leading to molecular bridging between interacting cells. In contrast, platelets are known to have membrane integrin IIb/IIIa glycoproteins highly specific to fibrinogen. This fact can be used for correction of platelets and, as we suppose, RBC aggregation by using integrin glycoproteins inhibitors.

The main goal of this work is to assess aggregation properties of blood drawn from patients suffering with AH and healthy donors as well as check the hypothesis of RBC aggregation reduction with integrin glycoproteins inhibitors in case of AH and enhanced RBC aggregation.

In this work, studies of aggregation properties of blood in patients suffering from AH were conducted by laser diffuse light scattering in vitro measurements on ensembles of cells. We used laser aggregometry technique [1], which are implemented in the laser aggregometer-deformometer device Rheoscan AnD-300 (Republic of Korea) [1]. Several parameters were measured: hydrodynamic strength of RBC aggregates - critical shear stress (CSS), characteristic time of RBC aggregates formation, aggregation index (percentage of the RBC participating in the aggregation during first 10 seconds of spontaneous aggregation process).

Double-channeled optical tweezers were used for in vitro measuring the aggregation speed as well as interaction forces during RBCs doublet formation on cellular level [2].

Studies of the effect of several commonly used inhibitors (RGDS, eptifibatide, tirofiban) on RBC aggregation for correction reason were performed with blood of patients with AH and control group.

All measurements were performed with human blood drawn from patients with AH (88 people) and practically healthy volunteers - control (18 people). Blood samples were stabilized with EDTA to prevent blood from clotting. The measurements were performed within two hours after blood sampling.

It was shown that in AH-patients, the ability of RBCs and platelets to aggregate is enhanced: the aggregation speed and forces of the cells interaction are significantly increased relative to the control group.

The hypothesis that cells aggregation can be corrected (reduced) in AH by integrin IIb/IIIa glycoproteins (IGP) inhibition of fibrinogen adsorption on RBC membrane was verified experimentally. IGP reduces nonspecific binding of fibrinogen molecules to cell membranes, which results in decreasing molecular bridging between interacting cells.

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### S9.624. Evaluation of locomotor activity induced by transcutaneous electrical spinal cord stimulation in patients with spinal cord injury

Militskova A.D.[1]*, Mukhametova E.R.[1], Yakovleva E.I.[1], Andrianov V.V.[1], Lavrov I.A.[1]
[1]*Kazan Federal University, Institute of fundumental medicine and biology, laboratory of Neuromodulation;*
* mamashotmilktea@gmail.com

The high prevalence of CNS pathologies caused by injuries and diseases of the spinal cord requires the development of new approaches of motor function rehabilitation. The method of the spinal cord electrical stimulation is one of the most promising in experimental and clinical practice, which makes available study the functions of various body systems. One of the recent years breakthrough is the identification of certain spinal cord areas, which electrical stimulation activates involuntary and voluntary movement in the lower extremities. There are two main methods of spinal cord stimulation to control locomotion – invasive stimulation using electrodes located on the dorsal surface of the brain (epidural stimulation) and non-invasive, when the electrodes are placed on the surface of the skin (transcutaneous electrical spinal cord stimulation (tSCS). Evaluation of the effectiveness of the electrical stimulation technique for inducing step-like movements with varying degrees of spinal cord injury arises interest.

The aim of this study was to evaluate the effects of transcutaneous electrical spinal cord stimulation on changes in motor activity parameters in patients with spinal cord injury (SCI).

The study involved 15 subjects (3 women and 12 men) with SCI at the level of C4-C5 and Th3-L2 vertebrae, with injury assessed on the AIS scale (American Spinal Cord Injury Association Impairment Scale), as A, B and C. Assessment of locomotor activity in subjects with SCI was carried out in the body weight unloading system (Redcord, Norway) in the supine position. tSCS was performed using two gel electrodes (24 mm) at the level of Th11-Th12 and Th12-L1 vertebrae. The stimulation frequency was 35 Hz, the stimulus intensity varied in the range from 20 to 115 mA. To register range of leg movements, a Vicon motion capture system (Nexus, UK) was used. Range of movements in the joints was calculated from the position of light-reflecting sensors located on the anatomical landmarks of the trunk and lower extremities along the axes of movement in the hip, knee, and ankle joints. The assessment of locomotor activity was carried out when the subjects attempted to perform stepping-like movements both without stimulation and during tSCS.

The assessment of locomotor activity caused by tSCS showed that the range of motion in the joints depends on the degree and level of spinal cord injury. Thus, in the case of incomplete SCI, locomotor activity in the lower extremities was initiated much more easily. In the group of subjects with SCI at the level of C4-5 vertebrae, there was a significant increase in angular movements in the hip, knee and ankle joints ($p \leq 0.05$). In the group of subjects with SCI at the level of Th3-Th9 vertebrae, a significant increase of range of movements was observed in the knee and ankle joints ($p \leq 0.05$). Similar stimulation in the group of subjects with SCI at the level of Th10-L2 vertebrae led to a significant increase in the values of range of movements only in the ankle joint ($p \leq 0.05$).

Thus, the observed effects of tSCS allows to increase the effectiveness of rehabilitation and opens up good prospects for the use of non-invasive transcutaneous stimulation in routine clinical practice for various groups of patients with spinal pathology.

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### S9.625. Evaluation of the effect of temperature receptors on the activity of meningeal afferent nerve fibers of the rat trigeminal nerve

Ananev A.[1], Fedorina A.[1], Telina E.[2], Gafurov O.[1]*
[1]*Kazan (Volga region) Federal University, Kazan, Russia;*
[2]*Kazan State Medical University, Kazan, Russia;*
* osgafurov@kpfu.ru

Maintaining body temperature is a vital process in homoiotherms. The fact that an increase in body temperature often leads to headache, led to discovery that thermoreceptors take part in migraine. It is believed that the trigger mechanism for migraine pain is the activation of trigeminal nerve fibers. It is known that channels from the TREK and TRP superfamilies serve as thermosensors in thermosensitive neurons, and different representatives of these channels are activated in different temperature ranges. Among TRP channels TRPV1 type is the most significant for migraine, and is activated at temperatures above 42°C; TRPV3, TRPV4, TRPM8, and TRPA1 types cannot be excluded from the possibility of their cooperative interaction and are activated at above 32°C, 27-34°C, 8-25°C, and below 16°C, respectively.

In this work, we investigated the effect of temperature changes on the electrical activity of the rat trigeminal nerve.

The experiments were carried out using a rat half-skull preparation. Action potentials (AP) were recorded from the trigeminal nerve isolated from the meningeal membrane and placed in a bath that was perfused with Krebs solution. Recordings were done using an extracellular electrode. During the experiment different conditions were applied for 10 minute as follows: control at a temperature of 22-23°C; increasing the temperature of the solution to 27°C; increasing the temperature of the solution to 37°C; lowering the temperature to 27°C, and adding capsaicin (1 μM).

Recorded APs were identified and grouped using cluster analysis that makes it possible to assess the contribution of individual nerve fibers to the overall electrical activity of the trigeminal nerve.

The average number of APs per 5 minutes in the control was 612±219 (mean ± sem; n=5). In 5 minutes after increasing the temperature of the solution from 22 to 27°C the amount of APs significantly increased up to 1616±347 (t-test; $p<0.01$). Within 5 minutes after the temperature was raised to 37°C the amount of APs increased from 1066±270 to 1421±314 ($p<0.05$). Subsequent application of capsaicin at a concentration of 1 μM slightly decreased frequency of AP from 1362±222 to 1271±281 ($p=0.86$).

A series of experiments with an increase in temperature from 22 to 37°C within first 5 minutes showed a significant increase in the amount of APs from 447±214 in control to 1764±717 (Wilcoxon T-test; $p<0.01$; n=9). Further use of capsaicin, after lowering the temperature down to 27°C, showed a non-significant increase in the amount of APs over a 5-minute interval from 931±388 to 959±358 ($p=0.75$).

Cluster analysis of APs recorded in the trigeminal nerve showed that the occurrence of APs in each cluster was different. Cluster analysis was carried out for a series of experiments with sequential increase in temperature to 27°C, and 37°C, followed by application of capsaicin. This regiment of several treatments was specifically designed in order to see how nerve fibers were able to "respond" to various conditions that lead to a change in electrical activity. A cluster was considered to being able to respond if the number of APs in this cluster doubled when the temperature increased to 27°C, 37°C, or after capsaicin was applied. As a result of the analysis, it was shown that 22% of the clusters did not respond to any of the actions; 16% of the clusters responded to an increase in temperature to 27°C; only 5% responded to temperature increase to 37°C;

only 10% of the clusters responded to the application of capsaicin, 32% of the clusters responded to both an increase in temperature to 27°C and a subsequent increase in temperature to 37°C. Thus, the presence of clusters with different activity profiles suggests that the nerve fibers of the trigeminal nerve can respond to temperature changes in different ranges and, therefore, can contain different types of thermoreceptors.

Assuming that different types of thermoreceptors or combinations thereof are present in Aδ- and C-type nerve fibers (with the distinct amplitude-time parameters of AP), we calculated the average AP amplitude for groups of clusters that responded to changes in temperature and capsaicin. It turned out that there was a significant difference between some groups. For example, the average AP amplitude of non-responding clusters was 8.7±0.76 a.u. (n=22) that was significantly lower ($p<0.05$) than in those that responded to an increase in temperature to 37°C (12.9±1.6 a.u., n=5). At the same time, the average AP amplitude for the largest group of clusters that responded to an increase in temperature to both 27°C and 37°C was 7.5±0.43 a.u. (n=41). That was significantly lower than in those that responded to an increase in temperature to 27°C (11.5±1.18 a.u., n=21, $p<0.005$), 37°C (12.9±1.6 a.u., n=5, $p<0.001$), or after capsaicin was applied (11.8±1.46 a.u., n=13, $p<0.05$).

Thus, the following can be concluded: 1) with an increase in temperature, an increase in the amount of APs occurs; 2) the fibers that make up the trigeminal nerve may contain a combination of TRP channels that is different in each type of nerve fibers and that determines their distinct response to different temperatures; 3) a different combination of TRP channels possibly is indicative of the Aδ or C fiber type.

We suggest that the activation of thermoreceptors, acting together with chemical agents, plays an important role in the occurrence of pathological activity in the nerve fibers of the meningeal sheath that can lead to the onset of pain in migraine.

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### S9.626. Exomeres are Extracellular Particles as Carriers of Cholesterol not Associated with Lipoproteins

Landa S.B.[1,2]*, Verlov N.A.[1], Fedorova N.D.[1], Filatov M.V.[1], Pantina R.A.[1], Burdakov V.S.[1], Varfolomeeva E.Yu.[1], Emanuel V.L.[2]
[1]*Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute»;*
[2]*Petersburg State Medical University, I.P. Pavlova of the Ministry of Health of the Russian Federation;*
* landa_sb@pnpi.nrcki.ru

Exosomes and exomeres are the smallest microparticles with a diameter of 20 to 130 nm. They are found in almost all biological fluids. Exosomes and exomeres are of considerable interest because they can be involved in intercellular signaling and are biological markers of cell state that can be used for diagnosis. The nomenclature of microparticles remains poorly developed to date. Most researchers try to classify them based on the method of formation, physico-chemical characteristics and the presence of tetrasporine markers CD9, CD63 and CD81.

The data presented in this work show that although exomeres carry biomarkers CD9, CD63 and CD81, characteristic of microparticles, they differ greatly from exosomes in size, buoyant density and lipid composition, especially in cholesterol content. The production of exomeres does not correlate with apoptosis, that is, exomeres are not products of cell death. The production of exomeres by cells is associated with the synthesis of cholesterol by cells and is inhibited by drugs of the statin class, which are regulators of the synthesis of mevalonate, an intermediate product of cholesterol metabolism, in contrast to exosomes, the formation of which is suppressed by ezetemib, which inhibits the pathways of cholesterol delivery to the cell. In addition, the work shows that the concentration of extracellular particles in the body strongly correlates with the concentration of total cholesterol in plasma, but weakly correlates with the concentration of cholesterol in lipoproteins. This suggests that not all plasma cholesterol is associated with lipoproteins, as previously thought.

Thus, exomeres are not a product of cell death and play an essential role in the transport of cholesterol in blood plasma.

### S9.627. Expression and Intracellular Localization of HDAC3 and Akt/GSK-3β in Dorsal Root Ganglion Neurons after Sciatic Nerve Transection

Dzreyan V.A.[1]*, Guzenko V.V.[1], Kalyuzhnaya Yu.N.[1]
[1]*Southern Federal University;*
* dzreyan2016@mail.ru

Pathological conditions, like ischemia or neurotrauma, decrease acetylation of histones and nonhistone proteins. This is probably due to the activation of histone deacetylases (HDACs), which disrupts protein synthesis. Since the activation of histone deacetylases and histone deacetylation result in the suppression of protein synthesis, the changes in the expression of these proteins can be considered the initial stages of the pathological process. However, the role of epigenetic processes in the regulation of cell death and survival in the first hours after nerve damage has not yet been studied.

One important experimental model of neurotrauma is the sciatic nerve transection in rodents, which leads to Wallerian degeneration of axons and death of neurons and glial cells. Sciatic nerve transection induces apoptosis of glial cells of the dorsal root ganglia (DRG) 2-3 cm away from the axotomy site. It is still unclear what primary signals are involved in the transmission of signals from the lesion site to the remote ganglion, resulting in cell survival or death.

In neurons, histone deacetylase HDAC3 is localized mainly in the cytoplasm and is activated via phosphorylation of the serine/threonine kinase GSK-3β, which is a mechanism that is normally activated by growth factor loss in PI3K/Akt signaling pathway. A number of works demonstrate a key neurotoxic role of HDAC3, but the specificity of HDAC3 activation in the induction of neuronal death is not fully understood. Therefore, it is of interest to investigate the Akt/GSK-3β signaling pathway involved in the phosphorylation of histone deacetylase 3 upon neurotrauma.

Work objective. To evaluate the intracellular localization of HDAC3 and Akt/GSK-3β kinases in neurons and glial DRG cells after sciatic nerve transection in rats using immunofluorescence microscopy study of co-localization of these proteins with the neuronal nuclear marker NeuN.

Materials and methods. Immunofluorescence microscopy was used to study axotomy-induced changes in the expression and localization of HDAC3 and Akt/GSK-3β kinases in rat DRG after sciatic nerve transection. For the detection of HDAC3 and kinases Akt/GSK-3β via immunofluorescence microscopy we used the anti-HDAC3 (SAB4503481 Merck, 1:250); anti-Akt (#9272 Cell Signaling, 1:250); anti-GSK3-3β (#9315 Cell Signaling, 1:250); anti-phospho-GSK-3β (Ser9) (#9323 Cell Signaling, 1:250) and anti-NeuN (MAB377 Merck, 1:1000) antibodies. The nuclei of all neurons and glial cells were images with Hoechst 33342 (Cat. No 14533). Co-localization of a target protein with the NeuN neuron marker was evaluated using the ImageJ application with the JACoP plugin. The co-localization coefficient M1 represents the ratio of pixels in the green channels (target protein) to the total signal recorded in the red channel (NeuN neuron marker). At least 100 cells per slice were used in the calculations. To calculate the mean level of fluorescence in experimental and control DRG samples, 10 control, and 10 experimental images were used for each of the 7 rats. The area-mean fluorescence of the cytoplasm and nucleus for each cell was estimated and the obtained values were

averaged. The rat DRG samples were photographed using an Olympus BX-51 fluorescent microscope equipped with OrcaFlash 4.0 V3 digital camera (Hamamatsu, Japan) at approximately 535 nm excitation wavelengths for Anti-Mouse IgG1 (γ1) labeled with CF555, 488 nm for anti-rabbit IgG (H+L) labeled with CF488A, and 365 nm for Hoechst-33342. Antibody fluorescence was recorded at wavelengths >580 nm and >460 nm, respectively. Protein level was estimated by fluorescence intensity in the ImageJ application (http://rsb.info.nih.gov/ij/, accessed on December 4, 2022)..

Results. The double immunofluorescent staining has shown that the level of HDAC3 in the cytoplasm of damaged DRGs was significantly overexpressed one hour after axotomy ($p<0.01$), but decreased in four hours agaist the control ganglia ($p<0.05$) and the level detected one hour later ($p<0.01$). No difference was observed 24 hours after the injury. In the nucleus of axotomized DRGs, HDAC3 levels increased in four hours after the transection compared to intact rat ganglia ($p<0.01$), but not in one or 24 hours. This indicates a redistribution of HDAC3 from the cytoplasm to the nucleus 4 hours after axotomy, which is also confirmed by the M1 coefficient ( $p<0.05$). According to the data obtained, the axotomy caused a significant decrease in the Akt level in 24 hours in the cytoplasm of the ganglion cells twofold relative to the control ganglion cells ($p<0.01$) and 1.5-fold relative to the four-hour group ( $p<0.01$). Akt level in the nucleus was unchanged and low. GSK3beta level was low and did not change throughout the study, both in the nucleus and cytoplasm of neurons of rat the ganglia. However, according to immunofluorescence analysis, the level of phospho-GSK-3β (Ser9) was significantly increased in 24 hours in the cell cytoplasm of axotomized ganglia ($p<0.01$).

Thus, our data indicate the involvement of HDAC3 and Akt/GSK3beta signaling pathway in axotomy-induced damage to neuronal and glial DRG cells. The double immunofluorescence staining has shown that sciatic nerve transection causes HDAC3 translocation from the cytoplasm to the nucleus in the first 24 hours after axotomy. The Akt antibody #9272 confirms silencing of protein expression in DRG of the rat spinal cord after sciatic nerve transection. The phospho-GSK-3β (Ser9) antibody #9323 was used to confirm downstream pathway activation with reduced Akt expression..

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### S9.628. Factors determining the efficiency of nitric oxide physiological effect

Titov V.Y.[1,2], Osipov A.N.[1]*
[1]*Russian National Research Medical University, Ministry of Health of the Russian Federation, Moscow;*
[2]*Moscow State Academy of Veterinary Medicine and Biotechnology, Ministry of Agriculture of Russian Federation ;*
* vtitov43@yandex.ru

There are two questions, the answers to which are necessary for clarification of the mechanism of NO physiological effect. These are mechanisms of specificity of NO physiological effects and prevention of NO oxidation to toxic nitrite. Living tissues contain many SH-groups. If NO is released spontaneously, its interaction with a physiologically significant target is low probable. Since NO is a short-living compound quickly oxidizing to nitrite, defense NO from oxidation by oxygen is necessary.

S-nitrosothiols (RSNO) and dinitrosyl iron complexes (DNIC) are considered as NO donors that prolong NO physiological lifetime [1]. But the ways of realization of the NO interaction specificity and the defense from its oxidation to nitrite it is not yet clear. One of the reasons is the absence of a method enabling a prompt monitoring of NO metabolite content of living tissues. [2, 3].

An enzymic sensor developed by us in collaboration with ICP RAS gives such ability. The method is based on the reversible inhibition of the catalase by all nitroso compounds and nitrite with an approximately equal efficiency that increases by two orders in the presence of chloride in plasma concentration as well as bromide and thiocyanate. DNIC lose the inhibiting ability in a system containing an iron chelator (o-phenanthroline, EDTA) and NO trap (hemoglobin) intercepting NO released from the complex. RSNO transform to DNIC under the action of ferrous ion and acquire DNIC properties. Nitrite (NO2-) and nitrosoamines practically produce no DNIC in a neutral medium and retain the inhibiting abilities under sequential addition of ferrous ion, glutathione, NO trap and iron chelator. The activity of catalase was measured calorimetrically by recording the kinetics of heat production accompanying that highly exothermic process (23.6 kcal/MH2O2). Such recording requires no preliminary sample purification. The sensibility of the method is 40 nM which exceeds that of EPR and photometric methods [3]. Thus, the content of basic NO metabolites can be promptly monitored.

According to the sensor data, the majority of living tissues contained nitrite in concentration below 50 nM in spite of the fact that, according to the data of the Griss test, they contained nitrite in a concentration of up to several hundred nM [3]. Nitrite, added exogenously to their samples, was determined by the sensor as nitrite. Therefore, the samples contained not nitrite but some compounds having NO-groups. It is shown that NO donors (RSNO, DNIC) produce a dyed product with the Griss reagent in acid medium that is necessary for the reaction. They appear to be decomposed in the acidic medium with the NO releasing and oxidation to nitrite. The concentration of NO donor compounds in living tissues varies from units to hundreds μM [3]. The intensification of NO synthesis after the introduction of arginine not result in the appearance of nitrite [4]. Therefore, living tissues have a mechanism to prevent NO oxidation to nitrite. It can be assumed that a synthesized NO molecule is immediately included in the donor compounds. The latter are considered to be short-living compounds [1, 2]. Nevertheless, the comparison of the sensor and EPR data with respect to DNIC, as the sensor and spectral data in respect to DNIC and RSNO, shows that these compounds can be modified, lose their spectral and EPR properties, but do not practically decompose spontaneously with the release of NO since they do not lose their specific inhibiting properties [3].

But, how the transition of NO from a donor molecule to a target can be occurs? NO donors do not lose their specific inhibiting properties during the incubation with NO trap hemoglobin. A DNIC completely loses the inhibiting property in the system containing NO trap and iron chelator exceeding by efficiency the ligands included in the complex. For instance, if the complex included glutathione (DNIC/GSH) it lost the inhibiting properties in the cysteine/hemoglobin system. DNIC/GSH is oxidized to nitrate in bird embryos, and a cysteine containing complex (DNIC/Cys) is not oxidized since it is not absorbed by tissues [4]. Therefore, the transition of NO from a donor to a target takes place in the presence of the target and destruction of the complex. It is supposed that the destruction may occur under the action of some groups included in the target. Endogenously synthesized DNIC have DNIC/GSH inhibiting properties. RSNO presumably perform the donor function by transforming to DNIC [3].

It is shown in our experiments that nitric oxide is synthesized with an approximately equal intensity in the poultry embryos of the same species. but the intensity of oxidation to nitate in breeds of meat direction productivity is many-fold higher than that of egg direction. In the latter, NO accumulates in the embryo as part of donor compounds [4]. Therefore, NO physiological efficiency is determined by the presence of a target that has chemical affinity to NO and induces decomposition of NO donors, but not by a spontaneous decomposition of the latter. It proposed that NO minimally stays in a free state when passes from a donor to a target.

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### S9.629. Features of Spreading Cortical Depression and Sensitivity to a Nitroglycerin-Induced Migraine Model in Rats with Prenatal Hyperhomocysteinemia

Sitdikova G.[1]*
[1]*Kazan Federal University;*
* sitdikovaguzel@gmail.com

Migraine is a neurological disease, the primary form of headache, the symptoms of which are periodic attacks of moderate to severe headache. Migraine attacks are accompanied by nausea, vomiting, phonophobia and/or photophobia. One of the main mechanisms of migraine development is an increase in neuronal excitability of both peripheral and central structures, the activity of which underlies the generation of pain in migraine. Homocysteine is a sulfur-containing substance that is formed from methionine in the diet. Disorders of methionine metabolism lead to an increase in the level of homocysteine in plasma and cerebrospinal fluid - hyperhomocysteinemia (hHCY) [1, 2]. Spreading cortical depolarization (CSD) is an electrophysiological phenomenon that underlies the aura in migraine, leads to disruption of ion homeostasis and changes in local vascular responses. hHCY according to clinical data is a factor in the development of migraine, however, there are no experimental data proving such a dependence. The aim of the work was to study the sensitivity to CSD in rats with prenatal hHCY. In addition, the mechanical sensitivity and anxiety of rats with hHCY were analyzed in a nitroglycerin (NTG)-induced migraine model.
The experiments were carried out on Wistar rats and were approved by the Local Ethical Committee of KFU (protocol No. 33 dated November 25, 2021). Prenatal hHCY was induced with a diet high in methionine. CSD was recorded in the somatosensory cortex of rats in vivo in response to the application of increasing concentrations of KCl (0.01–1.0 M). In addition, we analyzed the features of the distribution of long-term CSD. After registration of CSD 2,3,5-triphenyltetrazolium chlorine (TTC) was stained to assess the metabolic activity of brain tissue cells. Mechanical sensitivity was studied using Frey's hairs, the level of anxiety was determined in the test dark-light chamber and open field.
In the control group, the threshold concentration of KCl for the induction of CSD was 100 mM, in the hHCY group it was 10–50 mM. In addition, in the hHCY group, CSD s were longer, and the depolarization rate of CSD was lower compared to the control group. The baseline indicator of neuronal activity (multiple action potentials, MUA) in the granular and infragranular layers was significantly higher in the Hz group, indicating greater excitability of neurons. In addition, the increase in the frequency of MUA in the hHCY group at the beginning of the CSD was significantly higher than in the control group. In the next series of experiments, we investigated the ability of the somatosensory cortex to carry out long-term CSD with the application of KCl for 2 hours. The number of CSD for 2 hours of application in the hHCY group was significantly higher than in the control. In the hHCY group, repeated CSD propagated mainly in the lower layers, which may be associated with a reduced resistance of neurons to KCl-induced depolarization. Indeed, staining of brain sections with TTC dye showed the presence of a "necrotic funnel" in animals of the hHCY group.
It is known that sensitivity to the generation of CSD correlates with such a behavioral phenotype as mechanical allodynia, anxiety, and photophobia. In the HHC group, the mechanical threshold was significantly lower than in the control group. The introduction of NTG reduced the mechanical threshold of the animals of the hHCY group during the first hour, and by the second hour of observation, the threshold reached its minimum level. In the control group, mechanical hyperalgesia developed only by the third hour of NTG administration. Repeated administration of NTG caused chronic basal mechanical hypersensitivity, which was observed on the 5th day in the control group, and on the third day of the experiment in the hHCY group. In the "dark-light chamber" test, the time to the first entry into the dark chamber and the total time spent in the light chamber were significantly longer in the control group than in the hHCY group. Two hours after NTG injection, both parameters decreased in both groups, however, more significant changes were observed in the hHCY group.
Thus, in rats with prenatal HHC, there is an increased excitability of neurons and a predisposition to the occurrence of the phenomenon of CSD associated with migraine with aura. At the same time, mechanical algesia, photophobia, and anxiety in rats with HHC are also associated with a high risk of developing CSD [3,4,5]. The work was supported by the Russian Science Foundation (project no. 20-15-00100)
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### S9.630. Features of the gliotransmitter interaction of neurons and astrocytes during the propagation of calcium signals

Verisokin A.Yu.[1]*, Verveyko D.V.[1], Brazhe A.R.[2], Postnov D.E.[3]
[1]*Kursk State University;*
[2]*Lomonosov Moscow State University;*
[3]*Saratov State University;*
* ffalconn@mail.ru

Intensive experimental studies of the brain have shown that exhaustive understanding of the basic mechanisms of signal transmission in the nervous tissue, is impossible within the limitation of modeling exclusively neural connections. Thus, it has been shown that a variety of extrasynaptic connections mediated by processes occurring both

in glia, in particular in astrocytes, and by diffusion of neurotransmitters in the intercellular space play no less important role. The recent understanding of the functioning of the nervous tissue has led to the necessarity of modeling the processes occurring in a neurogliovascular unit, implying the result of the coordinated interaction of its elements as the required condition for the normal physiological state of the nervous tissue as a whole. The presented model study is based on currently available, but still far from complete, knowledge about the effect of the neurotransmitter norepinephrine on the functioning of the neurogliovascular unit and the effect of gliotransmitter pathways on the nervous tissue activity in general. We are based on experimental data, according to which, in the presence of norepinephrine, the astrocyte becomes more sensitive to near-synaptic glutamate, responding with bursts of calcium activity to weaker synapse activity [1], according to the GANE hypothesis ("glutamate amplifies noradrenergic effects") [2], the release of norepinephrine in some places increases with the release of glutamate in the case of increased activity of nearby synaptic terminals. The study of these processes is of key importance for understanding the processes of removal of harmful metabolites from the brain parenchyma and, thus, determining ways to reduce the risks of development and progression of neurodegenerative diseases [3].

Accounting for these mechanisms requires an adequate construction of the spatial structure of the mathematical model. The essential features of the proposed model are: two-dimensional representation of the astrocyte morphology based on experimental data; randomized in space and time distribution of activity of synaptic terminals; spatially heterogeneous distribution of the level of norepinephrine, including both scattered levels and localized sources; the action of the gliotransmitter secreted by the astrocyte during calcium activity. As a basis, we took the model with complex cell morphology that we have proposed earlier, which describes calcium dynamics on a two-dimensional astrocyte template [4].

A computational study of the proposed model was carried out, which made it possible to confirm the compliance of the results of numerical experiments with the available experimental data. A high degree of regularity in the formation of calcium waves was shown, which is associated with the fact that with a spatially irregular morphology of the astrocyte, there is inevitably an area of predominant wave initiation, and the presence of a refractoriness time for the calcium oscillator fixes this location as a pacemaker for future waves. It has been shown that the addition of norepinephrine causes an immediate surge in the level of IP3 and the birth of a calcium wave (for biological oscillators, this effect is known as a "phase reset"), depending on the pathways of stimulation with norepinephrine, a different level of change in the concentration of IP3 occurs, which determines the different frequency of generation of calcium waves. The performance of the GANE hypothesis was tested: it was shown that the "successful" mutual arrangement of sources of norepinephrine and synaptic terminals makes it possible to enhance both glutamate and noradrenaline activity and, thus, create cites of high neuronal activity. It has been shown that norepinephrine, in addition to a direct effect on the astrocyte, also enhances presynaptic activity. In turn, the distribution of glutamate leads to the activation of N-methyl-D-aspartate (NMDA) receptors, the release of gliotransmitters (for example, D-serine) accelerating the further release of norepinephrine. Together, these pathways form a double positive feedback loop that can drastically enhance the neuromodulatory effect in a small area of the astrocyte.

The proposed model can be used as a template model for further theoretical studies of the effects associated with the action of norepinephrine on the synapses and astrocytes, taking into account gliotransmitter dynamics, to analyze situations which are difficult or impossible to reproduce and control experimentally.

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## S9.631. Free radicals and signal transduction in cells

Martinovich G.G.[1]*, Martinovich I.V.[1], Voinarouski V.V.[1]
[1]*Belarusian State University;*
* martinovichgg@mail.ru

The formation of free radicals in cells is induced by many physical and chemical factors, including ionizing and non-ionizing radiation, mechanical stretching, temperature changes, nanomaterials, etc. Initially, the formation of free radicals in the organism was associated with the development of chronic and degenerative diseases. At present, it has been shown that free-radical products of oxygen metabolism participate in the regulation of a wide range of biochemical and physiological processes, including regenerative and adaptive processes, cell differentiation and apoptosis [1]. During signal transduction in cells with the participation of free radicals (redox signaling), in a series of electron transport processes, a directed of electrons transfer from proteins to oxygen occurs, which changes the conformation and activity of biological molecular "machines". However, the principles of recording and reading information in cells, which are realized with the participation of free radicals, have not yet been fully studied. This is largely due to the grand complexity of information processes that involve intracellular oxidizing and reducing agents.

Transduction of the regulatory redox signal occurs not by a single messenger molecule, but by a group of interacting oxidizing and reducing agents that form electron transport chains [2,3]. An increase in the content of oxidants as a result of a disruption of redox signaling processes causes a whole range of pathological processes and cell responses leading to the development of oxidative stress and pathology. On the other hand, a disruption of redox signaling by an increase in the intracellular concentration of reductants causes the development of such a pathophysiological state as reductive stress.

The necessary balance between oxidizing agents and reducing agents (redox homeostasis) in mammalian cells is regulated by the Nrf2 transcription factor, whose activity is controlled by the redox-dependent protein Keap1 [4]. Under normal conditions, Keap1 noncovalently binds Nrf2 and targets it to degradation in the 26S proteasome. Moderate oxidative stress and electrophilic agents disrupt the Keap1-Nrf2 interaction and the Nrf2 activates transcription of hundreds of genes involved in cell protection and adaptation to oxidative stress. Due to the key role of the Keap1-Nrf2 system in cell adaptation under stressful conditions, it is considered as a potential target for the treatment of a wide range of diseases [5,6]. However, in response to elevation of cellular oxidants above a critical threshold, Nrf2 stimulates expression of Klf9 transcription factor, resulting in further Klf9-dependent increases in oxidants and subsequent cell death [7]. Thus, the regulation of free radical processes in cells is carried out by a complex network of interactions between oxidizing and reducing agents, which determines the specificity of the response of a biological system.

Quantitative characterization of the unique network of interactions that determines the species and individual features of redox homeostasis is a necessary step for creating approaches for differentiation of physiological and pathophysiological processes involving free radicals. The task is to develop new concepts and physical models that do not currently exist in their finished form. One of the key problems is the establishment of fundamental physicochemical mechanisms that determine the

interaction of structural components in the network processes of regulation of redox homeostasis.
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### S9.632. Functional state of motor systems during simulated hypogravity and during the readaptation period. Effects of spinal cord stimulation

Fedianin A.O.[1,2]*, Baltin M.E.[1,2], Zaytceva T.N.[1], Shulman A.A.[1], Baltina T.V.[1], Eremeev A.A.[1]
[1]*Kazan (Volga region) Federal University;*
[2]*Volga Region State University of Physical Culture, Sport and Tourism;*
* artishock23@gmail.com

The main factors that determine the characteristics of motor systems are assumed to be exogenous gravitational forces and endogenous muscle forces. However, the specific ways and mechanisms of their action remain unclear. The wide prevalence of pathologies accompanied by changes in motor qualities and the intensive exploration of outer space makes it necessary to obtain new knowledge about the mechanisms of reorganization of motor skills, detailing the role and contribution of both peripheral and central structures of neuromotor systems to these processes. No less important is the understanding of the processes of restorative readaptation of the motor apparatus after the normalization of functioning conditions. Therapeutic approaches proposed to increase the speed and efficiency of recovery of motor function.

The aim of the work was to evaluate the effect of electrical and non-invasive magnetic stimulation of the spinal cord on the functional state of the neuromotor apparatus of the soleus (SM) and tibialis anterior muscles (TA) of the rat tibia during gravitational unloading and during the period of posthypogravitational readaptation.

Model experiments were carried out on laboratory male rats weighing 190-210 g in strict accordance with accepted bioethical standards. Animals were divided into the following experimental groups: "HU" - animals with simulated gravitational unloading of the hind limbs (7, 35 days; n=11); "HU+MS" - animals with simulated gravitational unloading of the hind limbs, combined with magnetic stimulation of the spinal cord (7, 35 days; n=10); “HU+ES” – animals with simulated gravitational unloading of the hind limbs, combined with electrical stimulation of the spinal cord (7, 35 days; n=9); "RD" - animals in conditions of readaptation to the action of the support reaction force and axial loads after simulated gravitational unloading (1, 3, 7, 14 days; n=18); "RD+MS" - animals under conditions of readaptation combined with magnetic stimulation of the spinal cord (1, 3, 7, 14 days; n=16); "RD+ES" - animals under conditions of readaptation combined with electrical stimulation of the spinal cord (1, 3, 7, 14 days; n=14).

Modeling of gravitational unloading was carried out by the generally accepted method of antiorthostatic hanging of the rat by the tail. To study the effects of readaptation to the action of the reaction force of the support and axial loads in animals, gravitational unloading of the hind limbs was modeled. Spinal cord stimulation was performed in the area of localization of the motor centers of the studied muscles (L4-S1 segments). Magnetic stimulation (HU+MS, RD+MS groups) was performed with a magnetic stimulator, an 8-shaped inductor. Electrical stimulation (HU+ES, RD+ES groups) was performed through pre-implanted electrodes. Stimulation parameters: daily for 90 minutes in series of 10 minutes with an interval of 10 minutes; amplitude of stimuli - threshold for contraction of the leg muscles; frequency - 3 Hz.

After the expiration of the experimental conditions, the reflex (H) and motor (M) responses of SM and TA were recorded. The threshold of occurrence, maximum amplitude, latency, and duration of evoked potentials were determined. The ratio of the maximum amplitudes of the reflex and motor responses was calculated.

The analysis of H response parameters indicated an increase in the reflex excitability of rat calf motor neurons both during 7-day (for SM) and 35-day (for SM and TA) simulated microgravity. The recorded decrease in the maximum amplitude of the M-response of the SM after prolonged unloading indicated a decrease in the total number of motor units, the development of atrophic processes. Activation of spinal structures under conditions of short-term (7 days) simulated unloading prevented changes in the reflex excitability of spinal motor centers, however, did not exclude hypogravitation-determined transformations during long-term unloading (35 days).

Under the conditions of posthypogravitational readaptation after 7 days of unloading, the reflex excitability of the corresponding motor centers approached the control level by 1 day already. After 35 days of simulated microgravity during readaptation for 1 day, a decrease in the reflex excitability of motor neurons of the SM and TA was observed, then the excitability increased. An increase in the latency and duration of the recorded potentials was noted. The restoration of the morphofunctional state of the muscle after unloading is obviously accompanied by a sharp increase in peripheral afferentation, including from the muscles of antagonists, the motor neuron pools of which are connected by reciprocal relationships; reinnervation processes and, as a result, desynchronization of recruitment of motor units. Under the conditions of spinal cord stimulation during the readaptation period, no sharp changes in the reflex excitability of the motor centers were observed.

On the 1st day of readaptation, an increased level of activity of motor neuron pools remained, however, by the 3rd day of the readaptation period, these indicators approached the control level, and no significant changes were recorded at the next studied stages of readaptation. Also, under conditions of readaptation combined with spinal cord stimulation, no changes in the threshold, latency and duration of the M-response were recorded, and the amplitude of the motor potential was restored to control values already 3 days later.

We conclude that spinal cord stimulation can activate the processes of neuronal plasticity, promote the reactivation of existing and, possibly, the formation of new intraspinal locomotor circuits. Data on the effectiveness of spinal cord stimulation can be taken as a basis for developing a therapeutic protocol for neurorehabilitation of patients after impaired/limited motor function.

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**S9.633. Fungi- and cytostatic properties of nanoscale potentially membrane-active 1,10-phenanthrocyanines (bi-1,10-phenanthrolylenes) of redox-inert Zn(II) and Cd(II)**

Demidov V.N.[1]*, Bogomolova E.V.[2], Sharoyko V.V.[3,4], Rochev R.D.[1], Badalyan A.G.[5]
[1]*I.V. Grebenshchikov Institute of Silicate Chemistry of RAS, St. Petersburg, Russia;*
[2]*V.L. Komarov Botanical Institute of RAS, St. Petersburg, Russia;*
[3]*St. Petersburg State University, Peterhof, Russia;*
[4]*Acad. I.P. Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia;*
[5]*A.F. Ioffe Institute of Physics and Technology of RAS, St. Petersburg, Russia;*
* vndemidov@mail.ru

Since the problems of treating fungal and oncological diseases are still far from their final solution, the search for new effective medicinal fungi- and cytostatic agents continues [1]. Among cytostatics, compounds of the dihydropyridine class attract attention [2]. The binuclear 1,10-phenanthrocyanine (bi-1,10-phenanthrolylene) complexes (PC) of d-elements contain their derivatives, dihydro-bi-1,10-phenanthrolines, as pharmacophore bridging ligands [3].
The fungi and cytostatic activity of diamagnetic (with strong field ligands) nanoscale binuclear complexes of electronic analogues Zn2+ [Ar]3d10 and Cd2+ [Kr]4d10 (phen)nM2+(μ-σH,πH-PCH)M2+(phen)n(–OAc)4 with pharmacophore N,N'–N',N"-bis-chelated 1,10-phenanthrocyanine (bi-1,10-phenanthrolylene) bridging ligands μ-σH,πH-PCH (phen–1,10-phenanthroline, -OAc–acetate anions, n=0-2), their initial purple-violet (and derivatives of yellow-brown chromophore forms μ-σH,πH-PCH'), soft colored colloidal glasses. PC synthesis was carried out using a thermal metal-assisted non-dehydrogenative C(sp2) H coupling of 1,10-phenanthroline [3] in the precursors of M(phen)n(OAc)2 (n=1-3). One of its central stages is the nucleophilic heteroaromatic substitution of hydrogen SNH. The PCs are characterized by IR spectroscopy, NMR spectroscopy, ESR, and elemental analysis data. Preliminary data on the fungistatic properties of yellow-brown forms (phen)nM(μ-PCH')M(phen)n(OAc)4 (M=Zn2+, n=1; Cd2+, n=2) with respect to fungi from the genera Aspergillus, Penicillium and Trichoderma show that the activity of Cd(II) PC is much higher than for the Zn(II) compound. Since Cd(II) PC is a thermodynamically stable coordination saturated compound, the bioavailability of Cd2+ ions is minimal. On the contrary, in Zn(II) PC, the bioavailability of Zn2+ ions is very high, due to the easy substitution of coordinated acetate anions for water molecules. On the other hand, the yellow-brown form of Cd(II) PC (like Zn(II)) It contains a redox-sensitive form of the bridge ligand μ-PCH', which can provoke significant redox processes in the biological environment. The redox-inert Zn2+ and Cd2+ ions in PC, unlike the complexes of redox-active Mn(II) and Co(II) studied together with them, cannot be initiators of biologically significant redox processes. The fungistatic activity of compounds progressively and unexpectedly increases in the series Co(II)<Zn(II)«Mn(II)<Cd(II). PC of redox-sensitive Mn(II) in its activity approaches the compound of redox-inert Cd(II). As follows from the cytotoxicity study (phen)nZn(μ-PCH')Zn(phen)n(OAc)4 (n=0, 1) on human MCF-7 (breast carcinoma) cell lines, the activity of the complex with n=0 is an order of magnitude higher than for n=1. This is consistent with lower coordination saturation and greater bioavailability of Zn2+ cations for the first substance. Study of DNA complexation with Zn(phen)2(OAc)2 and (phen)Zn(μ-PCH')Zn(phen)(OAc)4 showed that intercalation takes place in both cases [4]. The tendency of more complex associates of Zn(II) and Cd(II) PC, as volumetric chromophores, to IMI of the dispersion type [5] should lead to their affinity with cell membranes and cellular organelles. Dihydropyridine fragments, which are part of their bridging ligands, can play a certain role in the cytostatic properties of compounds. For PC of Zn(II) [6] and Cd(II), temperature- and photo-accessible electronic biradical triplet states were studied by the ESR method, since it cannot be excluded that such reactive forms may be the cause of thermo- and photo-activation of fungi and cytostatic action of compounds.
The study of EPR complexes was performed in the Lab. microwave spectroscopy of crystals of the Dep. of Solid State Physics of IPT of RAS and RC MRMI of St.-Pt. State Univ., NMR – in RC MRMI of St.-Pt. State Univ. and in St.-Pt. State TI (TU), fungistatic properties – in the BIN of RAS, within the framework of the topic «Biodiversity, ecology and structural and functional features of fungi and fungi-like protists» (AAAAA-A19-119020890079-6).
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**S9.634. Genetic mechanisms associated to ataxia**

Rodríguez-Labrada R.[1]*, Velazquez-Perez L.[1], Baryshev M.G.[3], Hernandez-Caceres J.[1], Svidlov A.A.[2,3], Dzhimak S.S.[2,3], Drobotenko M.I.[2]
[1]*Centro para la Investigación y Rehabilitación de las Ataxias Hereditarias;*
[2]*Kuban State University;*
[3]*Southern Scientific Centre of the RAS;*
* roberto.rodriguez@cneuro.edu.cu

Type-2 spino-cerebelar ataxia (SCA2) is characterized by progressive impairments in gait incoordination, speech and eye saccades, which involves different regions in the cerebellum. Its worldwide prevalence is estimated in about 5–7 cases per 100,000 people, although higher figures have been reported in particular populations, as in the Baguanos municipality in Eastern Cuba, where it reaches 142 cases per 100,000 inhabitants, the highest prevalence in the world. The underlying mutation of SCA2 is an unstable expansion of a polyglutamine domain within ataxin-2. The size of the polyglutamine expansion has a strong influence on the age at onset as well as the severity of disease [1].
The expansion of CAG trinucleotide repeats leads to disruption of protein function and neurodegeneration. Currently, the physiological function of ataxin-2 is not well understood, but several evidences suggest that ataxin-2 is involved in RNA metabolism and translational regulation. In particular, polymerase slippage while reading from CAG expansions has been suggested as an important pathophysiological mechanism [2].
In the framework of a mathematical model for angle DNA dynamics it was studied the influence of the torsion influences at the area of the promoter region of the DNA molecule corresponding to the ATXN2 gene, upon the appearance of areas of open states (OS) [3,4].
Defining M1 as the minimal – value of the torsion moment (TM) leading to the appearance of an OS in the promoter zone, and M2 as the minimal value of the TM leading to the appearance of an OS in the region of protein coding. It was obtained that the value of M2-M1 depends upon the length of the CAG-repeat and is reduced as the CAG-repeat becomes longer.
Thus, for a sufficiently long CAG-repeat, even a small increase in the torsion influence needed for OS formation in the promoter zone

may lead to an almost simultaneous appearance of a zone with OS in the coding region, which expands towards the promoter region. This process may lead to impairments in the genetic information reading process.

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### S9.635. Hepatoprotective effect of peroxiredoxin 6 in renal ischemia

Kurganova E.A.[2,1]*, Gordeeva A.E.[1], Nososelov V.I.[1]
[1]*Institute of Cell Biophysics of RAS;*
[2]*Pushchino State Natural Science Institute;*
* kurganova.ezhena@yandex.ru

There are multilevel connections between the kidneys and the liver, which leads to their joint damage if only one of them is injured. So ischemia-reperfusion (I-R) damage to the kidney leads to damage to the liver. It is believed that oxidative stress plays a key role in this process. To eliminate the damaging effect of kidney reperfusion on the liver, the use of antioxidant agents is required. In the present work, an antioxidant, the enzyme peroxiredoxin 6 (Prx6), was chosen as a protector of the liver tissue from oxidative stress in renal IR lesions. Prx6 is a unique multifunctional enzyme that performs a key protective antioxidant function in the cell. Prx6, along with peroxidase activity, has peroxynitrite reductase and phospholipase activities and is involved in intracellular cell signaling.

The aim of this work is to study the effect of exogenous Prx6 on the state of the liver in the initial reperfusion period after renal ischemia. Male Wistar rats were used in the experiments. Exogenous Prx6 was administered intravenously 15 minutes before renal ischemia. The period of ischemia is 45 minutes, reperfusion - 2, 5 and 24 hours. Recombinant Prx6 was obtained in the Laboratory of Reception Mechanisms of the Institute of Cell Biophysics of RAS.

Damage to the liver was noted after 2 hours of kidney reperfusion - expansion of the sinusoidal capillaries and vessels of the portal tracts, focal infiltration of leukocytes in the portal zone. With an increase in reperfusion time, there is an increase in the vascular reaction, the development of foci of hydropic dystrophy of hepatocytes, edema in the portal zones, with a pronounced focal-diffuse leukocyte infiltration, and cell apoptosis. Morphological changes in the liver with kidney damage affected the activity of liver enzymes in the blood. Their activity in the blood increased as much as possible after 5 hours of reperfusion: the activity of alanine aminotransferase - 3 times, aspartate aminotransferase - 9 times relative to the control values. On the contrary, when Prx6 was used, hepatocyte dystrophy was not observed, a mild vascular reaction and leukocyte infiltration were detected, apoptosis was reduced and liver enzymes activity were normalized.

I-R kidney damage leads to an increase in the level of interleukins in liver tissue within 24 hours, on the contrary, under conditions of protein use, there is no increase in the level of IL-6 and IL-10 cytokines in the liver. In addition, with renal I-R, an increase in the concentration of TBA-reactive products in the liver tissue was noted by 10 times after 5 hours of reperfusion of an ischemic kidney. The use of Prx6 does not lead to an increase in the concentration of TBA-reactive products in the liver during the entire period of reperfusion of an ischemic kidney. Their concentration remains at the level of control values.

Thus, I-R of the kidney leads to the development of pathological processes in the liver, which are maximally manifested after 5 hours of reperfusion. The use of Prx6 reduces liver damage, which is associated with its powerful antioxidant properties, which make it possible to neutralize the hyperproduction of reactive oxygen species already in the initial period of reperfusion.

The work is done in the framework of the state assignment of Pushchino Scientific Center for Biological Research of RAS (No 075-01512-22-00).

### S9.636. Hypothermic preservation of rat heart in Custodiol solution under pressure of carbon monoxide and oxygen gas mixture

Gurin A.E.[1]*, Gagarinsky E.L.[1], Fesenko (Jr.) E.E.[1]
[1]*Institute of Cell Biophysics, Russian Academy of Sciences;*
* gurinae@pbcras.ru

Organ transplantation in today's world remains the only way for many people to prolong life and improve its quality. Despite the year-on-year increase in the number of transplants in Russia, the number of patients on the waiting list exceeds the number of operations performed. Thus, the total number of heart transplants in Russia in 2021 was 290, while the waiting list for 2021 included 736 potential recipients. [1] This discrepancy is explained by the fact that the shelf life of the heart using standard methods of static cold preservation is 4-6 hours. Such a short storage period imposes significant restrictions on the availability of transplant material, in particular, on the possibility of collecting this organ in remote regions. To prolong the storage time of donor organs, such areas of research as the optimization of existing preservative solutions, oxygen persufflation, and normothermic apparatus perfusion have recently been developed. One of the new methods promising a serious prolongation of the deadlines for storage of a donor heart is gaseous hypothermic preservation under pressure of gas mixtures based on carbon monoxide and oxygen.

We have conducted a study on the restoration of the functional activity of the rat heart after hypothermic preservation under excessive pressure of a CO + O2 gas mixture washed with a Custodiol solution, which is the gold standard in transplantation practice in Russia. Experimental groups included: "Control", "Blood+Gas", "Custodiol+Gas", with subgroups according to organ storage time of 6, 12 and 24 hours. After preservation, the hearts were reperfused on an isolatedHEART-SR stand (Hugo Sachs Elektronik-Harvard Apparatus GmbH, Germany) and data on the developed pressure in the left ventricle and heart rate were recorded.

It has been shown that Custodiol, which in Russia is the gold standard for heart preservation, does not exert synergistic effects when used in combination with a protective gas mixture. In the rat hearts after being preserved with the use of Custodiol solution under high pressure of gas mixture for 12 hours, left ventricular contractility fell to 34 ± 9% of the intact control, taken as 100%. At the same time, in the hearts preserved without the use of Custodiol, this value was significantly higher, 61 ± 9%. After 6 and 24 hours of preservation, there were no significant differences in pressure generated by the left ventricle between rat hearts

preserved with and without the preservation solution: contractility reduced by about 75% (6 hours) and 30% (24 hours) as compared to control. It was shown that the heart was preserved better if the blood was not washed out. This fact can find its application in long-term gas hypothermic preservation with the use of blood cardioplegia or plegia with addition of blood elements to crystalloid solutions [2].

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### S9.637. Impact of High-Energy Proton Radiation on Visuomotor Perfomance in Nonhuman Primates

Tereshchenko L.V.[1]*, Borodachyova Yu.V.[1], Zhiganov L.S.[1,2], Shamsiev I.D.[2], Latanov A.V.[1]
[1]*Lomonosov Moscow State University, Faculty of Biology;*
[2]*Institute for Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences;*
* lter@mail.ru

Abstract: Two male monkeys (Macaca mulatta) were trained to perform a conditioned instrumental task consisting the execution of visually guided saccades to peripheral stimuli and manual responses. The task performance accuracy, saccadic and manual latencies before and after cranial irradiation with high-energy protons (170 MeV, 3 Gy) were analyzed. Within three months after irradiation, the animals demonstrated a stable level of task performance accuracy, but at the same time, saccadic and manual latencies increased. The results testify to the stability of the systemic mechanisms that control the instrumental performance to radiation exposure to protons. However, a slowing in the initiation of eye and hand movements, presumably, indicates early disorders in the processes of visuomotor integration and executive control as components of attention systems.
Key words: nonhuman primates, instrumental performance, ionizing radiation, protons, saccades, manual reaction, attention

### S9.638. Implementation of a colorimetric method of determining the mycobacterial growth and drug's MIC with new portable microbiological analyzer

Sychev A.V.[1]*, Lavrova A.I.[2,3], Dogonadze M.Z.[3], Postnikov E.B.[4]
[1]*Kursk State University, Research Center for Condensed Matter Physics;*
[2]*St-Petersburg University, Medical Department;*
[3]*St-Petersburg State Research Institute of Phthisiopulmonology;*
[4]*Kursk State University, Department of Theoretical Physics;*
* sychev1113@gmail.com

The usage of an indicator's colour change for the minimum inhibitory concentration (MIC) determining was proposed a sufficiently long time ago as a cheap alternative to radiometric and fluorometric instrumentation methods [1]. The key indicator substance for this application is resazurin (also known as Alamar Blue), which has blue colour changing to pink when resazurin reduces to resorufin due to the mitochondrial respiratory activity of viable microorganisms. However, in such a way it remained a qualitative method. On the contrary, in our work we address this approach quantitatively, revealing the possibility to determine parameters for both the mycobacterial growth curve and the functional response of M. tuberculosis (MbT) on a drug's concentration.

The work was carried out with a newly developed portable microbiological analyser [2] (patent RU2779840C1, priority date 2021-04-26) using the standard MbT strain H37Rv under the condition of the treatment-free growth and the action of the essential first-line antituberculosis drug isoniazid. The setup allows automated recording of light transmittance of the microbiological plate's cells in an adjusted spectral window with 15 min time steps. Such a discretization provides an opportunity for highly accurate data fitting to sigmoidal curves describing two-dimensional maps of the growth dynamics.

A special discussion will be devoted to the biochemical and biophysical mechanisms underlying the process quantification. It includes: (i) establishing a correspondence between concentrations of coexisting chemicals determined by spectral methods and components of photometric colour space; (ii) effects of the cell growth and division synchronization in mycobacterial colonies as reflected in a staged character of photometric curves discussed in the comparison to spectrofluorimetric ones [3].

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### S9.639. Importance of exposure sequence of neutron and proton radiations on the tumor response and skin reactions under irradiation of solid Ehrlich carcinoma on mice

Balakin V.E.[1], Rozanova O.M.[2]*, Belyakova T.A.[1], Smirnova E.N.[2], Strelnikova N.S.[1], Shemyakov A.E.[1,2], Smirnov A.V.[1]
[1]*Physical Technical Center, Lebedev Physical Institute of RAS, Protvino, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* rozanova.iteb@gmail.com

Introduction. Proton therapy is successfully used to treat the most common and complex types of cancer. For further extension and improvement of this type of therapy, methods and approaches to enhance the effect of proton radiation are being developed using FLASH therapy, nanoenhancers, and combined action with other types of radiation with synergistic or additive properties. In recent years, an idea has been advanced on the complex cell structure in cancer tumors as well as the presence of cancer stem cells in them, not only alleged to be capable of forming a tumor and maintaining its growth but also is responsible for the radioresistance of aggressive tumors and the occurrence of relapses after radiotherapy. Since there is substantial evidence indicating a contribution of cancer stem cells to the formation of secondary malignancies at later terms at the location of the primary tumor as well as in other sites, in order to increase the effectiveness of proton therapy, it is necessary to increase the impact on this population of tumor cells. Purpose. Impact of exposure sequence of combined action of proton radiation (PR) at a total dose of 80 Gy and neutrons at a dose of 5 Gy on the tumor response of the solid form of Ehrlich ascites carcinoma (EAC), reactions of the skin in tumor-bearing mice, duration of remission, incidence of tumor regrowth, and the life span was studied.

Materials and methods. The experiments were carried out using 8–9-week-old white outbred SHK male mice. The mice were kept in a vivarium at the ITEB RAS, under standard conditions and had free access to the standard dry ration and water. A total of 120 mice were used, with 15–30 mice in each group. As a tumor model, a fast-growing non-metastatic solid form of EAC was chosen. With the aim of inducing the solid form of EAC, the animals received intramuscular injections of $2 \times 106$ cells into the hips of the left hind legs. At this concentration of tumor cells, the frequency of tumor induction after 5 days is was 100%, and the death of animals occurs occurred after $47 \pm 5$ days. The first irradiation of EAC with PBSP in the Bragg peak or neutrons was carried out on the 5th day following the inoculation of EAC when the tumor nodule was palpated in each animal. In order to immobilize the mice at the time of irradiations, they were intraperitoneally injected with an anesthetizing mixture of Zoletil/Xylazine (0.7/3.4 mg/kg).

EAC in the first group of mice was irradiated only with PR in two fractions of 40 Gy with the interval of 24 hours ("protons"); EAC in the second group was irradiated once with neutrons at a dose of 5 Gy 3 h prior to the first fraction of PR ("neutrons + protons"); EAC in the third group was exposed to neutron irradiation at a dose of 5 Gy 3 h following the last PR fraction ("protons + neutrons"); and EAC in the fourth group of animals was exposed once to neutron irradiation at a dose of 5 Gy ("neutrons"). The fifth, sham control group consisted of unirradiated tumor-bearing mice, which were transported to radiation sources, anesthetized, and maintained under conditions imitating irradiation.

Results and discussion. In the "protons", "neutrons + protons" and "protons + neutrons" groups, a regression of primary tumor nodes in all mice a week after the irradiation and the absence of tumor regrowth within a month were observed. In the "neutrons" group, the delay of tumor growth induction was observed for 15 days in all animals, but then the tumor growth increased with a similar rate as in the control group. The delay in the tumor growth rate after irradiation with neutrons increased the maximum life span of mice to 77 days compared with the control where it was 54 days, and the average lifespan (AL) of irradiated animals increased by 15 days.

Since the palpable volume of the tumor was comparable with the size of the paw, and the tumor node was close to the skin, the skin received a high dose during irradiation. In the "neutrons" group, the development of grade 4 skin lesions was observed in 40% of mice, almost the same as in the "protons" group, and combined irradiation with neutrons delivered before protons increased almost twofold the frequency of the most severe skin reactions compared with the "protons" group. In the "protons + neutrons" group, the most favorable post-irradiation picture was observed: a low percent of mice with the 4th grade of skin damage and grade 1 skin damage in 30% of mice.

In mice exposed to combined irradiation, all cases of tumor regrowth were observed within 5–12 weeks after irradiation, by contrast to mice irradiated only with PR in which the resumption of tumor growth was observed at later terms, after 19–35 weeks. Throughout the observation period, the percentage of mice with the resumed growth of tumor of the same localization as the primary was 36% in the group "protons" and 50 and 43% in the groups "neutrons + protons" and "protons + neutrons", respectively.

The AL of mice without tumor regrowth in the groups with the combined exposure to neutrons and protons was significantly less (118 and 124 days) than in the group "protons" (278 days). The AL of mice with tumor regrowth in the group "protons" was 115 days after irradiation; in the groups "neutrons + protons" and "protons + neutrons", it was significantly less, 65 and 80 days after irradiation, respectively.

Cured mice, in which the tumor node did not develop at the site of irradiation throughout the life span, died earlier in the groups after the combined exposure than in the group irradiated with protons only. The maximum lifespan in the group "neutrons + protons" was 7 months, in the group "protons + neutrons" 11 months, and in the group "protons" 21 months.

Thus, the data on the duration of remission, incidence of tumor regrowth, and the life span of tumor-bearing mice demonstrate that additional exposure to neutrons on EAC both before and after PR significantly impairs the long-term effects of therapy.

### S9.640. Influence of the 8-oxyguanine-dna-glycosylase hogg1 gene polymorphism on the sensitivity of donor peripheral blood to the action of electromagnetic field

Tekutskaya E.E.[1]*
[1]*Kuban State University;*
* tekytska@mail.ru

The most common product of DNA oxidative damage under conditions of oxidative stress (OS) is 8-oxo-7,8-dihydroguanine (8-oxoG), which is the main biomarker of genome conformational rearrangements, an indicator of its destabilization [1]. According to ESCODD (European Standards Committee on Oxidative DNA Damage), the level of endogenous 8-oxoG in DNA is about 1 8-oxoG per 106G. Under genotoxic stress, this indicator can increase several times [1]. The appearance of 8-oxoG in the hereditary material of the cell indicates the destabilization of the genome as a result of the OS of the organism or its individual tissues and cells. During excisional repair, damaged areas are cut out of the DNA strand, then the resulting gaps are filled with intact material. In humans, 11 glycosylases have been isolated and described. These are 8-oxoguanine-DNA-glycosylases (OGG1, EC 3.2.2.23), which remove 8-OH-guanine, leaving behind a single-strand break [2]. Accumulating in biological fluids, 8-oxoG is one of the best biomarkers of genotoxic OS in various pathophysiological conditions, as we have shown previously [3].

To study the effect of gene sensitivity to the action of a low-frequency electromagnetic field (LF EMF), we analyzed the polymorphism of the 8-oxoguanine-DNA-glycosylase hOGG1 gene in healthy individuals. The polymorphism of the hOGG1 gene was assessed in blood serum in a sample of 18 apparently healthy donors (men aged 20–25, non-smokers). Blood was collected in 2.5 ml plastic tubes with the addition of EDTA as an anticoagulant at a final concentration of 2.0 mg/ml. When studying the rs1052133 polymorphic variants of the hOGG1 gene, a ready-made commercial kit developed by Sintol LLC was used. The reaction mixture for determining the Pro332Ala polymorphism of the 8-oxoguanin DNA glycosylase OGG1 gene was prepared according to the protocol. After completion of the amplification, the results of the study were analyzed using the Rotor-Gene Q software, setting the threshold line to 0.1 and highlighting the areas of the corresponding genotypes (Define Genotype, Wild Type, Heterozygous, Mutant). When interpreting the results, the flanking sequence was taken into account, the frequency of the minor allele G was taken equal to 30.21%, which corresponded to 1000 Genomes.

The results of PCR of blood serum samples of donors, carried out using genotyping and distribution analysis, showed that in healthy donors, there is mainly a homozygous C/C genotype of the hOGG1 gene, but along with this, there are heterozygous C/G- and homozygous for the G/G allele genotypes.

According to the results of PCR analysis, donors were divided into two groups: those with C/C genotype hOGG1 (11 people) - group 1 and C/G- and G/G genotypes (7 people) - group 2. To determine the resistance of the genomic material to oxidative modification under conditions of oxidative stress, whole blood samples from donors of two groups were treated with EMF at frequencies of 3, 30, and 50 Hz according to the procedure described in [3].

The degree of oxidative damage to DNA was assessed by the level of 8-oxoG concentration in blood serum, the content of which was determined by the method described in [3]. Treatment of blood samples of donors with EMF at frequencies of 3 and 30 Hz led to a significantly

higher content of oxidative damage 8-oxoG in the blood serum DNA of donors of the second group compared to the first. The initial level in control samples without exposure to EMF was 7.4 ng/ml for the 1st group and 6.3 ng/ml for the 2nd group. When exposed to EMF with a frequency of 3 Hz, the content of 8-oxoG significantly increased, reaching 14.8 ng/ml and 25 ng/ml, respectively. This indicates the maximum susceptibility to oxidative modification of DNA upon exposure to EMF under conditions of a normally functioning antioxidant defense system. Thus, donors with C/G- and G/G-polymorphism of the 8-oxoguanin DNA glycosylase OGG1 gene are more sensitive to the effects of low-frequency EMF, while donors with C/C polymorphism hOGG1 are much less susceptible to EMT, as evidenced by elevated levels of 8-oxoG in the blood serum of donors of the second group compared to the first after treatment of their blood samples in vitro with EMF.

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### S9.641. Investigation of the cytotoxic and radiosensitizing effect of pluronic-coated bismuth oxide nanoformulations on l929 mouse fibroblast cell cultures in vitro

Kolmanovich D.D.[1]*, Popov A.L.[1], Shemyakov A.E.[1], Zavestovskaya I.N.[2]
[1]*Institute of Theoretical and Experimental Biophysics of RAS;*
[2]*P.N. Lebedev Physical Institute of RAS;*
* kdd100996@mail.ru

Increasing interest in increasing the therapeutic efficacy of proton beam therapy is becoming increasingly relevant. Proton therapy is a well-established method of radiation therapy for the treatment of various types of cancer and other diseases. Numerous studies have shown an increase in the frequency of cell death along the proton Bragg curve, which is explained by the value of relative biological effectiveness (RBE), compared with standard photon beam therapy. The variability of the RBE of protons depends on the change in linear energy transfer (LET). [1] The promising ability of nanoparticles of various materials as effective radiosensitizers for local increase in tumor dose is being actively studied by various research groups. Nanoparticles with elements with high atomic number are considered as a strategy for improving tumor targeting and increasing the effectiveness of radiation therapy with proton and ion beams [2] due to a localized increase in the radiation dose in tissue filled with such nanoparticles compared to normal tissue without them. Charged particles can activate nanoparticles, and form radicals by the interaction of electrons emitted by nanoparticles. Clusters of emitted electrons and reactive oxygen species can lead to complex damage and enhance cell death. Bismuth compounds can serve as such nanosized radiosensitizers.

The relatively low toxicity of bismuth compounds allows them to be used for medical purposes. Thus, in radiation therapy, bismuth subcitrate has been used to date for the treatment of gastrointestinal diseases in clinical settings [3]. In addition, high atomic number bismuth is used for X-ray computed tomography (CT) due to its large X-ray attenuation coefficient (bismuth: 5.74 keV) [4]. We synthesized nanoformulations of bismuth oxide, followed by coating them with pluronic. After, a study was made of the cytotoxic and radiosensitizing properties of the synthesized nanoparticles using the methods of the MTT test and clonogenic analysis. The studies were carried out on cell cultures of mouse fibroblast line L929, which were supplemented with weighed portions of bismuth oxide nanoformulations coated with Pluronic. The final concentrations of nanoformulations were 1, 10, 25, and 50 μg/mL; after that, they were divided into non-irradiated groups and groups of cells irradiated with a proton beam in the Bragg peak at doses of 1.5, 3, and 5 Gy.

According to the results of the study using the MTT test method, it was shown that bismuth oxide nanoparticles coated with pluronic did not show a toxic effect without irradiation even at the maximum concentration (50 μg/ml). In the irradiated groups, already at a concentration of 1 μg/ml ($P < 0.0001$****), a sharp concentration-dependent decrease in the metabolic activity of cells was revealed. The maximum effect of radiosensitization of bismuth oxide nanoparticles coated with pluronic manifested itself at a concentration of 50 μg/mL after irradiation with a proton beam at a dose of 5 Gy. It should be noted that the optical density of formazan did not reach the lethal dose of 50% (LD50) and the maximum effect of radiosensitization was within ~ 35%.

The results of a parallel study using the analysis of clonogenic activity of cells revealed that bismuth oxide nanoparticles coated with pluronic at a concentration of 50 μg/ml caused a decrease in clonogenic activity of cells by 1.74 times compared with the control group. At the same time, after irradiation of L929 cells at a dose of 5 Gy, pre-incubated with nanoparticles at a concentration of 50 μg/ml, a complete cessation of cell proliferative activity was revealed.

Thus, the synthesized pluronic-coated bismuth oxide nanoparticles can be considered as a promising nanodispersed radiosensitizer for radiotherapeutic purposes.

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### S9.642. Investigation of the effect of phenolic substances under oxidative stress on nerve fiber

Syusin I.V.[1]*, Konusova D.A.[1], Karpova M.V.[1], Pinyaev S.I.[1], Revin V.V.[1]
[1]*National Research Mordovia State University;*
* ilya.sysin@gmail.com

Disruption of the nerve impulse can cause impairment of the body's functioning and death. Disruption of the proper functioning of nerve conductors can be observed during the development of neurodegenerative diseases in the body, in which the death of nerve cells progresses and gradually occurs atrophy of the corresponding tissues, with the onset of strong cognitive disorders.

The main factors leading to neurodegeneration include oxidative stress, disturbance of trophic function in the nervous tissue, insufficient blood supply, excessive release of glutamate and inflammatory factors.

Currently, the main methods aimed at nerve regeneration include surgical intervention and the use of stem cells. But their effectiveness remains

low. New therapeutic remedies are being sought to restore nerve fiber functions. Such tools should address issues such as enhancing growth factor expression, activating proliferative signaling pathways, reducing oxidative stress and proinflammatory cytokines, and improving neurovascular processes. And with such tasks successfully coped compounds secreted from plant sources: alkaloids and flavonoids. Therefore, plants are subject to pharmacological research to find new therapeutic remedies.
In connection with the above, the aim of the work was to study the effect of quercetin on oxidative stress processes in peripheral nerves.
The study used sciatic nerves isolated from rats of the Wistar line (200±20 g). Oxidative stress was initiated by hydrogen peroxide at a concentration of 20 mMol. Quercetin was used in a concentration of 10 mg/ml. Peripheral nerve incubation was conducted in a Ringer solution with the addition of active substances at a temperature of 38 0C.
After incubation, lipids were extracted from control and experimental samples using the Blye-Dyer method. The accumulation of Dienon conyugats (DC) and Malon dialdehyde (MDA) has been investigated to estimate the lipid peroxidation intensity. The amount of DC was determined using a spectrophotometric method at 233 nm. The amount of MDA was determined by the reaction between Malon dialdehyde and Thiobarbituric acid (TBC).
As a result of the conducted studies, changes in the quantitative content of DC and MDA in the nerve fiber were found during the development of POL processes and after the action of quercetin.
The content of DC and MDA in the intact nerve was 0.75 μMol/mg lipid and 0.4 μMol/mg protein, respectively.
The DC content in the test sample was 48% higher than the reference value after 30 minutes of incubation. The maximum change was recorded after 90 minutes of incubation and exceeded the reference value by 3 times, indicating an intensive oxidation process.
When the sample was incubated in a solution containing hydrogen peroxide and quercetin, there was a 65% decrease in DC compared to the experimental probe, indicating a decrease in the oxidation rate of lipids.
The amount of MDA after 30 minutes of incubation in the presence of hydrogen peroxide in the incubation medium increased by 63%. The largest change was recorded after 90 minutes and exceeded the reference value by 2 times. Quantitatively, the MDA content when using quercetin for 30 minutes was reduced by 24.6%, compared to the trial 1. With an increase in exposure time, the index decreased by 69.5%.
At 10 mg/ml of quercetin, DC and MDA levels were found to be significantly lower than those of the experimental group.
Based on the results obtained, it can be concluded that quercetin can have a protective effect, to combat oxidative stress due to its antioxidant and neuroprotective action.
It is obvious that quercetin, having antioxidant and neuroprotective properties, will inhibit the formation of lipid peroxidation products such as low-level MDA and DC.
Thus, the study of the molecular mechanism providing strong antioxidant ability of quercetin is of great importance for biomedical purposes. Therefore, research in this field, related to the action and properties of this substance, will come closer to the creation of a new class of regenerative drugs for the treatment of injuries, burns and various damage to the peripheral nervous system.

### S9.643. Investigation of the formation of a spiral wave on the cardiac tissue with various anisotropic properties of the substrate

Scherbina S.A.[1]*, Slotvitsky M.M.[1], Kalinin A.I.[1], Berezhnoy A.K.[1], Tsvelaya V.A.[1], Agladze K.I.[1]
[1]*MIPT;*
* scherbina.sa@phystech.edu

At the moment, according to various international studies, it has been established that the most common diseases leading to mortality in the population are cardiovascular diseases. For the correct diagnosis of the type of arrhythmia, a deep understanding of the basic mechanisms of cardiac activity is necessary both in normal conditions and in its various disorders. The most frequent disturbances are associated with the occurrence of spiral reentry waves. With the circulation of excitation and fragmentation of the spiral wave into chaotic chains, fibrillation of the heart tissue occurs. In an excitable medium, such spiral waves can form multiple rotating centers. The duration of reentry in the excitable tissue is maintained by reducing the refractory period of the medium and a long time of their holding. Such spiral waves can form on heterogeneous areas of the tissue, for example, on post-infarction scars.
In the work, cardiolayers were modeled, which have non-conductive sections in the form of an acute angle, provoking the appearance of spiral reentry waves. The aim of this work was to expand the range of stimulation frequencies that cause reentry formation depending on the degree of anisotropy.
In this work, an integrated approach was applied to the study of the formation of cardiac tissue depending on external conditions. Electrospinning (Nanon-01) was used to develop cell structures, depending on the different environment, microfibers were made from a solution of polycaprolactone. To improve the adhesive properties of the substrate, the fibers deposited on the carrier (cultivated glasses) were coated with the fibronectin protein. The treated polymer polydimethylsiloxane (PDMS) was used to create non-conductive areas on the samples in the form of right angles. We used a cell line of neonatal cardiomyocytes isolated from laboratory rats. The electrophysiological activity of the obtained cardiomyocytes, including the visualization of the conduction of their excitation waves through the tissue, was checked by optical mapping using calcium-dependent dyes. Using immunocytochemical analysis to obtain structural characteristics, cell samples were stained for the protein of the cell cytoskeleton f-actin and for the protein of the contractile apparatus of cardiomyocytes α-actinin, and cell nuclei were stained for DNA (DAPI).
First of all, a system was modeled that gave a wide corridor of critical frequencies, at which a reentry wave could potentially occur. Such a system was created on the basis of structural inhomogeneities. The usual thin obstacle gives an extremely narrow range of such frequencies in the control.
To increase the corridor of critical cases in which reentry occurs, in this work, we changed the structure of the sample itself with an angular obstacle, making the sample anisotropic due to directed fibers. For such samples, a jump of the excitation wave is formed on the obstacle and a reentry is formed already for a frequency of 3.3Hz. Statistically, the reentry on such samples is extremely stable, since it can catch on and include random inhomogeneities in its core, which are often found on anisotropic samples due to the difference in conduction rates.
2 types of angular obstruction sample were introduced: with parallel fibers and directional fiber patterns. For the first case, the angle was set in such a way that one of the walls of the angle was perpendicular to the direction of the fibers. For the second case, the patterns were made in such a way that the patterns were perpendicular to each other, and within each pattern the fibers were parallel and co-directed, by analogy with the letter "G". The angle for the case with different directions of parallel fibers was set at the border of the patterns. For the case with anisotropy created by parallel fibers alone, the reentry frequency corridor increased: the highest probability of occurrence was at 5 Hz stimulation. With a probability of 5.6%, reentry also occurred at a frequency of 3.3Hz.
In the case of samples with different directions of parallel fibers, the frequency corridor of reentry occurrence in comparison with simply parallel fibers became larger, but the reception of reentry became more stable: with a probability of 7% for a frequency of 3.3Hz. It is also worth emphasizing that for some of the samples (with a probability of 11%), the assimilation of the stimulation rhythm became impossible even for 2.5Hz. Potentially, such data may demonstrate an increase in the corridor of critical cases, especially for patients with mutations. More than 3 cases were tested for each frequency and for samples with different anisotropy.

Also, experiments were carried out on the appearance of reentry at the corner on anisotropic samples when an external electrical stimulus was applied from different sides of the corner: along the fibers and perpendicular to them. When the sample was stimulated in the direction along the fibers, the ratio of the velocities of the excitation wave fronts was 1.8, and when the stimulus was applied in the direction across the fibers, a jump and turn of the wave was observed over a larger frequency range, while the ratio of the velocities was Val/Vac=0.65. In the first case, that is, when the impulse was applied along the direction of the fibers, the reentry twisted at frequencies of 160–180ms, otherwise, that is, when the stimulus was applied perpendicular to the fibers, the reentry frequency corridor was from 180 to 250ms.
Thus, in this work, an environment as close as possible in terms of physiological characteristics was selected for the development and formation of excitable tissue from cardiomyocytes for the occurrence of pathologies and critical cases of excitation waves. These differences include an increase in the frequency corridor for the manifestation of critical cases in the case of the appearance of anisotropy, as well as anisotropic patterns, similar to the fibrous structure of the heart muscle.

**S9.644. Investigation of the influence of the CTAB coating the physicochemical magnetite nanoparticle's properties**

Shilova E.V.[1]*, Koltakov I.A.[1], Korchagina E.E.[1], Artjuhov V.G.[1]
[1]*Voronezh State University;*
* zinkovae@list.ru

Today, the use of magnetic nanoparticles (MNPs) in various fields of medicine is of great interest. Magnetite particles are preferred as magnetically controlled components of nanosystems due to its pronounced magnetic properties, as well as its lower toxicity compared to iron, cobalt and nickel. However, the aggregative stability of nanoparticles in complex body environments such as blood, tissue fluid, and lymph is a very important property. To prevent aggregation and flocculation, magnetite is modified with various stabilizing agents on its surface [4]: the coating allows increasing the circulation time in the bloodstream. An example of such surface-active stabilizing agent is cationic surfactant cetyltrimethylammonium bromide (CTAB) [2]. In this work, the objective was to investigate the effect of the coating of CTAB on the physicochemical properties of magnetite nanoparticles.
Experimental Methodology. Magnetite was obtained by co-precipitation. A mixture of salts of 2- and 3-valent iron with a total concentration of 30 mmol, in a stoichiometric ratio of 1:2, was used to prepare magnetite nanoparticles. A 1% ammonia solution was used as a precipitating agent. The obtained magnetite particles were washed 2 times with distilled water by placing in a magnetic field. To the magnetite obtained earlier, a 0.03% solution of CTAB was added and stirred for 5 minutes on a Microspin minicentrifuge-vortex. Then the supernatant was removed and the resulting precipitate was washed with distilled water. The obtained magnetite was dried in a FreeZoneTriad lyophilic dryer (Labconco).
The composition of the obtained magnetite was monitored using an X-ray diffractometer ARL X'TRA (Thermo Scientific). To assess the qualitative composition of the synthesized magnetite, X-ray diffraction patterns of the nanoparticles were recorded.
The ζ-potential of the nanoparticles before and after the addition of DTAB was measured using the electrophoretic light scattering method. A Zetasizer NanoZSP analyzer (Malvern Instruments) was used.
To obtain hemoglobin solution we used D.Drabkin method [1]. We used hemoglobin solution at a concentration of 10-5 mol/L. Hemoglobin was incubated with nanoparticles (1 mg nh per 1 ml of hemoglobin solution) for 30 minutes and 1 hour. Then, the MNPs was precipitated by centrifugation (8000 rpm, 10 min) on a MiniSpin centrifuge.
The size of hemoglobin molecules was measured using the dynamic light scattering method on a Zetasizer NanoZSP analyzer (Malvern Instruments).
Results and Discussion. To assess the qualitative composition of the synthesized magnetite nanoparticles, X-ray diffraction patterns of the nanoparticles were recorded. [3] The peaks 2θ = 18; 30.20; 35.53; 43.1; 57.1; detected on the X-ray radiographs correspond to those of the standard magnetite sample Fe3O4 .
To confirm the sorption of CTAB molecules, the ζ-potential of the MSF before and after the addition of CTAB was investigated. The synthesized magnetite nanoparticles had a ζ-potential equal to: -15.10 ± 4.51 mV. After coating with CTAB molecules the ζ-potential value was: 13.6 ± 3.99 mV. The values of the obtained ζ-potential suggest that, initially, based on Coulomb interactions, the sorption of single molecules of CTAB on the magnetite surface occurs with the formation of an extremely saturated monolayer of molecules of CTAB, the hydrophobic "tails" of which face outward. As the CTAB concentration increases, the second layer builds up due to hydrophobic interactions (formation of admocells) with positively charged CTAB groups facing outward.
To assess the possibility of practical application of the synthesized nanoparticles, their interaction with the main blood transport protein, hemoglobin, was studied. It was found that after a 30-minute incubation, the hydrodynamic diameter of the control hemoglobin sample was 5.01 ± 0.42 nm, and the modified MNPs and MNPs-CTAB was 5.44 ± 0.40 and 7.23 ± 0.33 nm, respectively. After 1 hour, the hydrodynamic diameter was 8.03 ± 1.08 nm (after MNPs exposure) and 73, 99 ± 4.41 nm (after MNPs-CTAB exposure).
The data obtained on the structure, properties and interaction of magnetite nanoparticles with proteins open up prospects for further study of the possibility of their practical application. From the data obtained, the need to develop methods to protect blood proteins from the action of magnetite nanoparticles follows.
The studies were carried out using the equipment of the Center for the Collective Use of Scientific Equipment of Voronezh State University
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**S9.645. Investigation of the role of chronic prenatal hypoxia in the functioning of neural-glial networks of the brain and their adaptation to acute stress in vitro**

Guseva E.A.[1]*, Vedunova M.V.[2], Mishchenko T.A.[3]
[1]*N. I. Lobachevsky State University of Nizhny Novgorod, Institute of Biology and Biomedicine;*
[2]*N. I. Lobachevsky State University of Nizhny Novgorod, Institute of Biology and Biomedicine;*
[3]*N. I. Lobachevsky State University of Nizhny Novgorod, Institute of Biology and Biomedicine;*
* eguseva655@gmail.com

Prenatal hypoxia is included in the list of the main causes of infant mortality and severe disability of newborns. Current adverse environmental factors, maternal stress, smoking and alcoholism lead to

insufficient supply of oxygen to the fetus. In addition, as a result of obstetric complications, the newborn may also be exposed to hypoxic effects. Oxygen starvation at an early stage of ontogenesis is one of the main risk factors for the development of psychopathologies, disorders in sensorimotor development and learning ability in adulthood. Oxidative stress induced by hypoxia is one of the main causes of epilepsy.

The aim of this study was to study the effect of chronic prenatal hypoxia on the functioning of neural-glial networks of primary hippocampal cell cultures and to assess the neural network adaptive potential to the effects of subsequent acute hypoxic stress. The object of the study was primary cultures of hippocampal cells obtained from 18-day-old embryos of mice of the hybrid line C57BL/6+C3H. Pregnant female mice from the 14th to the 18th day of gestation were placed for 2 hours in a sealed pressure chamber to create conditions corresponding to the rise to an altitude of 6500-7000 m above sea level. The control group of animals was not subjected to hypoxia modeling. Primary cultures of hippocampal cells were obtained from embryos on the 19th day of gestation, which were cultured for 21 days. On the 14th day of cultivation (DIV), part of the cultures were subjected to modeling of acute normobaric hypoxia by replacing the conditioned culture medium with a medium with a low oxygen content for 10 minutes. Spontaneous Ca2+ activity of primary hippocampal cultures was evaluated on the 1st, 3rd and 7th days of the posthypoxic period by calcium imaging with subsequent processing of the data obtained by original mathematical algorithms. Both the main parameters of spontaneous calcium activity (the number of functioning cells, the frequency and duration of the calcium signal) and the network characteristics of neural-glial networks were analyzed.

As a result of the conducted studies, it was shown that the effect of chronic prenatal hypoxia leads to a violation of the formation of the functional architecture of the neural-glial networks of primary hippocampal cell cultures, characterized by a significant decrease in the speed of signal propagation through the network and the number of functional relationships between the cells of the network. The effect of chronic prenatal hypoxia modulates the response of the neuron-glial network to the action of acute hypoxia, which manifests itself in the preservation of the main parameters of spontaneous calcium activity, such as the duration and frequency of Ca2+ oscillations, with a deterioration in the connectivity of cultures and the rate of propagation of the calcium signal, which is probably due to the restructuring of the functional architecture of the neuron-glial network. Further study of the molecular and cellular mechanisms induced by hypoxia at the neural network level will not only clarify and supplement the existing knowledge about the features of perinatal brain damage, but also improve the methods of diagnosis and treatment of hypoxic CNS injuries.

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**S9.646. Involvement of muscarinic receptors of M1 subtype in the regulation of neurosecretion in mouse motor synapses at different levels of intracellular ROS**

Kovyazina I.K.[1,2]*
[1]*Kazan Institute of Biochemistry and Biophysics;*
[2]*Kazan State Medical University;*
* irina.kovyazina@list.ru

One of the most effective forms of maintaining the stability of synaptic transmission is the modulation of evoked neurotransmitter release including autoregulation of the secretory process. In the neuromuscular synapse of vertebrates, autoregulation of acetylcholine release involves different types of cholinergic receptors, nicotinic and muscarinic, which suggests the possibility of modulating of neurosecretion through a number of signaling pathways.

It is known that nicotinic and muscarinic cholinoreceptors are targets for free radicals, which production can be elevated in nerve and muscle cells during increased activity and affect various targets in the area of synaptic contact. Although intracellular ROS are traditionally associated with oxidative stress, under normal physiological conditions they are necessary, and play the role of signaling messengers in a wide range of cellular processes, for example, participating in the maintenance of homeostatic neuronal plasticity to couple cellular metabolism with synaptic activity (Accardi et al., 2014; Diebold and Chandel, 2016).

The effects of blockade of M1 muscarinic receptors on the amplitude and temporal parameters of endplate potentials (EPPs) at low and high frequency of motor nerve stimulation were studied. The studies were carried out on isolated neuromuscular preparations of the BALB/c mice diaphragm muscle. EPPs were recorded intracellularly using standard microelectrode technique. Muscle contractions were blocked with μ-conotoxin GVIB (Peptide Institute Inc., Japan).

In the presence of selective M1 blocker VU-0255035 (0.1 μM, Tocris), a more pronounced depression of the EPP amplitudes was observed when the nerve was stimulated at a frequency of 50 and 70 Hz compared to the control. Thus, upon 70 Hz stimulation, the decrease was $43.0 \pm 3.6\%$ by the 15th stimulus in the train compared to $26.0 \pm 2.1\%$ in control. However, by the 40th stimulus, the difference in the severity of depression became insignificant. It is interesting to note that the decrease in EPP amplitudes during high-frequency stimulation was accompanied by an increase in the duration of the EPP rise time in intact preparations, and after the inactivation of M1 receptors, this increase in the EPP rise time was absent, and for the first ten EPPs in the pulse train, even a slight shortening of the EPP rising phase was observed. Nerve stimulation at 10 and 20 Hz led to the same depression of the EPP amplitudes in the control preparations and after the inactivation of M1 receptors, as well as to the same change in the duration of the EPP rise time.

Antimycin A, an antibiotic that selectively blocks the cytochrome-bc1-complex, the central enzyme in the respiratory electron transport chain, was used to increase the level of intracellular ROS, in particular, superoxide (Accardi et al., 2014). Incubation of the neuromuscular preparation for 15 min in antimycin A containing solution (5 μM) did not cause any changes in the parameters of EPPs during low-frequency nerve stimulation. With short-term rhythmic stimulation at 10 Hz frequency (1 s), synaptic depression after incubation with antimycin A was less pronounced than in control (by the 40th EPP in the train: $79.1 \pm 2.3\%$ of the amplitude of the 1st EPP compared with $73.9 \pm 0.9\%$). At 70 Hz, no significant difference in the development of synaptic depression between intact preparations and preparations incubated with antimycin A wase observed. After preliminary incubation of mouse diaphragm with antimycin A, blockade of M1 receptors led to a less pronounced changes in the EPP amplitude during the pulse train. The EPP rise time during the train changed in the same way as in control preparations. Thus, an increase in the production of intracellular ROS prevented the development of the effects of M1 receptors blockade on the amplitude and rise time of EPPs during high-frequency activity. A possible explanation for this phenomenon can be both the direct effect of intracellular ROS on the muscarinic receptor, and the fact that M1 receptors activation can regulate neurosecretion through the modulation of the redox status of the nerve ending. Elucidation of this mechanism is the subject of further research.

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### S9.647. Lactate in saliva is an indicator of an athlete's physical performance

Guk P.V.[1], Stepanova L.V.[1]*, Vyshedko A.M.[1], Sutormin O.S.[1], Kolenchukova O.A.[1,2], Zhukova G.V.[1], Kratasyuk V.A.[1,3]
[1]*Siberian Federal University, Krasnoyarsk, Russia;*
[2]*Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russia;*
[3]*Institute of Biophysics SB RAS, Krasnoyarsk, Russia;*
* slyudmila@mail.ru

One of the most important indicators of the athlete's body performance is the content of lactic acid (lactate) in the blood.
Saliva, which contains a large number of proteins, enzymes, and genetic molecules, is excellent as a diagnostic material for rapid analyses. Many compounds contained in blood are also present in saliva, therefore, it is functionally equivalent to blood serum, and can determine the physiological state of the body. Saliva, unlike blood, is a very dynamic biological fluid and changes in its component composition under physical stress of the body occur much earlier than in blood [1, 2].
Saliva testing is a simple, safe and non-invasive way to identify an athlete's performance and has high potential for the development of diagnostic methods in sports medicine as an invasive technology for use and decision-making about body fatigue. Currently, there is no comparative analysis of the lactate content in saliva and in the blood of athletes engaged in different types of physical activity. We believe that the lactate content in saliva is more stable and acceptable for analysis.
The purpose of the study: to identify the lactate content in saliva as an indicator of an athlete's physical performance.
The study involved athletes (n=31) engaged in luge (n=5), cross-country skiing (n=9), speed skating (n=7) and bobsleigh (n=10), qualified 1st category, candidate for master of Sports (CMS), master of sports (MS). The subjects performed a load with increasing power on the bicycle ergometer (the duration of the load is 5 minutes). At the beginning and end of the load (the last 30 seconds of work at a certain power level), the heart rate (HR) was recorded by an electrocardiogram, blood and saliva were taken for lactate content.
Lactic acid (lactate) in saliva was determined by spectrophotometric method (Shimadzu, Japan) by colorimetry of saliva with iron III chloride at a wavelength of 440 nm.
The lactate content in the blood before and after the load on the bicycle ergometer was provided by the staff of the Center for Physical Therapy and Sports Medicine (Krasnoyarsk).
Mathematical data processing was carried out in the Statistica 10 program (StatSoft Inc., USA) with the calculation of median (Me) and interquartile ranges (C25-C75 percentiles). The correlation was calculated by Spearman's criterion, the reliability of the differences between the indicators was evaluated by the nonparametric Wilcoxon criterion. The level of statistical significance was considered reliable at $p<0.05$.
The results of testing the functional state of the athletes' body showed that the heart rate after exercise is significantly higher than at rest ($p= 0.00001$), regardless of the qualification and type of load (aerobic or anaerobic) with which athletes train.
There was no significant difference in the lactate content in saliva and blood before exercise. The lactate content before physical exertion was the lowest for highly qualified athletes both in saliva and in blood, and the highest for athletes with qualifications of the 1st category and CMS, both in saliva and in blood.
The lactate content also depended on the type of physical activity that athletes are engaged in. Athletes engaged in aerobic physical activity (cross-country skiing) had the lowest lactate content in saliva and the highest lactate content in blood. Athletes engaged in anaerobic physical activity (luge, speed skating, bobsleigh), on the contrary, had the highest lactate content in saliva and the lowest content in blood.
After physical exertion, regardless of the type of physical exertion, the lactate content in the saliva of athletes increased slightly, in the blood it was much higher and significantly higher ($p=0.00001$).
For novice athletes, after physical exertion, the lactate content increased both in saliva and in blood ($p= 0.01$). The lactate content in the saliva of athletes with CMC and MS qualifications did not change after exercise, but it significantly increased in the blood ($p=0.0006$).
Thus, the lactate content in saliva at rest is comparable to its indicator in the blood only for highly qualified athletes engaged in aerobic sports. The lactate content in saliva at rest for athletes with CMS qualifications and novice athletes (1st category) is higher compared to its content in the blood mainly for athletes engaged in anaerobic sports. Physical exertion increased the lactate content in the saliva of novice athletes, and the change in lactate was insignificant for athletes with CMC and MS qualifications, regardless of the sport they are engaged in. The lactate content in the blood, on the contrary, sharply increased for highly qualified athletes and significantly for athletes with the qualifications of the CMC and the 1st category, regardless of the type of sport.
Apparently, in the mode of operation of an athlete with a high heart rate, the blood buffer system has accumulated the maximum concentration of lactate. At the same time, the lactate content in saliva changes only for novice athletes. It is known that highly qualified athletes have a low concentration of lactate, which characterizes their high athletic performance of the body in relation to a novice athlete whose body is not adapted to physical exertion. Consequently, the lactate content in saliva can determine the efficiency of the body.
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### S9.648. Link between conformation of cytochrome C heme, mitochondrial membrane potential and respiratory chain activity under normal and pathological conditions

Brazhe N.A.[1]*, Nikelshparg E.I.[1], Baizhumanov A.A.[1], Grivennikova V.G.[1], Semenova A.A.[2], Novikov S.M.[3], Volkov V.S.[3], Arsenin A.V.[3], Yakubovsky D.I.[3], Bochkova Z.V.[1], Sosnovtseva O.[4], Maksimov G.V.[1], Rubin A.B.[1]
[1]*Moscow State University;*
[2]*MSU, Faculty of Materials Science;*
[3]*MIPT;*
[4]*Copenhagen University;*
* nadiya.brazhe@gmail.com

The redox state of cells is closely related to the activity of the respiratory chain (electron transport chain, ETC) of mitochondria. Regulation of the mitochondrial respiratory chain is important for providing cells with ATP, as well as for optimizing the amount of superoxide anion radical formed in the ETC. The generation of the superoxide anion radical followed by the formation of other reactive oxygen species can be used in signaling processes or can lead to the development of oxidative stress. Cytochrome C is an important component of the ETC, which carries electron between complexes III and IV in the intermembrane space of mitochondria. Under certain conditions, cytochrome C can exit mitochondria and initiate apoptosis. Despite numerous studies of mitochondria, it remains unknown whether there are mechanisms of regulation of cytochrome C activity that affect its conformation and electron transport properties.
We have developed a new methodological approach based on surface-enhanced Raman spectroscopy (SERS) with the use of silver plasmonic nanostructures, which makes it possible to study the

cytochrome C heme conformation in intact functioning mitochondria. We found that the SERS spectra recorded from isolated mitochondria of cardiomyocytes represent a set of peaks characteristic of atomic vibrations in the pyrrole rings and methine bridges of the heme of oxidized cytochrome C and depend on the conformation of the heme. We have shown that cytochrome C heme reversibly changes its conformation between planar and ruffled forms during ETC activity, while depolarization of the inner mitochondrial membrane and the decrease in the concentration of protons in the intermembrane space increase the probability of the planar heme conformation, while hyperpolarization of the inner mitochondrial membrane and the increase in proton concentration decrease the probability of planar conformation. It is known that for the transfer of an electron between cytochrome C1 and cytochrome C, the correct orientation of the hemes relative to each other is necessary, while in the case of cytochrome C, the planar conformation of the heme is optimal. We assume that the depolarization of the inner mitochondrial membrane, which occurs during the active synthesis of ATP in ATP synthase with an increase in cellular activity, the cytochrome C heme acquires the planar conformation that accelerates the electron transfer between complex III and cytochrome C, increasing the activity of the entire ETC and promoting the synthesis ATP. We found that under hypertension in isolated mitochondria of cardiomyocytes the potential on the inner mitochondrial membrane does not affect the cytochrome C heme conformation, which can lead to a deterioration in the regulation of electron transport activity. We believe that the proposed approach to the study of cytochrome C in mitochondria can be a promising tool for studying the mechanisms of regulation of cytochrome C properties in intact mitochondria.

### S9.649. Machine learning methods for forecasting outcome of COVID-19 disease in children

Kuznetsova A.V.[1,2]*, Voronin E.M.[1], Samitova E.R.[3]
[1]*FSBI Central Research Institute of Epidemiology (CRIE) of Federal Service for the Oversight of Consumer Protection and Welfare (Rospotrebnadzor);*
[2]*N.M. Emanuel Institute of Biochemical Physics (IBCP);*
[3]*GBUZ Children's City Clinical Hospital named after Z.A. Bashlyaeva;*
* azforus@yandex.ru

A database of clinical and laboratory parameters in children with Covid-19 disease was carried out in the work. The multiparametric analysis was carried out using machine learning methods based on original methods of optimally reliable partitions and statistically weighted syndromes.
The incidence of Covid-19, according to general opinion, was more concerned with the adult population. But the children also got sick. A database of clinical and laboratory parameters in children with Covid-19 was collected at the Tushino Hospital. The tests were taken at admission to the hospital, on day 7 and on day 11. The number of indicators is 55. The number of patients with a fatal outcome was small - the first class was 10 people. The number of patients discharged from the hospital after recovery - second class – 45. The total number of patients is 55.
We used machine learning methods from the Data Master Azforus complex. On a set of the best methods, an ensemble was used to improve the recognition result.
Comparison of machine learning methods for the use of clinical and laboratory indicators in patients on admission to the hospital gave the following results (in parentheses, the results of Roc Auc): Statistically weighted syndromes - 0.647, Decision trees - 0.644, Linear discriminant analysis - 0.578, Gradient boosting - 0.533, Nearest neighbor method - 0.522. These are rather low recognition values. The ensemble on these 5 MO methods showed the value of AUC = 0.678. This corresponds to 42 out of 55 correctly recognized outcomes of the disease (76.4%).
After three days of hospital stay, recognition by machine learning methods increased slightly. Linear discriminant analysis - 0.656, Decision trees - 0.644, Statistically weighted syndromes - 0.630. The ensemble on these three methods recognized 41 correct outcomes (74.5%) on a sliding control (Leave-One-Out). The ROC AUC=0.693 slightly increased.
On the 7th day of hospital stay, recognition was already better: Decision trees -0.744, Statistically weighted syndromes - 0.739, Linear discriminant analysis - 0.689. The ensemble on these three best methods has already shown ROC AUC= 0.822. In the group of deceased, 7 (70.0%) out of 10 people were correctly recognized. In the group of recovered, 39 (86.7%) out of 45 people were correctly recognized. The total number of correct recognitions is 46 (83.6%).
On the 11th day after hospitalization, the recognition result on the sliding control is as follows: Statistically weighted syndromes - 0.897, Adaptive boosting-0.850, Decision trees - 0.850, Gradient boosting - 0.799, Linear discriminant analysis - 0.644. The results of the ensemble on these three best methods are ROC AUC=0.9433. In the class of the deceased, the number of correct recognition has not changed - 7 (70.0%). In the class of patients discharged from the hospital, recognition was 100.0% - 45 people were correctly assigned to their class. The total recognition result is 52 people (94.5%).
The following are significant indicators from the point of view of recognizing the class of deceased from the class of recovered patients (indicator with the designation of the point of study, the division boundary, significance): 1) CRP B - 30.24 - $p<0.0005$; 2) Urea B - 13.15 - $p<0.0005$; 3) CRP 7 - 49.325 - $p<0.001$; 4) Glucose B - 6.325 - $p<0.001$; 5) Total protein B - 56.35 -$p<0.007$; 6) Glucose 7 - 5.75 - $p<0.008$; 7) albumin B - 35.9 - $p<0.008$; 8) LDH B - 952.95 - $p<0.014$; 9) Creatinine 3 - 78 -$p<0.019$; 10) AST 0 - 177.7 - $p<0.022$; 11) Creatinine B – 66 - $p<0.023$; 12) Urea 7 - 11.6 - $p<0.023$; 13) LDH 7 - 805.6 - $p<0.047$; 14) Platelets B - 190.5 - $p<0.048$.
Thus, the most significant indicator was the C-reactive protein (significance on the permutation test $p<0.0005$). According to publications, this has already been noted in articles. If the value of this indicator is higher than 49.325 on the 7th day of the hospital and higher than 30.24 on the 11th day, there is a high risk of death in the hospital.
Next, we list the indicators with their division boundaries, indicating the class.
Above the border, the unfavorable class prevails in the following indicators: Urea B, Urea 7, Glucose B, Glucose 7, LDH B, LDH 7, Creatinine B, Creatinine 7, AST 0.
Below the border, the unfavorable class prevails in the indicators: Total protein B, Albumin B, Platelets B.
Machine learning methods make it possible to predict the threat of death in children with Covid-19 disease and identify the most significant indicators with their division boundaries, which allows doctors to take intensive treatment measures in patients with a poor prognosis at an early stage of the disease.

### S9.650. Magnetic and gold nanoparticles in living systems

Khomutov G.B.[1]*
[1]*M.V. Lomonosov Moscow State University, Faculty of Physics;*
* gbk@mail.ru

More than sixty years ago, L.A. Blumenfeld et al. were the first in the world to obtain experimental data indicating the presence of ferromagnetic or superparamagnetic iron oxide nanoparticles in cultures of unicellular organisms and in DNA preparations (the so-called broad lines of the EPR signal) [1]. To date, biogenic magnetic structures based on iron oxide (mainly quasi-linear chain ensembles of magnetic single-domain iron oxide nanoparticles) have already been found in a

wide variety of living organisms from bacteria and plants to insects, fish, birds and animals, including humans. Such structures can play an important physiological role (provide the orientation of the corresponding organisms in the Earth's magnetic field) and are formed as a result of biomineralization processes, or they can be a product and, accordingly, a biomarker of certain pathologies (for example, in humans, the presence of magnetic iron oxides correlates with neurodegenerative diseases). Nanophase metallic gold can also be generated by living systems (for example, bacteria) and is formed in the course of redox processes as a result of the biogenic reduction of Au(III) ions in the aqueous phase.

Currently, magnetic and gold nanoparticles, as well as functional nanosystems based on them, are the main and most important objects of research in a number of fundamental and applied fields of science, including nanoengineering and practical biophysics [2]. In view of a certain "biogenicity" and the corresponding low toxicity, it is magnetic iron oxide nanoparticles and metallic gold nanoparticles that are of the greatest interest for modern and promising biomedical applications, including highly effective diagnostic tools and new drug therapy technologies based on controlled spatiotemporal conjugation of drug delivery and activation processes. drugs in local target areas of the body [3]. This report presents an approach to solving the problem of controlled targeted delivery of drugs in living systems, based on the principles of biomimetics and involving the use of the most biocompatible and nontoxic functional materials found in living systems (lipids and biogenic amphiphilic molecules, polymers, nanoparticles of magnetic oxides of iron and metal gold) to create new biocompatible colloidal systems that are sensitive to external controlling physical and/or chemical influences, to encapsulate diagnostic tools, drugs and other biologically active substances for the purpose of their targeted targeted delivery to a specific target area of the body, as well as for their controlled release in the target area at the right time and in the right amount. The colloidal drug carriers being developed are biomimetic biocompatible liposomal and polymer constructs. The advantages of using liposomes as a basis for creating encapsulation agents are associated with their biomimetic structure and biocompatibility, as well as with the versatility due to the ability to encapsulate substances of a very different nature in liposomes - low and high molecular weight, hydrophilic, hydrophobic and amphiphilic compounds. [4, 5]. The use of biocompatible and biodegradable polymers opens up additional possibilities for creating and modifying drug carriers.

An important and relevant task at present remains the development of effective and safe methods for controlling the structural and functional characteristics of colloidal encapsulation agents through external physical and / or chemical influences to ensure controlled release of encapsulated substances in the target area of the body at the right time and in the right amount. In our approach, as the safest external control physical actions that provide selective remote control of the permeability of nanocomposite lipid vesicles, non-thermal effects of ultrashort electrical pulses are used, which provide the effect of selective electroporation of lipid membranes containing electrically conductive nanoparticles polarized in an external electric field, as well as external magnetic fields, causing the effects of controlling the spatial localization of magnetic colloidal drug carriers and magnetic deformation of such nanocomposite materials [5]. Of particular interest are combined external influences, in which synergistic effects and an increase in the overall efficiency of controlling the localization and state of colloidal drug carriers are possible.

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### S9.651. Magnetotactic Bacteria: Magnetic parameters of magnetosomes inside Magnetospirillum spp

Ryzhov V.A.[1]*, Deriglazov V.V.[1], Grouzdev D.S.[2], Koziaeva V.V.[3,4], Kiselev I.A.[1], Larionov I.I.[1], Gareev K.G.[4,5], Sitkov N.O.[4,5], Zimina T.M.[4,5], Marchenko Ya.Yu.[1], Fedorov V.S.[4,7], Shevtsov M.A.[4,6,7]

[1]*Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра "Курчатовский институт", Гатчина, Россия;*

[2]*SciBear OU, Таллинн, Эстония;*

[3]*Научный центр биотехнологии РАН, Институт биоинженерии, Москва, Россия;*

[4]*Институт цитологии Российской Академии Наук, Санкт-Петербург, Россия;*

[5]*Кафедра микро- и наноэлектроники, Санкт-Петербургский государственный электротехнический университет «ЛЭТИ», Санкт-Петербург, Россия;*

[6]*Центр трансляционных исследований рака (TranslaTUM), Klinikum Rechts der Isar, Технический университет Мюнхена, Мюнхен, Германия;*

[7]*Центр персонализированной медицины, Национальный медицинский исследовательский центр имени Алмазова, Санкт-Петербург, Россия;*

* ryzhov_va@pnpi.nrcki.ru

Magnetotactic bacteria are a group of organisms deeply studied in the last years due to their interesting magnetic behavior and potential applications in theranostics, hyperthermia and biosensor devices due to intracellular chains of submicron-sized membrane-enclosed magnetic particles called magnetosomes [1]. Magnetic parameters of magnetosomes inside the bacteria of the genus Magnetospirillum fixed by 5% formalin in the nutrient medium were estimated by the measurements: (i) of nonlinear longitudinal response to a weak ac magnetic field (NLR-M2) with registration of the second harmonic of magnetization [2] for MSR-1, LBB-42, AMB-1, SP-1, BB-1, and SO-1 strains; and (ii) of electron magnetic resonance (EMR) spectra with the special X-band spectrometer for wide-line registration [3] for the BB-1, MSR-1 and AMB-1 strains. To trace the evolution of the magnetic state of magnetosomes during long-term storage, freshly prepared samples (S1) and samples after a year of storage at 4o C (S2) were studied. The stable single-domain state of magnetic centers in magnetosomes indicating their proximity to superparamagnetic (SPM) regime was found by NLR-M2 at the scan frequency 0.02 Hz of the steady magnetic field. This allowed for a semi-quantitative analysis of M2 data with the formalism based on the numerical solution of the kinetic Fokker–Planck equation for SPM particles. Processing the NLR-M2 data evidenced the presence of two kinds of magnetosomes: (i) with the large magnetic moment ("heavy", monodispersed mode) and (ii) with the comparatively small magnetic moment ("light", dispersed mode), in both the S1 and S2 samples. The EMR spectra are formed mostly by the "heavy"

fraction for both samples due to the formation of magnetosome aggregates in the "light" fraction, accompanied by the suppression of their magnetic moment by dipolar correlations, in accordance with the M2 data. The presence of two peaks in the spectra evidences the presence of conventional uniaxial magnetic anisotropy in the magnetosomes. The appearance of one or two additional peaks in the spectra in the S2 samples of some strains instead of a broad diffuse line, on the one hand, suggests their instability at long storage even being fixed by formalin and sealed in the nitrogen atmosphere and, on the other hand, evidences that destruction of the magnetosome chains at long storage occurred not randomly. The Atomic Force Microscopy assessment of the state of the ensemble of bacteria in the medium after the long-term storage carried out for one typical strain (BB-1) confirmed this finding and showed partial degradation of the native magnetosomal chain into shorter chains forming aggregates.

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### S9.652. Mechanisms of cortical depression aggravation in barrel cortex of rats with prenatal hyperhomocysteinemia

Yakovlev A.[1]*, Shakhmatova V.[1], Gataulina E.[1], Sitdikova G.[1]
[1]*Kazan Federal University;*
* alv.yakovlev@gmail.com

Homocysteine, a sulfur-containing amino acid formed during the metabolism of methionine, has neurotoxic effects in high concentrations. Genetic mutations of enzymes of homocysteine metabolism, deficiency of B vitamins, excess of methionine and violations of the excretory function, taking certain drugs leads to an increase in the level of homocysteine in the body - hyperhomocysteinemia (hHCY). It has been shown that a high level of homocysteine in the blood as a result of oxidative stress, mitochondrial disorders, leads to neuronal damage and activation of endothelial cells, and increases the risk of thrombosis and ischemia, causes an increase in the permeability of the blood-brain barrier and, as a consequence, to the development of neurodegenerative diseases. Clinical data indicate that elevated homocysteine levels may be a risk factor for migraine. It has been shown that the frequency of cortical depression (CSD) underlying migraine with aura is associated with high levels of homocysteine. The aim of this work is to analyze the development of spreading cortical depression in the somatosensory cortex of rats with prenatal hyperhomocysteinemia. The experiments were carried out on animals aged P14-26 (P0 – birthday). In thalamocortical sections (400 μm) of the somatosensory cortex of rats, using an extracellular electrode, the EC and the internal optical signal (IOS) were recorded in the light transmission mode. CSD was induced using two protocols: equimolar replacement of NaCl with KCl in extracellular solution at concentrations of 50 mM for 5 min or local application of 50–100 mM KCl for 5 s. To assess changes in synaptic activity, excitatory postsynaptic potentials (EPSPs) of layer 4 neurons were recorded. An analysis of the experimental data under control conditions showed that the application of KCl caused the development of DCM after 88±6 s (n=20). The amplitude and duration of the hHCY were 5.9±1.1 mV and 342±24 s (n=20), respectively. Pre-incubation of sections in the homocysteine metabolite homocysteine-thiolactone (HCY-Th, 500μM) for 60 min did not cause significant changes in the amplitude and duration of the CSD, but the latent period of the CSD decreased by 1.5-2 times relative to the control (n=6, $p<0.05$ ). In sections of the cortex of rats with prenatal hHCY, a decrease in the latent period of the development of CSD to 36±5 s (n=7, $p>0.05$) was recorded, the amplitude and duration did not change significantly. Application of KCl resulted in complete inhibition of evoked synaptic responses in layer 4. Restoration of synaptic responses was observed after 15–20 min. By 60 minutes after the induction of CSD, the EPSPs amplitude recovered to 84±9% (n=14) relative to the control. In sections of rats with prenatal hHCY or after pre-incubation in HCY-T, a slowdown in the recovery of the EPSPs amplitude was observed, and by 60 min after the induction of the EC, it was 65±8% (n=6, $p<0.05$) relative to the control. In the next series of experiments, IOS was analyzed, which consisted of 2 peaks: the first one reflecting the depolarization of neurons, and the second one, the general swelling of cells. In sections of the cortex of rats with hHCY, there was only a decrease in the amplitude of 1 peak by 2 times (n=7, $p<0.05$) relative to the control and an increase in the recovery time of light transmission from 236±31 s (n=29) to 346±47 s (n=27 , $p<0.05$). Pre-incubation of thalamocortical sections (10 min) in the presence of a selective inhibitor of AMPA-receptors, CNQX (10μM), did not lead to changes in the amplitude and latent period of hHCY in sections of rats, both control and hHCY groups. Blocking of NMDA-receptors (20μM d-APV) led to a decrease in the amplitude and an increase in the latent period of the hHCY, both in control and in sections of the cortex of rats with hHz. Under the conditions of inhibition of NR2A (3μM TCN) and NR 2B (3μM Ro-256981) subunits of NMDA receptors in rats of the control group, suppression of the amplitude of the HHCY was recorded. In sections of the cortex of rats with hHCY, a significant decrease in the amplitude of the H hHCY was observed only when the 2B subunit of NMDA receptors was blocked. The time to peak CSD in the presence of 2A/B subunit blockers in the control group of rats did not differ significantly, respectively, relative to control conditions. In sections of rats with hHCY, blocking of the NR2A/B subunits led to an increase in the time to the peak of the hHCY, which is especially pronounced when the NR2B subunit is inhibited. Blocking of various subunits of NMDA receptors in the cortex of rats in both the control and experimental groups did not lead to a significant change in the duration of the hHCY. Thus, it was shown that the incubation of slices in HCY-T, as well as in rats with prenatal hHCY, leads to a decrease in the latent period and an increase in the amplitude of CSD, as well as a decrease in the recovery rate of evoked synaptic responses recorded in the somatosensory cortex. Also, it can be assumed that NMDA-type glutamate receptors are involved in the development of hHCY in thalamocortical sections of rats both in control and under conditions of prenatal hHCY, while the effect of blocking NMDA receptors and especially the NR2B subunit was more pronounced in the hHCY group. 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### S9.653. Mechanisms of regulation of airway smooth muscle contractile activity in metabolic disorders

Birulina Yu.G.[1]*, Ivanov V.V.[1], Buyko E.E.[1], Volkhina M.O.[1], Nosarev A.V.[1], Gusakova S.V.[1]
[1]*Siberian State Medical University, Tomsk, Russia;*
* birulina20@yandex.ru

One of the factors causing bronchopulmonary pathology is the metabolic syndrome (MS). There is evidence that visceral obesity, hyperinsulinemia, hyperglycemia and dyslipoproteinemia contribute to the development of bronchial hyperreactivity. The mechanisms underlying such disorders are associated with a disorder of the regulatory mechanisms of the contractile activity of the smooth muscle cells of the airway wall.

The contractile activity of airways isolated segments with removed epithelium of Wistar rats of the control (15 animals) and experimental

(18 animals) groups was studied by the mechanographic method using a Myobath II multi-channel tissue bath system. The control group was fed with standard laboratory rat diet (protein:fat:carbohydrate ratio 24%:6%:44%) with a free access to food and water. The rats of the experimental group at the 12th week were administrated with a high fat and high carbohydrate diet (protein:fat:carbohydrate ratio 16%:21%:54%), drinking water was replaced with a 20% fructose solution. Contractions of airway smooth muscle segments were induced by 30 mM potassium chloride solution (KCl, 30 mM). The amplitude of contractile responses to KCl served as the control (100%). The pharmacological effects of carbacholin (0.1-100 μM), salbutamol (0.1-100 μM), forskolin (0.1-10 μM) were tested.

As a result of the airway smooth muscles contractile activity study in experimental animals, it was found that the action of the nonselective cholinergic receptors agonist carbacholin (0.1–100 μM) causes a dose-dependent contraction of bronchial segments in control and experimental rats. The contractile responses amplitude in airway segments with removed epithelium in animals of the experimental group was higher than in the control group in the concentration range of 1–100 μM ($p<0.05$). Probably, the removing of the epithelium leads to a decrease in the action of dilatation factors released during the activation of cholinergic receptors. It was shown that in obesity caused by a high-fat diet, carbacholine enhances the contraction of the smooth muscles of the airways due to the calcium mobilization from cytosolic reserves, which stimulates the phosphorylation of myosin light chains. Activation of beta2-adrenergic receptors with salbutamol (0.1–100 μM) against the background of precontraction of segments by carbacholin (1 μM) caused a dose-dependent relaxation of the airway smooth muscle segments in rats of the control and experimental groups. The dilatation reactions of the segments with the removed epithelium from experimental rats decreased in the concentration range of 1–100 μM ($p<0.05$) when compared to the control group. One of the mechanisms for relaxing effect reducing on the bronchodilator action is the desensitization of beta2-adrenergic receptors, which occurs in obesity, due to increased expression of type 4 phosphodiesterase, which destroys cAMP. At the same time, contractile activity modulation in bronchial smooth muscles also depends on the acting mediator. It is possible that the activation of cholinergic mechanisms cancels the cAMP-dependent relaxation in smooth muscles. Against the background of the action of the adenylate cyclase activator forskolin, a dose-dependent relaxation of segments with the removed epithelium from rats of the control and experimental groups ($p<0.05$) occurred, more pronounced in the group of rats with MS.

Thus, the data obtained indicate that in animals with MS induced by a high-fat and high-carbohydrate diet, there are functional changes in the wall of the airways associated with a change in cAMP-dependent intracellular regulation.

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### S9.654. Membrane activity of three-finger cobra cytotoxins depends on the critical amino acid residues in the N-terminal and central loops

Dubovskii P.V.[1]*, Utkin Y.N.[1]
[1]*Institute of Bioorganic Chemistry named after Shemyakin M.M. and Ovchinnikov Yu. A.;*
* pvdubov@ya.ru

Cobra cytotoxins (CT) are the main component of their venom, responsible for necrosis of the affected tissues. It is believed that this is based on the membrane activity of CT. Therefore, it is not surprising that CT possess cytotoxic and antibacterial activities, like many membrane-active peptides [1].

The database of protein structures (https://www.uniprot.org) contains more than 80 members of the CT family. These polypeptides, 59-61 amino acid residue long, belong to the three-finger protein family. Their characteristic structural feature is the presence of three beta-structural hairpins held together by 4 disulfide bonds. CT interact with lipid membranes through the termini of beta-hairpins, or loops. These regions are the most variable. Which substitutions are most favorable for membrane activity of CT?

For the first time, a group of researchers from Taiwan tried to answer this question in 1994 [2]. They found that the presence of either proline (Pro30) or serine (Ser28) residue at the terminus of the central loop of CT significantly influences their membrane activity. Therefore, all CT were divided into 2 groups: P-type (with Pro30) and S-type (with Ser28). And P-toxins exhibit stronger membrane activity than S-toxins. However, relatively recently, we found that the antibacterial activity and cytotoxicity of a number of CT, as well as their capability to induce calcein leakage from phospholipid liposomes, depend on the presence of a single proline residue (Pro8) or a pair of prolines (Pro8–Pro9) at the extremity of the N-terminal loop [3]. At the same time, CT with two prolines are significantly inferior in activity to those with one proline. The total number of CT with two prolines is at least 20. It should be noted that the dependence of activity on P- and S-substitutions in the second loop also remains.

With this in mind, all CTs, according to their membrane activity, can be divided into 4 groups, depending on the presence of critical amino acid residues at the extremities of the N-terminal and central loops of the molecule. Group-1 consists of CT with Pro8-Pro9 and Ser28 residues. Group-2 is represented with CT possessing Pro8-Pro9 and Pro30. Group-3 includes CT with Pro8 and Ser28. Group-4 - with Pro8 and Pro30. At the same time, the membrane activity of CT increases in the following order: group-1 < group-2 < group-3 < group-4. In many cases it is the membrane activity of CT that determines their antibacterial activity and cytotoxicity [2]. Thus the proposed classification of CT will allow to predict and compare their antibacterial/cytotoxic properties.

Currently, we are validating the proposed classification by expanding the number of CT for which antibacterial/cytotoxic activities have been determined.

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### S9.655. Membrane characteristics of premotor interneurons of the defensive reflex after formation of anxiety-like behavior in snail

Chumarina A.I.[1,2]*, Arslanov A.I.[1,2], Silantyeva D.I.[1,2]
[1]*Institute of Fundamental Medicine and Biology;*
[2]*Kazan Federal University;*
* chumarinaadilya@yandex.ru

Anxiety, depending on the situation, can be a natural defensive reaction of the body or a serious pathology. Anxiety is often viewed as an exaggerated reaction of the body to an external threat, occurring in the absence of a life-threatening stimulus. The anxiety in mammalian is deeply rooted in their evolutionary past and can be found in invertebrates. So, in mollusks, an anxiety-like behaviour is expressed as an

unpredictable change in defensive and motor activity. It was determined that during the formation of long-term sensitization, which is close reaction such as fear, there is an increase in the excitability of the main elements of the neural network: sensory and command neurons, as well as an increase in the amplitude of EPSP. (Hochner et al., 1986). At the same time, it seems interesting how change the characteristics of neurons involved in the performing of defensive and motor responses in mollusks during anxiety. The aim of present study was to investigate how the anxiety-like behaviour affected the membrane characteristics of premotor interneurons of defensive reflex in snails.

The experiments were carried out on the terrestrial mollusk Helix Pomatia. To develop an anxiety-like behavior, 4 electrical stimuli were applied to mollusk on the area of the head each day with an interval of 1.5 hours within 3 days. Then the animals had 3 days of rest and after the same series of electrical stimulations were repeated. Anxiety-like behaviour was determined by behavioral tests such as locomotion, ommatophore and pneumostom retraction time. The electrophysiological activity of premotor interneurons was recorded on an isolated preparation of the nervous system of snails. The animals of the control group were kept in identical conditions, as well as the animals of the group when an anxiety-like behaviour was formed. The following parameters of the membrane characteristics of premotor interneurons were studied: membrane potential (Vm), amplitude and duration of excitatory postsynaptic potentials (EPSP). The registration of electrophysiological characteristics in snails that did not undergo the formation of anxiety-like behaviour was a control.

The study of the membrane characteristics of premotor interneurons showed that the membrane potential of premotor interneurons of snails with anxiety-like behaviour significantly shifted towards depolarization: Vm of the interneurons of animals with anxiety-like behaviour was -52.95±1.7 mV (n=9 ), while the Vm of the interneurons of animals in the control group was 59.2±2 mV (n=6) (P<0,05).

An analysis of the EPSP activity of premotor interneurons in snails showed that the total amplitude of EPSP recorded from these neurons in anxiety snails (1,01±0,1 mV) was slightly reduced compared to the total amplitude of EPSP in the premotor neurons of animals in the control group (1,3±0,3 мВ). The frequency of EPSP appearance in premotor interneurons in the group of animals with anxiety-like behaviour did not differ from that in the interneurons of control animals.

Thus, obtained data showed that, the rest membrane potential of premotor interneurons of defensive reflex in snails with anxiety-like behaviour was more depolarized compare with rest membrane potential in premotor interneurons in control snails, while the parameters of EPSP which reflected the activation of synapses from sensory neurons do not change significantly. The tendency to decreasing EPSP amplitude after the formation of an anxiety-like behavior can be explained by the depolarization of the membrane potential against which a part of the EPSP signal is lost. The mechanisms of the depolarization shift of the resting membrane potential during the formation of an anxiety-like state requires further study and is the goal of our next work.

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### S9.656. Membrane correlates of learning in molluscs: the role of serotonin, glutamate, and nitric oxide in the formation of conditioned defensive reflexes in the grape snail

Gainutdinov Kh.L.[1]*
[1]*Kazan Federal University;*
* kh_gainutdinov@mail.ru

Processes of learning and memory underlie behavior change, and memory is one of the main cognitive functions of the brain. The mechanism for storing and/or remembering the received information constitutes memory. The issues of memory consolidation, including the formation of conditioned reflexes, remain relevant. Although the question about the mechanisms of learning and memory arose a long time ago, it has not yet been fully studied. Neuromodulation can have a significant impact on the formation of long-term memory [1]. Examples of neuromodulators in the simple nervous system of mollusks are serotonin, nitric oxide, and glutamate. The literature demonstrate that serotonin (5-HT) is the main mediator that modulate defensive behavior in mollusks. 5-HT, applied to the surrounding solution, causes several cellular changes that lead to an increase in the defensive reflex. In addition to the well-known role of 5-HT as a mediator in synaptic transmission, it was shown that it can perform integrative functions when released into the extracellular environment [1]. These results served as the basis for the application of 5-HT washing solution as a reinforcing stimulus for the purpose of creating cellular analogs of learning. By applying 5-HT to the solution washing the central nervous system, it is also possible to reproduce the electrophysiological correlates of plasticity.

Nitric oxide (NO) is known as one of the most important signaling molecules regulating the physiological functions of the body and cell metabolism. Much attention is drawn to the study of the role of NO in the mechanisms of learning and memory. NO-synthesizing neurons have been found in the nervous system of invertebrates, including mollusks. In mollusks, as in mammals, NO plays the role of an intercellular messenger and a signaling molecule in various parts of the nervous system. We have shown that both the NO donor sodium nitroprusside and the NO-synthase blocker L-NAME have a direct effect on the electrical characteristics of the premotor interneurons of the terrestrial snail.

It is known that an essential role in the regulation of brain activity, particularly in memory processes, is played by L-glutamate, the main excitatory neurotransmitter in both vertebrates and many invertebrates. On the one hand, we studied the effect of changes in the content of serotonin, nitric oxide and glutamate on the formation of conditioned defensive reflexes of aversion to food and changing the environment, as well as on the reconsolidation of memory of these reflexes. On the other hand, we have conducted studies of the membrane mechanisms of the formation of conditional defensive reflexes in a mollusk with a simple nervous system – the terrestrial snail. To do this, we analyzed changes in the excitability of the premotor interneurons of the defensive reflex LPa3 and RPa3: the values of the membrane potential (Vm) and the threshold of action potential generation (Vt).

It was found that the application of 5-HT and the precursor of its synthesis 5-hydroxytryptophan (5-HTP) into the washing solution caused a decrease in the membrane potential (Vm) of LPa3 and RPa3 neurons, in both intact and trained animals. At the same time, in trained and sensitized snails, unlike intact snails, this application caused an increase in threshold potential (Vt). The results show that the responses (sensitivity) of premotor interneurons to extracellularly applied 5-HT or 5-HTP change after associative learning and long-term sensitization.

It has been demonstrated that the reconsolidation of this contextually dependent memory of the situational conditioned reflex (CR) during reminder and simultaneous inhibition of protein synthesis does not occur if serotonin transmission is disrupted in the nervous system. It is shown that the development of the CR to the situation is accompanied by a depolarization shift and a decrease in the Vt of LPa3 and RPa3 neurons. No further Vm changes were detected after the reminder (initiation of reconsolidation) both with the subsequent injection of a protein synthesis blocker or saline solution. The Vt of these neurons decreases after learning and remains unchanged after the initiation of reconsolidation.

It was found that blocking the NMDA receptor with the MK-801 blocker in terrestrial snails accelerates the process of aversive learning.

It has been shown that the application of a NO sodium nitroprusside donor into a solution washing the preparation of intact snails causes an increasing hyperpolarization of the membrane of premotor interneurons at 5.5 mV by the 10th minute. The application of the L-NAME

NO-synthase blocker into the solution washing the isolated snail preparation caused a gradual decrease in the membrane potential by 5.0 mV for 30 minutes. Thus, we have demonstrated that in certain neurons NO synthesis inhibition (i.e. a decrease in its amount) can cause depolarization of the membrane whileincreasing NO causes hyperpolarization. This allows us to assume the correlation of the NO level in a neuron with its membrane potential. The obtained results also indicate the need for 5-HT for the process of reconsolidation of memory in the example of a grape snail.

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### S9.657. Metabolic imaging of liver tissue in the process of regeneration with pathology

Rodimova S.A.[1,2]*, Bobrov N.V.[1,3], Shchechkin I.D.[1,2], Krylov D.P.[1,2], Kozlov D.S.[1,2], Elagin V.V.[1], Karabut M.M.[1], Mozherov A.M.[1], Zagainov V.E.[1,4], Zagaynova E.V.[1,2], Kuznetsova D.S.[1,2]
[1]*Privolzhsky research medical university;*
[2]*N.I. Lobachevsky Nizhny Novgorod National Research State University, Nizhny Novgorod, Russia ;*
[3]*The Volga District Medical Centre of Federal Medical and Biological Agency, Nizhny Novgorod, Russia ;*
[4]*Nizhny Novgorod Regional Clinical Oncologic Dispensary, Nizhny Novgorod, Russia;*
* srodimova123@gmail.com

More than 1 million new cases of primary and secondary liver cancer are registered annually. The only type of therapy remains surgical treatment of the liver. However, 5-year survival after liver resection reaches 14-61%. Despite modern advances in surgical technique and the improvement of methods for preoperative assessment of liver function, there is still a high risk of postoperative liver failure, associated with the presence of background hepatic pathology, as well as with the difference in the regenerative potential of the liver of each patient. Standard clinical methods for assessing the state of the liver tissue are not sufficiently informative and do not allow a predictive assessment of the regenerative potential of the liver remnant. Thus, the search for new methods and criteria for intraoperative rapid assessment of the structure and function of the liver remains an urgent task, which would make it possible to detect the presence of pathological changes already in the early stages, as well as to assess the regenerative potential of the liver remnant. Modern label-free methods of multiphoton microscopy with time-resolved microscopy (FLIM) and optical second harmonic generation (SHG) expand the possibilities of studying the structural and functional state of liver tissue at the cellular level.

A series of experiments were carried out on Wistar rats. Normal regeneration was induced by 30% and 70% partial hepatectomy (PH). Fibrosis was induced by administration of CCl4 for 8 weeks. Steatosis was induced with a 60% high fat diet for 12 weeks. At different stages of the pathology, we induced regeneration by 70% PH. Monitoring of the regenerating liver was carried out on days 3rd and 7th after PH. Using multiphoton microscopy, we performed analysis of the liver tissue structure. The assessment of collagen content in the tissue was carried out by the SHG. Using FLIM, we analyzed the contributions of the fluorescence lifetimes of bound forms of NADH (a2,%) and NADPH (a3,%). Morphological and morphometric analysis and a standard biochemical blood test were performed as control methods.

As a result, we revealed that the regeneration of normal liver is characterized by a uniform distribution of the NAD(P)H autofluorescence signal and the absence of collagen accumulation in the tissue. The FLIM method revealed the main optical criterion for successful regeneration - a sharp increase in the contributions of a2 and a3 on the 3rd day of regeneration, which is associated with an increase in the intensity of oxidative phosphorylation and biosynthetic processes in hepatocytes. In case of induced steatosis and fibrosis, we identified zones with a reduced NAD(P)H autofluorescence signal associated with foci of fibrosis and lipid infiltration. Using FLIM we showed a decrease in the contributions of a2 and a3 in the early stages of pathology, with a subsequent increase in these parameters in the later stages. With the induction of regeneration at the later stages of the pathology, there is no jump in a2 and a3 on the 3rd day of regeneration, which is associated with lipotoxicity and mitochondrial dysfunction in hepatocytes. Thus, we identified the optical criteria for successful liver regeneration: a uniform distribution of the NAD(P)H autofluorescence signal, as well as a sharp increase in the contributions of the fluorescence lifetimes of the bound form of NADH and NADPH. The criteria for violated liver regenerative potential in the presence of pathology defined by zones with a reduced NAD(P)H autofluorescence signal, as well as the absence of a sharp jump in the contributions of the bound form of NADH and NADPH at the later stages of pathology. The results obtained could be useful for the developing criteria for a predictive express-assessment of the state of the liver tissue in the clinic.

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### S9.658. Mitochondrial pore (mPTP) and cell death

Kritskaya K.A.[1]*, Stelmashchuk O.A.[2], Berezhnov A.V.[1,2], Abramov A.Y.[2,3]
[1]*Institute of Cell Biophysics RAS;*
[2]*Cell Physiology and Pathology Laboratory, Orel State University, Orel, Russia;*
[3]*Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK;*
* kritskayak96@yandex.ru

Various pathological conditions such as neurodegeneration, cardiovascular diseases, cancer and aging are associated with impaired mitochondrial function and, in particular, mitochondrial pore (mPTP). The opening of mPTP leads to a acute increase in the permeability of the mitochondrial membrane, loss of mitochondrial potential and triggering programmed cell death – apoptosis or necrosis. In turn, the opening of mPTP may be caused by ROS overproduction, calcium overload, low substrate availability, inhibition of mitochondrial respiratory complexes, etc. It is assumed that the mechanism of triggering apoptosis mediated by mPTP is disrupted in tumor cells, while in neurodegeneration, the toxicity of protein aggregates, such as a-synuclein, is mediated by the opening of mPTP.

It is known that the opening of mPTP is a universal mechanism of induction of apoptosis for various types of mammalian cells, however, the quantitative analisis of mitochondria with opened mPTP vs. total pool of mitochondria of the cell necessary to trigger cell death has not yet been established.

To study this parameter, we selected 4 mammalian cell types: neurons, astrocytes, breast cancer cells, and fibroblasts. The cells were loaded with a potential-sensitive TMRM dye (25 nM) and a fluorescent substrate of caspase 3 NucView (2 uM). Next, a step–by-step application of ferutinin (an electrogenic ionophore inducer of mPTP opening), mediating an increase in the permeability of the mitochondrial membrane and calcium overload of mitochondria, was carried out. The opening of mPTP occurs if the addition of ferutinin is accompanied by a rapid loss of mitochondrial potential and the extinction of TMRM fluorescence. The percentage of mitochondria with opened mPTP during the induction of apoptosis (which was evidenced by a sharp increase in NucView

fluorescence) to the total pool of mitochondria at the initial moment of recording (initial mitochondrial area, 100%) was calculated.
It was found that the lowest percentage of mitochondria with opened mPTP (25±1.4%) is required for the induction of apoptosis in fibroblasts. In neurons, 64 ±4% of mitochondria with opened mPTP were required to induce apoptosis, while in astrocytes this value was 77± 5%. In tumor cells, despite the large heterogeneity of the data, the induction of apoptosis required the opening of mPTP in more than 90 ± 8% of mitochondria.
Since the evolutionary role of neurons and astrocytes is strongly associated with calcium signaling, it is likely that such differences in this indicator can be explained by the lower sensitivity of neuronal and astrocytic mitochondria to calcium overload compared to fibroblast mitochondria. Tumor cells, on the other hand, are characterized by a high rate of mitophagy and autophagy, which may restrain the induction of apoptosis, despite the opened of mPTP in most mitochondria of the cell.
Thus, in this paper, the threshold of sensitivity to the induction of apoptosis through the opening of mPTP and calcium overload in different types of cells is described. Since substances that modulate the work of mPTP are considered as a potential target for the treatment of various diseases, it is necessary to study in detail the sensitivity of mitochondria in various cell types to their action.
This study was conducted in the framework of the State assignment of PSCBR RAS № 075-01512-22-03 «New generation neuroprotective drugs» № 1022080100047-5-1.6.4.

### S9.659. Model of organization of physiological functions of the human body based on the spectral analysis of the acoustoencephalogram

Shabanov G.A.[1], Rybchenko A.A.[1], Lugovaya E.A.[1]*
[1]*Scientific Research Center Arktika Far Eastern Branch of the Russian Academy of Sciences;*
* elena_plant@mail.ru

Vibration sensitivity appears to be one of the biggest forms to reflect connections between the human body and a wide variety of environmental influences. Nerve cells, epithelial cells, muscle fibers are seen to perceive sound stimuli and vibrations directly, without specific receptors. The evolution of pre-nervous types of information transmission could not avoid cell mechanical microvibrations since 0.1 to 30 Hz frequencies information is transmitted over long distances with virtually no attenuation; it is the least energy-consuming in comparison with chemical and nervous transmission, and has the property of diffusely spreading throughout the body. Being one of the founders of comparative physiology and evolutionary approach to information transmission, H.S. Koshtoyants (1950) claimed that absolutely everything that exists in a living organism works together: new developed mechanisms of information transmission and simpler, more primitive ones. Not only the new mechanisms replace the simpler ones, which continue to function together, each in its place, they also integrate into them [1].
Since H. Magoun summarized it in his monograph [2], there has been an idea that the physiological approach to assessing the behavior of functions requires the study of regulatory or modulating influences that come from the brain spontaneously active reticular activating structures with their functional heterogeneity considered as an independent complex neurophysiological mechanism. In our research we concluded that, being the simplest form of energy accumulation, the oscillation energy is naturally used by the brain non-specific structures to form a background adaptive potential as a multi-frequency matrix of multiple arousals. Such a system should include quite long periodic regimes that form the spatial organization and functional state of the overlying brain structures, as well as the dynamic tone of peripheral effectors and the entire body [3]. For each function, from organismal to cellular, a frequency cluster is built based on the needs. The cluster is made up of nerve and somatic cells with one frequency as an organizing factor, and the functional state determined by the amplitude of microvibrations. Such a model works as "the bigger access" and synchronizes the work of cells in the frequency cluster, regardless of their location in space, the presence and trajectory of nerve conductors. Representing a potentially huge set of competing needs and behavior vectors, neural clusters are closely associated and provide a hierarchy of functions and the body integrity.
The spectral analysis of the brain acoustic signal had certain unique features: 0.1 to 27 Hz band of the analyzed signal, the number of spectral harmonics equal to 8400, 160 seconds as the optimum integration time to isolate long-running oscillations of the brain activating system, and the accuracy of determining spectral harmonics with the decimal point going up to the fourth place which was provided by the F1-1013 frequency standard rubidium.
Any function can be represented in time as a set of consecutive measurement points. A cross-correlation analysis is used to synchronously select one frequency harmonic of the brain oscillations for such a function with its envelope of the spectrum that gives the maximum correlation coefficient. Examples of frequency clusters for the functions at different levels of the hierarchy: protein S100 – 0.8270 Hz; protein TRPM8 – 6.1655 Hz; ferritin FTH1 – 0.4046 Hz; blood glucose – 0.2465 Hz; blood WBC – 3.2323 Hz; depression index – 6.3961 Hz; personal anxiety – 6.8588 Hz, etc.
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### S9.660. Modulating effect of polyphenols on growth factors of damaged somatic nerve

Otryaskin Y.S.[1]*, Pinyaev S.I.[1]
[1]*Ogarev Mordovia State University;*
* otryaskin.yaroslav@mail.ru

Peripheral nerve injuries are common injuries with a wide range of symptoms depending on the severity and the affected nerves. In the process, if neurotrophin support is not obtained, the proximal segment will rapidly degenerate and the cell body will die. Therefore, studies of the growth, development, and protection of nerve cells by the family of neurotrophic factors are of great value from the point of view of fundamental and clinical research.
Polyphenols are an important group of phytochemicals that are found in abundance in food. Polyphenols are reported to have therapeutic value in neurodegenerative diseases. They are also known to play an important role in the prevention of various types of cancer. Cellular survival signaling is important in suppressing the mechanism of cell death and balancing apoptosis signaling in the nervous system.
Polyphenols, phenolic compounds, flavanoids and terpenes are well known for their antioxidant activity. Phenols have been defined as a group of polyphenols that are an important secondary metabolite found in plants. They are responsible for the antioxidant effect of flavanoids and their positive effect on many diseases. Flavanoids are powerful antioxidants against free radicals and they are described as free radical scavengers. The ability to absorb free radicals is mainly attributed to the high reactivity of the hydroxyl groups. This activity is attributed to their hydrogen donor capacity, and the phenyl group of the flavanoids undoubtedly serves as a source of readily available hydrogen atoms.
Resveratrol (3,4',5-trihydroxystilbene) is a naturally occurring polyphenol found in various plant matter, grapes and red wine, which is

naturally produced by some plants in response to injury or pathogens. Resveratrol has many beneficial properties for brain tissue homeostasis, mainly related to its ability to scavenge free radicals, inducing antioxidant pathways and exerting anti-inflammatory effects.
Dihydroquercetin (taxifolin) is a compound that belongs to flavononols. These are compounds containing flavan - 3 - it, with a structure characterized by 2 - phenyl - 3,4 - dihydro - 2H - 1 - benzopyran containing a hydroxyl group, and a ketone with carbon atoms C2 and C3, respectively. Dihydroquercetin is a natural antioxidant of plant origin and is a bioflavonoid.
Nerve growth factor (NGF) was the first discovered member of the neurotrophic factor family and is an important neurotrophic factor for the development and maintenance of the central and peripheral nervous system. Several studies have suggested that NGF and its receptors, the tropomyosin-related kinase receptor 1 and the NGF receptor, are involved in the wound healing process and are important contributors to the healing of several wounds both in vivo and in vitro.
Scientists of the Faculty of Biotechnology and Biology are working with the use of resveratrol and dihydroquercetin as a stimulator of regenerative processes in damaged nerve tissues. It has been experimentally shown that with the introduction of resveratrol and dihydroquercetin, the content of myelin proteins increases, the phospholipid and fatty acid composition of damaged nerve conductors is restored.

### S9.661. Morphological substantiation of stiffness decreases of tumor tissue during therapy

Plekhanov A.A.[1]*, Gubarkova E.V.[1], Sirotkina M.A.[1], Elagin V.V.[1], Sovetsky A.A.[2], Pavlov M.V.[3], Vorontsov D.A.[3], Bogomolova A.Y.[1], Vorontsov A.Y.[3], Gamayunov S.V.[3], Zaitsev V.Y.[2], Gladkova N.D.[1]
[1]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia;*
[2]*Institute of Applied Physics of RAS, Nizhny Novgorod, Russia;*
[3]*Nizhny Novgorod Regional Oncologic Hospital, Nizhny Novgorod, Russia;*
* strike_gor@mail.ru

Currently, the opinion of radiologists on the reliability and diagnostic significance of breast tissue stiffness studies using ultrasound diagnostics has not been fully formed due to the ambiguous results of clinical trials. However, a few recent studies show on a relationship between a significant decrease in stiffness values after the first course of neoadjuvant chemotherapy (NACT) and a complete / pronounced pathomorphological response of the tumor after treatment. Thus, the tumor node often remains the outlines of a large-scale after the first course of NACT, despite a pronounced decrease in stiffness values. The presented research is aimed to study morphological substantiation of changes in breast cancer tissue stiffness during NACT using the method of Compression Optical Coherence Elastography (OCE), which allows determining stiffness of individual structural components of biological tissues.
The study protocol being approved by the Research Ethics Board of the Nizhny Novgorod Regional Oncologic Hospital (REB#12, granted December 23, 2021). All patients provided written informed consent. For patients with an established diagnosis of invasive ductal breast cancer, core needle biopsy samples were taken before and after the first course of NACT (for clinically justified tasks of determining the appropriateness of further therapy). In addition, the tumor node was examined using ultrasonic compression elastography (Samsung RS80A, South Korea). The Young's modulus of elasticity of tissue structural components of core needle biopsy samples was studied by OCE using a multimodal optical coherence tomography device (IAP RAS, Nizhny Novgorod). Then, a histological examination of tissue was carried out. Ultrasound examination after the first course of NACT showed a stiffness decrease of individual tissue areas in the projection of tumor node, while the high stiffness areas were also detected. At the same time, the size of tumors did not significantly decrease, which was considered the stable disease. OCE study of core needle biopsy samples revealed a general stiffness decrease due to three pathologically confirmed changes: (1) reduction of high stiffness areas due to decrease of viable tumor cells, (2) registration of low stiffness areas due to the appearance of necrotic tumor cells, (3) and increase of low stiffness areas due to the formation of stromal fibers (which replace the necrotic tumor cells). In summary, our results demonstrate the features of changes in the stiffness and morphology of breast tumor tissue during therapy. It has been shown that stiffness decrease in tissue occurs due to a reduction of viable tumor cells, the appearance of necrotic tumor cells, and then replacing necrotic cells by stromal fibers. Further monitoring and data collection can allow to develop of new predictive criteria for early efficiency evaluation of neoadjuvant tumor therapy.
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### S9.662. Multimodal optical coherence tomography for clinical evaluation of vulvar skin repair after PDT therapy for lichen sclerosus

Potapov A.L.[1]*, Loginova M.M.[1,2], Plekhanov A.A.[1], Moiseev A.A.[3], Matveev L.A.[3], Sedova E.S.[4], Gamayunov S.V.[4], Radenska-Lopovok S.G.[5], Gladkova N.D.[1], Sirotkina M.A.[1]
[1]*Privolzhsky Research Medical University;*
[2]*Lobachevsky State University of Nizhny Novgorod;*
[3]*Federal research center Institute of Applied Physics of the RAS;*
[4]*Nizhny Novgorod Regional Oncologic Hospital;*
[5]*I.M. Sechenov First Moscow State Medical University (Sechenov University);*
* Arseniy1109@gmail.com

Multimodal optical coherence tomography (OCT) is a modern non-invasive imaging technology that allows obtaining 3D images of biological tissues with a spatial resolution of 10–15 micrometers to a depth of up to 2 mm without additional contrast [1]. At the same time, data on the structural and functional characteristics of the tissue are obtained from the same area of interest in real time, which certainly increases the information content and specificity of the study. The overall structure of the tissue and the condition of the collagen fibers are assessed on cross-polarization OCT images; the state of microcirculation (blood and lymphatic vessels) - on OCT angiographic images. It is important that all types of information received can be assessed both visually and quantitatively. As a result of scanning, in less than 30 seconds, a 3D data set is obtained, from which B-scans and en face images can be analyzed at different depths within the scanned area. The scanning depth is an important limiting factor for OCT technology, which imposes restrictions on the objects of study. In case of direct access of the OCT probe to biological tissue, the objects of study can be: skin, mucous membranes of the gynecological tract, oral cavity, malignant neoplasms of the skin. With intraoperative access, the objects of study can be brain tumors, breast cancer, abdominal organs. Multimodal OCT has shown its effectiveness in diagnosing different molecular subtypes of breast cancer [2]. OCA angiography is promising for evaluating the effectiveness of photodynamic therapy for basal cell carcinoma [3].
An important property of OCT angiography is the visualization of the vascular network at different depths. This principle can be used to assess the depth of connective tissue damage, for example in vulvar lichen sclerosus.
Vulvar lichen sclerosus (VLS) is a chronic skin inflammatory disease that predominantly affects the vulvar area in women and severely reduces quality of life. Photodynamic therapy (PDT) is an effective treatment for VLS, targeting collagen fibers and the dermal vasculature. Due to the relapsing nature of the disease and abnormal collagen synthesis, tissue repair may not be complete. For a non-invasive

assessment of tissue repair, the use of the multimodal optical coherence tomography method is proposed.
The purpose of this study was to search for optical criteria for the effectiveness of PDT and early recurrence of lichen sclerosus.
Materials and methods. Total 12 patients diagnosed with VLS and 10 patients without vulvar pathology were studied. PDT was performed with a medical laser "Lakhta-Milon" 662 nm at a dose of 0.16 W/cm2 with intravenous administration of Photoditazine (0.7 mg/kg). The study was carried out using MM OCT developed at the Institute of Applied Physics Russian Academy of Sciences (Nizhny Novgorod). A 3.4 x 3.4 x 1.25 mm3 3D dataset was acquired within 26 s, from which tissue structure and microcirculation information was extracted. Dynamic observation was carried out before PDT, immediately after PDT, after 24 hours, 1-4 months and 6 months after PDT. Histological examination was performed before PDT and 4 months post PDT.
Results. Multimodal OCT in VLS demonstrates a change in the scattering and polarization properties of the vulvar tissue due to atrophy of the epidermis, hyperkeratosis, edema, and formation of a sclerotic dermis, as well as a decrease in blood and lymphatic vessels relative to the norm. After PDT, a decrease in the density of the vascular network was observed, up to their complete disappearance after 24 hours. Starting from 1 month after PDT, the density of blood and lymphatic vessels gradually recovered, reaching a maximum level 2–3 months after PDT, but at the same time not reaching the density values characteristic of normal tissue. The restoration of scattering and polarization properties simultaneously with the restoration of the layered structure on OCT images was observed on the 2nd month after PDT. A biopsy at 4 months after PDT shows a normotrophic scar formation.
Conclusions. Multimodal OCT is a promising in vivo method for monitoring the dynamics of both vulvar vascular component and the connective tissue collagen fibers recovery. This may allow assessing the effectiveness of treatment and fixing the occurrence of a recurrence of VLS.
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### S9.663. NO production intensity and rat myocardial contractility during hypokinesia

Zaripova R.I.[1]*, Sungatullina M.I.[1], Dikopolskaya N.B.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1], Gainutdinov Kh.L.[1,2]
[1]*Institute of Fundamental Medicine and Biology;*
[2]*Kazan E. K. ZavoiskyPhysical -Technical Institute (KPhTI);*
* ratno1992@mail.ru

Deficiency of movements in human life is an important medical and social problem caused by lifestyle, professional activities, prolonged bed rest, etc. There is a decrease in the load on the muscular apparatus, which leads to changes in functional and morphological changes to pathological conditions, depending on the duration and degree of hypokinesia.
Nitric oxide (NO) is an important biological mediator involved in many physiological and pathophysiological processes. The NO synthase system is widely represented in different heart structures. NO is able to exert both an activating and an inhibitory effect on various metabolic processes occurring in the body of mammals and humans. It has also been shown that the NO system plays an important role in the adaptation of the body to various changes in the external environment and external conditions, for example, during significant physical exertion. Prolonged restriction of motor activity causes changes in contractile function and weakening of the heart muscle, as well as weakening of venous and arterial vessels.
An EPR study of the intensity of nitric oxide (NO) production was carried out in the simulation of movement deficit in rats by analyzing the amount of NO - containing paramagnetic complexes in the tissues of the heart. Also, a study was made of the force of contraction of the isolated heart of rats according to Langendorff. Experimental animals were in conditions of deficit of movements, starting from the age of 3 weeks: the first two days, the time of hypokinesia was 1 hour, and then increased by 2 hours every 2 days. By the 25th day of hypokinesia, the residence time of the animals in the cages reached 23 hours, and remained constant until the end of the experiment.
The amount of NO was estimated from the intensity of the characteristic EPR signal belonging to the (DETC)2-Fe2+-NO complex. The contractile (or inotropic) function of the heart was assessed by the pressure developed by the left ventricle using the Langendorf PowerLab 8/35 device (ADinstruments, Australia) using the LabChart Pro program (Australia).
It has been established that the presence of rats in conditions of deficit of movements for 2 months leads to an increase in the content of NO in the tissues of the heart by 2 times. With a deficit of movements, a decrease in the force of contraction of the heart was recorded, which is possibly associated with a change in the level of NO. Since excessive formation of NO can significantly reduce the tone of smooth muscle cells, impair endothelial function and directly suppress myocardial contractility.

### S9.664. Neuroprotective potential of intracellular acidification in a toxic model of Parkinson's disease

Nadeev A.D.[1]*, Kritskaya K.A.[1], Fedotova E.I.[1], Berezhnov A.V.[1]
[1]*Institute of Cell Biophysics of the Russian Academy of Sciences;*
* madeev1987@gmail.com

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the loss of midbrain dopaminergic neurons. The mechanism of neurodegeneration is associated with the accumulation of pathological protein aggregates, oxidative stress, and mitochondrial dysfunction, including impaired mitophagy. It is assumed that the activation of mitophagy avoids cell death and returns them to normal functioning. We have previously demonstrated that lactate and pyruvate are able to restore mitochondrial function by inducing mitophagy through a decrease in intracellular pH. The concentration of lactate in the body can increase dramatically during physical activity, while the brain absorbs lactate in proportion to the concentration in the arterial blood. Based on this, we hypothesized that physical exercise could induce mitophagy in midbrain neurons. In addition, an increase in the concentration of carbon dioxide (5-20%) in the inhaled air causes a reversible acidification of brain cells. Therefore, it may promote mitophagy. Thus, we hypothesized that forced inhalation of a CO2-rich gas-air mixture could activate mitophagy and promote neuronal survival.
In this study, we aimed to test two experimental therapeutic approaches: forced moderate physical activity and high CO2 inhalation for the treatment of PD in a rodent model treated with the model toxicant rotenone. 12-month-old CD-1 mice were used in the experiments. Rotenone dissolved in olive oil was administered intraperitoneally 5 times a week for 6 weeks at a dose of 2 mg/kg of body weight. Treatment was carried out starting from the third week of toxic modeling. Moderate physical activity consisted of forced running on a treadmill; for forced inhalation, the animals were placed in a closed glass cylinder filled with 20%

CO2 for 2 minutes. PD-like symptoms were assessed in behavioral tests for impaired locomotion and coordination: "beam travel" and "cylinder" tests. Paraffinized brain sections were stained according to Nissl. After 2 weeks, rotenone caused a significant decrease in motor activity and impaired coordination.
In our experiments, treatment with moderate exercise did not lead to significant restorative changes in the studied neurobehavioral parameters.
On the contrary, our second therapeutic procedure, consisting of high CO2 forced inhalation, has shown promising results. Mice exposed to 20% CO2 three times a week showed no signs of impaired coordination in the beam travel test during 4 weeks of experimental therapy, with a significant difference from pre-treatment levels at week 2. In addition, in the cylinde" test, we observed a recovery and even an increase in the 1st week of the total locomotor activity, which was reduced after the administration of rotenone. Taken together, these tests demonstrate the efficacy of CO2 on sensorimotor function. Also, on histological preparations of the mice brain, lesions of the substantia nigra neurons were not observed, in contrast to the control group that received rotenone. However, a survival analysis did not reveal any difference between this therapy and the no-treatment group.
Thus, we conducted a primary experimental verification of our proposed, previously unused therapeutic approach. In a toxic model of PD, we have demonstrated for the first time that inhalation of high CO2, presumably through transient acidification of the brain and thus initiation of mitophagy, can eliminate the symptoms of PD.
The work was supported by the Russian Science Foundation grant 22-24-01043.

**S9.665. New colloidal systems based on biomimetic polycomplexes**

Grigoryan I.V.[1,2]*, Spiridonov V.V.[1], Adelyanov A.M.[1], Koksharov Yu.A.[1,2], Potapenkov K.V.[1], Taranov I.V.[2], Khomutov G.B.[1,2], Yaroslavov A.A.[1]
[1]*Lomonosov Moscow State University;*
[2]*Institute of Radio-engineering and Electronics of RAS;*
* grigorian.iv19@physics.msu.ru

Currently, one of the most urgent interdisciplinary problems at the intersection of biophysics, chemistry and medicine is the creation of new effective drug therapy systems based on selective and targeted delivery of drugs directly to the areas of the body that are the goals of therapeutic action.The solution to this problem is associated with the development of biocompatible colloidal means of encapsulating drugs that can provide controlled targeted delivery and release of drugs to target areas of the body. At the same time, the localization of colloidal carriers and drugs in biological liquid media of the body can be controlled by chemical, biochemical and physical (in particular, magnetic) influences.
This paper describes new colloidal magnetic polycomplexes based on biocompatible polymers modified with biogenic polyamine spermin, which can be used for encapsulation and drug delivery.
During the work:
1. New colloidal polycomplexes based on polyacrylic acid molecules of various molecular weights modified with spermine were obtained. The main characteristics of the polycomplexes were determined: zeta potentials, hydrodynamic diameters, the aggregative stability of the complexes was studied and the optimal ratio for further modification between the number of polyanion units and the number of cationic "stapler" molecules was determined.
2. The possibility of including medicinal compounds in polycomplexes was demonstrated. antitumor drug doxorubicin was used as such a compound in the work
3. The magnetic properties of polycomplexes functionalized by magnetic nanoparticles were investigated by the EPR method. Data indicating the nature of the nanoparticles formed in the biopolymer matrix have been obtained.
The obtained results indicate the possibility of creating new magnetic polycomplexes based on biocompatible polymers modified with biogenic polyamine spermin, capable of including medicinal preparations in their composition.
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**S9.666. Noopept modulation of snail neuronal membrane excitability**

Murzina G.B.[1]*
[1] *Institute of Higher Nervous Activity and Neurophysiology of RAS;*
* gbmurzina@mail.ru

Noopept (ethyl ether of N-phenylacetyl-L-prolylglycine) has found wide application in medical practice in memory and attention disorders caused by traumatic brain injury, cerebral vascular insufficiency, asthenic disorders. Along with the effect of noopept on long-term processes (which is the dominant effect of the drug), its effect on the excitability of the neuronal membrane in a shorter period of time was found. It has been shown that this drug changes the amplitude of the acetylcholine-induced incoming current (ACh-current) of the command neurons of the snail [1]. The dose curve of noopept exposure is bell–shaped, and the ACh-current amplification is observed at low concentrations (0.1nmol – 0.01μmol). The analysis of the amplitude of the induced ACh-current in order to clarify the mechanisms of the cholinopositive effect of noopept was carried out using a mathematical model. The model describes the main processes from the application of the mediator to the registration of the induced Ach-current [2]. The simulation results showed that the greatest correspondence between the calculated and experimental curves under the influence of noopept is observed when changing the model parameters describing an increase in the total number of receptors on the neuronal membrane and an increase in the lifetime (or probability of transition) of membrane receptors to the open state. An increase in the number of acetylcholine receptors on the membrane of neurons may be a consequence of the effect of noopept on the processes of endo- or exocytosis of receptors by interacting with proteins of the under membrane matrix or due to the effect on the kinases or phosphatases involved in the process of membrane or intracellular transport of acetylcholine receptors.
In order to specify which processes of endo- or exocytosis of acetylcholine receptors noopept has an effect on, studies have been conducted on the effects of the drug on depression caused by acetylcholine incoming current on the command neurons of the grape snail. Our previous studies on the effect of protein kinase and protein phosphatase inhibitors and a number of transport process blockers on ACh-current depression [3] allowed us to correlate their effects with the effect of noopept, which made it possible to specify the intracellular targets of the drug. To analyze the experimental data, a modified mathematical model was used, taking into account the different location of receptors (on the cell membrane and in the formed and recycled vesicles), the transport of receptors from the endoplasmic reticulum to the membrane and the process of degradation of receptors. The parameters of the model equations were the rates of change in the location of the receptors. Conclusions about which processes the drug affects were made based on

which parameters were changed to obtain the simulated curves. Studies were conducted for two concentrations of noopept - 1nmol and 10μmol, because, as previously shown, the drug has a different effect on the depression of the incoming ACh-current: at the first concentration, the greatest increase in the incoming current was observed, and at the second, its unreliable decrease. Comparison of the curves of depression production under the influence of noopept with the previously obtained curves of the effect of various blockers on ACh-current depression made it possible to conclude that noopept at a lower concentration - on the activity of protein phosphatase 2A, and at a higher concentration – also on the activity of protein phosphatase 1.

The additional calculations made and the existing literature data made it possible to clarify which intracellular processes associated with the activity of these protein phosphatases can determine the effect of noopept on the excitability of the membrane of neurons of the snail.

In the detected area of exposure to noopept, there is an effective average concentration of the drug in the blood for a person, therefore, when taking the drug, it is necessary to take into account the possibility of its short-term exposure.

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**S9.667. On Radioprotective Properties of Chlorophyll-Based Drugs**

Romodin L.A.[1,2]*
[1]*State Scientific Center of the Russian Federation – Federal Medical Biophysical Center named after A. I. Burnazyan of the FMBA of Russia;*
[2]*BIOTECH University;*
* rla2904@mail.ru

Due to the growing threat of radioactive contamination of the environment caused by potential accidents at radiation industry facilities, the increasing amount of radioactive waste, and the likelihood of using or testing nuclear weapons, the development of effective and safe radioprotective drugs remains a very critical task. These drugs are also needed to alleviate the effects of radiation therapy in cancer patients, as well as to prevent radiation injuries in astronauts. Even though people have been working on this problem since the mid-20th century, it cannot be considered resolved at present.

This is due to the poor radioprotective effect of the used radioprotective agents with low chemical toxicity and the high toxicity of more effective radioprotective agents used in Russia and in the West, respectively: indralin and amifostine [1].

However, it is reported in the literature that chlorophyll-based drugs can have significant radioprotective properties (see the review [2] for more details). In other countries, a lot of attention has been focused on chlorophyllin, a water-soluble chlorophyll derivative [3–5]. There is information about its protective effect on the genetic apparatus of animals exposed to radiation [3, 4] and its potential ability to suppress the metabolism of lipid radiotoxins [5]. The ability of chlorophyllin to suppress lipid peroxidation was proven using the method of recording of chemiluminescence [6].

In Russia, the concept of using chlorophyll-based drugs as radioprotective agents was suggested by Professor N.P. Lysenko independently of his foreign colleagues. He focused on the use of chlorophyll itself. A doctoral dissertation [7] written under his supervision showed that the use of this substance resulted in an increase in the survival of mice exposed to γ-radiation and a decrease in the content of lipid peroxidation products in their liver and blood serum.

To be able to use chlorophyll-based drugs successfully, we need to determine the most effective clinically acceptable form of the drug. The use of water-soluble chlorophyllin seems promising. Therefore, it was chosen for further research.

In an experiment conducted in cooperation with M.A. Ignatov, a junior research fellow of the Radiation Biophysics Laboratory of the A.I. Burnazyan Federal Medical Biophysical Center, a suspension of human lymphocytes pre-incubated in the presence of 5 μM to 100 μM of Na-Cu-chlorophyllin was exposed to an X-ray radiation dose of 2 Gy. Then the degree of DNA damage was evaluated using alkaline single-cell gel electrophoresis which showed no significant differences between the samples incubated with different concentrations of chlorophyllin and control samples.

This finding was the reason for conducting a study on the ability of Na-Cu-chlorophyllin to penetrate lymphocytes. During the study, a suspension of lymphocytes was incubated in a chlorophyllin solution with a concentration of 300 μM, after which the lymphocytes were separated from the incubation medium by centrifugation at 200 g for 20 minutes and were lysed with 0.5 % Triton X-100. Spectrophotometric determination of chlorophyllin content in the lysates and the incubation medium showed a higher chlorophyllin concentration in lymphocyte lysates compared to the medium. This suggests that it penetrates the cytoplasm by an active transport mechanism.

However, it is unlikely that chlorophyllin penetrates the nuclear membrane. This can explain the fact that it did not mitigate the destructive effect of X-ray radiation on the lymphocyte DNA in the radiation-exposed suspension, and the studies [3, 4] that evaluated its effect in bone marrow cells and spermatogonia of radiation-exposed mice showed a gene-protective effect of chlorophyllin. These cells constantly undergo mitosis which destroys the nuclear membrane. Therefore, chlorophyllin from the cytoplasm easily found its way to their nuclei, where it was able to protect their genetic apparatus.

Since it is the rapidly dividing cells that are usually the most radiosensitive, chlorophyllin can be considered a substance worthy of further investigation as a means to prevent radiation injury.

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### S9.668. On the molecular mechanisms of nitric oxide and ascorbic acid action in peritoneal macrophages

Kuropteva Z.V.[1]*, Belaya O.L.[2], Zhumabaeva T.T.[3]
[1]*N.M. Emanuel Institute of Biochemical Physics of the Russian Academy of Sciences ;*
[2]*Moscow State A.I Evdokimov University of Medicine and Dentistry of the Ministry of Healthcare of Russia;*
[3]*Osh State University, Kirgizstan;*
* zv.kuropteva@gmail.com

In the 1980s, it was found that activated macrophages (MP), cultured together with target tumor cells, kill target cells. And the agent responsible for the death of tumor cells is nitric oxide (NO) synthesized by macrophages. The participation of NO in the cytotoxic activity of macrophages to tumor cells was one of the first functional properties described for this molecule. It was shown that inhibition of NO synthesis in macrophages leads to a decrease in the antitumor resistance of the body, and the discovery of this basic fact made a great contribution to the increased number of studies devoted to the formation and function of NO in normal and pathological conditions. Although there are various, sometimes contradictory data on the role of NO in the development of tumors, it has definitely been established that NO synthesized by leukocytes and macrophages plays a main role in their ability to destroy tumor cells. It was shown that sustained continuous production of NO in macrophages ensures their cytotoxic/ cytostatic activity not only to tumor cells, but also to viruses, bacteria, fungi, protozoa, and helminths.
On cell lines and mouse, lines were found that resistance to microbes is often associated with the expression of the induced NO synthase (iNOS). More direct evidence of the iNOS role was obtained first by Hibbs J. and his colleagues (1987), and then by many other researchers. They used iNOS inhibitors and showed that inhibitors worsen the course of diseases caused by many viruses and bacteria. The mechanisms of nitric oxide action were established. They included inhibition of mitochondrial respiration (MR) and DNA synthesis, iron loss by cells, and inhibition of enzymes involved in the Krebs cycle. NO is obviously not the only anti-pathogenic immune defense effector, but in most cases, it is either an inducer or an executor of a bactericidal program. Therefore, the increased (NO) synthesis in the cells of the immune system is important in raising the body's resistance to infectious diseases, and, possibly, due to the available literary data, the prevention of tumor diseases.
Peritoneal macrophages were isolated by the standard method of washing the contents of the abdominal cavity of SHK mice with a 0.9% NaCl solution. The collected cells were washed twice with the same solution and centrifuged at 250 g for 10 min. The precipitate was suspended in saline. The cell concentration was 2x109 in 1 ml. To feed the cells, 2% serum solution of cattle was added (Biowest, France).
But it turned out that peritoneal MP isolated in a standard way, incubated at room temperature, can also synthesize a small amount of NO without additional stimulation, which could be detected by the presence of nitrites and nitrates, which are stable products of NO oxidation and are registered by UV spectroscopy upon absorption of 330-340 nm. This MP ability was used in this work to study the effect of AA on NO synthesis in these cells. UV and ESR spectroscopy methods were used for recording NO formed in MP suspension incubated at room temperature with and without AA. When studying the formation of NO by ESR, Hb was used as an NO trap, which has a high affinity to NO, and interacting with it, forms stable nitrosyl Hem-NO complexes (Heme-NO NCs), which determine the well-known ESR signal - a wide singlet with g-factor 2.02 and characteristic triplet splitting of 17 G with a g-factor of 2.01.
Suspension of peritoneal macrophages (MP) specimens of mice incubated with AA (10 mM) and without it within 48 hours. By 48 h, the intensity of the ESR signal of Heme-NO NC and, consequently, the amount of NO formed in the samples incubated with AA was more than three times higher than the intensity of this signal in samples without AA. A quantitative assessment showed that in these conditions, 1.5 x 109 NO molecules are produced in macrophages per cell by 48 hours under the action of AA.
UV absorption in samples incubated with AA was significantly higher (more than twice) than in supernatant samples of macrophages incubated without AA. It should be noted that the amount of nitrite formed in the samples in which the absorption spectrum is actually should really be more than registered, since AA has not yet been used completely, part of the formed nitrite decomposes again under the action of AA. Therefore, in experiments using the ESR method, when the all formed NO molecules are accepted by hemoglobin, by 48 hours, there was a more than 3-fold increase in NO amount compared to control samples.
To answer the question on the mechanisms of AA action further studies are necessary. However, according to the data obtained and those available in literature, it is possible to suggest that the main role of AA is to restore iNOS cofactors. NO-synthase is a carefully regulated enzyme and for its normal functioning 6 cofactors are necessary: FMN, FAD, NADH, BH4, NADPH and calmodulin connected with the active center. Moreover, tetrahydrobiopterin (BH4), which is considered a necessary participant in the synthesis of NO as a redox active cofactor, is a key cofactor of iNOS. AA is necessary to prevent the irreversible oxidation of the key cofactor of the NO-synthase of tetrahydrobiopterin BH4 to dihydrobiopterin BH2 and thereby maintain the iNOS activity and increases NO synthesis.

### S9.669. Once again about aging, stability and copies of mitochondrial dna, the level of reactive oxygen species

Koltovaya N.A.[1]*, Christova R.[2], Gospodinov A.[2]
[1]*Joint Institute for Nuclear Research, 6 Joliot-Curie St., 141980 Dubna, Russia;*
[2]*Institute of Molecular Biology BAS, 21 Acad. George Bonchev St., 1113 Sofia, Bulgaria;*
* koltovaya@jinr.ru

Dysfunction of mitochondria and the mitochondrial (mt) genome is often the cause of not only disease, but also aging. With age, there is a decrease in the total number of mitochondria, a violation of their internal structure, a decrease in the total amount of mtDNA, and various mtDNA damage accumulates. The study of the mechanisms of stabilization of the mt genome is of undoubted scientific and practical interest. A good model is the yeast Saccharomyces cerevisiae, for which even deletion of mtDNA (rho0 mutants) is not fatal. In our studies, we use strains derived from the collection strain X2180-1A (the reference yeast strain S288c is also its derivative). This strain is characterized by a high frequency of mt deletion mutations in respiratory deficiency (petite, rho-). Genetic analysis showed that high rho-- mutability is due to the simultaneous presence of at least five genes, each of which causes only a relatively weak increase in spontaneous rho--mutability (Devin, Koltovaya, 1987). Subsequently, whole genome sequencing revealed four defective alleles of the MIP1, SAL1, CAT5, and MKT1 genes, which are responsible for high mt mutability (Dimitrov et al., 2009). These genes encode mtDNA polymerase, an ATP/ADP mt transporter, mt monooxidase, and DNA endonuclease. The mutations have an additive effect and add up to a high frequency of petites. On the background of increased mt mutability, we were able to isolate several srm (spontaneous rho--mutability) nuclear mutations that decrease mutagenesis and stabilize the

mt genome (Devin et al., 1990; Koltovaya et al., 2003). Each of the srm mutations suppressed the overall additive effect of four defective alleles. Three of the srm mutations are localized in the SRM5/CDC28-CDK1, SRM12/HFI1-SAGA, and SRM8/NET1 (Sir2) genes encoding subunits of complexes responsible for phosphorylation, acetylation, and deacetylation. The remaining two mutations, srm1 and srm2, are being identified. Whole genome sequencing allowed the identification of preliminary candidates SRM1/TOP2 (topoisomerase) and SRM2/HUL4 (ubiquitin ligase).

Using the PCR–RT amplification method, we analyzed the copy number of mtDNA. Of the five analyzed mutants, in all but one (srm1), the copy number of mt DNA increased approximately twofold. Thus, stabilization of the mt genome occurred on the background of an increase in the copy number of mtDNA.

Analysis of chronological aging showed that srm1 did not affect, srm2 increased, and srm5, srm8 and srm12 reduced the lifespane of stationary cultures compared to the initial culture. It is known that the ada1/srm12 mutation also accelerates replicative aging (Sinclair et al., 1997). However, deletion of mtDNA (rho0-mutations) led to an increase in the lifespan of SRM, srm2, and srm5 cells, but significantly reduced the lifespan of srm1, srm8, and srm12 cells. Thus, against the background of stabilization of the mt genome, both increasing and decreasing of lifespane can occur. The main cause of chronological aging is the accumulation of acetic acid in the medium, a product of yeast metabolism, which induces apoptotic cell death. Indeed, upon treatment with peroxide, which also induces apoptosis, the picture for rho+ cells is the same, but for rho0 mutants they diverge, apparently, the effect of srm mutations on aging for rho+ and rho0 is different.

Yeast aging is accompanied by increased formation of reactive oxygen species (ROS), which, in turn, activate the apoptotic pathway leading to cell death. The level of ROS may also affect the copy number of mtDNA. We assessed the effect of srm mutations on the oxidative activity of strains using flow cytometry and specialized ROS dyes (H2DCFDA, DHR123, DHE). The strains used by us obtained the hap1 mutation from the original X2180-1A strain, which disrupts the functioning of the transcription factor that regulates the oxygen response, namely, the synthesis of respiratory enzymes, heme, ergosterol, and proteins involved in oxidative stress (Kwast et al., 1998). By the way, hap1 slightly increases the frequency of petites (HAP1 - 2.8%, hap1 - 5.8%). hap1 causes a decrease in the synthesis of all cytochromes (aa3, b, c+c1) and accumulation of the Zn-porphyrin pigment. Respiration imbalance can increase ROS levels. The hap1 strains used have reduced synthesis of ergosterol and antioxidant enzymes, including cytoplasmic catalase Ctt1, superoxide dismutase Sod2, and flavohemoglobin Yhb1, which can increase membrane permeability and ROS levels. The accumulation of Zn-porphyrin, whose fluorescence maximum is observed at 585 and 540 nm, affects autofluorescence. On this complex biochemical background, experiments have shown that srm2, srm8, and srm12 reduce autofluorescence. When stained, the fluorescence maxima are slightly shifted, which indicates a low level of ROS. However, upon treatment with peroxide, the srm1 mutant is characterized by a higher peroxide concentration in the cytoplasm and mitochondria, while srm5 is lower. On the background of rho0, the level of peroxide in mitochondria is high in srm1 and srm8, and low in srm2 and srm12. Thus, against the background of an increase in the concentration of peroxide in cells as a result of various mechanisms, a multidirectional effect of srm mutations is observed. More research is needed to understand the processes in which the SRM genes are involved. In the future, it is planned to analyze the cell transcriptome, catalase and superoxide dismutase synthesis, telomere length, as well as directly test apoptosis (AnnexinV-FITC/PI) and membrane potential (JC-1 and Rh123) using fluorescent dyes.

**S9.670. Oxidative stress markers in healthy pregnant women and pregnant women with urogenital infection in the early stages of gestation**

Zhambalova B.A.[1]*, Osipov A.N.[1], Teselkin Yu.O.[1]
[1]*N.I. Pirogov Russian National Research Medical University;*
* zhambalovaba@inbox.ru

The aim of the study. Investigation of oxidative stress markers in women in the early stages of healthy pregnancy and pregnancy complicated by urogenital infection.

Materials and methods. The study involved 90 women aged 20-45 years, who were divided into 3 groups: group 1-healthy non-pregnant women (n=30); group 2-healthy pregnant women (n=30); group 3-pregnant women with urogenital infection (n=30). All pregnant women were in the first trimester of gestation (8-10 week). Determination of the functional activity of phagocytes whole blood was performed by luminol-dependent chemiluminescence (LCL). Chemiluminescent method was also used to measure the total antioxidant capacity of blood plasma. Content in blood plasma one of the products of lipid peroxidation, malondialdehyde (MDA), was determined spectrophotometrically.

Results of the study. It was found that in pregnant women of the 2nd and 3rd groups, the intensity of whole blood LCL was higher than in women of the 1st group. So, during stimulation with zymosan, the average values of this indicator in the 2nd and 3rd groups increased by 2.8 and 8 times, respectively, and during stimulation with opsonized zymosan, by 2.4 and 4.6 times compared with the 1st group ($p<0.05$). When comparing the intensity of LCL in whole blood of women of the 2nd and 3rd groups, significant differences were also found. In particular, upon stimulation of phagocytes of whole blood of patients of the 3rd group with zymosan, the intensity of the registered luminescence exceeded the intensity of luminescence of the whole blood of women of the 2nd group by 2.9 times ($p<0.05$), and upon stimulation with opsonized zymosan - 1.9 times ($p<0.05$). The observed changes in the intensity of whole blood LCL in women of the 2nd and 3rd groups compared with the 1st group can be due to both the properties of the phagocytic cells themselves and their large number in the blood. To exclude the latter, we compared the LCL intensities normalized to the content of phagocytes in the studied blood volume. However, in this case, the differences between the intensity of normalized LCL of whole blood in women of these groups remained significant. It is important to note that during stimulation with zymosan or opsonized zymosan, the intensity of normalized LCL of whole blood phagocytes in patients of the 3rd group was 3.5 and 2 times higher, respectively, than in women of the 1st group ($p<0.05$) and 1.8 and 1.2 times higher than in women of the 2nd group ($p<0.05$). The obtained results show that already in the first trimester of physiological pregnancy there is a significant increase in the functional activity of blood phagocytes. The presence of urogenital infection (UGI) in pregnant women is accompanied by an even greater increase in the ability of phagocytes to produce reactive oxygen species (ROS). To assess the level of oxidative stress in women of the examined groups, the content of MDA in blood plasma, as well as its antioxidant activity (AOA), was determined. It was found that the content of MDA in blood plasma in women of the 2nd and 3rd groups was 1.3 and 1.5 times higher, respectively, compared with its content in women of the 1st group ($p<0.05$). As for the AOA of blood plasma, it was 1.4 and 1.5 times lower, respectively ($p<0.05$). An increase in the content of MDA in the blood plasma of women of the 2nd and 3rd groups indicates the activation of the free radical process in their body, while a decrease in plasma AOA indicates a decrease in the level of its antioxidant protection. Based on the results obtained for women

in each group, an oxidative stress index was calculated, which is the ratio of MDA/AOA. In this regard, the numerator reflects the intensity of free radical reactions, and the denominator characterizes the state of the antioxidant system. The calculated index of oxidative stress in healthy pregnant women and pregnant women with urogenital infection exceeds its value in healthy non-pregnant women by 1.8 and 2.2 times, respectively ($p<0.05$). This suggests that in women of the 2nd and 3rd groups there is a pronounced imbalance between the production of prooxidants and the state of antioxidant protection in the direction of increasing the production of prooxidants. At the same time, there is a tendency to increase free radical reactions in women of the 3rd group compared with the 2nd group. A significant contribution to the total production of reactive oxygen species by blood phagocytes in the first trimester of pregnancy complicated by urogenital infection can be made by the activation of TLR-dependent signaling pathways as a result of the expression of Toll-like receptors on the cytoplasmic membrane of cells. This assumption is supported by the fact that the correlation coefficient between LCL of whole blood phagocytes stimulated by zymosan and the content of MDA in blood plasma in women of the 3rd group was equal to +0.71 ($p<0.05$), while in women of the 1st and 2nd groups it was significantly lower and amounted to +0.15 and +0.23, respectively ($p<0.05$).
Conclusion. Comparison of oxidative stress markers in healthy pregnant women and pregnant women with urogenital infection with those of healthy non-pregnant women leads to the conclusion about the development of oxidative stress in the first trimester of pregnancy. The presence of urogenital infections in pregnant women contributes to the strengthening of imbalance between the production of pro-oxidants and the activity of the antioxidant system.
Keywords: healthy pregnancy, urogenital infection, phagocytes blood, reactive oxygen species, oxidative stress, total antioxidant capacity of blood plasma, chemiluminescence.

### S9.671. Participation of M5 muscarinic cholinoreceptors in the regulation of neurosecretion in mice motor synapses in normal and under oxidative stress conditions

Khamidullina A.A.[1,2]*, Kovyazina I.V.[2], Teplov A.Y.[1]
[1]*Kazan State Medical University, General Pathology Department;*
[2]*Kazan State Medical University, Medical and Biological Physics with Computer Science and Medical Equipment Department;*
* aliyakham21@gmail.com

Muscarinic cholinergic receptors (mAChRs) are a subtype of receptors sensitive to muscarine and acetylcholine. There are five subtypes of mAChRs (M1-M5), which are superfamilies of G protein-coupled receptors. Previous studies show that all known mAChR subtypes play a significant role in the regulation of cardiovascular, gastrointestinal, motor functions, and are also involved in the processes of learning, memorization, and the formation of behavioral responses. A more detailed study of mAChRs functions is hampered by the small number of pharmacological agents specific to each type, which can be used to influence the molecular mechanisms associated with these receptors.
M5 subtype mAChRs are of particular interest. There is evidence that the dysfunction of the M5 subtype is associated with schizophrenia, Alzheimer's disease and the drug addiction and alcoholism. The presence of this mAChRs subtype on muscle fibers has also been shown, but their role in the regulation of synaptic functions at neuromuscular junction has yet to be studied. New developments in pharmacological agents have led to the creation of the compound VU-0238429, the first M5 selective positive allosteric modulator (PAM).
It is known that cholinergic receptors are the target for free radicals, including reactive oxygen species (ROS), which are formed both during the normal functioning of the skeletal muscle and in a number of pathological situations (inflammatory processes, prolonged lack of muscle activity, heavy metal poisoning). In turn, the activation of some mAChRs subtypes also leads to an increase in the production of nitric oxide and ROS.
The aim of this study was to analyze the effects of positive modulation of M5 mAChRs under normal conditions and under conditions of increased ROS production in mouse skeletal muscle synapses.
Materials and methods. The studies were carried out on isolated neuromuscular preparations of the BALB/c mouse diaphragm muscle using extracellular endplate potentials (EPPs) recording technique. EPPs induced by the motor nerve stimulation with paired pulses (14 ms period followed by a rest period of 2 s) and spontaneously occurring "miniature" EPPs (MEPPs) were recorded. The preparation was perfused with physiologically relevant, Ringer-Krebs saline. For positive modulation of M5 mAChRs, compound VU-0238429 was used at a concentration of 1 μM. To increase the level of ROS, the neuromuscular preparation was incubated for 20 min in an iron containing solution (Fe2O12 S3 xH2O, 0.1 mM). The amplitude and temporal parameters of EPPs and MEPPs, as well as the EPP quantum content (estimated by the ratio of the amplitudes of evoked and spontaneous signals) for the first and second stimuli were evaluated.
Results. The parameters of spontaneously occurring MEPPs, namely the frequency of signals, their amplitude, as well as the 10-90% rise time and 10-90% decay time in the presence of a prooxidant did not change significantly. The EPP quantum content for the first and second stimuli and the temporal parameters of the signals also did not change after incubation in a Fe2+ containing solution.
Previously, we found that the EPP quantum content in the presence of PAM (compound VU-0238429) increased by 22%. However, after incubation of the preparation in a prooxidant containing solution, the addition of VU-0238429 did not lead to a change in the quantum content of postsynaptic responses to either the first or second impulses during paired stimulation. The MEPP parameters also did not change after the addition of PAM.
Conclusions. The effects of positive modulation of M5 cholinergic receptors on EPP parameters in mouse synapses suggest that these receptors are present on the membrane of motor nerve endings and are involved in the regulation of neurosecretion. Simulation of oxidative stress conditions for an isolated neuromuscular preparation caused by incubation of a skeletal muscle in a solution with a prooxidant – Fe2+, does not affect the amplitude and temporal parameters of EPPs and MEPPs, but prevented the effects of the M5 mAChRs modulator on the quantal content of EPPs. Thus, M5 mAChRs can be a target for reactive oxygen species, which should be taken into account when developing drugs based on muscarinic agents.

### S9.672. Patient-derived 3D models of glioma with immune microenvironment to study the efficacy of immunotherapy by FLIM

Yuzhakova D.V.[1]*, Sachkova D.A.[2,1], Izosimova A.V.[1,2], Yashin K.S.[1], Mozherov A.M.[1], Yusubalieva G.M.[3], Kulemzin S.V.[4], Shirmanova M.S.[1]
[1]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia;*
[2]*Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
[3]*Federal Research and Clinical Center, Federal Medical and Biological Agency, Moscow, Russia;*
[4]*Institute of Molecular and Cellular Biology SB RAS, Novosibirsk, Russia;*
* yuzhakova-diana@mail.ru

The main problem in the field of glioma immunotherapy is insufficient knowledge about the individual characteristics of immune mechanisms for each individual patient. Successful application of

immunotherapeutic approaches, such as check-point inhibitors and CAR therapy, to glial tumors requires the implementation of a personalized approach.
The studies were carried out using material from patients with glioma Grade II-IV, operated at the PIMU University Hospital.
A new patient-specific 3D model of glioma with an immune microenvironment has been developed. For this purpose, a library of primary glial and lymphocyte cultures of patients has been created, and an original approach has been developed, including optimized methods of cell extraction, a technology for cultivating a three-dimensional tumor structure (based on a spheroid or a cultured tissue fragment), growth conditions, and a cell co-cultivation scheme. The BD FACSAria III cell sorter was used to analyze the subpopulation and activation profile of lymphocytes. To simulate a microenvironment close to the real one, we additionally analyzed the subpopulation composition of tumor-infiltrating lymphocytes.
It has been established that immune cells in the presence of tumor antigens remain viable and actively proliferate.
It has been demonstrated that this model is able to reflect the response of immune and tumor cells to various types of check-point immunotherapy. As an example, the addition of an anti-CTLA4 therapeutic antibody to a 3D model of a particular patient results in a significant increase in the number of immune cells and a decrease in the number of tumor cells compared to an untreated control, as well as an increase in the expression level of CD25 in the pool of CD4+ T cells and CD8+ T cells compared to control without treatment. However, the addition of an anti-PD-1 antibody to this patient model did not result in any therapeutic effect.
The second block of experiments is devoted to cellular immunotherapy using new modified/targeted linear NK cells. A high cytotoxic activity of "enhanced" YT cells with overexpressed VAV1 protein, which enhances cytotoxic activity, and knockout of the CISH gene, which modulates susceptibility to IL-15, as well as a line with a CAR receptor specific for EGFRvIII, was demonstrated on the model of glioblastoma spheroids. In addition to standard methods, the response of cells to therapy was assessed using advanced optical metabolic imaging FLIM in the NAD(P)H coenzyme channel (ex. 375 nm, em. 435 – 485 nm) on a confocal microscope LSM 880 (Carl Zeiss, Germany) with FLIM prefix TCSPC (Becker & Hickl, Germany). It was shown that cell therapy with these lines led to a statistically significant increase in the mean lifetime of NAD(P)H fluorescence in tumor cells, which may be associated with a shift in metabolism towards oxidative phosphorylation and, accordingly, with a decrease in glial cell proliferation.
The work was supported by Grant of the President # MK-2092.2022.3 (3D model of glioblastoma with immune microenvironment, check-point therapy) and Russian Science Foundation # 22-64-00057 (cell therapy).

### S9.673. Peritumoral white matter state evaluation using optical coherence tomography

Achkasova K.A.[1]*, Moiseev A.A.[2], Yashin K.S.[1], Kiseleva E.B.[1], Bederina E.L.[1], Gladkova N.D.[1]
[1]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia;*
[2]*Institute of Applied Physics of RAS, Nizhny Novgorod, Russia;*
* achkasova.k@bk.ru

Introduction. Assessment of peritumoral white matter morphological state and the ability to differentiate damaged areas from tumor and normal pathways is an important scientific and practical task. It may significantly improve the quality of brain tumors resections by two ways: preventing the accidental damage of healthy white matter and avoiding the preservation of damaged non-viable areas, abundantly infiltrated with tumor cells. Optical coherence tomography (OCT) is a promising tool for brain tissue visualization and in the present study, we for the first time show the ability of cross-polarization (CP) OCT to detect white matter with damaged myelinated fibers and delineate it from healthy pathways and tumors.
Materials and methods. 215 samples of brain tissue obtained from patients with different brain tumors were included in the study. Evaluation of obtained OCT data arrays was performed in three steps: 1) visual analysis of B-scans based on the assessment of three main parameters (signal intensity, signal attenuation rate, uniformity of attenuation); 2) quantitative assessment based on attenuation coefficients estimation in co- (Att(co)) and cross-polarizations (Att(cross); 3) building of en-face color-coded maps representing the distribution of optical coefficients values with subsequent visual analysis. For each studied tissue type (normal white matter / damaged white matter / tumor), we determined the defining characteristics of structural CP OCT images and color-coded maps, and then classification tests containing the set of 100 CP OCT images and two sets of 100 color-coded maps in co- and cross-polarizations were given to 8 blinded respondents after training. To determine the diagnostic ability of visual assessment of OCT data to distinguish between studied tissue types we calculated the F-score as it allows measuring a test's accuracy in case of multiclass classification.
Results. Based on the assessment of three main parameters of B-scans, it is possible to detect white matter areas with damaged myelinated fibers and differentiate them from normal white matter and tumor tissue. Usage of attenuation coefficients in co- and cross-polarizations also allow distinguishing all studied brain tissue types. It was demonstrated, that alteration of myelinated fibers in the study area causes statistically significant decrease in the values of attenuation coefficients compared to normal white matter; at the same time, the values remain statistically higher than those of a tumor. Nevertheless, the assessment of a single numerical value obtained from an 3D OCT data array may not be sufficient, as it does not represent the structural heterogeneity of studied area. In this regard, the use of color-coded optical maps looks more promising combining, on the one hand, the objectivity of attenuation coefficients and, on the other hand, visibility of the visual assessment that leads to increase of the diagnostic accuracy of the method compared to visual analysis of structural OCT images.
Conclusions. Damage to myelinated fibers leads to the decrease in the scattering properties of the white matter, reflected in the nature of received CP OCT signal that can be detected using qualitative and quantitative approaches. Visual assessment of B-scans and en-face color-coded maps allows differentiating areas of damaged white matter, normal white matter, and a tumor from each other, while usage of color-coded maps demonstrate the higher diagnostic accuracy compared to structural images (F-score = 0.84 and 0.79, respectively). Additional usage of optical coefficients numerical values demonstrate the significant differences between different tissue types with high accuracy ($p<0.0001$). Thus, the results of the study confirm the promise of using OCT as a tool for searching the resections margin in brain tumors surgery.

### S9.674. Phagocytic activity of dendritic cells cultured with photodynamic treated glioma cells by tetracyanotetra(aryl)porphyrazine (pz IV)

Sleptsova E.E.[1]*, Saviuk M.O.[1], Turubanova V.D.[1], Redkin T.S.[1], Vedunova M.V.[1], Krysko D.V.[1,2,3]
[1]*Lobachevsky Univercity;*
[2]*Cancer Research Institute Ghent, Ghent, Belgium;;*
[3] *Cell Death Investigation and Therapy Laboratory, Ghent University, Ghent, Belgium;*
* ees222@list.ru

Previous studies have shown the presence of immunogenic potential in dying and dead glioma GL261 cells exposed to photodynamic effects (PDT) based on pz IV. These cells actively emit DAMP's, such as ATP

and HMGB1, and also exposure calreticulin on the cell membrane, which is a signal for antigen-presenting cells.
In this study, we showed that cells dying as a result of PDT attract dendritic cells (DC) and are actively phagocytized by them.
To demonstration the immune response to tumor antigens, bone marrow dendritic cells were co-cultured with GL261 glioma cells. GL261 cells, pre-stained with CellTracker Green CMFDA were subjected to photoinduced cell death based on pz IV. After that, they were collected and co-cultured with bone marrow-derived dendritic cells for 2 hours. Next, the cells were collected from the culture plates, stained with PE-Cy-anti-CD11c antibodies and analyzed by flow cytometry. Dendritic cells co-cultured with live non-induced tumor cells were used as a control.
It has been shown that cells undergoing photoinduction based on pz IV are actively phagocytized by antigen-presenting cells, unlike living cells and cells killed by several freeze-thaw cycles. Thus, glioma cells that have undergone photoinduced cell death based on pz IV can cause a more active immune response. However, in order to form a final conclusion, it is also necessary to consider the activation of dendritic cells, to analyze the process of release of the main signaling molecules by activated DC.

### S9.675. Photobiomodulation in myofascial syndrome therapy

Shchelchkova N.A.[1,2]*, Bavrina A.P.[1], Belousova I.I.[1], Vasyagina T.I.[1], Pchelin P.V.[1,2], Lapshin R.D.[1]
[1]*Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation;*
[2]*Lobachevsky State University of Nizhny Novgorod ;*
* n.shchelchkova@mail.ru

Nowadays photobiomodulation is considered as one of the promising non-invasive therapeutic approaches to the treatment of myofascial pain syndrome (MPS). MPS is based on the formation of myofascial trigger points (MTrPs) - muscle fibers with discrete overcontracted groups of sarcomeres [1,2]. An increased amount of intracellular calcium and violation of myocyte oxygenation leads to the destruction of mitochondria and increased tissue hypoxia. The effects of photobiomodulation are mainly associated with the use of red light (600-700 nm), the targets for which are proteins containing metals of variable valence.
The aim of this study was to study the effect of low-intensity red light on the morphological and functional characteristics of mitochondria in a model of myofascial pain syndrome.
The study was carried out on outbred male Wistar rats (age 18 months). Animals were divided into 3 groups. Control (group 1) - intact animals of the same age. Group with MPS modeling (group 2) and group with MPS modeling and subsequent photobiomodulation (group 3). MPS modeling was performed according to the protocol [3]. Photobiomodulation was carried out daily for animals of group 3 after MPS modeling during 4 days using LED (light-emitting diode) lasers (Spectr LC-02, Russia) at a wavelength of 650±30 nm. Ultrastructure analysis of thigh muscle sarcomere in the injury zone was performed by electron microscopy. The functionality of the mitochondrial membrane was assessed by high-resolution respirometry. Statistical analysis was performed using the SPSS Statistics (v.27) software package. Samples were compared using the Mann-Whitney test for independent samples.
After MTrPs modeling a tight muscle cord was found at the site of a chronic bruise. In MTrPs myocytes mitochondria with an enlightened matrix and fragmented cristae predominated, but condensed forms of mitochondria are also visible. MTrPs photobiomodulation led to an increase in the heterogeneity of the mitochondrial population in muscle fibers with the predominance of organelles with a normal structure compared to the group without irradiation by 17%.
The presence of MTrPs caused a significant decrease in oxidative phosphorylation with the participation of NADH dehydrogenase (OXPHOSCI) and complex IV (cytochrome oxidase). Multiple photomodulation activated basal respiration by 2 times even compared to intact group (669.5±111.1 and 326.0±64.16 pmol O2/s*mg, respectively), as well as respiration during oxidative phosphorylation with the participation of CI+CII. The activity of complex IV after photomodulation increased by 1.9 times relative to the intact group (7791.0±1298.0 and 4107±607.0 pmol O2/s*mg, respectively). The normalization of mitochondrial membrane potential index to the level of intact animals was also noted.
The results obtained showed that the course low-intensity LED irradiation of MTrPs with red light normalizes the structure of skeletal muscle mitochondria population in modeling myofascial pain syndrome (MPS) and significantly stimulates the activity of respiratory chain enzymes, which may indicate an increase in ATP production and relief of hypoxic processes in the muscle. Thus, the results of the study substantiate the possibility of therapeutic application of photobiostimulation in MPS.
The research was carried out within the framework of the State task 121030100281-9 at EGISU NIOKTR.
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### S9.676. Photobiomodulation of the human gut microbiota in vitro using red and near infrared LED radiation

Khramov R.N.[2]*, Zalomova L.V.[1], Fesenko (Jr.) E.E.[1]
[1]*ICB RAS;*
[2]*ITEB RAS;*
* khramov30@mail.ru

Background and goals. It is no coincidence that the totality of metabolite molecules (metabolome) in the blood is figuratively called the molecular "mirror" of our health. Recent data have shown that 69% of metabolite associations were exclusively microbiome-driven, 15% were exclusively genetic, and 16% were under hybrid genome-microbiome control (Diener et al., 2022). The human microbiome is an excellent predictor of changes in host phenotype and, more generally, phenome, explaining up to 20% of host adaptation and associated cellular/molecular phenomena, while the genome can explain up to less than 2% of host-related modifications (Puce et al., 2022). These findings are considered promising because they provide the keys to successful development of the correction of metabolic disorders if we can manage the microbiome, since a large number of metabolites can be manipulated with diet and probiotics. At the same time, metabolites under strict genetic control will not respond to lifestyle changes, and therefore they should be considered as targets for pharmacological and non-drug interventions, which, in particular, include human photobiomodulation (PBM). PBM is used widely enough to relieve pain, heal wounds and much more. A rather young area of PBM is external (through the surface of the body) irradiation of the gut microbiota (GM), which is even assigned a role as the most important organ of the body (Bicknell et al., 2022.), and GM is defined as a key component of health and a decrease in microbial composition, are recognized as one of the main factors of many diseases and disorders.
Study design, materials and methods. For an exogenous significant impact on the GM ecosystem through the surface of the abdomen, it is

advisable to use light in the transparency window of biological tissues (600–1500 nm), which was justified theoretically (Khramov, 2021). We have attempted for the first time to evaluate the efficacy of direct PBM in an isolated, as far as possible, complete microbial ecosystem of GM in vitro derived from human stool samples. At the same time, try to compare the efficiency of PBM for LED radiation in two wavelength ranges: in red (K) and near infrared (NIR) with max-ima of 660 nm and 940 nm, respectively. We sought to obtain more information about the integral parameters of the entire isolated GM ecosystem, as well as one of the most important GM strains, bifidobacteria (Bifidobacterium breve), in terms of cell survival after damaging cryoloading in liquid nitrogen and the effect on growth curves of microorganisms during PBM in these ranges at different radiation doses. Growth curves were measured from the optical density of cultured GM cells under anaerobic conditions, pre-exposed to light with a maximum of 660 or 940 from LED sources. Resistance to damage from the stress test of cryopreservation of microorganisms was assessed by analyzing the ratio of the number of living and dead cells.
Results. GM microorganisms reacted differently to exposure at 660 nm and 940 nm. The maximum significant increase in GM survival after the cryo-preservation stress test was more than three times greater at 940 nm compared to 660 nm (up to 40% relative to control) in the dose range from 10 to 600 J/m2. A similar picture was observed for Bifidobacterium breve microorganisms: at 940 nm, the increase in survival reached 14%, and at 660 nm, no significant changes were observed in the range of 10–160 J/m2. PBM 660 nm did not have a significant effect on the growth curves of Bifidobacterium breve, while PBM 940 nm caused significant changes in growth curves at 4 and 6 hours of cultivation: both suppression by 3.5% and an increase by 9% compared to the control, but only on cell cultures after the loading procedure of cryopreservation. Also, only in the culture of GM after cryopreservation, radiation from 940 nm caused significant changes in a wide range of doses, both at a very low dose of 1 J/m2 and at sufficiently high doses up to 67800 J/m2, on which the effects were positive and were of a non-thermal nature. . It has been shown that the addition of cryoprotectant DMSO reduces the protective effects of PBM.
Conclusion. To the best of our knowledge, this is the first demonstration of different in vitro susceptibility of GM bacteria in response to PBM using red and near-infrared LED light. It turned out that testing for survival is a more sensitive method than the analysis of growth curves of microorganisms in response to PBM. We also found cell-type-specific differences in response to PBM exposure in vitro with red and near-infrared LED light. These results confirm that different response pathways are involved after exposure to 660 and 940 nm LED light and that 940 nm near infrared light can produce a greater beneficial effect on GM microbes than red light. The effects of PBM are highly likely to have a stabilizing effect on the cell membrane. The fact that the effects of PBM are more pronounced either during damage or during cultivation after damage caused by a non-invasive cryopreservation procedure, it can be assumed that PBM can be especially effective in various diseases or stress factors leading to dysbiosis with damage and death useful human GM.

### S9.677. Photoinactivation of Mycolicibacterium smegmatis by using tricarbocyanin-based photosensitizers

Kozobkova N.V.[1]*, Samtsov M.P.[2], Lugovsky A.P.[2], Tarasov D.S.[2], Belko N.V.[2], Savitsky A.P.[1], Shleeva M.O.[1]
[1]*A.N. Bach Institute of Biochemistry, Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, Russia;*
[2]*A. N. Sevchenko Institute of Applied Physical Problems of Belarusian State University;*
* natalia.cosolapowa@gmail.com

The causative agent of tuberculosis (TB) - Mycobacterium tuberculosis has the ability to form dormant forms under unfavorable conditions, which not only acquire resistance to all known antibacterial drugs, but also has the ability to maintain viability in the human body for decades and cause the resumption of the disease. The spread of mycobacterium strains with multidrug resistance dictates the need to develop new approaches to combat these diseases. One of those opportunities is the method of photodynamic inactivation (PDI), the non-specificity of the damaging effect of which prevents the development of resistance. To a large extent, the application of this method is limited by the shallow penetration of light used for excitement of porphyrins, chlorins, methylene blue. The wavelength of the radiation required for their activation is limited to 690 nm, which corresponds to the visible range. Experiments with the use of photosensitizers activated by the near infrared range (740 nm) have not been carried out against mycobacteria before. The aim of this work was to develop a new strategy for combating mycobacteria by the PDI ofmethod using photosensitizers (PS) based on tricarbocyanin dyes which are capable to cause a photodynamic effect using light with longer wavelength that result to deeper penetration into animal and human tissues.
A non-pathogenic and fast-growing close genetic relative of the causative agent of tuberculosis, Mycolicibacterium (basonym: Mycobacterium) smegmatis, was used as the object of the study. Hydrophilic (PC 220) and hydrophobic (PC 154) forms of indotricarbocyanine dyes were used as photosensitizers. The maximum absorption band of photosensitizers is corresponded to 742 nm and the half-width is 70 nm, the maximum of the fluorescence spectrum is corresponded to at 758 nm, the half-width is 41 nm.
A daily culture of M. smegmatis cells (OD590 > 1) was diluted with NB medium to an optical density of OD590 = 0.2 and incubated with dyes for 2 hours at a temperature of 37 ° C in the dark with constant stirring. The illumination was carried out by 740 nm LED radiation (SOLIS-740C, Thorlabs, USA) at a power density of 260 mW/cm2. The exposure dose for the samples was 78, 234, 468 J/cm2, which corresponded to the illumination time of 5, 15 and 30 minutes. The viability of bacteria was analyzed by counting the number of colony forming units on agar medium.
As a result, it was revealed that at a concentration of 40 μM of the PC220 dye and a light dose of 468 J/cm2, cause a significant death of M. smegmatis cells (89%). At a light dose of 78 J/cm2, 50% of bacterial cells died. The use of the hydrophobic form of this photosensitizer demonstrated a more pronounced photodynamic effect. At a concentration of 40 μM of the PC154 dye and a light dose of 468 J/cm2, the number of M. smegmatis dead cells was 97%, probably due to better binding of PS to the hydrophobic wall of mycobacteria.
Thus, the conducted study demonstrates for the first time the possibility of in vitro photoinactivation of mycobacteria on the example of a fast–growing relative of the causative agent of tuberculosis - M. smegmatis using photosensitizers activated by radiation in the near infrared range. The development of this direction is promising to combat tuberculosis along with antibiotic therapy.
This study was funded by Russian Science Foundation grant 19-15-00324.

### S9.678. Photoinduced immunogenic cell death as a base for effective dendritic cell vaccination against mouse glioma

Turubanova V.D.[1,2]*, Saviuk M.O.[1,2], Sleptsova E.E.[1], Redkin T.S.[1], Mishchenko T.A.[1], Balalaeva I.V.[1], Lermontova S.S.[1,3], Klapshina L.G.[3], Vedunova M.V.[1], Krysko D.V.[1,2]
[1]*Lobachevsky State University of Nizhny Novgorod;*
[2]*Ghent University;*
[3]*Institute of bioorganic chemistry;*
* vikaturu@mail.ru

The initial stage of gliomas treatment is surgical resection, which provides tumor volume reduction and obtaining a sample for histological analysis and tumor genotyping. There are difficulties with complete

removal of the tumor nidus during surgery due to the invasiveness of gliomas in the surrounding tissues, in addition, the features of their microenvironment are the cause of frequent recurrences.
Clinical studies have shown that dendritic cell (DC) vaccines attenuate immune suppression and activate CD8+ T cells. In relation to gliomas, the use of dendritic cell vaccines is a promising treatment option, given the disloyal microenvironment and propensity to metastasize.
Current research on dendritic cell vaccines is focused on enhancing the immunogenic potential of vaccination through various strategies. It can be the induction of immunogenic cell death (ICD) of tumor cells, which is accompanied by the release of DAMPs danger molecules and triggering the antigen-presentation process.
One way to induce ICD is through photodynamic exposure. Dye photoactivation leads to stress and activates a cascade of reactions leading to a regulated form of tumor cell death. Different photoagents have different mechanisms of triggering immunogenic death due to the primary target of photosensitizer action.
We investigated a tetra(aryl)tetraianoporphyrazines compounds with different aryl substituents as well as a commercially available photosensitizer Photosens (Niopik, Russia). All of them are immunogenic cell death inducers and dying photoinduced cells using these agents effectively protect mice and syngeneic tumor models.
Immunized C57BL/6 mice were well protected against brain glioma growth and demonstrated high overall survival with all photoagents compared to control animals. In addition, MRI slides analysis showed that the tumor foci of the immunized animals were significantly smaller than those of the control groups of mice.
Thus, all these data indicate that tetra(aryl_tetracyanoporphyrazines and photosens can act as effective ICD inducers, potentially broadening the prospects for the development of effective immunotherapeutic strategies for the treatment of brain tumors.
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**S9.679. Physico-chemistry of nitrogen monooxide (nitric oxide) and its compounds as a determinant of their biological activity**

Vanin A.F.[1]*
[1]*N.N. Semenov Institute of Chemical Physics of Russian Academy of Sciences;*
* vanin.dnic@gmail.com

Principal physico-chemical feature which determines nitrogen monooxide (nitric oxide – NO) function as an universal regulator of biological processes in all living organisms is proposed to be capable of NO molecules to bind in pairs with two valence iron ion following with their disproportion reaction and the formation of dinitrosyl iron complexes (DNIC) with thiol-containing ligands. NO including into the complexes ensures its protection against harmful action of superoxide ions thereby the opportunity NO molecule transfer inside and between cells and tissues (thereby providing paracrynic action of NO). Moreover, NO molecule inclusion into DNICs is accompanied with their transformation into nitrosonuium cations (NO+). Thus, the DNICs with thiol-containig ligands can function as both NO and NO+ donors in living organisms. The first are responsible mainly for positive (regulatory) biological action of DNICs while the latter – for negative (cytotoxic) activity of the complexes. The examples of positive action of DNICs on human and animal organisms will be considered (hypotensive action, wound healing acceleration etc.). Regarding negative biological activity of DNICs their capability of inhibiting proliferation of bacteria, viruses (in particular, coronavirus Covid-19), endometrioms and malignant tumours will be demonstrated.

**S9.680. Physiological significance of the white gene encoding g the subunit of the ABC transporter in Drosophila melanogaster: lifespan control, effects on the CNS, and other effects of mutation in an isogenic genetic environment**

Bylino O.V.[1]*, Dobrovolskaya K.E.[2], Bikeev A.I.[3], Bekbulatov D.A.[2], Prokofiev D.Y.[3,4], Dzhelad S.S.[5], Alekseev A.A.[6], Velikanova A.V.[3], Shidlovskii Y.V.[7,8], Batin M.A.[3]
[1]*Department of the Control of Genetic Processes, Institute of Gene Biology Russian Academy of Sciences, Moscow, Russia;*
[2]*Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia;*
[3]*Open Longevity, Los Angeles, USA;*
[4]*INO CPE «Yandex EdTEch», Moscow, Russia;*
[5]*Shemyakin-Ovchinnikov Institute of bioorganic chemistry, Russian Academy of Sciences, Moscow, Russia;*
[6]*Faculty of Physics, Laboratory of Engineering Physics, Lomonosov Moscow State University, Moscow, Russia;*
[7]*Department of Gene Expression Regulation in Development, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia,;*
[8]*Department of Biology and General Genetics, I.M. Sechenov First Moscow State Medical University, Moscow, Russia;*
* bylino@gmail.com

The white gene is the first gene discovered in Drosophila, but despite its more than 100-year history, the physiological significance of this gene is still not fully understood. white encodes a subfamily ABCG subunit of the ABC transporter superfamily that carries out ATP-dependent active transport (Influx/Efflux) of substances across the membrane against their concentration gradient. Overexpression of ABCG proteins causes multidrug resistance, and their mutations lead to hereditary syndromes and a wide range of proliferative and nonproliferative diseases in humans. All this determines the importance of studying the functional properties of ABCG genes and proteins.
The closest homologues of Drosophila White are ABCG4/White2 (81% similarity of the amino acid sequence with White) and ABCG1/ABC8/White (51% similarity). Like human ABCG4, white is expressed predominantly in the brain and eyes. In recent years, the role of the white gene for a number of important processes in Drosophila, such as stem cell maintenance, glucose tolerance, learning processes, etc., has been discovered. Moreover, there is some evidence regarding the possible involvement of this and similar genes in the control of lifespan and healthspan (HS). Based on the above, it is of great interest to elucidate the role of the white gene in controlling HS on the Drosophila model, as well as to study the mechanism of the effect of the white mutation on HS.
A shortcoming of the works that previously studied the effect of white on HS in Drosophila is the lack of genetic background alignment between the experimental (w1118, extended deletion of the white locus with unmapped boundaries) and control lines. When studying quantitative traits such as HS, fecundity, etc., studies should be performed using as close a genetic background as possible. In this work, using recombination of chromosome y1w67c23 with wild-type Oregon RC and Canton S, we separated mutation w67c23 (short deletion of the promoter and first exon) from mutation y1. Next, 10 backcrosses were made with the w67c23 line to the parental lines. Thus, isogenic control lines carrying the w67c23 mutation on the Canton S and Oregon RC genetic background were obtained.
In a series of tests, we analyzed the effect of the white mutation on HS, central nervous system (CNS) function, and general biological functions of Drosophila. In the tests for CNS and general biological functions, differences between the groups were detected by analysis of variance (ANOVA). Kaplan-Meier survival curves and mortality

curves for male (M) and female (F) mutant and control lines were constructed to study the effect on HS. Survival curves were analyzed using the Kolmogorov-Smirnov two-sample test. The rate of aging was estimated from the mortality curves using the MRDT criterion.
We found that the control lines differed in maximal HS (maxHS) (age of 90% mortality Canton S: M=65 days, F=73 days; Oregon RC: M=61, F=60), and also showed significant differences in mean and median HS (MHS) between M and F (Canton S: M=51, F=59.5, p=<0.0001; Oregon RC: M=48, F=36.5, p<0.0001), which correlated well with differences in mortality and aging rate curves in M and F. Thus, in isogenic laboratory lines, the MHS between M and F is different. It can be assumed that the set of alleles of the genes controlling MHS are different in these lines.
The introduction of the white mutation into the Canton S and Oregon RC genotypes did not practically change the maxHS of the flies (age of 90% mortality Canton S: M=65, F=73; wCanton S M=66, F=70; Oregon RC: M=61, F=60; wOregon RC: M=61, F=60). However, quite unexpectedly, it turned out that sexual dimorphism by MHS in the white mutants completely disappeared; MHS in M and F was comparable and did not differ significantly (wCanton S: M=54.5, F=55.5, p=0.558; wOregon RC: M=42, F=41, p=0.275). The effect of the white mutation was characteristic of both genetic backgrounds, but its direction depended on the line genotype: in the Canton S genetic background, the mutation significantly increased MHS in M (Canton S: M=51, wCanton S M=54.5, p=0.001), but significantly decreased MHS in F (Canton S F=59.5, wCanton S F=55.5, p=0.01), while in the Oregon RC background, conversely, significantly decreased MHS in M (Oregon RC M=48, wOregon RC M=41, p=0.001), but significantly increased MHS in F (Oregon RC F=36.5, wOregon RC F=41, p=0.05). Thus, (i) introducing a sex-linked gene mutation into the genotype equalizes MHS in M and F, (ii) the effect of the white mutation depends on modifier genes affecting the MHS trait, between which there are obviously epistatic interactions.
To assess the effect of the white mutation on the CNS, locomotion and sexual behavior (the success of copulation was evaluated) were studied. Differences were detected in both genetic backgrounds. With the mutation, there was a flattening of the two-humped circadian rhythm of fly activity and a significant decrease in total locomotion (P<0.05). Copulation in such flies was delayed, not only in males, but also in females (the mechanism of courtship perception was impaired) (P<0.05). Thus, changes in the functioning of the CNS in white mutants, apparently, can be the reason for the decrease in the activity of flies.
To assess the general biological effects of the white mutation, the rate of development, fecundity, and survival of the zygotes were studied. These parameters were significantly reduced in white mutants in both genetic backgrounds (P<0.05). The competition index, which characterizes the overall fitness of the line (estimated as the number of offspring in relation to the tester and control lines), showed that white mutants showed very low fitness, which was lower than in the control and tester lines (the line with linked second chromosomes) (P <0.05). The findings demonstrate the role of the CNS and ABCG genes in the control of MHS. The work was supported by the Russian Science Foundation 18-74-10051.

### S9.681. Pore formation in liposome membranes in the presence of cytochrome C complexes with phosphatidic acid

Blagova A.V.[1]*, Stepanov G.O.[1], Osipov A.N.[1]
[1]*Pirogov Russian National Research Medical University;*
* annablagova2000@mail.ru

The key link in the pathogenesis of many diseases is disruption of apoptosis. When studying the molecular mechanisms of apoptotic processes, the mitochondrial mechanism, which is characterized by entrainment of peroxidase activity of cytochrome c. However, this event is preceded by the interaction of cytochrome c with anionic phospholipids of mitochondrial membranes [1]. It has been well studied that pores form in cardiolipin containing liposomes that interact with cytochrome [2], but the effect of cytochrome on other anionic phospholipids was not previously known.
The aim of this work was to investigate pore formation in liposomes containing an admixture of phosphatidic acid in the presence of cytochrome C and hydrogen peroxide.
The study was performed on a fluorimeter in time recording mode at fixed excitation (560 nm) and emission (590 nm) wavelengths and slits of 5 nm. Three types of liposomes were made: containing only phosphatidylcholine, containing phosphatidylcholine with 20% phosphatidic acid admixture, and containing phosphatidylcholine with 20% cardiolipin admixture. The liposomes contained the dye sulforhodamine B in high concentration. At this concentration and the self-quenching effect, the initial fluorescence intensity was low. However with cytochrome c adding, pores formed in the membranes containing the phosphatidic acid admixture, and, as a result of the dye exit and distribution over the cuvette volume, the fluorescence intensity increased.
For samples containing liposomes with an admixture of phosphatidic acid, cytochrome C, and hydrogen peroxide, the change in fluorescence intensity was 250% relative to the initial value. Similar samples containing cardiolipin instead of phosphatidic acid showed a 220% increase in intensity, and the samples with cardiolipin and phosphatidic acid admixture did not differ statistically, indicating that these two phospholipids contribute equally to membrane pore formation. For samples that contained only phosphatidylcholine among the phospholipids, this ratio was 43%.
Thus, it is shown that cytochrome c in interaction with both phosphatidic acid and cardiolipin in the presence of hydrogen peroxide leads to pore formation in liposome membranes. Membrane pore formation is necessary for apoptosis to occur. That is, the interaction of phosphatidic acid with cytochrome c can trigger apoptotic processes, which has not been previously studied.
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### S9.682. Possibilities of fluorescence time-resolved microscopy in differentiation of glioma from normal brain tissues - a pilot study

Komarova A.D.[1,2]*, Yashin K.S.[2], Kiseleva E.B.[2], Mozherov A.M.[2], Lukina M.M.[2], Shcheslavskiy V.I.[2], Shirmanova M.V.[2]
[1]*Lobachevsky State University of Nizhny Novgorod;*
[2]*Privolzhskiy Research Medical University;*
* komarova.anastasii@gmail.com

Gliomas are primary tumors of the central nervous system and are the most common malignant neoplasm of the brain in adults. About 70% of primary brain tumors are represented by various gliomas, of which more than half have a high degree of malignancy at the time of diagnosis. The treatment of gliomas includes microsurgical removal of the tumor, while the volume of tumor resection significantly correlates with the life expectancy of patients. Due to the aggressive growth of gliomas with diffuse invasion into healthy brain tissue, it is difficult to determine the boundaries of tumor tissue resection. An urgent task in this area is the development of new highly sensitive methods for rapid intraoperative diagnostics. FLIM (Fluorescence Lifetime Imaging) of intrinsic (or auto-) fluorescence of tissues is considered as a promising technology

(Lukina et al. Frontiers in Oncol. 2019; Yuzhakova et al. Frontiers in Oncol. 2022).

The aim of this work was to study the autofluorescence of glial tumors and normal brain tissues ex vivo using FLIM microscopy.

Native samples of glial tumors of patients were studied using FLIM microscopy in the spectral channel of the metabolic cofactor NAD(P)H. Twelve samples of gliomas of various grades of malignancy were explored: Grade II (diffuse astrocytoma) n=5, Grade III (anaplastic astrocytoma) n=3 and Grade IV (glioblastoma) n=5, as well as samples of white matter, peritumoral area, and cerebral cortex. Samples were transported to the laboratory in 10% BSA (bovine serum albumin) on ice, within 30 min after surgical removal of the tumor. Autofluorescence analysis by FLIM was performed on a laser scanning microscope LSM 880 (Carl Zeiss, Germany) equipped with a FLIM module based on time-correlated single photon counting TCSPC (Becker&Hickl GmbH, Germany). Autofluorescence was excited in the two-photon mode at a wavelength of 750 nm by a Mai Tai HP femtosecond laser (Spectra-Physics, USA). Fluorescence was detected in the range of 450-490 nm. The laser excitation power on the sample was 6 mW. The signal acquisition time was 120 s. An oil-immersion C-Apochromat W Korr lens with x40/1.2 was used in the experiments. The fluorescence decay parameters were estimated using the SPCImage program (average lifetime $\tau$m, lifetimes of the short $\tau$1 and long $\tau$2 components, and the relative contributions of the short a1 and long a2 components).

FLIM microscopy showed that glial tumors of different grades of malignancy have differences in autofluorescence lifetime. It has been demonstrated that glioblastomas (Grade IV) differ statistically significantly from astrocytomas (Grade II and Grade III) in terms of the mean lifetime of NAD(P)H fluorescence: $\tau$m (Grade IV) = 1.02(0.88;1.14), $\tau$m (Grade II) = 1.05(0.93;1.14) (p=0.042), $\tau$m (Grade III) = 1.04(0.95;1.11) (p=0.00075); by the relative contribution of the short component: a1 (Grade IV) = 71.42(68.78;73.83), a1 (Grade II) = 69.62(66.59;73.76) (p=9.5* 10-6), a1 (Grade III) = 70.40(67.95; 73.02) (p=0.0096). The recorded values of fluorescence decay parameters in tumor samples ($\tau$m ~1 ns, $\tau$1 ~0.4, $\tau$2~2.5, a1~72%) corresponded to typical values of the metabolic cofactor NAD(P)H. Therefore, based on the data obtained, it can be assumed that the metabolic status of glioblastomas shifts towards glycolysis, which leads to an increase in the contribution of free NAD(P)H. Samples of the white matter of the brain and peritumoral areas were characterized by a large contribution of the long component: 69.08±3.72% and 68.74±3.46%, respectively, compared with samples of astrocytomas and glioblastomas.

Thus, the FLIM method makes it possible to distinguish between glial tumors of different grades of malignancy and to distinguish tumor tissue from normal (white matter of the brain) and from peritumoral areas by the parameters of NAD(P)H autofluorescence decay.

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### S9.683. Production of reactive oxygen species by leukocytes stimulated by zymosan and lipopolysaccharide in women with early gestation miscarriage

Zhambalova B.A.[1]*, Osipov A.N.[1], Teselkin Yu.O.[1]
[1]*N.I. Pirogov Russian National Research Medical University;*
* zhambalovaba@inbox.ru

Purpose of the study. The production of reactive oxygen species by peripheral blood polymorphonuclear leukocytes stimulated with zymosan and lipopolysaccharide, recognized by Toll-like receptors, in women with miscarriage of infectious genesis in the early stages of gestation compared with healthy pregnant women was studied.

Materials and methods. We examined 75 women aged 18–42 years, who were divided into 3 groups: group 1 - healthy non-pregnant women (n = 25); 2nd group - healthy pregnant women (n = 25); Group 3 - women with infectious miscarriage (n = 25). All pregnant women were in the first trimester of gestation (weeks 5–12). Determination of the functional activity of polymorphonuclear leukocytes (PNL) of peripheral blood was carried out by the method of luminol-dependent chemiluminescence (LCL).

Research results. At the first stage of the study, to determine the maximum effective concentration of ligands, we used the blood of healthy non-pregnant women to study the dependence of the intensity of LCL PNL on the concentration of the added ligand with a constant number of cells. In various studies, different amounts of zymosan and lipopolysaccharide (LPS) are used to study the functional activity of cells by the LCL assay. A dose-dependent change in the intensity of LCL PNL was detected. The maximum intensity was observed at a zymosan concentration of 100 μg/ml, and LPS at 200 μg/ml. On the basis of the obtained results, the following concentrations of zymosan and LPS were selected: 5, 50, and 100 μg/ml to identify significant differences in the responses of the cells of the studied patient groups. Further, in healthy pregnant women and women with infectious genesis miscarriage, the effect of these ligands on the intensity of LCL PNL was studied. The intensity of LCL PNL increases in both groups of patients with an increase in zymosan concentration from 5 to 100 μg/ml. At the same time, in women with miscarriage of infectious genesis, the intensity of LCL cells, when stimulated with zymosan at a concentration of 100 μg/ml, is 1.5 times ($p<0.05$) more than in healthy pregnant women. In the case of stimulation of LPS cells with an increase in the concentration of ligand in the studied groups of patients, a marked difference in the cell responses is observed. An increase in the intensity of LCL PNL with an increase in the concentration of LPS from 5 to 100 μg/ml is observed only in women with a physiological pregnancy. In women with miscarriage of infectious genesis, the intensity of LCL PNL increases only at concentrations from 5 to 50 μg/ml, and a further increase in the concentration of LPS to 100 μg/ml leads to a decrease in the intensity of LCL PNL. At the same time, the intensity of LCL PNL stimulated by LPS at a concentration of 100 μg/ml in women with miscarriage of infectious genesis is 3.1 times less ($p<0.05$) than in women with a physiological pregnancy. It can be assumed that one of the reasons for a decrease in the production of ROS PNL stimulated by large concentrations of LPS in women with miscarriage of infectious genesis compared with healthy non-pregnant women and women with physiological pregnancy is reduced expression of TLR4 (Toll-like receptors) on the membrane of these cells. In chronic infection, constant stimulation of the receptor by infectious agents, possibly, causes TLR4 inhibition, decreases expression of this receptor, and already for the next stimulus with these same ligands, only the inhibition of the cellular response is observed, and reactive oxygen species production decreases. As a result, there is a decrease in the PNL bactericidal activity in women with miscarriage of infectious genesis, which may be one of the mechanisms for the development of miscarriage. When conducting a comparative analysis of the effect of TLR2 and TLR4 ligands on the production of reactive oxygen species, the following differences are observed: the maximum intensity of LCL PNL in women with physiological pregnancy while stimulating their luminescence with zymosan at a concentration of 100 μg/ml is 2.2 times higher ($p<0.05$), compared with the stimulation of LPS cells. In women with infectious genesis miscarriage, this difference is significant: the intensity of LCL PNL stimulated by zymosan at a concentration of 100 μg/ml is 10.7 times higher ($p<0.05$), compared with stimulation of LPS cells of the same concentration. It cannot be ruled out that TLR2-mediated production of ROS may make a significant contribution to the overall production of ROS by blood phagocytes in women with infectious diseases miscarriage.

Conclusion. In women with miscarriage of infectious genesis and in healthy pregnant women against the background of the development of oxidative stress in the first trimester of gestation, the production of reactive oxygen species by polymorphonuclear leukocytes of peripheral blood stimulated by zymosan or lipopolysaccharide recognized by Toll-like receptors may depend on the expression of these receptors on cell membrane.
Key words: polymorphonuclear leukocytes, zymosan, lipopolysaccharide, chemiluminescence, Toll-like receptors

### S9.684. Promising nuclear physics methods of binary proton therapy

Zavestovskaya I.Z.[1,2]*
[1]*Lebedev Physical Institute of RAS;*
[2]*MEPHI;*
* zavestovskayain@lebedev.ru

The report is devoted to a review of the work on the project "Development of new technologies for the diagnosis and radiation therapy of socially significant diseases with proton and ion beams using binary nuclear physics methods", implemented at LPI jointly with the MEPHI and the NMIC of Radiology of the Ministry of Health of the Russian Federation under an agreement with the Ministry of Education and Science within the framework of the FSTP "Development of synchrotron and neutron research and research infrastructure for 2019-2027.
Despite the significant progress made in the diagnosis, treatment and prevention of oncological diseases, these diseases remain a serious threat to humanity and are the second leading cause of death in the world. Radiation therapy is an effective method of treating oncological diseases of various pathologies. High-tech and promising methods of radiation therapy include hadron therapy - radiotherapy using particle beams, such as neutrons, protons, alpha particles, charged ions, etc. Proton and ion therapy have received the greatest development in the world among hadron therapy technologies. This is due to the fundamental properties of the interaction of proton and ion beams with matter, which allow us to obtain unique characteristics of the deep distribution of the absorbed dose of hadrons. Protons and ions rapidly lose energy in the last few millimeters of penetration into tissues, which gives a pronounced maximum dose distribution (Bragg peak) in a localized area, which can be adjusted in depth by changing the initial energy of the proton beam. The Bragg peak can be precisely localized anywhere in the patient's body due to the choice of proton energy, and several Bragg peaks can be shifted in depth to create a distributed Bragg peak, which is used to irradiate the pathogenic area. This advantage can be used both to achieve tumoricidal dose values and its high-conformal delivery, and to significantly reduce doses in normal tissues, proximal and distal with respect to the target volume.
The proton therapy Complex (CPT) "Prometheus", on which work is being carried out in the project, is a compact proton synchrotron capable of accelerating protons in the energy range of 30-330 MeV. According to its characteristics, the installation is significantly superior to the world's leading leaders: low weight (15 tons), low power consumption (up to 100 kW) and compact dimensions (outer diameter – 5 m) allow the complex to be placed in ordinary hospitals without erecting separate buildings. The energy selection step at the accelerator is 0.1 MeV, which makes it possible to plan the irradiation procedure in the direction of beam propagation with submillimeter accuracy. At the same time, the beam size in the orthogonal plane is no more than 3 mm at a beam energy of 150 MeV, one of the most commonly used energies for irradiation. The project provides for the development, testing and implementation of promising radiotherapy technologies on the upgraded Prometheus CPT:
- Development of the fundamentals of new binary proton therapy technologies based on targeted technologies using promising nanoparticles, nanocomposite materials and multifunctional systems based on them as radiosensitizers of therapy and/or active agents for visualization.
- Development of the fundamentals of new technologies of radiation therapy based on the combined action of rare-ionizing (proton) and dense-ionizing (neutron / carbon ions) radiation, allowing to create a high dose gradient between tumor and normal tissues, and at the same time, to increase the damaging effect (including radioresistant tumors) due to exposure carbon ions/neutrons with high relative biological efficiency (OBE).
- Mathematical modeling of the processes that determine the effectiveness of radiation therapies: the dynamics of changes in the size and possible movement of the tumor during irradiation, irradiation modes (fractionation, intensity modulation, sensitization by nanoparticles, etc.) and spatio-temporal changes in the radiosensitivity of tumor cells.
- Development of a proton radiography and tomography method based on the proton synchrotron of the Physics and Technology Center (FTC) of the FIAN, which allows determining the length of the proton path inside the patient's body with millimeter and submillimeter accuracy and significantly increase the image contrast and thereby significantly increase the efficiency of using proton therapy.
- Development of proton and ion therapy technologies that take into account during a proton therapy session the movement and displacement of the tumor and internal organs arising from palpitations and breathing or involuntary movements of the patient, based on a comparative analysis of various methods of irradiation of intrafractionally moving tumors of the abdominal and thoracic regions during studies on a dynamic water phantom.
- Improvement and modernization of the proton synchrotron FTC FIAN – proton therapy complex (CBT) "Prometheus" based on the solution of the major applied tasks set in the project and the techno developed in the project
New technologies of proton therapy will make it possible to give specific recommendations to the medical community aimed at improving the effectiveness of proton therapy.

### S9.685. Protective effect of intracellular acidification in toxic cellular models of Parkinson's disease

Fedotova E.I.[1,2], Kritskaya K.A.[1], Berezhnov A.V.[1,2]*
[1]*Institute of Cell Biophysics of RAS;*
[2]*Orel State University named after I.S. Turgenev;*
* g_56@rambler.ru

Parkinson's disease (PD) is based on the selective death of dopaminergic neurons in the midbrain. Understanding the causes of these cell death is the first step towards developing neuroprotective agents and curing or slowing down a disease that currently has no effective treatment. Although the exact mechanisms of neuronal damage remain unclear, abnormal mitochondrial function appears to be a convergent point in processes of cell death. Accumulation of alpha-synuclein aggregates, oxidative stress, reticulum stress, disorders of auto/mitophagy processes, and disorders of calcium signaling are considered as disorders associated with mitochondrial damage. It has been hypothesized that moderate activation of mitophagy may protect cells from death as a result of disorders associated with PD. At the same time, the question of how to induce this process in cells remains open. Previously, we suggested that acidification of the intracellular environment activates the processes of autophagy and mitophagy in cells. In this regard, the aim of this work was: to evaluate the protective potential of short-term acidification of the intracellular environment in toxic cellular model of Parkinson's disease.
The experiments were carried out using confocal, fluorescent microscopy and real-time PCR on toxic cellular model of PD, a neuroblastoma culture SH-SY5Y treated with neurotoxin MPP+.

In this work, we assessed the basal level of intracellular pH in a neuroblastoma culture and showed that when cells were treated with 500 mkM MPP+ for 24–48 h, intracellular pH did not change significantly. The level of auto/mitophagy was studied by the degree of colocalization of mitochondria and lysosomes, and it was shown that this parameter was increased in the MPP+-induced model compared to the control culture. The expression of some mitophagy genes is also increased.
The morphology and dynamics of the mitochondrial reticulum (network) in PD cellular models were assessed and an increase in the number of individual mitochondria was found, as well as a decrease in the average relative length of mitochondria in the network in cells treated with MPP+ compared with the control. The data were confirmed by analysis of gene expression, which resulted in an increase in the mRNA level of genes responsible for mitochondrial dynamics.
The degree of mitochondrial reactive oxygen species (ROS) production was quantified and it was shown that in the MPP+-induced PD model, the rate of ROS production was increased compared to the control.
The differences in the bioenergetic state of cells in the toxic model of PD were revealed, namely, a decrease in the mitochondrial potential, the total pool of NADH and redox index, the level of reduced glutathione in MPP+-treated cultures was observed, which indicates disorders associated with cell bioenergetics.
The cell viability in SH-SY5Y culture was assessed: the number of dead cells increased when cells were treated with MPP+ compared to control. The study of apoptosis genes also revealed an increase in expression.
Thus, it was shown in the work that the level of intracellular pH did not change in the toxic MPP+-induced cellular model. However, the degree of mitophagy in such cells was increased, as well as an increase in the number of individual mitochondria and a decrease in their relative length, the following were also observed: ROS overproduction, disturbances in the state of mitochondria (mitochondrial potential, NADH redox index, reduced glutathione level) and an increase in non-viable cells by compared with a control culture of neuroblastoma.
In the work, short-term acidification of the intracellular environment was carried out by replacing the extracellular solution with a solution with a low pH value (pH 6.6 for 60 min), as well as adding agents that lower the intracellular pH value, such as sodium pyruvate (10 mM) and sodium lactate (10 mM ), in a toxic cellular model of Parkinson's disease. It was found that acidification triggers auto/mitophagy, changes the morphology and dynamics of the mitochondrial network, improves the bioenergetic state of cells, and also increases the level of viable cells, thereby protecting cells from death in SH-SY5Y neuroblastoma culture treated with neurotoxin MPP+.
The work was performed within the Government Contact No. 075-01512-22-03 on the topic: "Neuroprotective drugs of new generation" No. 1022080100047-5-1.6.4.

**S9.686. Receptor specificity and transmissibility as elements for quantitative assessment of zoonotic influenza viruses pandemic potential**

Onkhonova G.S.[1]*, Kosenko M.N.[1], Gudymo A.S.[1], Molchanova M.L.[1], Vasiltsova N.N.[1], Marchenko V.Yu.[1], Tran T.Nhai.[2], Ryzhikov A.B.[1]
[1]*Federal Budgetary Research Institution State Research Center of Virology and Biotechnology "Vector" Rospotrebnadzor;*
[2]*Joint Russian-Vietnamese Tropical Research and Technology Centre;*
* onhonova_gs@vector.nsc.ru

Beyond the need to monitor seasonal and zoonotic strains, fundamental research remains important for a comprehensive study and understanding of the mechanisms for emergence of new influenza virus variants, including strains with epidemic and pandemic potential. All respiratory viruses, including influenza virus, are characterized by the ability to airborne transmission. Despite the high morbidity and mortality, the molecular mechanisms of influenza virus transmissibility are not well understood. The role of receptor binding in the influenza virus life cycle is important, as it is the initial stage of viral particle penetration into the infected organism cell. With the emergence of strains that are able to overcome the interspecies barrier, as well as collective immunity formed to already circulating variants of the influenza virus, there is a danger of both sporadic infections and epidemics. In 2016, WHO introduced the Tool for Influenza Pandemic Risk Assessment (TIPRA). The present study is concerned with two elements of TIPRA, the receptor binding capacity of influenza viruses and the transmissibility of the virus in animal models. During this investigation avian influenza virus strains circulating in Russia and Socialist Republic of Vietnam were studied. The studied avian influenza viruses of subtypes A/H5Nx and A/H9N2 retain their specificity for the "avian" type of sialic receptors. Some A/H9N2 subtype viruses have receptor specificity for both "avian" and "human" receptor types. Avian influenza virus A/chicken/Primorsky Krai/1771/2018 (H9N2) with "double" specificity demonstrated transmissibility in a ferret model. The constant circulation of such viruses among avian populations and occasional human infection make it possible for viruses to evolve, with likely changes in receptor specificity and transmissibility, which could lead to the emergence of a new pandemic strain. A quantitative assessment of the pandemic potential of zoonotic influenza viruses is necessary for a timely response and the adoption of anti-epidemic protective measures

**S9.687. Role of NADPH oxidase 2 in the development of oxidative stress and microglyosis in the mice hippocampus during induced amyloid toxicity**

Osypov A.A.[2,1]*, Lyubanskaya A.D.[1,3], Mukhina K.A.[1,3], Korchagina V.M.[1], Popova I.Yu.[1]
[1]*ITEB RAS;*
[2]*IHNA&NPh RAS;*
[3]*MSU;*
* aosypov@gmail.com

The development of early therapy for Alzheimer's disease (AD) is an urgent problem of modern biology and medicine. The main risk factors for AD are related to oxidative stress, to which 2 isoform of NADPH oxidase (NOX2) contributes significantly. The aim of this study was to elucidate the role of NOX2 in the development of oxidative stress and microglia in the hippocampus of mice during the initial stages of AD development.
The experiments were performed on BALB/c mice, which were preliminarily implanted with cannulas to inject drugs into the brain ventricles. The mice were divided into three groups: a control group with ACSF administration, n = 5; a group with beta-amyloid (Aβ) administration, n = 4; and a group with Aβ and the selective NOX2 inhibitor GSK2795039 (GSK) administration, n = 4. The drugs were injected into awake mice using a Hamilton syringe through a cannula (1 μL, at a rate of 1 μL/min) daily for 3 days. Twelve hours after the last drug injection, the brains were extracted, the left half was fixed in 4% paraformaldehyde for immunohistochemical analysis, and the right half was analyzed by biochemical methods. Because the greatest amount of NOX2 is found on the microglia and activation of these cells is observed in the development of Alzheimer's disease, immunohistochemical analysis of the microglia in the hippocampus was performed in this work. The right hemisphere of the brain was homogenized and oxidative stress markers were analyzed in different fractions.
The results showed that the Aβ group significantly increased microglial cell area in the hippocampus (by 50% in the dentate fascia and 58% and the CA1 field of the hippocampus). NOX2 blockade significantly decreased microglial cell area to 47-49% compared with control values. Biochemical studies showed that the membrane fraction of the brain

homogenate of Aβ group mice had increased peroxidized lipids and decreased content of reduced sulfhydryl groups, indicating a prolonged local effect of AFC. GSK largely prevented these changes. The mitochondrial fraction showed no differences between the groups, indicating the absence of mitochondrial pathology in the early stage of pathology development.
Principal component analysis by biochemical parameters (even more so when combined with glial parameters) allows us to clearly separate the group with amyloid from the control and GSK groups, which do not differ between themselves. Multicomponent cross-correlation analysis between various biochemical and glial parameters revealed a significant difference between the groups in the patterns of correlated parameter distribution, with an increased number of correlated parameters in the amyloid and especially GSK groups, which may indicate a coordinated change in metabolism in the development of pathology. At the same time, cross-correlation analysis of the combined group showed a lower number of correlated parameters than even in the control, indicating the antidirectional effect of GSK on the development of pathology.
NADPH oxidase 2 blocker prevents both microgliosis in the hippocampus and oxidative stress in the mouse brain during induced amyloid toxicity and, thus, may represent a pharmacological target for direct and effective therapy of Alzheimer's disease.

**S9.688. Role of calcium-permeable AMPA and kainate receptors in plasticity**

Dolgacheva L.P.[1]*, Zinchenko V.P.[1]
[1]*Institute of Cell Biophysics of the RAS;*
* dolgacheva@mail.ru

Glutamate is the main excitatory neurotransmitter in the mammalian brain and acts through ionotropic (NMDA, AMPA and KA) and metabotropic (mGlu) receptors. Ionotropic glutamate receptors play a central role in cell development and in the regulation of synaptic plasticity - the ability of synapses to rapidly change transmission intensity depending on neuronal activity. Synaptic plasticity is considered as basis of learning and memory processes. Calcium-permeable NMDA receptors play an important role in these processes. However, for activation, these receptors require preliminary depolarization of the postsynaptic membrane by AMPA receptors. AMPA receptors are ligand-gated channels permeable to Na+ and K+. However, certain subtypes of these receptors are also permeable to Ca2+ (CP-AMPARs). Fast interneurons expressing CP-AMPARs practically do not have NMDA receptors. Activation of CP-AMPARs in these interneurons provides rapid postsynaptic Ca2+ entry, which induces the process of synaptic plasticity (Hainmuller et al., 2014; Lalanne T., et al., 2016). Ca2+ influx into cells through CP-AMPARs plays an important role in synaptogenesis and the neural networks formation in early brain development (McDonald and Johnston, 1990; Stubblefield and Benke, 2010). In the adult brain, CP-AMPARs are mainly located in the postsynaptic membrane of inhibitory neurons. Many studies have shown that modulators of CP-AMPARs potentiate plasticity, improve memory and learning, and are involved in the genesis of neurodegenerative diseases such as ischemia, stroke, and seizures. Selective blockers of CP-AMPARs have a neuroprotective effect and prevent neuronal death during ischemia. Using antagonists of CP-AMPARs, it has been shown that not only NMDA receptors, but also CP-AMPARs are involved in the induction of long-term potentiation (LTP) in the hippocampus (Yu et al., 2021), mainly due to changes in the composition of receptors in synapses (Yu et al, 2021). The number of receptors in synapses is regulated by endocytosis, exocytosis, and endosomal sorting. The ratio of transported Ca2+-permeable and Ca2+-impermeable AMPA receptors depends on synaptic activity. With a decrease in the efficiency of synaptic transmission in LTD, a decrease in the number of AMPARs in the synapse occurs due to the transport of AMPARs into lysosomes and subsequent degradation, while in LTP, a return and an increase in the number of AMPARs occur in synapses. The movement and incorporation of CP-AMPARs into the synaptic area is regulated by phosphorylation of the GluA1 subunit by protein kinase PKA and dephosphorylation by phosphatase PP2B (Guire et al, 2008; Wang et al, 2020). It is assumed that during hyperexcitation and learning processes, CP-AMPARs are transported into synapses and modify synaptic plasticity towards the formation of neuronal connections. It has been shown that CP-AMPARs have a stronger effect on plasticity than NMDARs due to their ability to increase the intracellular Ca2+ concentration voltage-independently. The fact that in a adult brain CP-AMPARs are predominantly localized mainly in GABAergic neurons, which, as has recently been shown, can innervate other GABAergic neurons containing CP-kainate receptors (CP-KARs) (Zinchenko et al., 2020), indicates a special role of these receptors in the plasticity of GABAergic neurons. Activation of CP-AMPARs localized in GABAergic neurons can enhance Ca2+-dependent GABA secretion and inhibit the activity of other GABAergic neurons and thus participate in the disinhibition of the neuronal network.
Kainate tetramer receptors (KARs) are formed by two families of subunits: subunits with low affinity for agonists (GluK1-GluK3) and subunits with high affinity for agonists (GluK4-GluK5). KARs are present in both pre- and postsynaptic membranes of neurons and play the role of modulators of neurotransmission and neuronal development (Chittajallu et al., MacDermott et al., 1999). KARs play a more complex role in synaptic plasticity than AMPARs due to their localization in both post- and presynaptic membranes, where they rapidly activate the secretion of GABA and other neurotransmitters for a long period. Activation of presynaptic CP-KARs leads to an increase of the neurotransmitter release, due to an increase in the calcium presynaptic concentration. Thus, activation of CP-KARs causes the release of GABA from interneurons, initiating the inhibition of neural network activity (Sakha, et al., 2016). Such a significant role of CP-AMPAR and CP-KAR in the modulation of synaptic transmission in GABAergic neurons is due not only to the calcium permeability of receptors, but also to their ability to respond earlier and faster than others neurons, which leads to inhibition of hyperexcitation in other neurons due to advanced GABA release (Zinchenko et al., 2021). The effect is due to increased excitability and weakened GABA(A)-dependent inhibition of CP-KARs and CP-AMPARs containing GABAergic neurons (Gaidin et al., 2022). Thus, glutamate CP-AMPARs and CP-KARs play a key role in both the regulation of neurotransmitter release and the synaptic plasticity of GABAergic neurons.

**S9.689. Role of erythrocyte hemolytic resistance in the detection of unstable atheromas**

Yastrebova E.S.[1]*, Maltsev V.P.[1], Karpenko A.A.[2]
[1]*Voevodsky Institute of Chemical Kinetics and Combustion;*
[2]*State Research Institute of Circulation Pathology;*
* kat30cer@gmail.com

Background: One of the main clinical manifestations of atherosclerosis is the formation of atherosclerotic plaques (AP), the instability of which determines the risk of fatal cardiovascular events. It is important to develop new factors of atheroma destabilization that would have predictive value. Hypoxic conditions in different parts of the vessels and intravascular hemolysis play an important role in formation of unstable AP. It regulates by oxygen exchange which is limited by erythrocyte band 3 membrane proteins and erythrocyte structure. This study aims to detect deviations in the erythrocyte parameters which capable of the formation and progression of unstable atheroma in the pathogenesis of atherosclerosis.
Methods: Carotid artery plaques were obtained by endarterectomy. Histological examination of AP microscopic sections by AxioCam MRc5.

Blood was drawn by venipuncture and analyzed at room temperature (22 °C). Totally 58 patients and 60 donors were included in this study. All donors were divided in three groups: patients with stable and unstable plaques and healthy donors. Each sample was 1000-fold diluted in 0.9% saline or lysis solution to obtain appropriate cell concentration (0.5•107÷2•107) for efficient system operation. Volume containing 100 µl of diluted blood was placed into a testing tube and measured with the scanning flow cytometer (SFC fabricated by CytoNova Ltd., Novosibirsk, Russia, http://cyto.kinetics.nsc.ru).
Results: We investigated the following parameters: erythrocyte morphology (diameter, thicknesses, volume, surface area, sphericity index and spontaneous curvature), erythrocyte functional membrane properties (elasticity, active and total anion exchangers) and hemoglobin content in individual cells. We combined these results with standard diagnostic parameters, including assessment of lipid metabolism disorders and glycated hemoglobin level in blood. It turns out that a statistically significant difference (according to the Mann-Whitney test with p-value = 0.029) between groups with different types of atheromas is found in the parameter responsible for resistance to induced isotonic hemolysis. Moreover, patients with stable plaque have the higher rate of anion permeability that appears in healthy group or patients with unstable plaque.
Conclusion: Observed erythrocyte behavior can be explain as attempt to satisfy the increased oxygen demand in AP and to eliminate hypoxic conditions, and, as a result, the growth and destabilization of the AP.

**S9.690. Role of the microbiota in neurodegeneration**

Sobol K.V.[1]*
[1]*I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry;*
* peep9@yandex.ru

The functional interaction of the gastrointestinal tract (GIT) and the central nervous system (CNS) includes the autonomic nervous system (ANS), enteric nervous system (ENS), immune and neuroendocrine systems [1-4]. One of the central roles in this interaction is played by the microbiota of the gastrointestinal tract. Moreover, the microbiota, in addition to useful substances, can synthesize amyloid proteins, lipopolysaccharides, endotoxins, and other active substances that can stimulate amyloidosis in the CNS, as well as inflammatory reactions that contribute to the development and/or progression of neurodegenerative diseases [4-8].
A large number of amyloid molecules synthesized by the microbiota increases the availability of amyloid in the central nervous system with age [9]. Moreover, microbiota amyloids (curli, Aβ42, etc.) are able to activate Toll receptors (TLR2/TLR1) and participate in pro-inflammatory reactions [10]. Microbial amyloids and lipopolysaccharides are significant activators of inflammatory responses, inducing the release of relevant cytokines and activating complement proteins. As a result, vascular permeability changes and free radicals are generated, thereby stimulating the processes of amyloid formation. These pathological processes are characteristic of Alzheimer's disease. It remains to be seen what percentage of microbial amyloid molecules are found in the senile plaques of patients with Alzheimer's disease. What is the evolution of amyloid formation associated with the microbiota, and how does it change with age?
There is evidence that cyanobacteria of the intestinal microbiota can produce an excess of the neurotoxin beta-methylamino-L-alanine (BMAA) [11], elevated concentrations of which were found in the brain of patients with amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease [9, 13].
Currently, the point of view that pathogenic microbes make a potential contribution to the aging of the body and, possibly, to the development of neurodegenerative diseases, in particular, Alzheimer's disease, is increasingly recognized [14, 15]. It should be noted that most of the changes observed in Alzheimer's disease, such as inflammatory reactions, atrophy of brain cells, amyloidosis, cognitive impairment, etc. may also be due to microbial infection.
Recently, it has been demonstrated in sterile genetically modified mice with artificially reproduced Parkinson's disease (with increased expression of alpha-synuclein) that the microbiota can stimulate synucleopathy, neuroinflammation and characteristic motor dysfunction [16]. Microbial metabolites have also been identified that may be involved in the development of Parkinson's disease [16].
Currently, studies are underway regarding the use of probiotics and nutritional products for the prevention and possible treatment of CNS diseases that can activate neuroendocrine, neuroimmune and humoral mechanisms [9]. For example, there is evidence of the effects of probiotics and various food ingredients on the development of multiple sclerosis [17], on cognitive processes [18], as well as on mental disorders, including anxiety, autism, depression and schizophrenia [9, 19].
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## S9.691. SERPA technology in the diagnosis of prostate adenocarcinoma

Gudkov G.V.[1], Tarasov Y.V.[2], Zolotavina M.L.[2]*, Fedorenko T.V.[1], Filippov E.F.[3], Demchenko L.S.[1], Faniev M.V.[1], Krutenko D.V.[1]
[1]*Children's city hospital of Krasnodar, Krasnodar, Russia;*
[2]*Kuban State University, Krasnodar, Russia;*
[3]*Kuban State Medical University, Krasnodar, Russia;*
* zolotavina_m@mail.ru

Prostate cancer is one of the most common neoplasms [1]. Altered immunoreactivity of patients with prostate cancer causes the appearance of autoantibodies to tumor-associated antigens (AAO), the determination of which is crucial in the diagnosis and prognosis of the disease. An effective search for an individual profile of the most immunogenic AAO allows the implementation of modern SERPA (Serological Proteome Analysis) technology [2], which includes a combination of 2D electrophoresis, immunoblotting and mass spectrometry (peptide sequencing) methods.
The purpose of the study. The use of SERPA technology in the determination of immunoreactive targets in the tumor tissue of patients with prostate adenocarcinoma (APJ), as an additional method of diagnostic strategy.
Material and methods. The study was conducted in the laboratory of cellular technologies of the Center for Reproductive and Cellular Medicine of the State Medical Institution "DGKB Krasnodar", 10 patients were examined: three of them had highly differentiated acinar APJ, the rest had foci of chronic inflammation with signs of benign hyperplasia. The study material was prostate tissue samples obtained during a fine needle biopsy, as well as autoserum of patients. The washed biopsies were homogenized in a rehydration buffer (7M urea, 2M thiourea, 4% CHAP, 30mM Tris/HCl pH 9.0, aprotinin) followed by centrifugation of cell lysate at 14000×g (30 minutes at 4oC). The supernatant proteins were precipitated with acetone (at -20°C) with further vacuum drying of the precipitate and dissolution in a rehydration buffer (ReadyPrep ™ 2D Rehydration/Sample Duffer, Bio–Rad). Two identical 2D gels were prepared for each sample, one of which was used for transfer to a PVDF membrane (Trans-Blot ® Turbo LF PVDF, Bio-Rad) and detection of immunogenic targets, and the other for their excision and mass spectrometric identification. After passive rehydration of two IPG strips (IPG strips ReadyStrip pH 3-10. 11 cm, Bio-Rad), on each of which 185 μl of the sample was applied, isofocusing (PROTEAN ® i12 ™ IEF System, Bio-Rad) was performed, followed by electrophoresis of the strips in polyacrylamide gels (AnykD ™ Criterion ™ TGX Stain-Free Protein Gel 11 cm IPG, Bio-Rad). 2D gels were stained (Bio-Safe ™ Coomassie Stain, Bio-Rad) and one of them was used for transfer (Trans-Blot ® TurboTM, Bio-Rad) to a membrane that was subjected to immunoblotting, where the patient's autoserum was used as primary antibodies (1:200), and the locations of immunoreactive targets were determined by colorimetric detection (Goat Anti-Humane IgG (H + L)-HRP Conjugate). The target spots on the membrane were carefully compared with the corresponding spots on the second colored 2D gel, after which they were cut out of the gel, trypsinolysis was performed (Bruker Daltonics, Germany), followed by identification on the MALDI-TOF Autoflex Speed mass spectrometer (Bruker), in reflex mode and in the mass range 800-4000 Da. The most pronounced peaks of the TOF spectrum were subjected to MALDI-TOF-MS/MS analysis (sequencing). The search was performed using Biotools v3.2 (Bruker) software in the MS and MS/MS measurement database and by accessing the MASCOT search engine (Matrix Science Ltd) with a threshold score of more than 56 points ($p<0.05$) on the MOWSE scale for SwissProt.
Results and discussion. SERPA technology made it possible to establish high autoimmunoreactivity against protein targets of APJ, among which there were changes in the activity of α-enolase (ENO1), aldolase (ALDO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the concentration of annexin A2 (ANXA2). A possible explanation for the autoimmune response against the pronounced activity of glycolytic enzymes (ENO1, ALDO, GAPDH) is the high expression in tumor cells associated with a radical restructuring of energy metabolism in favor of "aerobic" glycolysis (Warburg effect) [3] for the needs of intensive proliferation and growth. In addition, ENO1, GAPDH and ANXA2 are involved in the receptor-mediated conversion of plasminogen to plasmin [4], which ensures the remodeling of the intercellular matrix and the creation of optimal conditions for active migration and metastasis of tumor cells. In patients with signs of chronic inflammation and hyperplasia, immunoreactivity with respect to these markers was insignificant. Thus, the use of SERPA technology allows for an effective search for an individual profile of the most immunogenic AAO.
Conclusions. SERPA technology makes it possible to specifically identify immunoreactive targets of APJ cells, which opens up additional opportunities for new diagnostic and therapeutic strategies.

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## S9.692. Software-hardware complex for prototyping of visual neuroprostheses

Kravchenko S.V.[1]*, Sakhnov S.N.[1,2], Myasnikova V.V.[1,2]
[1]*Krasnodar branch of S.N. Fedorov National Medical Research Center "MNTK "Eye Microsurgery", Krasnodar, Russia;*
[2]*Kuban State Medical University, Krasnodar, Russia;*
* ksv.1991@yandex.ru

Blindness due to various causes is a serious medical and social problem. One of the solutions is vision prosthetics. There are different types and constructions of visual prostheses, but they have a common principle, which consists in converting an image received from a video camera into a pattern for multichannel electrical stimulation of the neural structures of the visual analyzer, like retina, optic nerve, visual cortex, etc. The visual scene perceived by the user of a visual prosthesis, has significant differences from biological vision of healthy person. Pattern of phosphenes has low spatial resolution and is not capable of transmitting information about the color and depth of the image [1]. Prototyping of bionic vision systems is an important step in their development, which can optimize this process.
The aim of this work is development of software-hardware complex for prototyping of visual neuroprostheses.
Designed system consists of video-camera, IBM PC-compatible laptop with GNU/Linux operating system and digital unit for stimulating pattern generation on ATmega2560 microcontroller. During operation of the device, the video stream from the camera is processed on a laptop by previously developed program "Video-Simplificator U" (Certificate of state registration of a computer program No. 2021660749 Russian Federation) [2], and in the form of a series of arrays via a serial port is transmitted to digital unit for stimulating pattern generation, the firmware of which (Certificate of state registration of the computer program No. 2021680407 Russian Federation) [3] converts the received data into stimulation patterns. An electrostimulation device is possible to be connected to unit for stimulating pattern generation for stimulation of the structures of the visual analyzer in animal's in vivo experiments

or neuron cultures for in vitro models. Also, generated patterns can be visualized by array of LEDs, which allows simulating a phosphenes pattern for testing various image pre-processing algorithms in visual prostheses.

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### S9.693. Spatiotemporal patterns of astrocytic calcium signaling: experiment and modeling

Brazhe A.[1,2]*, Fedotova A.[1,2], Verisokin A.[3], Verveyko D.[3], Postnov D.[4], Semyanov A.[2]
[1]*Lomonosov Moscow State University;*
[2]*IBCh RAS, department of molecular neurobiology;*
[3]*Condenced Matter Physics Center, Kursk State University;*
[4]*Department of Optics and Biophotonics, Saratov State University;*
* brazhe@biophys.msu.ru

The role of astrocytes, the most common type of brain glial cells, in cognitive processes from sensory information processing to memory formation, behavior patterns, regulation of circadian rhythms is actively researched. The amount of rapidly growing body of new experimental data on calcium imaging of astrocytes, however, has not yet turned into the quality of understanding the basic laws of their function. This qualitative leap in description and understanding of the role of astrocyte calcium signaling in the regulation of brain function requires synthesis of new approaches to the processing and analysis of experimental data as well as data-driven and mechanism-based theoretical models. Experimental data indicate the importance of astrocyte calcium signaling for CNS function, and one should distinguish between spontaneous activity of astrocytes and that associated with the release of neuromodulators such as norepinephrine.

Here we present a synthesis between experimental data and modeling of calcium activity in astrocytes. Processing and analysis of experimental data is based on the approaches we are developing for denoising and reconstruction of individual episodes of activity. The algorithm relies on dimensionality reduction, signal factorization into spatial and temporal components, and partial reconstruction after discarding insignificant components. The observed episodes of spontaneous calcium activity can be interpreted as a stochastic process, but the question of the presence or absence of certain regularities or repetitive patterns of activation at the level of individual cells or astrocytic syncytium remains open. We consider approaches to looking for such patterns using simplified data-driven dynamic models. Further understanding of the mechanisms involved in the initiation and propagation of calcium signaling events demands creation of nonlinear mathematical models that describe the putative biophysical mechanisms of calcium dynamics in astrocytes. We consider such distributed models implemented on realistic spatial templates, as well as the patterns of calcium dynamics in these models, determined by the local morphology of the astrocyte. Calcium imaging of astrocytes in the cortex in vivo during spontaneous locomotion of the animal on a mobile platform indicates reliable activation of astrocytes following initiation of movement. A characteristic pattern of activation of individual astrocytic domains was found, in which the response is initiated at the periphery of the domain and propagates centripetally, gradually reaching the cell body and activating it. Individual astrocytes in different regions of the visual field can be activated non-simultaneously, however the general pattern is reproduced within each spatial domain. At the same time, the ongoing calcium responses in the soma are characterized by calcium fluctuations, which is consistent with the concept of the IP3-dependent mechanism as a calcium oscillator. The main characteristics of calcium response patterns are reproduced in data-driven generative models (DMDc). Computer simulations of the mathematical model of calcium dynamics on realistic spatial templates have been implemented as well. Mechanism-based modeling allows to simulate basic of spontaneous and noradrenaline-driven calcium responses in astrocytes.

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### S9.694. Study of EEG Spectral Correlation Characteristics in Post-COVID-19 Patients

Kulbaeva M.S.[1]*, Mustfin M.[1], Mustafina G.[1], Shvetsova Y.V.[1]
[1]*Al-Farabi Kazakh National University ;*
[2]*Al-Farabi Kazakh National University ;*
* Elenna4444@mail.ru

Coronavirus infection (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus.

Most people with COVID-19 have mild or moderate symptoms and recover without specific treatment. However, some people have a severe disease and require medical attention.

Neurological complications have become an important cause of morbidity and mortality in the context of the ongoing COVID-19 pandemic. In addition to respiratory failure, many hospitalized patients experience neurological manifestations ranging from headache and loss of smell to confusion and disabling strokes. COVID-19 is expected to affect the nervous system in the long term

Loss of smell is known to be one of the earliest symptoms of covid infection. This was established on the basis of an increase in the magnetic resonance imaging signal in the olfactory cortex of the brain, which indicates an infection of the nervous system. The virus enters the central nervous system through the bloodstream, infecting endothelial cells. Second, the virus can enter the peripheral nervous system through retrograde neuronal pathways. The virus can be internalized in nerve endings by endocytosis, transported retrogradely, and spread transsynaptically to other areas of the brain.

Research hypotheses:

1. The power of the alpha rhythm, the main EEG rhythm in a state of calm wakefulness with eyes closed, in people who have had COVID-19 is reduced compared to people who did not have a history of COVID-19.
2. The ratio of the power of the EEG theta rhythm to the power of the EEG alpha rhythm in the background EEG is increased in people who have had COVID-19 compared to people who did not have a history of COVID-19.
3. In a state of calm wakefulness with eyes closed, the EEG correlation between different areas of the cerebral cortex is lower in people who have had COVID-19 compared to people who did not have a history of COVID-19.

Methods: recording of an electroencephalogram of the brain on the device "Neuronspectr-4", comparative spectral-correlation analysis of the EEG during statistical data processing in the SPSS program.

Results: Signs of a decrease in the functional state of the brain in survivors of COVID-19 were revealed, which manifested itself in an increased content of the slow-wave theta rhythm in the EEG, reduced reactivity of the alpha rhythm, and a reduced level of functional interconnection between different areas of the brain in survivors of COVID-19 compared to those who did not. sick individuals.

Conclusions:

1. According to the results of spectral analysis, there were no changes in the alpha rhythm when the eyes were closed. Theta waves in the

closed eye were higher in people with covid compared to normal background. The reason for this is that people with covid are more likely to experience a decrease in mood, stressful situations.
2. On the basis of spectral analysis, the characteristics of the ratio of alpha and theta rhythms were determined. In patients with covid, alpha waves are weakly expressed when the eye is opened due to a low reaction to light. In relation to alpha to theta, both groups had relatively the same index.
3.According to the results based on the Correlation analysis, in people who had covid-19, the functional ratio of the cortical areas in the background was lower than in those who were not ill. This means that in people suffering from covid, the contact reaction of the areas of the cerebral cortex with each other is weakened. In terms of statistical accuracy, these data were relatively comparable.
4. Correlation and spectral analyzes have shown that in people who have had covid-19, the functional connection of the central nervous system is relatively lower than in healthy people. This means that COVID-19 infection can affect the nervous system through the cerebrospinal fluid or through a decrease in oxygen entering the CNS.

### S9.695. Study of endometrial tissue elastic properties in hyperplastic and neoplastic processes by compression OCT elastography

Loginova M.[1,2]*, Plekhanov A.[1], Gubarkova E.[1], Grechkanov G.[3], Avetisyan E.[3], Sovetsky A.[4], Zaitsev V.[4], Gamayunov S.[3], Gladkova N.[1], Sirotkina M.[1]
[1]*Privolzhsky Research Medical University;*
[2]*Lobachevsky University;*
[3]*Nizhny Novgorod Regional Oncologic Hospital;*
[4]*Institute of Applied Physics of the RAS;*
* pandaagron@ya.ru

Endometrial cancer is a malignant epithelial tumor of the uterus and is a common gynecological malignancy worldwide. The precursor of endometrial cancer is endometrial hyperplasia, which also requires treatment and regular monitoring. The most accurate method for diagnosing hyperplastic processes is a histological analysis of tissue, which involves curettage of uterine cavity. This procedure is traumatic for women and can adversely affect reproductive function. It is known that benign lesions of the uterus are represented by a soft structure due to hyperplasia of the glands and the content of a small amount of fibrous components. The onset of malignant transformation, on the contrary, will be associated with increased stiffness. Therefore, a new non-invasive method of optical coherence elastography (OCE) makes it possible to determine the morphological components and structural changes of tissue by elastic properties and can become a promising tool for monitoring hyperplastic processes in the uterus. The aim of the research was to reveal the elastic properties of normal endometrial tissue and endometrial tissue in hyperplastic and neoplastic processes. The research was carried out on ex vivo samples of normal endometrial tissue and endometrial tissue changed by hyperplastic and neoplastic processes. The elastic properties (stiffness) of the endometrial tissue were studied using the compression OCE method based on the visualization of deformations created in the tissue by pressing by the OCT probe and the assessment of the interframe variation of the signal phase gradient between adjacent B-scans. The use of a calibration silicone layer (layer stiffness 100 kPa) on the tissue surface made it possible to quantify its elastic properties (Young's modulus, kPa) with a level of detail of ~30-50 μm. For quantitative evaluation and comparative analysis of OCE-images of endometrial tissue, a standardized pressure range (4±1 kPa) was used. The obtained data were compared with the results of histological examination, which was carried out by staining with hematoxylin and eosin and picrofuchsin according to Van Gieson. The ability of OCE to visualize differences in normal endometrial tissue and endometrial tissue changed by hyperplastic and neoplastic processes was demonstrated. The elastographic images revealed that the normal postmenopausal endometrium has a homogeneous distribution of low stiffness values (~200-250 kPa), which correspond to a thin atrophic layer with small and rare glands. With endometrial hyperplasia on elastographic images, there was a decrease in the stiffness value (below 100 kPa) compared to normal endometrium, which indicates tissue growth and the predominance of glands over single connective tissue fibers. In endometrial cancer, a heterogeneous distribution of high stiffness values (above 500 kPa) from large glandular structures merging with connective tissue fibers is observed.
The obtained OCE data correlate well with histological images, which proves the promising use of this method in distinguishing between benign and malignant lesions of the endometrium. In the future, the result obtained can be used for real-time non-invasive assessment of the state of the endometrium in hyperplastic and neoplastic processes.
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### S9.696. Study of functional activity peculiarities of neuron-astrocytic brain networks under hypoxic state and blockade of connexins 43

Mishchenko T.A.[1]*, Yarkov R.S.[1], Saviuk M.O.[1], Perenkov A.D.[1], Krivonosov M.I.[1], Vedunova M.V.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
* saharnova87@mail.ru

Brain hypoxia continues to head the list of urgent global health problems that need to be resolved. The launching of hypoxia-induced pathological mechanisms is not only the cause of cell death and significant changes in brain functioning, but also one of the key factors predisposing the development of severe CNS pathologies, including ischemic stroke, neurodegenerative and oncological processes. Hypoxia has a detrimental effect on both neurons and glial cells, but the role of the latter in the brain's adaptation to oxygen deficiency has not been sufficiently investigated. Glial cells, in particular astrocytes, form bidirectional communications with neurons allowing the organization of plastic and tightly regulated networks. Astrocytic networks formed via gap junctions have signaling activity and take an active part in the regulation of exchange between the cytoplasm and the extracellular space. Gap junctions of astrocytes consist of six protein subunits, connexins (Cx), among which connexin 43 (Cx43) mainly provides the connection between astrocytes in the brain. Recent studies have shown that astrocytic gap junctions not only provide rapid intercellular exchange of ions and metabolites, which is crucial for the buffering of K+ ions and glutamate, Ca2+ wave propagation and synaptic plasticity but also are participants in the development of damage in hypoxia-ischemic states. The goal of this study is focused on the detailed characterization of astrocytic Cx43 influence on the functional reorganization of neuron-glial networks in the hypoxia modelling in vitro. Primary cortical cultures obtained from the brains of C57BL/6 mice at day 18 of gestation were used as an object of the research. Acute normobaric hypoxia was modeled on day 14 of cultures development in vitro by replacing conditioned culture medium with a medium with a low-oxygen content for 10 min. Selective Cx43 inhibitor - Gap19 (10 μM, Sigma-Aldrich, Germany) was added to the culture medium 20 min before hypoxia modelling. The expression levels of metabotropic glutamate receptors mGluR2 and mGluR5 in primary cortical culture cells were assessed by quantitative real-time PCR method (RT-qPCR) on days 1 and 3 after hypoxia simulation. In late post-hypoxic period, spontaneous calcium activity was recorded by calcium imaging technique and the network characteristics of primary cortical cultures were evaluated using original mathematical algorithms.
We showed that in normoxia, the blockade of astrocytic Cx43 caused an increased expression of mGluR2 и mGluR5 receptors expression

as well as long-term modulation of spontaneous calcium activity of primary cortical cultures, primarily characterized by the restructuring of the functional architectonics of neuron-glial networks via reduction the correlation level between cells in the network and the proportion of existing correlated cell-to-cell connections. Blockade of Cx43 in the modeled hypoxia had a pronounced neuroprotective effect. In the late post-hypoxic period under the background of elevated mGluR5 receptor expression, the values of mGluR2 expression were found to decrease to physiological level, suggesting the activation of the alternative molecular mechanisms of cellular adaptation to hypoxic damage. Moreover, Cx43 blockade maintained the main parameters of spontaneous calcium activity of primary cortical cultures such as the percentage of functionally active cells, duration and frequency of Ca2+ oscillations and the functional architectonics of the neuron-glial network with preserved profile of calcium oscillations and high correlation level of calcium signals between cells in the network. The results demonstrate the crucial importance of astrocytic networks in functional brain adaptation to hypoxic damage, which could be a promising target for developing a strategy for rational antihypoxic therapy.
Research was carried out in the frame of the scientific program of the National Center for Physics and Mathematics (project "Artificial intelligence and big data in technical, industrial, natural and social systems").

**S9.697. Study of functional architectonics restructuring of brain neural networks under hypoxic damage and blockade of astrocytic connexins 43 (Cx43)**

Vedunova M.V.[1]*, Mishchenko T.A.[1], Kalyakulina A.I.[1], Yarkov R.S.[1], Ivanchenko M.V.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
* mvedunova@yandex.ru

Investigation of pathogenetic aspects of brain hypoxia and peculiarities of the development of systemic responses and adaptation to oxygen deficiency remain of extreme relevance. Of particular interest is the study of the mechanisms of hypoxic damage at the level of neural networks which considers as the main functional units of the CNS responsible for the implementation of information processing, storage and transmission of information as well as for realization of higher cognitive functions, including memory, consciousness, emotional reactions. Such approach, using modern electrophysiological methods and mathematical algorithms of data analysis, allows the analysis of fine mechanisms of restructuring the functional architectonics of neural networks under hypoxic damage, as well as the contribution of each network element to the development of the functional network response to the stress. However, accumulating evidence suggested that physiological and pathological processes in the neural networks critically depend on the activity of glial cells, and particularly astrocytes. Following in the formation of their own networks, astrocytes perform not only homeostatic and trophic functions for neurons, but, more importantly, being in close bidirectional dynamic communication with neural networks, they can regulate their functional activity and, therefore, take an active participation in signal transmission affecting brain functions, including cognitive function and processing information.
The goal of this study was to assess the influence of astrocytic Cx43 on the spontaneous bioelectrical activity of primary hippocampal cultures in the hypoxic damage in vitro. Primary hippocampal cultures obtained from C57BL/6 mice embryos (day 18 of gestation) were served as a study object. The cells were cultured on multielectrode arrays MEA 60 (Multichannel Systems, Germany) at an initial density of 9000 cells/mm2. On day 14 of cultivation in vitro, the cultures were subjected to acute normobaric hypoxia by replacing normoxic culture medium with a medium with a low-oxygen content for 10 min. In order to uncouple interastrocyte communications, the selective Cx43 inhibitor Gap19 (10 μM, Sigma-Aldrich, Germany) was added to the culture medium 20 min before the hypoxia modelling. Electrophysiological signals from primary hippocampal cultures cultivated on multielectrode arrays were recorded using a MEA2100-2x60-System-E system (Multichannel Systems, Germany). The data obtained were then analyzed using an original software package of mathematical algorithms. In addition to the basic parameters of spontaneous bioelectrical activity (ex., the number of network busts and spikes in a burst), we investigated the specifics of reorganization of the internal functional structure of the neural network response by constructing correlation graphs and activation patterns, determined by the time of the appearance of the spike sequence within a network burst.
We showed that in normoxia, the blockade of astrocytic Cx43 resulted in modulation of spontaneous neural network activity of primary hippocampal cultures. Significant decrease in the number of bursts and spikes forming the network burst was detected 2 hours after addition of Gap19. In early (24 hours) and late (7 days) post-hypoxic periods all analyzed parameters of spontaneous bioelectrical activity in the "Gap19" group exceeded the values of the baseline (14 DIV); on the other hand, the number of network bursts was reduced relative to the values of the intact group. Analysis of the activation patterns and correlative interactions revealed that the development of primary cultures against the background of Cx43 blockade was similar to the intact group and characterized by the restructuring of the internal functional architectonics of the neural network mainly focused on the preservation of the existing and formation of new active groups of neurons. Blockade of Cx43 in hypoxia modelling contributed to the partial preservation of spontaneous bioelectrical activity of primary hippocampal cultures. In the remote post-hypoxic period (7 days), the values of the "Hypoxia+Gap19" group were comparable with the baseline activity (14 DIV) and significantly exceeded the values of the "Hypoxia" group. However, in the "Hypoxia+Gar19" group, the number of network bursts and spikes in a burst was decreased relative to the intact group on average by 1.6 and 2.2 times, respectively. Maintenance of the main parameters of spontaneous bioelectrical activity of primary hippocampal cultures contributed to partial preservation of functional architectonics of neural networks with subsequent positive restructuring in the remote post-hypoxic period. Against the background of the loss of some active cell groups due to hypoxic damage, new functional network elements involved in the formation of the network burst are observed to appear.
Overall, our findings demonstrate crucial role of interastrocyte communications in neuroprotective mechanisms involved in the functional adaptation of brain neural networks to hypoxic damage, a more detailed study of which could lay the basis for developing an effective antihypoxic strategy.
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**S9.698. Study of mass concentration of ions in urine at different stages of urolithiasis formation in an in vivo experiment on mice**

Gulina L.S.[1]*, Emanuel V.L.[3], Burdakov V.S.[2]
[1]*Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute»;*
[2]*ГБУЗ «Санкт-Петербургский клинический научно-практический центр специализированных видов медицинской помощи (онкологический)»;*
[3]*Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова;*
* c.lupus@mail.ru

Urolithiasis is one of the most common urological diseases affecting the entire urinary system as a whole. Urolithiasis is characterized by

the formation of stones in the organs of the urinary system that interfere with the release of urine and have a traumatic effect on the organs. Thus, it can be said that practical and high-quality modeling of urolithiasis in vivo will help for further studies of therapy and prevention of this disease.

One of the most common factors of crystallogenesis is glomular and tubulointerstitial damage to the kidneys. Ethylene glycol, sodium oxalate and 1-hydroxyproline are used as lithogenic agents in modeling. In our study, to simulate oxalate urolithiasis, we use intraperitoneal administration of sodium oxalate (Na2C2O4) once at a dosage of 7 mg per 100 g. body weight of the animal. The advantage of the chosen method is the rapidity of the formation of oxalate urolithiasis 2-4 hours after the administration of the drug. At the moment, the changes of mass concentration of ions in urine are not described, which does not allow to fully represent the processes occurring in the urine at different stages of urolithiasis formation. Capillary electrophoresis method was used to determine the ionic composition of urine and blood serum.

Rodents are most often used as a test system, although they are not prone to the formation of stones in the organs of the urinary system. However, the use of the most suitable dogs and pigs for this is considered impractical. In our study, a model of oxalate urolithiasis was performed on male outbrand ICR laboratory mice (CD-1).

For the study, two groups of animals were formed, each consisting of 12 male outbrand mature mice. Mice at the beginning of the experiment were clinically healthy and were kept under the same conditions in cages of 12 heads with a 12-hour day-night regimen at a temperature of 20-22ºC, a humidity of 60-70% and received a standard diet that did not differ from the usual one. The study was conducted in accordance with the rules for working with laboratory animals and in compliance with the rules of bioethics.

On the day of the start of the experiment, the animals were weighed, marked and divided into two groups, after urine was collected for data collection at the "0 hours" point. Then the first group, the control group (n=12), was injected intraperitoneally with 100 µl of sodium chloride 0.9% once, while the experimental group was injected with 100 µl of sodium oxalate (Na2C2O4) once at a dosage of 7 mg per 100 g. body weight of the animal resuspended in 0.9% NaCl solution. Urine collection for general clinical and biochemical analysis, studies of sediment microscopy, conductivity and ionic composition was carried out at the time of formation of the experimental groups, 4 hours after the start of the experiment and 24 hours after the start of the experiment. Taking samples of blood serum and materials for histological examination of the kidney was carried out at control points 4 and 24 hours. Mice were euthanized by decapitation.

On day 0, no crystals were observed in the urine sediment of the animals of the control and experimental groups, however, already 4 hours after the administration of the drugs, single crystals of calcium oxalate monohydrate and dihydrate were found in the urine of the animals of the experimental group. 24 hours after the administration of the drug in the urine of experimental animals, a decrease in the amount of oxalates is observed throughout the field with sediment microscopy. Crystals in the urine of animals of the control group were not detected during the experiment.

Damage to the renal glomeruli was confirmed by the results of morphological changes in the kidneys during histological examination (dilatation of the tubules, the presence of oxalate crystals in the tubules), biochemical examination of blood serum and urine (increased creatinine concentration), the study of the ionic composition of blood serum and urine, the study of urine conductivity, as well as the presence of crystals in the urine sediment.

In conclusion, the results obtained provide up-to-date information on the modeling of urolithiasis induced by intravenous administration of Na2C2O4 in an in vivo experiment and demonstrating changes in the ionic composition of urine and blood serum.

**S9.699. Study of synaptic input in parietal ganglia interneurons of defensive behavior of terrestrial snail**

Arslanov A.A.[1]*, Andrianov V.V.[1], Deryabina I.B.[1], Chihab A.[1], Silantyeva D.I.[1], Gainutdinov Kh.L.[1]
[1]*Kazan (Privolzhsky) Federal University;*
* arslanov-1999@mail.ru

The nature of learning and memory is based on the cellular mechanisms of synaptic and non-synaptic plasticity[1]. It is well established that both enhancements of individual synaptic connections [2] and changes in the endogenous properties of the neuron and its membrane [3] are what underpins the learning process. One of the most relevant issues of modern neurobiology is the plasticity mechanisms associated with changes in the state (excitability) of neurons involved in the convergence of sensory information and transmitting their signals further along the network [4,5,6]. This work aimed to study changes in the subthreshold background electrical activity of snail command neurons after associative learning. Recording the subthreshold background activity of silent neuronsallows us to infer the total electrical activity of incoming synapses. To that end, a method has to be developed that allows the qualitative and quantitative assessment of the background electrical activity during intracellular recording of a nerve cell and analysis of its changes during the formation of a conditioned reflex of aversion to a certain type of food in the snail.

Electrophysiological measurements were carried out using an improved technique for recording the transmembrane potential, which allows the detection ofexcitatory postsynaptic potentials (EPSPs) with an amplitude of 0.2 mV. EPSPs were determined visually by the characteristic shape of the change in the membrane potential. The recording technique was associated with achieving minimal noise when registering potentials and smoothing signals. To describe the observed changes in the background activity of neurons, the average amplitude and the number of EPSP were analyzed.

It was found that the development of a conditioned defensive reflex of food aversion in the terrestrial snails is associated with a significant increase in the number of low-amplitude single EPSPs in the giant interneurons of defensive behavior. In this case, an increase in the number of low-amplitude single EPSPs may indicate either an increase in the number of action potentials in the corresponding presynaptic neurons or an increase in the amplitude of previously unmeasurable EPSPs (the amplitude is below the threshold of 0.5 mV chosen by us). Unfortunately, despite the fact that giant interneurons of defensive behavior have rather wide sensory inputs, there is very little information in the literature about specific presynaptic sensory neurons [7]. Our analysis of the subthreshold background activity of silent interneurons of the defensive behavior of the terrestrial snail made it possible to find changes in the synaptic input associated with the development of a conditioned defensive reflex of food aversion.

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**S9.700. Study of the dose curve of survival of B16/F1 mouse melanoma cells after their irradiation with proton beams at the Bragg peak**

Romanov M.V.[1]*, Shemyakov A.E.[1,2], Popov A.L.[1]
[1]*ITEB RAS;*
[2]*PTC LPI;*
* rmvya@yandex.ru

Melanoma is one of the most difficult types of cancer to treat [1]. One of the methods to increase the effectiveness of therapy is the use of proton irradiation [2]. Unlike photons, which fade away exponentially and irradiate everything in their path, particles (protons and carbon ions) have a finite path length and emit most of their energy at the end of their path. Such an energy distribution along the path is described by the Bragg peak [3]. Due to this, it is possible to increase the delivered radiation doses and achieve their better localisation.

The effect of a 165 MeV proton beam in the dose range from 0 to 8 Gy in 1 Gy increments on the B16/F1 mouse melanoma cell line was evaluated to construct a survival curve. A dose-dependent effect of colony formation was revealed according to clonogenic analysis. When melanoma cells were irradiated at a dose of 1 Gy, the number of colonies relative to the control was 75%. Irradiation at a dose of 3 Gy of melanoma cells led to the formation of 13.8% of colonies, and with a further increase in the dose, the number of formed colonies was less than 5.5%. According to the Live/Dead test, it was found that proton irradiation does not cause significant death over the entire range of radiation doses studied (the ratio of dead cells to their total number did not exceed 13%). In addition, it was found that with an increase in the dose of radiation, a decrease in the membrane mitochondrial potential occurred, however, a statistically significant difference compared to the control was observed only in cells exposed to radiation from 5 Gy and above. In the future, it is planned to study the radiosensitizing properties of the Ce0.9Gd0.1O2-x nanocomposite on the B16/F1 cell line, and the above results will be used to evaluate its effectiveness upon irradiation with a proton beam.

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**S9.701. Study of the effect of clobetasol on the conduction of excitation, changes in the content of proteins and nerve growth factor in damaged nerve conductors**

Kuzmenko T.P.[1]*, Revin V.V.[1], Parchaykina M.V.[1], Revina E.S.[1], Kachanova K.V.[1], Krygina M.A.[1]
[1]*Faculty of Biotechnology and Biology National Research Mordovia State University, Saransk, Russia;*
* zyuzina-tatjana@mail.ru

Currently, one of the most important problems in the field of modern regenerative medicine is the problem of restoring the functioning of damaged somatic nerves. Pathologies of the peripheral nervous system make up a significant proportion in the structure of morbidity in the adult population, since the degree of restoration of nerve function remains low, and the treatment time is long. In recent years, the most relevant methods for the effective flow of regeneration processes are the imposition of conduits [1], exogenous administration of physiologically active substances [2], electrical stimulation [3], and their combination [4]. Nevertheless, despite numerous studies, the search for the most optimal ways to stimulate regenerative processes, as well as the study of factors that minimize degeneration processes in the nerve conductor after its damage, is still underway. In this regard, of particular interest are glucocorticoids, in particular, clobetasol, one of the side effects of which is the ability to stimulate regeneration processes in damaged nervous tissue [5]. Recently, more and more attention has been paid to nerve growth factors (NGFs), which regulate the survival of sympathetic and sensory neurons in the peripheral nervous system [6]. However, the role of clobetasol in triggering signaling pathways involving NGF, which are responsible for the survival and restoration of the physiological functions of the nerve fiber, remains insufficiently studied.

The object of the study was the sciatic nerves of Wistar rats. In animals of the 1st experimental group, the sciatic nerve was transected, in the 2nd group, after transection of the sciatic nerve, clobetasol was intramuscularly injected daily at a concentration of 0.5 mg/kg, the 3rd group served as a control. Bioelectrical activity was recorded for an isolated nerve during its extracellular retraction with the following stimulation parameters: amplitude 1.5 V, duration 0.3 ms, stimulation frequency 100 pulses/s. The total protein content was quantified by the Lowry method [7]. Quantification of NGF molecules was performed by ELISA using special commercial kits (Cloud-Clone Corp., China).

After an injury to the nerve conductor, degeneration of nerve fibers is observed over a small extent of the proximal and throughout its distal segment. Such processes are associated with a violation of the central innervation, due to which a clear regulation and control over the functional state of the somatic nerves is carried out.

Mechanical trauma to the sciatic nerve of a rat caused by its cutting leads to a decrease in the amplitude of the action potential by the 7th day of the experiment and a partial restoration of conduction by the 14th day after the injury. The introduction of clobetasol at a concentration of 0.5 mg/kg is accompanied by a significant increase in the amplitude of the action potential by the 14th day of observation compared with damage.

The data obtained are consistent with changes in the protein composition of the nerve after injury. Thus, in the first seven days after injury in the process of degeneration, there is an increased breakdown of the protein fraction of the nerve conductor, in particular, in the proximal section, the content of proteins decreases by an average of 0.3 times, and in the distal by 2.0 times compared with the control. However, with an increase in the experiment to 30 days, as a result of the activation of regenerative processes in the proximal part of the nerve, the synthesis of proteins necessary for restoring its structure increases, the level of which practically reaches the control values.

The introduction of clobetasol at a concentration of 0.5 mg/kg leads to a decrease in the rate of protein breakdown in the proximal nerve during its degeneration. Most likely, due to the mechanism of action of clobetasol through glucocorticoid receptors [5], there is an increase in the synthesis of structural proteins of the nerve, namely, neurofilaments H, M, L and tubulin.

In addition, recent studies have shown that clobetasol activates the expression of NT-3 and BDNF in oligodendrocytes, thereby promoting their differentiation and proliferation [8]. Based on this, at the next stage, the change in the content of nerve growth factor was studied in case of damage to somatic nerves and against the background of drug administration. It has been shown that the use of clobetasol in peripheral nerve injury is accompanied by an increase in NGF expression.

Thus, on the 30th day of observation, its level increased by an average of 1.7 times in the proximal part of the nerve and 3.8 times in its distal segment compared with the control.
Thus, clobetasol at a concentration of 0.5 mg/kg contributes to the restoration of the conduction of the action potential in the damaged nerve conductor and a less pronounced breakdown of the protein fraction of nerve fibers. Enhanced synthesis of nerve growth factor against the background of clobetasol administration indicates its participation in the launch of regenerative signaling pathways responsible for restoring the structural integrity and functional activity of nerve fibers after injury.
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**S9.702. Study of the effect of high-energy proton irradiation in high power mode on mice in vivo**

Shemyakov A.[1,2]*, Dyukina A.[2], Zaichkina S.[2], Agapov A.[3], Mitsyn G.[3], Shipulin K.[3]
[1]*LPI Physico-technical Centre;*
[2]*Institute of Theoretical and Experimental Biophysics;*
[3]*Joint Institute for Nuclear Research;*
* alshemyakov@yandex.ru

Proton beam therapy becomes more accessible every year for use in many countries of the world. With the development of this technology, scientists and engineers are working in search of the most optimal conditions to improve the effectiveness of this type of radiation therapy. One of the directions is the study of dose delivery in high power mode, (more than 50 Gy/sec). Exposure of a biological object to high-intensity proton beams for an ultrashort period of time (up to 100 ms) and subsequent biochemical processes cause the so-called FLASH effect. A number of studies have shown that increasing the dose delivery rate can reduce toxicity to healthy tissues, because tumor cells are more sensitive to this type of radiation. The study of the effect of the dose delivery rate was previously carried out both on photon beams and on electrons, however, due to their greater mass and damaging ability, protons are again becoming an object of study for doctors and biologists. The nature of this effect has not yet been studied in detail and its investigation is of great importance for the development of clinical radiotherapy in the future. Currently, flash irradiation has begun to be tested in the treatment of osteosarcomas on the first patients. However, there is still no understanding of the exact mechanism of the selective increase in cell death of tumor cells. On the contrary, a number of researchers have shown no effect of increasing cell death in comparison with standard radiation intensity.
In this work, we investigated the effect of the proton beam of the Phasotron of the Laboratory of Nuclear Problems of the JINR (Joint Institute for Nuclear Research) on mice in vivo. The irradiation of animals was carried out on a special stand for radiation research with proton beams with an energy of 600 MeV. The dose rate for irradiation in the FLASH mode was 80 Gy/s; for the control group, the dose rate was reduced to the standard used in radiation therapy - 2 Gy/min. The object of the study were two-month-old male SHK mice. Irradiation was carried out by one animal for the whole body at a dose of 1 to 8.5 Gy. We have studied the yield of micronuclei in erythrocytes of the bone marrow, the mass index of the thymus and spleen, as well as the average life expectancy depending on the power of the applied radiation dose.

**S9.703. Study of the effect of plasma treatment on the morphology of biomedical polylactide matrices and neuronal cell adhesion**

Mikhutkin A.A.[1]*, Azieva A.M.[1], Yastremsky E.V.[2], Patsaev T.D.[1], Kirillova D.A.[1], Sharikov R.V.[1], Sharikova N.A.[1], Antipova K.G.[1], Grigoriev T.E.[1], Vasiliev A.L.[1,2]
[1]*National Research Center "Kurchatov Institute";*
[2]*Shubnikov Institute of Crystallography of FSRC "Crystallography and Photonics" RAS;*
* Alex.Mikhutkin@gmail.com

Extracellular matrices adhesive properties mainly depend on chemical and structural features of their surface and play an important role in tissue engineering. The matrix surface morphology and structure are crucial for the cell adhesion and proliferation. Thus, development of artificial extracellular matrices from biopolymer materials with optimal characteristics for the cell adhesion processes is of great importance for regenerative medicine.
Primary neurons are used not only for the basic research, but also for the treatment of neurological pathologies and injuries, while nerve tissue regeneration remains a major challenge for medicine. Implantation, adhesion and growth of neurons requires appropriate matrices. Currently, one of the most promising synthetic polymers for implants is polylactide (PLA), which has good biocompatibility even despite the hydrophobic nature of the compound. Various types of biopolymer materials - films, sponges and nonwoven fibrous materials - can be used as artificial matrices. It was proposed the treatment of the samples with low-energy plasma in order to optimize the matrices surface microstructure and consequently to improve the surface hydrophilicity and interaction with cells.
In the present work we studied the effect of the plasma treatment of polylactide matrices of different types - films, sponges and nonwoven fibrous materials - on their morphology, surface hydrophilicity and adhesion of newborn mouse neuronal cells. Samples were subjected to air plasma treatment (treatment duration of 30 and 60 minutes) and were compared with control (raw) samples. The matrices morphological properties were determined by scanning electron microscopy (SEM). SEM images obtained during the study allowed us to see the evolution of polymer matrix morphology caused by plasma treatment (surface relief formation). For numerical evaluation of these changes three-dimensional surface reconstruction method using two SEM images (stereo pair) based on the stereophotogrammetric approach [1] was applied. The obtained three-dimensional models of the specimen surfaces allowed us to calculate a number of quantitative parameters characterizing the change in the relief (roughness), including the real surface area, during plasma modification, in comparison to the control specimens [2]. To confirm the results, the specific surface area was also measured by the BET method (Brunauer, Emmet and Teller method [3]). In addition, the surface wettability after plasma treatment was investigated by environmental SEM. The matrices general three-dimensional morphology was studied by confocal laser scanning microscopy [4,5].
We present the study of mouse primary neuronal cells adhesion on PLA matrices of various types, namely, oriented and non-oriented nonwoven fibrous and sponge matrices before and after plasma treatment. Fluorescence and environmental scanning electron microscopy were used to study the adhesion of dissociated neuronal cells on isotropic and anisotropic nonwoven fibrous and sponge PLA matrices.

As a result, it was found that plasma treatment leads to the formation of surfaces relief on all types of matrices, significantly increasing the roughness, as well as improving their hydrophilicity. The surface modification parameters can be controlled by changing the plasma exposure time. The neurons obtained from the brain of newborn mice showed improved adhesion on matrices of all types after plasma treatment, with the most pronounced effect observed on non-oriented matrices. Thus, we have shown that changing the surface morphology and hydrophilicity of the polymer makes it possible to enhance neuronal cell adhesion.

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### S9.704. Study of the effectiveness of combination therapy using micrometer carriers for the treatment of lung cancer

Rogova A.S.[1,2,3]*, Matiushevskaia A.O.[4], Timin A.S.[1,2]
[1]*Peter the Great St.Petersburg Polytechnic University ;*
[2]*ITMO UNIVERSITY;*
[3]*St. Petersburg State Chemical and Pharmaceutical Academy;*
[4]*St. Petersburg National Research Academic University Named After Z.I. Alferov of the Russian Academy of Sciences;*
* anna.aroo@mail.ru

Introduction: One of the most dangerous and severe types of cancer is lung cancer. World statistics show that among malignant tumors, it annually occupies a leading place in the number of deaths. The disease is difficult to treat, and existing therapies: chemotherapy, immunotherapy and radiation therapy - inevitably affect not only the affected, but also healthy tissues. That is why it is important to find an effective therapeutic method that combines the advantages of existing ones, and, in addition, would reduce the impact on healthy organs. The most effective methods of lung cancer therapy are chemotherapy and radiotherapy. Thus, the purpose of this study is to study the effectiveness of the combination of these two methods.
Materials and methods: BALB/C mice were injected intravenously with CT26 cells at a concentration of 10^5 cells/100 μl to produce lung metastases. On day 7 after tumor inoculation, mice were divided into four groups: (i) PBS (0.2 ml, intravenous injection), (ii) Particles with 177Lu (4 MBq, 0.2 ml, intravenous injection), (iii) Cisplatin (4 mg/kg intraperitoneal injection), (iv) Particles with 177Lu (4 MBq, 0.2 ml, intravenous injection) + Cisplatin (4 mg/kg intraperitoneal injection). After 21 days of therapy, mice were sacrificed and lung tissues and major organs were collected for histological studies. A visual count of metastatic nodes in the lungs was also performed. In addition, a survival assessment test was conducted.
Results: According to the results of histological studies and visual examination of lung tissues of mice with CT26 tumors, a significant positive effect of combination therapy in comparison with monotherapy was revealed. Mice treated with a combination of 177Lu and Cisplatin particles showed a significant reduction in metastatic nodules, as well as longer survival (80%, day 27) compared to mice that received monotherapy (50-60%, day 27) or control animals (0%, day 27).
Conclusions: Thus, micro-sized particles with 177Lu have demonstrated their effectiveness in the combined use of radio and chemotherapy for the treatment of lung metastases.
The work was carried out with the support of the state task (FSEG-2022-0012).

### S9.705. Study of the physicochemical properties of breast milk in a patient on hemodialysis therapy

Filat'yeva A.E.[1]*, Kondakova E.V.[1], Lobanova N.A.[1], Nagaev E.I.[2], Sarimov R.M.[2], Gudkov S.V.[2], Vedunova M.V.[1]
[1]*National Research Lobachevsky State University of Nizhny Novgorod;*
[2]*Prokhorov General Physics Institute of the Russian Academy of Sciences;*
* filatjevaanastasia@yandex.ru

Currently, according to the achievements of modern medicine and the proper management of patients with end-stage renal disease (ESRD) who are on renal replacement therapy (RRT), the likelihood of a successful pregnancy and the birth of a healthy child is increasing. In this regard, the issue of recommendations for breastfeeding in this group of patients is relevant.
The material for the study was milk samples from a patient with ESRD receiving RRT by the method of program hemodialysis, whose conscious decision was breastfeeding. Samples were collected before the hemodialysis procedure, as well as immediately after and 4,7,13 hours after its completion. The control was the milk of healthy women with a similar period of feeding. The content of creatinine, urea, total protein, albumin and glucose was assessed using an automatic biochemical analyzer DIRUI CS-T240. The method of multi-angle dynamic light scattering (MADLS) was used to determine the size of milk particles, reflecting the diffusion properties of its constituent molecules. The fluorescence spectroscopy was used to determine the protein concentration and the number of aggregates.
As a result, a significant increase in the content of creatinine and urea was shown both before and after RRT compared with control samples of milk from healthy women. At the same time, the lowest content of uremic toxins was observed 4 hours after the completion of the procedure. Based on the data on the MADLS and fluorescence spectroscopy, it can be concluded that there are low molecular weight compounds in the milk samples of a patient with ESRD, which may be associated with the presence of toxic substances, as well as a low protein content compared to control samples. The results obtained call into question the applicability of breastfeeding in this group of patients.
The work was supported by the federal program of academic leadership «Priorities 2030» (H-470-99).

### S9.706. Study of the spectral properties of donor red blood cells modified by the drug "Mexiprim"

Korvyakova P.V.[1]*, Sokolova L.O.[1], Putintseva O.V.[1]
[1]*Voronezh State University;*
* lyudmila.sokolova.94@mail.ru

Mexiprim is an original domestic drug produced by NIZHPHARM (Nizhny Novgorod, Russia). The main active ingredient is ethylmethylhydroxypyridine succinate (EMHPS) belonging to 3-oxypyridine class (3-OP), and the auxiliary element is sodium bisulfite[1].The drug has a wide range of biological action.It is used to eliminate antihypoxic, anticonvulsant and stress-protective effects. EMGPS accelerates metabolic processes and blood supply to the brain, decreases blood coagulation

cell aggregation and stabilizes erythrocyte membranes.However, we previously found that the effect of Mexiprim drug on human erythrocyte suspensions contributed to changes in their cytoarchitectonics and reduced the number of biconvex discocytes[2]. One of the highly sensitive methods to study the structural state of erythrocytes to understand the biophysical basis of interaction of blood cells with modifiers is spectrophotometry in the UV- and visible regions. In view of the above, the aim of this work was to study the effect of the drug Mexiprim on the spectral properties of erythrocyte suspension of donor blood over different time periods.

Erythrocytes were obtained from donor blood on the day of sampling according to the method [3]. The drug Mexiprim (JSC NIZHPHARM, Nizhny Novgorod, Russia) with 3.18•10-2mol/L concentration was added to 9 ml of erythrocyte suspension with 5 - 106 μl/ml concentration and incubated in TS-1/80 SPU dry-air thermostat (Russia) at +37 °C for 1 and 24 h. Electronic absorption spectra (EAP) of erythrocytes before and after exposure to Mexiprim were recorded by UV-2401 PC (Shimadzu, Japan) in wavelength range from 230 to 700 nm at 1 nm with spectral slit width of 1 nm.

The EAP of native donor erythrocytes were characterized by two peaks in the UV-region (273-275 and 341-347nm) and three absorption bands in the visible part of the spectrum (418, 543 and 578nm). After incubation of erythrocytes at 370°C for 1 h we didn't observe shifts of absorption maxima and statistically reliable difference of samples optical density in comparison with control. 24 h the modification of erythrocytes with Mexiprim induced distinct changes both in UV- and visible regions of ESP. Thus, values of erythrocyte optical density in all ESP maxima markedly decrease, indicating a decrease in light scattering of analyzed samples in the presence of the modifier. Moreover, the maximum at 341-347 nm was shifted to 329-334 nm due to changes in the contact area of heme and globin protein components. Simultaneously, there was a decrease in the optical density in the Sore band from 0.816±0.046 to 0.675±0.034 and its shift to 409 nm, caused by accumulation of oxidized forms of heme protein - methemoglobin in samples.

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### S9.707. Study of urine colloid fractional composition in experimental animals at different stages of nephrolithiasis modeling in vivo

Verlov N.A.[1]*, Gulina L.S.[1], Burdakov V.S.[1], Landa S.B.[1], Bogdanov A.A.[2], Emanuel V.L.[3]

[1]*NRC «Kurchatov Institute» - PNPI;*

[2]*Saint-Petersburg clinical scientific and practical center for specialized types of medical care (Oncology);*

[3]*Pavlov First Saint Petersburg State Medical University;*

* verlov_na@pnpi.nrcki.ru

Many species of animals, as well as humans, greater or lesser extent, are prone to formation of kidney and urinary tract stones. Despite the fact that laboratory rodents are not normally predisposed to urolithiasis, several models based on use of lithogenic agents have been developed and successfully applied in research practice. At present time it is considered that most suitable animals for investigation of process kidney stone formation are rats, mice, dogs and pigs. Ethylene glycol is most widely used as lithogenic agents, but recently one can often find works in which oxalate urolithiasis was modeled by intraperitoneal administration of sodium oxalate (NaOx) solution. In our study, we studied the fractional composition of urine colloid in the context of its biophysical properties, such as ionic composition and pH, and biochemical composition. Two groups of male outbred ICR laboratory mice (CD-1) of 12 animals in each group participated in the experiment. Animals in control group received intraperitoneal injection of 100 μl physiological solution once, animals in experimental group received 100 μl of NaOx solution once in a dosage of 7mg per 100 grams of animal weight. Urine samples were taken at control points at 4 h and 24 h. Analysis of fractional composition at 4-hour point showed an increase in concentration of particles in urine colloid of animals in experimental group (7.56±0.57E8 particles per ml, 3.51±0.24E8 particles per ml in control group). Twenty-four hours after beginning of experiment, concentration of particles in urine of animals in experimental group decreased to a level of 2.47±0.27E8 particles per ml, concentration in animals in control group remained at same level of 3.62±0.25E8 particles per ml. In all cases, the mode of distribution was in region of 100-110 nm, which corresponds to peak of the oligomeric form of 7-MDa uromodulin protein. Uromodulin is a unique renal glycoprotein, synthesized in thick ascending segment of the Genle loop, and constitutes up to 60% of protein in urine. Uromodulin tends to form oligomers; most common oligomers in urine have a mass of 7 MDa and 28 MDa. Microscopy analysis of sediment revealed oxalate crystals at 4 hr in urine of experimental group mice. Also, sediment microscopy revealed that crystals were surrounded by protein filaments that stained well with bromophenol blue. General clinical urinalysis revealed a decrease in urine density (1.000 g/cm3 at 4 hours, 1.020 g/cm3 at 24 hours, control 1.030 g/cm3), an increase in protein content (>500 mg/dL at 4 hours, 100 mg/dL at 24 hours, 30 mg/dL in control) and an increase in pH (pH = 8 at 4 hours, at 24 hours and control pH = 5) in animals in experimental group. Histological analysis 24 hours after induction of pathology revealed following changes in kidney of experimental animals: multiple crystals in nephrons, localized mainly in thick ascending segment of Genle loop, multiple hemorrhages. On the basis of the obtained data, it can be concluded that during first stage of nephrolithiasis development up to 4 hours, against background of crystallogenesis processes in secondary urine formation, uromodulin protein plays a major role in stabilization, which inhibits growth of crystals, preventing them from reaching 10 microns in size and more, when they overlap lumen of nephron tubule. In process of stabilization of urine colloid, in a constant stream of fluid, protein concentration gradually decreases, which leads to an increase in rate of crystallogenesis and eventually to blockage of the lumen of tubule of the nephron.

### S9.708. Study the mechanisms of the death of nigral dopaminergic neurons in parkinsonism

Kucheryanu V.G.[1]*, Bocharov E.V.[2], Voronina N.A.[1], Goloborshcheva V.V.[1], Bocharova O.A.[2]

[1]*Institute of General Pathology and Pathophysiology;*

[2]*National Medical Research Center of Oncology named after N.N. N.N. Blokhin of the Ministry of Health of Russia;*

* vkucheryanu@mail.ru

Parkinson's disease (PD) is a common neurodegenerative disease that often leads to the development of disability. PD develops as a result of the death of dopaminergic neurons in the substantia Nigra pars compacta (SN) of the brain, which leads to a sharp decrease dopamine level in striatum. The mechanisms of the neurons death at the PD are currently not completely disclosed. Parkinson's disease is modeled in

C57BL/6J mice, and in some cases, rats using neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is introduced systematically, it easily penetrates through the blood-brain barrier. In the glial cells of the brain using the monoaminoxidase B enzyme, MPTP is metabolized to the 1-methyl-4-phenylpyridin (MPP+). MPP+ causes the division of the process of oxidative phosphorylation in mitochondria due to the inhibition of complex I, which leads to the cessation of ATP synthesis, the deficiency of energy and the death of dopaminergic neurons in SN.

The state of oxidative stress (OS) striatum was studied on a PD model in rats caused by the intraperitoneal administration of the MPTP, intracaudate or nigral introduction of MPP+. The severity of parkinsonic symptoms: oligokinesia, rigidity and tremor correlated with the level of malondialdehyde (MDA) in striatum. With the development of a parkinsonian simptomes, caused by the introduction of MPP+ in SN, a reliable and maximum increase in the level of MDA in striatum was observed [1]. In the blood of patients with PD, an increase in the level of MDA was found and a decrease in the activity of enzymes of the antioxidant system: superoxiddismutase, catalase and glutathione-S-transferase [2]. The results indicate the participation of OS in the mechanisms of the death of nigrostriatal neurons in PD.

MPTP injection in rats for 6 days led to the development of oligokinesia and rigidity. The infusion of glutamate to SN increased the development of MPTP -induced parkinsonic symptoms - oligokinesia and rigidity in old rats. Glutamate exacerbated rigidity in animals with MPTP-induced parkinsonism. These data reveal the ability of glutamate to potentiate PS. Pre-parallel with MPTP, the introduction of antagonist of glutamate NMDA receptors of Memantin reduced the animals oligokinesia and rigidity. The results indicate an important role of glutamate in the mechanisms of degeneration and the death of nigral neurons and the development of parkinsonism and the prospect of using antagonists of NMDA receptors for the correction of PD [3].

It was found that patients with post mortem PD in emergencies decrease the level of basic fibroblast growth factor (FGF-2) to 4 %. Intranasal administration of the FGF-2 in a dose of 3μg per mouse 3 times before and 3 and 5 days from the beginning of the injection of MPTP reduced the development of oligokinesia, muscle rigidity in animals. The intranasal infusion of the acidic fibroblast growth factor (FGF-1) reduced the severity of oligokinesia, rigidity and tremor, and also prevented a sharp decrease in the level of dopamine and its metabolites: 3,4-dihydroxyphenylacetic acid and homovanillic acid in the striatum. At the same time, a decrease in the number of damaged nigral neurons to $22.1 \pm 4.5\%$ ($p < 0.01$) in the case of the introduction of the FGF-2 and up to $27.6 \pm 4.1\%$ ($p < 0.01$) when using the FGF-1. Thus, FGF can weaken or delay the degenerative process, prevent death or to restore reversibly damaged neurons in the SN in PD.

One of the main mechanisms of the death of SN DA neurons in PD is neuroinflammation as a result of activation of microglia, which leads to increased release of pro-inflammatory cytokines in the brain, which activate the enzyme cyclooxygenase-2 and the transcriptional factor of NF-κB, which causes the nucleus apoptosis. When modeling the early clinical phase of PD in mice caused by the injection of MPTP (12 mg/kg, 4 times), an increase in the level of pro-inflammatory cytokines IL-1β, IL-6, interferon-gamma, tumor necrosis factor-alpha (TNF-alpha) in the nigrocaudate complex compared with control [4]. In the blood of patients with PD, an increased content of IL-6 and TNF-alpha was found [2]. Prior administration of the derivative of adamantan, hemantane (20 mg/kg) or the inclusion in the complex therapy of the PD multiphytoadaptogen, which has neuroprotective properties, led to a decrease in the level of studied pro-inflammatory cytokines [4;5]. Clarification of the mechanisms of the death of nigrostriatal dopaminergic neurons in parkinsonism can serve as the basis for finding effective neuroprotective agents that can stop or reduce the degree of neurodegeneration and progression of Parkinson's disease.

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**S9.709. Sublingual glycine impact on cerebrovascular reactivity**

Mashkovtseva E.V.[1,2]*, Rudnikova N.A.[3], Kopylova V.S.[1], Nartsissov Y.R.[1,4]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Moscow, Russia;*
[2]*Pirogov Russian National Research Medical University, Moscow, Russia;*
[3]*Postgraduate Education Academy of FGBU FNKC FMBA, Moscow, Russia;*
[4]*Biomedical Research Group, BiDiPharma GmbH, Siek, Germany;*
* mashkovtseva@icmph.ru

Cerebrovascular reactivity (CVR) – resistive vessels ability to dilate and constrict in reaction to dynamic functioning conditions to optimize the cerebral blood flow, maintain the pH levels and delivery of oxygen and nutrients to the brain. CVR is an integrative indicator of blood flow system adaptation so its evaluation helps to look at increased risk of cardiovascular problems, cognitive decline and to predict unfavorable outcomes of brain injury or ischemic stroke. Cerebrovascular reactivity measurement dynamically in healthy people in different conditions or after the use of various chemical compounds is promising.

1 g of glycine amino acid taken sublingually is well-known to effect on acute brain ischemia, long-term application regulates autonomic nervous system and corrects vascular tonus. Direct glycine application on brain arterioles leading to their dilation was experimentally shown. Meanwhile its direct impact on CVR adjustment is still underexplored.

Transcranial Doppler of middle cerebral arteries and duplex ultrasound study of extra cranial brachiocephalic vessels of 30 healthy volunteers was performed using US scanner Mindray DC-80. Vascular permeability, artery diameter, alterations, perivascular tissues, linear blood flow velocity, resistance index (RI) and pulsation index (PI) were evaluated. Cerebrovascular reactivity was discovered along with 1 g glycine/placebo sublingual intake and after hypercapnia functional test (voluntary breath holding) before and after the application.

Linear blood flow velocities and indices before and after 30 days of glycine intake differed significantly in the study group and between study and control group. Most valuable changes occurred in volunteers with left/right hemisphere asymmetry that can indicate decrease of hypercompensation requirements after blood flow normalization. Peak flow velocities in hypercapnia functional test increased more significantly after 1g glycine intake reflecting its direct impact on CVR.

### S9.710. Synthesis and application of calcium carbonate nanoparticles for combined therapy of oncological diseases

Yakubova A.A.[1,2]*, Darwish A.[1], Mitusova K.A.[1], Ahmetova D.R.[1], Timin A.S.[1]
[1]*Peter the Great St.Petersburg Polytechnic University;*
[2]*Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences;*
* yakubova.nastya@bk.ru

Optimization of existing treatment protocols, as well as the use of a personalized approach to prescribing therapy to patients with oncology are among the priorities in healthcare. The development of new forms of targeted delivery of chemotherapy drugs and photosensitizers for combination therapy will significantly increase the effectiveness of treatment and improve the quality of life of patients suffering from cancer.
Thus, in the field of cancer treatment, there is a need to develop innovative approaches to creating dosage forms to increase the effectiveness of therapy. Thus, the development of nanotechnology has made it possible to expand the possibilities of cancer therapy by influencing the body at the cellular and subcellular levels. The main agents in this field are nanoparticles that have a high potential in the field of diagnosis and therapy of a wide range of diseases due to the size due to which they can circulate through the body and the possibility of encapsulating various drugs or combinations thereof in them.
Thus, it is necessary to create nanocarriers for systemic administration that allow encapsulating and combining drugs, protecting them from external influences, and prolonged release of the drug from nanoparticles allows circulating nanosystems to accumulate in the foci of the disease and, consequently, achieve the necessary therapeutic dose at lower initial dosages. To do this, they must have a number of important properties: stability, non-toxicity, bioavailability. At the same time, the size of these particles should not exceed 100 nm in order to freely spread throughout the body and accumulate at the necessary points.
Thus, calcium carbonate particles stabilized by the organic agent polyacrylic acid were created, the size range of which is from 80 to 110 nm. Further, the stability of particles in three different media with different pH (4.1, 6.2, 10.1) was analyzed for 24 hours on DLS Malvern equipment. The shape and structure of the particles were studied using a scanning electron microscope (SEM) and a transmission electron microscope (TEM). The particles are stable over a wide pH range, changing their size slightly in a strongly acidic and strongly alkaline environment.
As drugs for combination therapy, the photodynamic drug "Radochlorin" was used, which, when irradiated with a laser, releases singlet oxygen that is harmful to cancer cells, as well as the cytostatic "Doxorubicin". The drugs were encapsulated by adsorption individually into nanoparticles, and the amount of the released therapeutic agent over time was studied. For this purpose, calibration graphs of the dependence of the optical density on the concentration of the medicinal substance in the linear range were constructed. Then the substances were encapsulated into nanoparticles by adsorption, after which, after a while, the particles were deposited and a superadding liquid was taken, which was analyzed for the amount of the substance. And so it was repeated for certain periods of time. Thus, it was possible to study the time of complete release of doxorubicin and radachlorin from calcium carbonate nanoparticles, as well as to calculate the adsorption capacity of the particles, which was 16.7% according to the formula:
((total drug added(mg)- unbound drug above precipitate(mg))*100)/(mass of particles (mg)+ total drug (mg) - unbound drug(mg))
At the same time, the encapsulation efficiency of the drug, calculated according to the following formula, was more than 90%.
(total drug added(mg)- amount of unbound drug above pellet(mg))/(total drug added(mg))
Thus, the information obtained allows you to plan further therapy: the amount needed for encapsulation to create a therapeutic dose and to calculate the time for which the concentration of drugs in the body will be maximum.
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### S9.711. Temporal patterns of spontaneous activity of auditory cortex neurons in lightly anesthetized and awake mice

Khorunzhii G.D.[1]*, Egorova M.A.[1]
[1]*I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian academy of sciences;*
* khorunzhii.gd@gmail.com

The high spontaneous activity is a key feature of neurons located in mammalian auditory cortical area, by which the cortical and brainstem levels of auditory system are distinguished (Egorova, 2005; Luczak et al., 2009; Sakata, Harris, 2012). The ordered temporal structure of spontaneous activity of primary auditory cortex single neurons was shown as well as the role of neuronal spontaneous firing for providing of functional template of excitation-inhibition synchronization in cortical-thalamic neural networks was suggested by the studies carried on the anesthetized animals (Egorova, 2005; Molnar et al., 2020). In addition, the probability assessment of forming of burst temporal patterns of auditory cortical neurons spontaneous activity, which we performed in anesthetized mice, showed that these patterns occurrence differs from random one (Khorunzhii, Egorova, 2022). However, the affection of auditory cortex single neurons spontaneous firing to the sound analysis processes remains poorly understood. The properties of temporal structure of auditory cortex neurons spontaneous activity in awake animals are also insufficiently studied. Thus, the present study is aimed to both the assessment and comparison of temporal patterns of spontaneous spikes recorded from single auditory cortical neurons in anesthetized and awake mice.
The spontaneous discharges were extracellulary recorded from single neurons located in the primary auditory field (AI) and anterior auditory field (AAF) of auditory cortex both in anesthetized and awake mice. All neurons both in anesthetized and awake animals demonstrated high and temporally ordered spontaneous activity.
The orderliness of neuronal spontaneous activity in anesthetized mice occurred as a grouping of its spontaneous spikes into bursts, which in different units included 4-9 spikes. The number of spikes in one burst varied not only between different units but also between different bursts in the same neuron (by 2 – 3 spikes). The analysis of the variance of mean spike number in bursts, undertaken for each neuron, showed that the variance values were about one spike in 49% of studied neurons and about two spikes – in 68% of neurons. Bursts of spontaneous spikes, as a rule, were grouped into longer temporal patterns up to several seconds long, consisting of 9-10 bursts. We found these patterns in spontaneous spiking of 70% of cortical neurons and preliminary called them “hyper bursts”. In the studied neurons, from 40 to 100% of spontaneous spikes were combined into bursts, and 90–95% of bursts were combined into “hyper bursts”. Thus, the actual probability of formation of both burst

and “hyper burst” ordered temporal structure of spontaneous activity of auditory cortex neurons in anesthetized mice was close to 1.0 value, which was dramatically higher than the probabilities of independent and random occurrence of the same number of events at time intervals corresponding to the recording period. The neurons in the AI and AAF fields did not differ significantly in the properties of the temporal patterns of their spontaneous activity ($p>0.5$).

An analysis of the spontaneous activity temporal structure of neurons in the primary auditory cortex of awake mice revealed a similar picture: individual spikes were combined into bursts containing 2–27 spikes in different neurons. In 47% of the studied neurons, the variance of mean spike number in bursts did not exceed 2 spikes. The values of the interspike intervals in bursts for different neurons were in the range of 6.5–41 ms. Bursts in different neurons included from 73 to 100% of spontaneous spikes, i.e., the probability of grouping of spontaneous spikes into bursts reached 0.7–1 for studied neurons.

Comparison of the properties of spontaneous firing of auditory cortical neurons in awake and anesthetized mice did not reveal significant differences in such characteristics as the percent of spontaneous spikes in bursts, the average duration of an individual burst, and the value of the interspike interval within a burst ($p>0.5$). The average frequency of firing in bursts of spontaneous spikes also did not differ significantly between the neurons of awake and anesthetized mice, but we noted a trend towards a higher frequency of spontaneous firing of cortical neurons in awake animals ($p=0.08$, Mann-Whitney test). Thus, the obtained data suggest that the ordered temporal structure of the spontaneous activity of neurons in the auditory cortex of the anesthetized mouse is highly stable and changes relatively little upon awakening of the animal.

The discovered strict orderliness of the temporal structure of the spontaneous activity of neurons in the mouse auditory cortex made reasonable the further analysis of its fractal properties. To evaluate them, we used indicators that describe both the variability and periodicity of a system consisting of a certain number of spontaneous spikes occurring at a given time interval - the so-called Fano and Allan factors. The obtained results indicate that the temporal structure of the spontaneous firing of auditory cortex neurons of both anesthetized and awake mice seems to demonstrate the signs of a periodic process. However, this aspect requires further detailed research.

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## S9.712. The anti-inflammatory effect of the polysaccharide from Helianthus tuberosus L

Generalov E.A.[1,2]*, Pyatigorskaya N.V.[2]
[1]*Lomonosov Moscow State University;*
[2]*I.M. Sechenov First Moscow State Medical University (Sechenov University);*
* generals1179@gmail.com

The anti-inflammatory effect of the polysaccharide from Helianthus tuberosus L.

Natural polysaccharides have high biological activity, coupled with the absence of toxicity, allergenicity and pyrogenicity. Due to this, carbohydrates are a very important object of research from a practical point of view. The polysaccharide from Helianthus tuberosus L. has a more prominent anti-inflammatory effect than Glycyram in the models of edema with subplantar formalin administration and in the "pocket granuloma" model.

Introduction

Natural polysaccharides have high biological activity, coupled with the absence of toxicity, allergenicity and pyrogenicity. Due to this, carbohydrates are a very important object of research from a practical point of view. Polysaccharides isolated from plants in many cases have been reported to posses immunomodulatory properties, stimulate phagocytosis, increase the number of immunoglobulins in the blood and activate humoral immunity.

Inflammation is a protective and adaptive process of the body. Nevertheless, there are pathological processes in which inflammation plays an important role in the disadaptation of an organism - autoimmune and autoinflammatory processes. For example, the cytokine storm in COVID-19, rheumatoid arthritis, systemic lupus erythematosus, and other similar pathologies cause the most damage precisely with the participation of inflammatory reactions. On the other hand, modern anti-inflammatory drugs have a set of negative side effects, which makes it necessary to search for new effective anti-inflammatory drugs with a minimum of negative side effects. This paper considers natural polysaccharides with anti-inflammatory activity.

Materials and methods

Polysaccharide from Helianthus tuberosus L. (HTLP) was obtained earlier [1]. The evaluation of the anti-inflammatory effect of HTLP was carried out in models of edema with subplantar administration of formalin and in the model of "pocket granuloma".

In the first case, acute inflammatory edema formed after subplantar injection of 0.1 ml of a 2% aqueous solution of formalin into the hind paw of a rat. The intensity of the inflammatory response was assessed by the increase in foot volume (a digital plethysmometer was used) and the percentage of inflammation inhibition (%) was calculated. HTLP was injected subcutaneously once at doses of 100 μg/animal as a sterile 0.9% NaCl solution. The control was 0.9% NaCl solution and Glycyram - 25 mg/kg. The test substances were administered to Wistar males weighing 180–250 g an hour after the development of inflammation [2].

In the pocket granuloma model, edema was induced by introducing 20 cm3 of air into the interscapular region of rats. In the resulting "air bag" was injected with 0.5 ml of a 50% oil solution of turpentine. HTLP was injected subcutaneously once at doses of 100 μg/mouse as a sterile 0.9% NaCl solution. The control was 0.9% NaCl solution and Glycyram - 25 mg/kg. The test substances were administered to Wistar males weighing 180–250 g an hour after the development of inflammation [2].

Results

As a result, it was found that in the “pocket granuloma” model, HTLP has an antiexudative effect at the level of 65.1%, while Glyciram has only 33.5%, the introduction of 0.9% NaCl did not significantly affect the volume of edema. In the formalin edema inhibition model, the data are shown in Table 1.

Table 1. Inhibition of edema, (%).
Таблица 1. Угнетение отека, (%).

| Group Dose Time, hr | 4 | 6 | 8 | 24 |
|---|---|---|---|---|
| Control - 0,0 | 0,0 | 0,0 | 0,0 | |
| Glyciram 25 mg/kg | 4,7 | 25,2 | 30,0 | 50,8 |
| HTLP 100 mcr/animal | 46,4 | 70,0 | 91,1 | 90,3 |

Conclusions and discussion

The results obtained demonstrate that the polysaccharide has more pronounced anti-inflammatory properties than glyceram in the selected models. HTLP was almost 2 times more effective in reducing the volume of inflammation in the Formalin Test and the Pocket Granuloma model after 24 hours. In addition, the rate of development of edema suppression, which is most important for reducing the volume

of affected tissues, was significantly higher in HTLP (already after 4 hours - 46%) than Glyciram (25% after 6 hours).
Given the previously obtained data on immunomodulatory activity [3] and interferon-inducing activity, it can be concluded that HTLP has anti-inflammatory activity mediated through the activation of cytokine cascades of immunocompetent cells. Anti-inflammatory activity and lack of side effects make HTLP an important subject for further study.
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### S9.713. The blockade of electrostatic interactions between virus and cell the novel way for protection against highly contagious SARS-CoV-2 and influenza strains

Onikienko S.B.[1]*
[1]*Sankt-Petersburgh Scientific Center of RAS;*
* sergeionikienko@bk.ru

Insertion of four positively charged amino acids into the spike protein molecule cause the significant increase of SARS-CoV-2 contagiousness. Fusion of coronavirus and target cell can be induced by the Coulumb force based on viral's own electric charge. Insertion of three new positively charged amino acids (arginine, histidine, lysine) dramatically increases positive electric charge of Omikron strain spike protein (five times higher than other SARS-Cov-2 strains).Electrostatic interactions between the positively charged virus spike protein and negatively charged target cell receptors induce virus-cell fusion and replication in the the target cells due to attractions of opposite electric charges. The Spike protein of highly contagious variant Kraken of Omicron strain (infects more than 20 persons) contains a great number of positively charged amino acids. The similar positive charge of SARS-Cov-2 and antiviral antibodies can dramatically reduce their electrostatic interactions and induce coronavirus immune escape. Highly contagious nature of Omikron strain can be based on the electrostatic interactions between the positively charged spike protein amino acids and negatively charged cell target receptors. The positively charged amino acids(arginine, lysine) were discovered in haemagglutinin molecule of highly contagious H1N1 virus strain. The protective compositions based on positively charged molecules were developed to fight against highly infective virus strains of SARS-Cov-2 and influenza H1N1. The positively charged protective electrostatic barrier can block electrostatic interactions between positively charged virus sites and negatively charged target cell receptors. Cationic liposomes with positively charged amino acids (arginine and its metabolite NO), low-grade oxidized microelements, electron acceptors (xanthohumol, thymoquinone, oxidized glutathione and sodium thiosulfate) can solve the problem. The protective composition against highly contagious SARS-Cov-2 strains has been developed. It includes positively charged molecules: arginine-based cationic lecithine liposomes, electron acceptors: xanthohumol(from hop extract), sodium thiosulfate. Myrrh essential oil, extract of Nigella sativa, medium-chain triglycerides were included to improve the target effect of the composition. Composition against H1N1 flu virus includes positively charged molecules:spermidin, cationic steroid squalamine and arginine based lecithin liposomes. Myrrh essential oil, hop and ginseng oil extracts, carrageenan, succinate and medium chain triglycerides were included into composition to improve its protective effect. Antiviral effects were studied in Vero and MDCK cell culture infected with coronavirus and H1N1 virus, dynamics of PCR-test and clinical manifestations of the disease were studied in patients infected with SARS-Cov-2 and H1N1 flu virus. Intranasal electrostatic barrier induced by drug composition inhibited virus entry and replication in target cells, blocked viral proteases, coronavirus and H1N1 haemagglutinin receptors, activated protective interferon synthesis, T-cell immune response, and decreased the level of proinflammatory cytokines. Coronavirus content was decreased in cell cultural environment by more than 1000 times 2 days after incubation with the protective drug composition. Antiviral spray inhibited SARS-Cov-2 replication by 92-94% two days after starting treatment and caused the completed virus dissapperanse for 3-5 day treatment (based on PCR-test).Increased loss of disease symptoms was stated based on the signs of rhinitis, pharyngitis and laryngotracheitis. Clinical signs of recovery were observed on 3-6 after starting treatment in 85% cases of the main group and in 13% of the control group. Comparable results were obtained using the composition to protect against highly contagious H1N1 influenza virus

### S9.714. The contribution of NOS to the increase in NO production in the heart during motor activity limitation

Zaripova R.I.[1]*, Jafarova G.G.[1], Andrianov V.V.[1,2], Gainutdinov Kh.L.[1,2], Sungatullina M.I.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1]
[1]*Institute of Fundamental Medicine and Biology;*
[2]*Kazan E. K. ZavoiskyPhysical -Technical Institute (KPhTI);*
* ratno1992@mail.ru

The significant role of nitric oxide (NO) in many processes, including heart activity, as well as insufficiency of information about the functions of NO during changes in motor activity predetermine the importance of studies in this direction. During the course of life, the level of motor activity often changes downward under the influence of some environmental requirements. If a person changes his lifestyle so that his motor activity becomes low by necessity, his organism must adapt to the new condition. In these cases, a specific adaptation develops, which boils down to structural and metabolic dysfunctions of many organs and body systems. Movement deficit is accompanied by the development in the body of phenomena unfavorable for health (detrained cardiovascular system, atrophy of skeletal musculature and atherosclerosis, and osteopenia, etc.).
One of the most effective and direct methods of detecting and quantifying NO in biological samples is the method of spin trap EPR spectroscopy. The spin trap method is based on the reaction of the NO radical with the spin trap. A complex of Fe2+ with diethyldithiocarbamate (DETC) was used to capture NO and form the stable ternary complex (DETC)2–Fe2+–NO. This complex is paramagnetic (SFe = 1/2 and IN = 3/2) and can be detected by the EPR method. The complexes are characterized by an easily recognizable EPR spectrum with a g-factor of g = 2.035 and a triplet hyperfine structure. The amount of NO was estimated from the intensity of the characteristic EPR signal belonging to the (DETC)2–Fe2+–NO complex. The signals were compared for the integral intensity values, since the integral intensity of the EPR signal is directly proportional to the concentration of paramagnetic complexes. Thirty minutes after drug administration, the rat anesthetized with urethane was fixed on the operating table and cut, and the removed organs were quickly dried and frozen in liquid nitrogen in capillaries for measurements. The EPR spectra of the prepared samples were recorded on an ER-200E-SRC Bruker EMX/plus X-band EPR spectrometer with an ER 4112HV temperature attachment at 77 K. The following parameters were kept constant in all experiments: microwave power of 30 mW, modulation of 5 G, amplification of 4 ×

104 , time constant of 100 ms, spectrum recording time of 50 s, and number of accumulations of 8. The computer of an Aspect 3000 spectrometer from Bruker was used to accumulate and record the spectra. Immediately before the measurement, the finished sample truncated according to the shape of the measurement cuvette is weighed. The weight of the samples needed to be about 100 mg. The amplitude of the EPR spectra was always normalized to the weight of the sample and to the amplitude of the EPR signal of the reference sample. Application of L-NAME after 1 month spent under conditions of movement deficit led to a decrease in NO levels to the values registered in the control group (natural motor activity). Consequently, the increase in the amount of NO in the heart of rats under motion deficit may be due to the contribution of NOS.

**S9.715. The dynamics of bursting activity determines the efficiency of intermodular connections in modular networks in vitro**

Pigareva Y.I.[1,2]*, Gladkov A.A.[1,2], Kolpakov V.N.[1,2], Bukatin A.S.[3,4], Zemlyanskov M.S.[1], Kazantsev V.B.[1], Pimashkin A.S.[1], Mukhina I.V.[1,2]
[1]*N.I. Lobachevsky National Research Nizhny Novgorod State University;*
[2]*Privolzhsky Research Medical University;*
[3]*Alferov Saint-Petersburg National Research Academic University of the Russian Academy of Sciences;*
[4]*Institute for Analytical Instrumentation of the RAS;*
* pigareva@neuro.nnov.ru

Numerous studies have investigated the complex organization of the brain's structural and functional networks, but the relationship between topology and information processing is still not well understood. In recent years, it has been shown that brain neural networks have a modular organization that promotes efficient integration and separation of information, resistance to damage, and rapid adaptation. Modular systems combine the properties of functional differentiation and integration. The segregation of neuronal cultures into related areas (modules) makes it possible to obtain the dynamics of spontaneous activity corresponding to the modular systems' properties. Each module has its own spontaneous burst dynamics, and some bursts propagate from one module to another, initiating a response burst in the Target module. Microfluidic techniques enable the formation of neural networks with a controlled number of modules and intermodule connections. Combination with microelectrode arrays these methods provide high spatial and temporal resolution for investigating functional interaction Our work aimed to investigate the relationship between the intramodular activity of neuronal cultures in vitro and the effectiveness of intermodular interaction.
Research methods. The study was performed on an experimental model of primary cell cultures of the hippocampus of C57BL/6 mouse embryos. A microfluidic chip was developed using soft lithography from polydimethylsiloxane (PDMS). Cell cultivation was performed in two chambers connected by asymmetric microchannels creating a directional connection between the Source and the Target networks. The chip was combined with a microelectrode array with 60 electrodes (Multichannel systems, Germany). Bioelectrical activity was registered using the MEA2100-2x60-System-E device (Multichannel systems, Germany) with a sampling frequency of 20 kHz. Spike and burst detection was performed using previously developed software and the MatLab software package [1]. Data are presented as median and percentile.
Results. Our study showed that the spontaneous activity observed in the modules can be classified into two types based on the distribution of burst's characteristics as the Spike rates in a burst and the Duration of a burst. The neural networks with a cluster of "large" bursts exhibited higher levels of intramodular activity, including longer bursts and a greater number of spikes per burst.
At the same time, such dynamics corresponded to increased levels of inter-module activity, which consisted of bursts propagating from the Source to the Target module. The percentage of bursts propagating was 23% (+16.75 -4; n = 11) in neural networks with a cluster of "large" bursts. In neural networks with activity without "large" bursts, the percentage of propagated bursts was less than 4% (+2 -12.25; n = 9; $p<0.05$; Mann-Whitney test).
Conclusions. The efficiency of module interaction, expressed as the percentage of Source module bursts that initiate a burst in the Target module, depends not only on the formed connection, but also on the intramodular activity.
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**S9.716. The effect of Uridine on physiological activity in blood lymphocytes in the Rotenone model of Parkinson's disease in rats**

Khunderyakova N.V.[2]*, Medvedeva V.P.[1,2], Mosentsov A.A.[1,2], Khmil N.V.[1,2], Mironova G.D.[1,2]
[1]*Puschino State Natural Science Institute, Pushchino, Russia;;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS;*
* nkhunderyakova@gmail.com

Parkinson's disease (PD) is a chronic neurodegenerative disease associated with the death of dopaminergic substantia nigra neurons. It is known that one of the causes of PD is mitochondrial dysfunction associated with a decrease in their energy efficiency and the development of oxidative stress. In our work, the effect of uridine on the activity of the key mitochondrial enzyme succinate dehydrogenase (SDH) and cytosolic -lactate dehydrogenase (LDH) in blood lymphocytes on smears and on serum malondialdehyde (MDA) levels in rotenone-induced parkinsonian model was investigated. Previous works by our group has shown that uridine leads to an increase in the formation of uridine diphosphate in the cell, an activator of mitochondrial ATP-dependent potassium channel (mitoKATP-channel). Activation of mitoKATP-channel leads to a decrease in oxidative stress [1, 2], which is why we used uridine in the PD model to treat mitochondrial dysfunction in rats.
Materials and Methods: The study was performed on male Wistar rats (n=25) weighing 280-320 g, kept at the ITEB RAS in compliance with the rules of the European Convention for the Treatment of Laboratory Animals. To simulate PD in rats, a rotenone solution (SIGMA) was used at a dose of 1.7 mg/kg animal weight, administered by subcutaneous chronic injection for 28 days, according to the scheme of 2 days of dosing, 2 days of rest. Rotenone is a cellular respiration inhibitor; it blocks electron transfer from glandular clusters of complex I to ubiquinone. Uridine was administered intraperitoneally at a concentration of 30 mg/kg animal weight with a frequency of 2 days of dosing and 2 days of rest.
The activities of SDH (a biomarker of aerobic mitochondrial respiration) and LDH (a biomarker of anaerobic respiration - glycolysis) were measured by the CBC method in immobilized blood lymphocytes on smears [3]. The CBC method is based on the reduction reaction of nitroblue tetrazolium chloride to dark blue diformazan (DF) at the second site of the mitochondrial respiratory chain. Thin blood smears were fixed for 30 sec with 60 % acetone and 10 mM HEPES, pH 5.2 - 5.4, then stained in incubation medium containing: 125 mM KCl, 10 mM HEPES, 1.22 mM NST for 1 hour at 37°C, pH 7.2 ±0.05 and additives; 5mM succinic acid was the main assay to characterize LDH activity and 5mM lactic acid, 5mM malonic acid and 0.5mM NAD - LDH activity. Smears were studied under 1000X magnification using a Leica DM-2000 microscope. Cytomorphological and quantitative analysis of color micrographs of lymphocytes was performed using

the "Bloodrunner" and "Cell Composer" programs to determine the amount and distribution of DF dye in each cell. The level of lipid peroxides in blood serum was determined by MDA with thiobarbituric acid using diagnostic kit "TBK-AGAT". Statistical data processing was performed using licensed Microsoft Office Excel-2007 and Statistica 10.0 software. The significance of differences between the groups was determined using Student's t-test. Differences were considered significant at values *p<0.05.

Results. Subcutaneous dosing of rotenone to rats to simulate PD resulted in a significant (p≤0.05) 2-fold increase in SDH activity (control 1.3 ±0.37 unit; PD 2.4 ±0.85 unit (unit - amount of DF)), and LDH activity, which also increased 2-fold (p≤0.01) (control 2.24 ±1.19; PD 3.9 ±1.0 unit). Uridine administration significantly reduced the increased SDH activity (mitochondrial hyperactivation) by 25 %, apparently due to the mechanism of mitoKATP-channel activation, without an effect on cytosolic LDH activity. Measurement of the radius of lymphocytes in sick and control animals, without dividing them into subpopulations, revealed no significant differences. However, the size of lymphocytes increased significantly in the group with uridine compared to the control animals (control 3.2 ±0.2; uridine 3.9 ±0.4 μm2). The range of variation in lymphocyte size was greater than in control animals against a background of uridine administration. The determination of MDA in blood serum was significantly 2-fold higher in animals with PD than in the control group (control 4.44 ±0.85; PD 7.68 ±1.34 mMol/L, p≤0.01), and administration of uridine to sick animals, reduced this index 2.5-foldto a level lower than that of the control group (PD + uridine 2.72 ±0.67 mMol/L, p≤0.01). A similar effect of uridine as a factor in reducing oxidative stress has been shown in other work by our group [1]. It is known that the rotenone model is one of the experimental models of PD, which causes nigrostriatal degeneration caused by inhibition of complex I of the mitochondrial respiratory chain and the appearance of Levy bodies. In our work, we first detected a significant increase in the activity of SDH and LDH in this model, i.e., an increase in both aerobic and anaerobic respiration in blood lymphocytes, as well as an increase in the serum MDA level, which confirms the development of oxidative stress in these animals. Administration of uridine, an activator of the mitoKATP-channel, resulted in a 25 % reduction in SDH hyperactivation in blood lymphocyte mitochondria and a significant decrease in serum lipid peroxide levels in animals with PD. These data support our hypothesis that uridine reduces oxidative stress and normalizes mitochondrial function in lymphocytes, so it can be recommended for the prevention and treatment of PD.

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## S9.717. The effect of infrared radiation on the energy metabolism state at neuropathic pain syndrome in the experiment

Didenko N.V.[1]*, Soloveva A.G.[1], Polyakova A.G.[1], Belyaeva K.L.[1], Peretyagin P.V.[1]
[1]*Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of Health of the Russian Federation, Nizhny Novgorod, Russia;*
* Natalika-nv@mail.ru

The urgency of the problem of neuropathic pain and regeneration of the peripheral nervous system is associated with a high degree of disability and reduced quality of life [1]. Neurodegenerative processes in nerve endings negatively affect the regulation of all metabolic processes of the human body. The infrared (IR) radiation is supposed to be a factor for accelerated regeneration of nerve tissues and improving metabolism in general, since it promotes the stimulation of angiogenesis, synthesis of growth factors and oxygen saturation in blood [2].

The aim of this study was to evaluate the effect of infrared radiation on the erythrocytes metabolism of rats with neuropathic pain syndrome on the 10th day after injury.

This study was carried out on Wistar rats weighing 300g in strict accordance with ethical standards and rules of good laboratory practice (GLP). Animals (n=30) were divided into 3 groups: group 1 - healthy animals, group 2 - control, group 3 - experimental. Rats of groups 2 and 3 under the anesthesia (Zoletil+Xyl) simulated neuropathic pain syndrome. Animals of the experimental group were exposed daily to radiation in the infrared range with a wavelength of 810 nm: the occipital mound area was irradiated (procedure time 10 minutes), then the sciatic nerve lesion area was irradiated (procedure time 10 minutes). A certified matrix device "Elmedlife M" (Russia) was used for non-invasive therapeutic effects in pulsed mode by optical radiation of the infrared range. The blood of the animals was taken after 10 days by decapitation under anesthesia using sodium citrate in the ratio (1:4). Activity of aldehyde dehydrogenase (AlDH, lactate dehydrogenase in direct (LDHd) and reverse (LDHr) glucose-6-phosphate dehydrogenase (G6PDG) was evaluated in the hemolysate of washed erythrocytes by spectrophotometry[3]. The measurement data were processed on the Statistica 6.0 software using nonparametric analysis methods for comparison.

Exposure of infrared radiation in the experimental group of animals with neuropathic pain syndrome led to normalization of energy metabolism in erythrocytes compared to the control group, so the specific activity of LDHr decreased by 18.551% (p<0.001). The specific activity of LDHd at the same time was reduced by 5.833% (p= 0.0024) compared to the healthy animals data. Such a shift of energy metabolism to anabolic processes helps to reduce the accumulation of lactate in the tissues of the rat body of experimental group and reduces the state of acidosis in general.

The specific activity of AlDH in the rat blood erythrocytes of the experimental group when exposed to IR radiation decreased by 9.849% (p< 0.001) compared to the healthy animals data and by 23.812% (p< 0.001) compared to the control. Thus, exposure of IR radiation reduced the accumulation of aldehydes in the blood of rats with neuropathic pain syndrome. The specific activity of G6PDH after exposure of IR radiation was reduced by 3.576% (p=0.046) compared to the healthy animals data, and increased by 31.966% (p< 0.001) compared to the control group, which means an acceleration of the pentose phosphate pathway, and acceleration of respiration processes in general.

Thus, it was shown that metabolic status of erythrocytes of rats with neuropathic pain syndrome improves under the influence of IR radiation with a predominance of anabolic processes. It was revealed that IR radiation exposure affected to the normalization of the parameters of all studied enzymes level (LDHd and LDHr, AlDH, G6PDG) was noted, which lead to the normalization of respiratory processes, as well as a decrease in intoxication in the blood of rats in experimental group.

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**S9.718. The effectiveness of passive targeting of nanoscale carriers for the treatment of breast cancer**

Postovalova A.S.[2]*, Istomina M.S.[1]
[1]*V.A. Almazov National Medical Research Center of the Ministry of Health of the Russian Federation;*
[2]*ITMO National Research University;*
* alisa_postovalova@mail.ru

According to data provided by the International Agency for Research on Cancer (IARC), breast cancer in women is the most frequently diagnosed disease with a high prevalence worldwide [1]. It is known that due to genetic disorders, intensive division of cancer cells, as well as concomitant active angiogenesis, tumor tissue actively grows during breast cancer. These features can be used to target various nanoscale carriers containing therapeutic agents to influence tumor tissue for therapeutic purposes. In this regard, it is especially important to study the EPR effect, a phenomenon that explains the passive targeting and accumulation of nanoscale carriers in the tumor focus. Due to the correctly selected characteristics of such carriers, their accumulation in the affected tissue is carried out, which avoids the toxic effects of the therapeutic compound on healthy tissues, and its prolonged release from nanoparticles is also achieved [2]. In this work, the effectiveness of passive targeting and retention of a therapeutic agent delivery system in tumors based on spherical nanoscale particles based on silicon oxide was investigated.

Particles based on silicon oxide (Si2O3) with a size of 80 -120 nm were obtained as a result of synthesis from 4 solutions: 99% ethanol, purified water, 99% tetraethylortosilicate (Teos) and ammonia (NH3) - during 2 hours of active stirring on a magnetic stirrer. Cy5 fluorophore-labeled nanoscale carriers were used to visualize their bio-distribution ex vivo. Nanoparticles were characterized using DRS, light microscopy, particle capture by cells of the 4T1 line (breast cancer), cytotoxicity were evaluated. In order to study the bio-distribution of particles in organs (heart, lungs, liver, spleen, kidneys, tumor), samples were administered intravenously, at the rate of 100 µl of particle suspension per animal, to balb/c mice with a breast cancer model. The animals were then sacrificed 2, 6, 24, 48 hours after injection, and the intensity of the fluorescent signal in the organs was analyzed using a fluorescent imaging system (IVIS Lumina II, PerkinElmer Inc., USA). Visualization of the distribution of nanoscale carriers was also evaluated using single-photon emission computed tomography and direct radiometry of organs on a gamma radiation counter (Triatler with a Final detector, Hidex Oy, Finland). To do this, the particles obtained during chemical synthesis were labeled with the 99mTc isotope. 15 animals were involved in the experiment. The experimental work was carried out in compliance with the ethical principles declared by the European Convention for the Protection of the Rights of Vertebrate Animals Used for Experimental Purposes. To determine the significant differences between several sets of experimental data, the Student's t-test and variance analysis were used. The values of $p < 0.05$ and $p^* < 0.005$ were statistically significant.

In females of the Balb/c mice line with model pathology 4T1 (breast cancer), the accumulation of 3% of particles in the tumor was noted. The greatest absorption and retention of particles by tumor cells, namely more than 80%, occurs in the liver. In addition, some of the smaller particles are excreted from the body through the kidneys and urinary system. Both methods of visualization of nanoscale particles showed a statistically identical percentage of their distribution. In this regard, each of the visualization techniques can be selected as diagnostic, depending on the relevance of its application.

Thus, the study of the bio-distribution of nanoscale carriers based on silicon oxide for therapy shows passive targeting, accumulation and retention (EPR effect) of particles in tumor tissue, as well as a significant accumulation of carriers in the main organs.

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**S9.719. The influence of resveratrol on the expression of functional proteins of axons and Schwann cells during damage and regeneration of the peripheral nerve**

Pinyaev S.I.[1]*, Pianzina A.E.[1], Otryaskin Y.S.[1], Syusin I.V.[1], Revin V.V.[1]
[1]*Ogarev Mordovia State University;*
* komrad.pinyaev2009@yandex.ru

The study of the mechanisms of damage to peripheral nerves is a key problem in physiology, biophysics, and medicine. To date, no mechanisms have been found that can effectively restore or enhance regeneration processes. Damage to peripheral nerves can lead to loss of neuronal communication along sensory and motor nerves between the CNS and peripheral organs, and these often lead to painful neuropathies due to reduced motor and sensory functions and can be disastrous for patients, dramatically affecting their life functions. Proteins such as myelin null and growth-associated protein-43 can act to monitor the regeneration process.

The aim of the work was to study the expression of axonal protein (GAP-43) and Schwann cell protein (P0) in rat sciatic nerve injury and under the action of resveratrol.

The object of the study was the sciatic nerves of mature Wistar rats. Animals were divided into 3 experimental groups: intact animals, animals with damaged sciatic nerve, withdrawn from the experiment for a month, with an injection of resveratrol.

In the course of the work, the following methods were used: preparation of the sciatic nerve, isolation of the myelin fraction, determination of the amount of protein according to Lowry, electrophoresis of myelin-specific proteins, Western blotting, enzyme immunoassay method for analyzing the amount of NGF, measuring the functional sciatic index and the action potential of the sciatic nerve.

Myelin null protein is the main myelin protein expressed by Schwann cells, constituting approximately 50% of all PNS myelin proteins and is required for both normal function and normal structure of myelin. Nerve injury promotes changes in the myelin sheath, which causes changes in the interaxonal interactions of Schwann cells and thus leads to axonal degeneration. Growth-associated protein-43 (GAP-43) is a cell membrane phosphorylation protein with a membrane of nerve endings, which belongs to the family of calmodulin-binding proteins. GAP-43 is a neurospecific protein that regulates many aspects of neuronal development, plasticity and regeneration. It is closely related to nerve growth and synapse formation, especially in nerve regeneration. These facts determined the choice of these proteins for investigation.

In the course of the study, we found that nerve injury contributes to a change in the amount of myelin null protein both in the proximal and distal parts of the nerve. At the same time, resveratrol has a positive effect on the restoration of the amount of myelin null protein. The change in the amount of null protein confirms the fact that it is a marker of Schwann cell recovery, and resveratrol affects the expression of Schwann cell proteins. Our results on the presence of such an axonal protein as growth-associated protein-43 in damaged nerves indicate the occurrence of intense regenerative processes after injury, since this protein regulates the actin cytoskeleton of neurons, thus being a marker of axon recovery. The increase in this protein found by us

upon injection of resveratrol suggests that this compound affects the synthesis of growth-associated protein-43, and thus affects the regenerative processes in the PNS. We also established the dependence of the amount of nerve growth factor (NGF) on the concentration of resveratrol. Analysis of the functional sciatic index of the damaged peripheral nerve in normal and after injury and action potential showed the restoration of innervation by the injured nerve of the muscle, and the use of resveratrol intensified this process.
Thus, based on our results, we can conclude that resveratrol affects the expression of functional proteins of the axon (GAP-43) and Schwann cells (P0) of rats during injury and regeneration of peripheral nerves, contributing to a more pronounced course of regenerative processes in the injured nerve. . Speaking about the mechanism of its action, we can say that the effect can be both from direct action and from indirect action, since it is known that many polyphenols, including resveratrol, have a modulating effect on various cell signaling pathways, but here it is required further, more detailed study of this fact.

### S9.720. The molecular mechanisms of hyperthermia through cytochrome C-dependent processes

Stepanov G.O.[1]*, Kharitonov D.V.[1], Alekseeva A.O.[1], Eremina Ya.V.[1], Badalov A.A.[1], Volkov V.V.[1], Vladimirov Yu.A.[1], Osipov A.N.[1]
[1]*Pirogov Russian National Research Medical University;*
* stepg@yandex.ru

The treatment of oncological diseases is one of the most difficult tasks for medicine. It is well known that it is difficult to initiate apoptotic processes in cancer cells, the inclusion of which is necessary for their elimination from the body. In this regard, the mechanisms of controlled cell death and ways of their regulation attract special attention of researchers and doctors today.
As you know, one of the methods that increases the effectiveness of cancer treatment is hyperthermia. In hyperthermia, the tumor tissues are selectively heated to temperatures ranging from 39 to 45 ° C. Recent studies based on the thermoradiobiological justification of hyperthermia indicate that it is a powerful radio and chemosensitizer [1]. In addition, the use of hyperthermia can be separated in time with a radiation therapy (according to the recommendations for about an hour) and, of course, the question how to explain the intensification of pro-apoptotic free radical processes remains. After a slight increase in temperature the effectivities therapy and especially the preservation of this effect when returning to the initial temperature values.
The purpose of this study: to investigate the structural and functional changes of cytochrome c (CytC) as one of the main proteins initiating apoptotic processes along the mitochondrial pathway, as well as the effect of temperature on cytochrome c–dependent apoptotic processes.
The study was performed at the molecular level using spectrophotometry, spectrofluorometry and chemiluminescence of cytochrome C complexes with cardiolipin (CL), phosphatidic acid (PA) and phosphatidylcholine (PC), at temperatures up to 45°C.
It was found that CytC complexes with PA or CL are characterized by an increase in the fluorescence intensity of tryptophan 59 by about 1.7 times. A change in the intensity of the 695 nm cytochrome c band is observed only in the presence of CL-containing liposomes (by ~13.7% at 25°C, ~19.4% at 37°C and ~21.4% at 45°C), but is absent both in the presence of PC and PA. The intensity of chemiluminescence increases by 26% when the temperature rises from 25 to 37°C in the presence of PA. During the transition from 37 to 45°C, no increase in the intensity of Cyt C chemiluminescence in the presence of phospholipids was detected. It is particularly interesting that both spectrofluorimetry methods and the evaluation of cytochrome c- induced POL (chemiluminescence with coumarin) have shown the phenomenon of hysteresis when heating CytC complexes with anionic phospholipids. This phenomenon was manifested in the fact that the structural and functional properties of cytochrome c when it was heated to 45C and then cooled to 25C significantly differed (up to about 26%) from similar samples that were not preheated, but simply measured at 25C.
Thus, it is shown that: 1. An increase in temperature changes the conformation and peroxidase activity of CytC; 2. Peroxidase activity increases in the presence of anionic phospholipids; 3. Temperature incubation of CytC with PA and CL has led to irreversible conformational changes, unlike the control PC, which can explain the molecular mechanisms of the hysteresis effect, which is delayed during hyperthermia even recommendations according to tht application.
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### S9.721. The role of arachidonic acid and its derivatives in the functioning of the neurogliovacular unit

Lukin P.O.[1], Verisokin A.Yu.[1], Verveyko D.V.[1]*
[1]*Kursk State University;*
* allegroform@gmail.com

Disturbances in the blood supply to the brain lead to a loss of energy balance in nerve tissues and subsequently to various neurodegenerative pathologies (in particular, Alzheimer's, Parkinson's diseases, stroke), accompanied by the formation of cortical spreading depolarization (CSD) waves, leading to neuronal death. Modern experimental studies show that the increase in blood flow after RCD significantly depends on the synthesis of arachidonic acid (AA) and its derivatives. Thus, in particular, some signaling molecules (EETs, PGE2) have a vasodilating effect; on the other hand, the synthesis and accumulation of the vasoconstrictor 20-HETE in cell membranes leads to disruption of the neurovascular interface, including the appearance of new CSD centers and long-term ischemic damage to the nervous system. Understanding the role of AA and its metabolites in the normal functioning of the brain has emerged in recent years due to the accumulation of a large amount of experimental data [1].
When modeling, we are based on a local model of calcium dynamics in the astrocyte [2], which was further extended by us to a spatial case, in which the morphological features of the astrocyte were taken into account [3]. We complement the glutamate interaction between astrocyte and neuron with vascular dynamics. Thus, the proposed local model contains neurogliovascular connections that describe a complex of interactions between neurons, astrocytes, and blood vessels: calcium dynamics in astrocytes with the possibility of taking into account morphological affiliation, synaptic activity, and vascular dynamics. The model is supplemented with equations describing the production of AA and its derivatives and their influence on the activity of the neurogliovascular unit as a whole.
As a result of a series of numerical experiments, we determined the effects that demonstrate the effect of AA and its derivatives on vascular tone, astrocytic dynamics, and synaptic activity. The relationship between the levels of EET and PGE2 concentrations, the characteristics of blood vessels and oxygenation level and calcium dynamics was determined. The model shows that an external effect aimed at increasing the level of 20-HETE leads to constriction of blood vessels and a decrease in calcium dynamics in the astrocyte, which is consistent with the known experimental data, while a short-term burst of calcium activity is possible under the condition of a long stimulation time.
Thus, the results of the model study confirm that for a full theoretical description of the key biophysical processes occurring in the nervous tissue of the cerebral cortex, it is necessary to include into the model all the interaction pathways for the key elements of the neurogliovascular unit – astrocytes, neurons and blood vessels, – including vasomodulatory effects of AA and its metabolites. The model study suggests a

high efficiency of indirect effects on blood flow and neuronal activity by controlling the synthesis of AA metabolites. This approach seems to be a promising pharmacological method for the treatment of a large number of neurodegenerative pathologies.

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### S9.722. The role of carbon monoxide in the regulation of nociceptive activity of the peripheral trigeminal nerve

Koroleva K.S.[1]*, Svitko S.O.[1], Ananev A.S.[1], Verhoturova T.M.[1], Buglinina A.D.[1], Sitdikova G.F.[1]
[1]*Institute of Fundamental Medicine and Biology;*
* k.s.koroleva@yandex.ru

Migraine is a widespread neurovascular disease characterized by complex pathogenesis and difficulties with its treatment. It is urgent to study the molecular mechanisms underlying the onset of migraine pain in order to find new methods of migraine prevention and treatment. The trigeminal vascular system is considered as a source of pain signal in migraine. Clinical and preclinical studies demonstrate increased excitability of the trigeminal vascular system during migraine attacks. According to the trigeminal-vascular theory of migraine pathogenesis, which combines neuronal and vascular mechanisms of migraine development, disruption of interaction between cranial vessels, trigeminal nerve and CNS leads to development of aseptic neurogenic inflammation of brain sheaths provoking nociceptive activity. An important role in this process is assigned to the trigeminal nerve as the initiator of neurogenic inflammation, on the one hand, and the as conductor of nociceptive information into the CNS, on the other hand. Neurons and afferents of the trigeminal ganglion, as well as mast cells, express various receptors and channels involved in the perception of the damaging stimulus and conduction of nociceptive information into the CNS. Recently, a genetic analysis of patients with migraine has shown a significant role for a mutation of the CO synthesis enzyme hemoxygenase in the pathogenesis of this disease. Carbon monoxide (CO) is an endogenously produced signaling gastransmitter involved in nociception, neurotransmission, and cerebral hemodynamics. However, the neuronal mechanisms of CO involvement in the development of migraine are virtually unstudied. Therefore, the aim of our research was to reveal the role of carbon monoxide (CO) in the nociceptive activity of rat trigeminal afferents.

Experiments were performed on male (4-8 weeks old) Wistar rats. We used an electrophysiological method of recording action potentials (APs) of the trigeminal nerve innervating the dura mater in a rat half-cranial preparation.

In our study, we used the CO donor, CORM-2 at a concentration of 30 μM, the CO blocker, zinc protoporferin (ZnPP) at a concentration of 10 μM, and a physiological solution saturated with CO.

Replacement of the physiological solution saturated with O2, commonly used in our experiments, with physiological solution saturated with CO with incubation of the preparation for 30 min resulted in a significant increase in AP frequency twofold (baseline AP frequency was 213.1±37.9 APs per 5 min; after addition the frequency was - 494.3±64.6 APs per 5 min; n=9; p = 0.009).

The use of the CO donor, SORM-2 (30 μM), also resulted in an increase in AP frequency. The baseline AP frequency was 126.3±35.5 APs per 5 min; after application of CORM-2, the frequency by 5 min was 280.3±36.5 APs per 5 min; by 10 min 251.2±28.5 APs per 5 min; by 15 min, 217.6±28.5 APs per 5 min (n=4; p = 0.04).

The endogenous CO synthesis blocker, zinc protoporferin, ZnPP (10 μM), did not affect the frequency of APs in trigeminal afferents. The baseline AP frequency was 141.25±43.9 APs per 5 min; after ZnPP application, the frequency by 5 min was 195.6±72.5 APs per 5 min; by 10 min, 201.6±66.6 APs per 5 min; by 15 min, 125.6±40.9 APs per 5 min (n=5). The data obtained indicate that the enzymatic systems of endogenous CO synthesis do not contribute to baseline electrical activity in trigeminal afferents. In addition, we have shown that the CO donor increases the nociceptive activity of trigeminal afferents, and it is reasonable to use the CO donor CORM-2 for further investigation of the receptor mechanisms of nociceptive activity formation.

According to the literature, the mechanisms of CO action may be related to the activation of soluble guanylate cyclase (sGC). Indeed, we have shown that the use of the sGC inhibitor ODQ at a concentration of 10 μM leads to a decrease in the pro-nociceptive effect of CO. Incubation of the half-skull preparation in ODQ for 20 min resulted in no change in the AP frequency (n=4; p=0.87). The baseline AP frequency in the trigeminal nerve was 187.5 ± 86.2 APs per 5 min and 172.5 ± 30.1 APs per 5 min after 20 min incubation in ODQ (10 μM) (n=4; p = 0.87). Subsequent addition of CORM-2 (30 μM) did not cause a significant change in AP frequency and by 10 min of application the AP frequency was 145.2 ± 47.3 APs per 5 min and by 15 min 129.7 ± 50.8 APs per 5 min (p = 0.62); by 20 min 127.1 ± 40.3 APs per 5 min (n=4; p = 0.12).

Our data indicate a leading role of soluble guanylate cyclase in the effects of exogenous CO in rat trigeminal afferents. Activation of guanylate cyclase leads to the synthesis of cGMP and activation of protein kinase G, which through phosphorylation processes can influence the activity of ion channels, receptors, and plays an important role in Ca2+ homeostasis. In addition, stimulation of soluble guanylate cyclase with VL-102 can directly increase the expression and release of CGRP from trigeminal ganglion neurons. The involvement of guanyl cyclase in pro-nociceptive effects is also supported by studies in which ODQ exerted antinociceptive effects after intrathecal injection in models of inflammatory and neuropathic pain.

Our findings contribute to the understanding of the neuronal mechanisms of CO involvement in the pathogenesis of migraine, which may play a role for the development of specific drugs aimed at migraine therapy in the future.

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### S9.723. The role of dysfunction of the mitochondrial transport systems of calcium and potassium ions in the progression of Duchenne muscular dystrophy. Correction paths

Dubinin M.V.[1]*, Belosludtsev K.N.[1,2]
[1]*Mari State University, Yoshkar-Ola, Russia;*
[2]*Institute of Theoretical and Experimental Biophysics of RAS, Pushchino, Russia;*
* Dubinin1989@gmail.com

Duchenne muscular dystrophy (DMD) is a recessive X-linked hereditary disease caused by mutations in the gene encoding the dystrophin protein. This is one of the most common forms of muscular dystrophy - DMD is diagnosed in an average of 1 in 3500 boys. Due to the absence of dystrophin, muscle fibers become brittle, which causes rupture of the sarcolemma, an increase in their permeability during muscle contraction, and causes the release of soluble enzymes, such as creatine kinase, from the cells and the penetration of calcium and other ions

into the interior. In addition, dystrophin and the dystrophin associated glycoprotein complex play an important role in coordinating the work of various signaling systems, including ion channels that ensure the normal functioning of skeletal muscles, and the loss of these structures leads to dysregulation of ion homeostasis [1].
Currently, research is ongoing aimed at creating a gene therapy that can restore the normal expression of dystrophin. However, such approaches often face multiple technical problems, primarily due to the delivery of the vector, and can only be effective if therapy is started early, before the irreversible replacement of muscle tissue with non-functional connective tissue. In this regard, much attention is paid to the correction of the secondary effects of DMD, primarily the disruption of Ca2+ homeostasis associated with an increase in the amount of reactive oxygen species (ROS), chronic inflammation, a decrease in regenerative capacity, and fibrosis [1]. Mitochondria deserve special attention, providing muscle cells with energy in the form of ATP, which is necessary for normal contraction. During the development of DMD, these organelles demonstrate a significant decrease in the intensity of oxidative phosphorylation and hyperproduction of ROS, a decrease in the biogenesis of organelles and a violation of their dynamics [1]. In addition, a number of our works demonstrated that the mitochondria of skeletal muscles of dystrophin-deficient mdx mice are characterized by rearrangements in the systems of calcium and potassium transport [2, 3]. In particular, such changes are accompanied by a decrease in the efficiency of calcium uniport and sensitivity to the induction of the mitochondrial calcium-dependent pore (known as the MPT pore) [2], as well as inhibition of the transport of potassium ions and the content of this ion in the matrix of organelles [3]. We found that improving the ability of mitochondria to accumulate calcium ions in the matrix by using the non-immunosuppressive MPT pore inhibitor alisporivir leads to the normalization of mitochondrial function and ultrastructure, as well as a decrease in the intensity of destructive processes in skeletal muscles [4]. In addition, we have recently found that the activation of potassium ion transport in the skeletal muscle mitochondria of mdx mice using uridine, a precursor of the ATP-dependent potassium channel (mitoKATP) activator UDP, leads to a significant decrease in the level of fibrosis in skeletal muscles [3]. A more pronounced effect was shown for NS1619, an activator of the mitochondrial calcium-activated potassium channel (mitoBKCa), which improved the transport and level of potassium ions in the skeletal muscle mitochondria of mdx mice, which also contributed to a decrease in the intensity of oxidative stress and an increase in the calcium capacity of organelles, and was also accompanied by an improvement in the ultrastructure of organelles and mitigation of degenerative processes in the skeletal muscles of animals [5]. This report discusses the role of dysfunction of calcium and potassium ion transport systems in skeletal mitochondria in the development of Duchenne dystrophy, as well as the possibility of correcting this pathology by improving the function of these structures.
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**S9.724. The role of gravitational and muscular forces in the bone tissue remodeling**

Baltina T.V.[1]*, Sachenkov O.A.[1]
[1]*Kazan Federal University;*
* tvbaltina@gmail.com

It is believed that the main regulators of mechanical bone transduction are exogenous gravitational forces and endogenous muscle forces. It's known, that changes in bone tissue are distinctive aspect of prolonged immobilization after injuries. In this case it is assumed that neuromuscular apparatus disorder leads to changes in the mechanical properties of muscle tissue and then to changes in the mechanical properties of bone tissue. But still, there is no convincing evidence of the muscle leading role in the regulation of bone tissue metabolism.
The H.M. Frost's mechanostat theory postulates a linear relationship between load and bone strength. According to the mechanostat theory local elastic deformation control bones adaptation processes. The origin of the local elastic deformation can be gravity or muscle strength. In case of gravity load some clarification should be mentioned. Gravity influence can be divided by mass and reactive loading. Reactive loading appears by foot support and can disappear in case of physical activity decrease. Meanwhile, mass loading can disappear only in case of weightlessness [1]. Then, H. M. Frost clarified the interrelation of the mechanostat system elements by including the influence of the nervous system, muscle contractions and mechanical loading (e.g. physical activity or foot support). Additionally, non-mechanical agents which affect modeling and remodeling were divided by systemic and local. So, the crucial question is what factor influences greater on activating mechanotransduction process? The answer can significantly improve the quality of clinical treatments. In this case, the physical medicine treatment can be designed for bone tissue restoration.
Bone loss is a common accompanying disease in clinical practice. E.g., patients with Duchenne muscular dystrophy or cerebral palsy suffer with bone mass loss and an increased risk of fractures [2,3]. In addition, significant bone loss occurs in patients with spinal cord injury [4,5]. Non-use of the hind limbs because of injury, immobilization (e.g. bed rest) or space flight lead to significant loss of bone and muscle tissue [6,7].
Bone loss appears in hind limb unloading models [8], additionally macromechanical and structural changes appears [9]. Studies of microgravity, unloading or non-usage (e.g. muscle tenotomy, denervation) models allows us to better understand the bone remodeling mechanism And leads to a new assumption - that biomechanical evolution can influence the remodeling process. Due to the modern data, it can be assumed that the signaling pathways responsible for influencing the morphology and function of muscles and bones are joint and consistent [10].
However, most gravity-related activities also require muscular effort (e.g. running, jumping). Opposite, some activities stimulate the skeleton almost exclusively due to muscle load (e.g. lifting weights, swimming), so it is important to evaluate the nature of the load. Determining the main stimulus for an adaptive response at the macroscopic level (muscle forces or gravitational loads) carries the potential for developing physical exercises and treatment methods aimed at more effective bone mass increase, as well as optimizing existing rehabilitation and prevention programs for osteoporosis.
The research is focused on systematization widespread experimental models, measured parameters and received correlation between external physical influence, its nature and bone tissue remodeling.
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### S9.725. The role of heat shock 70kda protein A1 in bipolar affective disorder

Seregin A.A.[1]*, Krotenko N.M.[2], Dmitrieva E.M.[1], Smirnova L.P.[1]
[1]*Tomsk National Research Medical Center of the Russian Academy of Sciences Mental Health Research Institute;*
[2]*Siberian State Medical University , SSMU;*
* apocalips1991@mail.ru

Today, studies related to the search for proteins specific to depression, bipolar disorder (BD) and other mental disorders are gaining popularity all over the world. The search for such proteins reflecting characteristic changes in the pathogenesis of these diseases is promising. (English J. et al., 2010; Lakhan S., 2006; Domenici E., 2010). The review of the literature shows that the works existing at this stage using proteomic analysis are mainly represented by works on schizophrenia; there are a few works done with depression and bipolar disorder, but mostly on posthumous material. As a result of a previous comparative mass spectrometric study of blood serum proteins in patients with depression and bipolar disorder, as well as in healthy donors, proteins were identified: heat shock protein 1A (Heat Shock 70kDa Protein1A), (HSPA1A) - 70.052, and alpha-actin -2 (Actin, aortic smooth muscle) (ACTA2), – 42.009 Da. In the present work, a comparative study of the amount of these proteins in the blood serum of patients with depression and bipolar disorder, presumably involved in the pathogenesis of these disorders, was carried out.
A clinical and biological examination of the blood serum of 74 people was carried out. The study groups were formed from 30 patients with recurrent depressive disorder (F33), and 28 patients with bipolar disorder (F31). Diagnostic assessment and clinical verification of the diagnosis in patients were carried out by doctors of the clinic of the Mental Health Research Institute in accordance with ICD-10. The mean age of the patients was 40.33±14.1 years. Blood was taken from all examined in the morning on an empty stomach before the start of therapy. As a control group, 14 mentally and somatically healthy individuals were examined, comparable in sex and age, with the examined patients (mean age 32.6±2.2 years).
To determine the amount of the studied proteins, commercial kits for enzyme-linked immunosorbent assay were used according to the manufacturer's protocol. The content of heat shock protein 1A was determined using the SEB081Hu Enzyme-linked Immunosorbent Assay Kit For Heat Shock 70kDa Protein1A(HSPA1A) from Homo sapiens (Human) (Cloud-Clone Corp., USA), and the amount of alpha-actin-2 was determined using Human α-Smooth Muscle Actin (α-SMA) ELISA Kit from Homo sapiens (Human) (Cloud-Clone Corp., USA). The statistical significance of differences between groups was determined using the nonparametric Kruskal-Wallis test and the Mann-Whitney U-test.
As a result of statistically significant differences in the content of ACTA2 between patients with bipolar disorder (164.85[151.05;187.95] ng/ml), depression (166.875[146.535;194.775]ng/ml) and healthy individuals (165.75[160.425; 178.575]ng/mL) is off-white, pairwise comparisons also showed no difference. However, significant differences between the studied groups were found in the content of heat shock 70kDa protein1A (HSPA1A). Pairwise comparison revealed that these differences arise due to an increase in the level of this protein in patients with bipolar disorder (0.8356 [0.5948; 1.098] ng / ml), in comparison with healthy individuals (0.6135 [0.5123; 0. 7722]ng/ml, Mann-Whitney U Test $p = 0.016$). The HSPA1A protein belongs to a family of highly conserved heat shock proteins expressed or induced in response to various stressors. They are involved in the synthesis and transport of proteins, and when exposed to stress factors, they prevent misfolding and aggregation of proteins (Benarroch 2011). It is known that these proteins are involved in the embryonic development of the central nervous system, and also participate in neuroprotection preventing the death of neurons (Reed-Herbert et al. 2006). According to literature sources, immune disorders were found in bipolar disorder during acute episodes of mania or depression (Barbosa et al., 2014; Brietzke et al., 2009; Cunha et al., 2008; Ortiz-Domínguez et al. 2007 Tsai et al. 2012). Also in the study by K. Becking et al. was found to overexpress HSPA1A in monocytes of patients with bipolar disorder during a depressive episode (Becking K, et all, 2015). In addition, based on the data of the model of protein-protein interactions common for HSPA1A and brain beams (A.M. Humyra et all, 2022), it is highly likely that HSPA1A is involved in the pathogenesis of BD. However, there are works on the association of the HSPA1A protein with paranoid schizophrenia, which may indicate common pathogenetic processes in these diseases, and this issue requires further study. Thus, the HSPA1A protein may be directly involved in the pathogenesis of BD and be proposed as an additional paraclinical criterion for BD in the further study of its role in this pathology.

### S9.726. The role of miRNA in the mechanisms of CNS plasticity and the possibility of using it for the protection of cognitive impairment

Grinkevich L.N.[1]*
[1]*Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia;*
* larisa_gr_spb@mail.ru

The main challenges in studying the molecular basis of long-term memory formation are associated with the multitude of signaling systems, the integration of which is necessary for successful learning, and the variety of regulatory processes that interact at the genome level. The latter include the regulation of gene expression through DNA-binding transcription factors, as well as epigenetic modifications that

regulate the structure of chromatin, or messenger RNA (mRNA) biogenesis through microRNA. MicroRNAs gained increased attention of researchers due to their importance in the development of the nervous system, in the formation of synaptic plasticity and in long-term memory, and due to high potential of the use of miRNAs for the treatment of diseases associated with cognitive impairment [1]. In addition, miRNAs entering the extracellular space can serve as intercellular communicators and potentially biomarkers for diseases diagnostics. MicroRNAs are highly conserved, small endogenous RNAs capable of repressing up to 70% of protein-coding mRNAs. At the same time, one microRNA is able to regulate the expression of an entire network of genes (often several tens) and, accordingly, its dysfunction can cause a large polygenic effect. The greatest amount of miRNA is expressed in the CNS, and the expression spectrum varies in different structures and cellular elements of the brain, and biogenesis is regulated by neuronal activity. In connection with the foregoing, and taking into account the significant complexity of the CNS structure, the studies of microRNAs functions have been studied very fragmentarily.

An important role in the study of epigenetic processes in the mechanisms of plasticity is played by animals with a relatively simple structure of the CNS, in particular, mollusks. We use the development of a conditioned reflex of food aversion in the Helix mollusk, which is based on a change in the efficiency of synaptic transmission between neurons that lie in the network of this reflex. We have shown that the formation of this reflex involves both microRNAs potentially necessary for the inhibition of mRNAs that negatively affect the mechanisms of plasticity, and microRNAs involved in the activation of genes necessary for plastic rearrangements. Further studies, carried out in collaboration with Institute of Cytology and Chemistry of the Siberian Branch of the Russian Academy of Sciences, showed that during Helix training, several dozens of different conserved microRNAs are differentially expressed, with expression increasing in half of the microRNAs and suppressed in the other [2]. Among the miRNAs found by us, there are several homologues involved in the formation of LTP in vertebrates and humans. In addition, we carried out a comparative analysis of microRNAs expression in well-learning and poorly learning animals with dysfunction of the serotonergic system subjected to the learning procedure. These studies allowed us to confirm the important role in the LTM formation of several miRNA families, including the MIR-10 family, which is the most represented in the CNS Helix, the MIR-33, MIR-133, MIR-153 families, and to show the important role of the serotonergic system in the regulation of microRNA expression [2]. Changes in the metabolism of a number of microRNAs in animals with dysfunction of the serotonergic system, along with epigenetic changes in the structure of chromatin and modification of transcription factors, through impaired expression of downstream genes, may underlie the impairment of long-term memory associated with defensive behavior in Helix. Our data on the involvement of a number of conservative microRNAs in the Helix memory formation, which play an important role in the mechanisms of plasticity in various animal species, including vertebrates, confirm and expand the idea that the molecular mechanism the of long-term memory formation, including epigenetic marking, is conservative phenomenon in the course of evolution. The development of various learning models, including those with cognitive impairments in different animal species, the use of the latest genome and epigenome editing technologies, allows us to hope for progress in this most complex area of research. Recent advances in this field, as well as prospects for the use of miRNAs as target targets for improving cognitive impairment associated with microRNA dysfunction in neurodegenerative, neurological and age-related dysfunctions, will be discussed in the report. Optimism in this area is associated with the advent of CRISPR/Cas genome-editing technologies aimed at RNA [3].

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**S9.727. The role of microviscosity of the cytoplasmic membrane of tumor cells during chemotherapy**

Shimolina L.E.[1]*, Khlynova A.E.[1], Druzhkova I.N.[1], Ignatova N.I.[1], Zagaynova E.V.[2], Kuimova M.K.[3], Shirmanova M.V.[1]
[1]*Privolzhsky Research Medical University;*
[2]*Lobachevsky State University of Nizhny Novgorod, Russia;*
[3]*Imperial College London, United Kingdom;*
* shimolina.l@mail.ru

Membrane microviscosity plays an important role in cell biophysics, controlling the rate of diffusion and transport, and the activity of many enzymes and receptors. A change in the value of microviscosity and/or lipid composition in a living cell can signal serious disorders, including the transformation of a normal cell into a cancer cell. Understanding the role of membrane microviscosity in the response of tumor cells to chemotherapy is important, since the cell membrane is actively involved in drug transport. Recent studies suggest that the response of a tumor to chemotherapy is determined not only by the interaction of the drug with the primary target (eg, DNA), but may include multiple physiological and physicochemical changes. Of particular interest is the study of the properties of the cytoplasmic membranes of tumor cells and the changes that develop during antitumor therapy. The study of the effect of chemotherapeutic drugs on the viscosity of living cells is important for a better understanding of the mechanisms of drug action and evaluation of the effectiveness of therapy.

This work is aimed to study the microviscosity of the plasma membrane of cancer cells using a fluorescent molecular rotor BODIPY2 and fluorescent lifetime imaging microscopy FLIM during chemotherapy with platinum drugs.

The study was carried out on cultured cancer cells CT26 (mouse colorectal cancer), HCT116 (human colorectal cancer) and oxaliplatin-resistant cell line - HCT116-OXAR. Animal studies were performed on Balb/c and nu/nu mice with subcutaneously transplanted tumors. The molecular rotor fluorescence lifetime was recorded using an LSM 880 confocal microscope (Carl Zeiss, Germany) equipped with a TCSPC-based FLIM module (Becker&Hickl Inc., Germany). Microviscosity was measured in the plasma membranes of individual cells using a BODIPY2 fluorescent molecular rotor (excitation 850 nm, reg. 500–550 nm). Cells were treated with cisplatin (Teva, Israel) at a dose of 2.6 μM (IC50) for CT26 and oxaliplatin (Teva, Israel) at a dose of 4.0 μM (IC50) for HCT116. To study the chemical composition of lipids, time-of-flight mass spectrometry of secondary ions was used on a ToF-SIMS 5 instrument (ION-TOF Gmbh, Germany). For each sample, 12 mass spectra were obtained in the mode of both positive and negative ions. The yield of secondary lipid ions was calculated as the intensity of the corresponding lipid peak, normalized to the total number of ions.

Protocols for imaging the microviscosity of membranes of living tumor cells were developed for models with different organization: monolayer cell cultures, spheroids, and subcutaneous tumors in mice. We have demonstrated for the first time that membrane microviscosity can be measured in subcutaneous tumors in vivo with subcellular resolution

using FLIM with a viscosity-sensitive water-soluble molecular rotor BODIPY2.
During the work, a significant increase in the microviscosity of membranes of viable CT26 cells was recorded 24 h after incubation with cisplatin from 322 ± 21 cP to 400 ± 27 cP. Incubation of HCT116 cells with oxaliplatin also led to an increase in membrane microviscosity from 437 ± 77 cP to 593 ± 139 cP after 24 h of incubation with the chemotherapy drug. The data are in good agreement with each other in different models, no dose-dependent effect was found. Using the ToF-SIMS mass spectrometry method, an increase in the amount of cholesterol and a decrease in the content of unsaturated fatty acids in the membranes were detected after 24 hours.
To test the obtained effects on the membrane viscosity, we analyzed the change in microviscosity in chemoresistant HCT116-OXAR cells under the action of oxaliplatin. Incubation with oxaliplatin did not affect the membrane microviscosity, the values were ~450 cP. We assume that the recorded increase in membrane microviscosity at the late stages of incubation with platinum-containing drugs (24 h) is part of the response of the tumor cell to exposure and is not associated with the direct interaction of the drug with the membrane.
The perfomed study expands the understanding of the mechanisms of tumor response to treatment and the actions of chemotherapy drugs, which is important for the search for new antitumor targets and methods for monitoring the effectiveness of therapy. The data obtained can be useful in the development of new types of anticancer treatment and improvement of existing ones. This work was supported by the Russian Science Foundation, grant no. 23-74-00045.

**S9.728. The role of the nitrergic system in the mechanisms of brain damage induced by long-term exposure to the pesticide rotenone**

Bashkatova V.G.[1]*
[1]*P. K. Anokhin Research Institute of Normal Physiology;*
* v.bashkatova@nphys.ru

In modern conditions, the human body is experiencing significant stress associated with a rapidly increasing number of adverse environmental factors. The latter include the growing use of genetically modified (GM) foods. It is known that GM plants lose sensitivity to pesticides, so a significant amount of pesticides accumulated by GM plants can enter the human body with food. Recently, there have been publications linking the increased prevalence of Parkinson's disease (PD) among agricultural workers with constant contact with pesticides. Rotenone (a mitochondrial complex I inhibitor) is one of the most commonly used broad-spectrum pesticides. It has been found that rotenone has the ability to selectively damage dopaminergic neurons in the brain [1]. Recent reports have shown that nitric oxide (NO) is involved in the formation of dopaminergic neurotoxicity [2; 3]. However, despite a significant number of studies, the possible involvement of NO the mechanisms of development of neurodegenerative processes is confirmed mainly by works that use substances - analyzers (metabolic precursors/donors of NO or inhibitors of NO synthase), as well as indirect methods for determining NO (nitrate content/nitrite, etc.). In our work, to determine NO, we used the method of direct electron paramagnetic resonance, developed by prof. A.F. Vanin [4;5]. The aim of this work was to study the role of the nitrergic system of the brain during repeated long-term administration of a low dose of the pesticide rotenone.
In our work it was found that with acute administration of rotenone in a wide range of doses (doses of 1 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg), there were no disturbances in animal behavior, which was recorded in the catalepsy test. A single exposure to the indicated doses of the pesticide did not change in the NO content in the brain structures of rats. Only 20 days after daily administration of rotenone at a dose of 2 mg/kg, a significant increase in NO formation in the striatum of the rat brain was found. At the same time, no signs of catalepsy were observed in these animals. On the 30th day of pesticide administration, the rats demonstrated akinesia in the catalepsy test. Along with this, a noticeable increase in NO production was also observed in the striatum and cerebral cortex of rats. After 60 injections of rotenone at a dose of 2 mg/kg, the maximum NO values were found in the striatum, which is considered one of the main dopaminergic structures of the brain. At the same time, the rats showed pronounced signs of stable catalepsy. Preliminary administration of the selective inhibitor of inducible NO synthase aminoguanidine partially prevented the increase in NO levels induced by rotenone in rat brain structures on the 50th and 60th days of drug administration. During these periods, aminoguanidine also reduced the intensity of catalepsy caused by prolonged administration of the pesticide.
Thus, as a result of the work, it was shown that long-term exposure to a low dose of the pesticide rotenone leads to the development of parkinsonism and increased NO generation in the striatum and cerebral cortex of rats. As a result of our studies, it was established that the rise in the level of NO in the striatum preceded the appearance of the first signs of catalepsy. These data indicate the prognostic value of determining the parameters of the nitrergic system system of the brain in the development of neurodegenerative processes. The data obtained indicate that the nitrergic system of the brain is one of the most important functional systems of the body, which ensures the regulatory processes of the body and the maintenance of homeostasis under the influence of environmentally unfavorable environmental factors.
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**S9.729. The sexual dimorphism in the morpho-functional organization of astrocytes in replicative "passage" aging model**

Shirokova O.M.[1]*, Vasilchikov P.I.[1,2], Korotchenko S.A.[1], Pershin V.I.[1,2], Chernov Ya.V.[1], Davydov D.I.[1,2], Kozlyaeva E.V.[1], Shchelchkova N.A.[1,2], Mukhina I.V.[1,2]
[1]*Privolzhsky Research Medical University, 603005, Nizhny Novgorod, pl. Minin and Pozharsky, 10/1;*
[2]*National Research Lobachevsky State University of Nizhny Novgorod., 603022, Nizhny Novgorod, Gagarin Ave., 23;;*
* shirokovaom@gmail.com

It was previously assumed that cells in male and female animals and humans have the same molecular pathways in ontogenesis, and therefore, in order to save money, all studies, including those that formed the basis of evidence-based medicine, were carried out mainly on males (Clayton, 2016). However, later it turned out that many differences in the response to various types of treatment in men and women lie not only in endocrine regulation, but also in genetic sexual dimorphism at the level of intracellular components of cells in organism (Mauvais-Jarvis et al., 2020). Since mitochondria-endoplasmic contacts (MERCs)

are not only highly conserved cellular components, but also central participants in most diseases, the study of their properties in the context of biological sex becomes important for further deep understanding of pathogenetic mechanisms.
The aim of the work was to search for the sexual dimorphism of brain glial cells during aging, depending on the presence of sex chromosomes, and to determine the role of MERC in this process.
Aging in the primary culture of cerebral cortex astrocytes was achieved by a replicative "passage" aging model. Functional, molecular, and structural differences between cell cultures containing XX- and XY-sex chromosomes were evaluated after genotyping of newborn mice from which the cortex cells were taken. The expression of a number of genes was assessed by real-time PCR, the functional state of cells was studied using calcium imaging using Fluo-4-AM calcium dye (Thermo Fischer, USA). Immunocytochemical labeling of astrocytic cells made it possible to identify the cells, the state of contacts between mitochondria and the endoplasmic reticulum was assessed using transmission electron microscopy, and the content of NSE, S100, BDNF, and estradiol was detected using enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone, China).
Quantitative determination of proteins and estradiol in the cell medium and cell lysates by ELISA showed no statistically significant differences between cells containing different sex chromosomes. Calcium imaging in vitro revealed a statistically significant difference in the calcium activity of astrocytic cells depending on the sex chromosomes at passages 8 and 9. The duration and frequency of calcium oscillations in the XX genotype was higher than in the XY genotype. Differences in the viability of glial cells between genotypes at late passages were found: in the XX genotype, the viability of cells is higher than in cells with the XY genotype. The difference in the expression of several genes was determined: snpH; stat3; MFN; IgF1; Il6; cGamp. The volume of astrocytic cells of the XX genotype was larger than that of the XY astroglia. At the same time, no ultrastructural differences were found in the qualitative analysis between astrocytes of different genotypes for sex chromosomes.
Thus, data have been revealed that prove the existence of differences in the functional properties (calcium activity, expression of a number of genes) and morphometric parameters (cell volume) of astrocytes during aging, depending on the presence of sex chromosomes, which indicates the need for further study of the sexual dimorphism of somatic cells.
The study was supported by the Russian Science Foundation, grant No. 22-15-20043.

### S9.730. The study of NO content in the skeletal muscles of rats in the deficit of movement by EPR-spectroscopy

Zaripova R.I.[1]*, Jafarova G.G.[1], Andrianov V.V.[1,2], Gainutdinov Kh.L.[1,2], Sungatullina M.I.[1], Ziyatdinova N.I.[1], Zefirov T.L.[1]
[1]*Institute of Fundamental Medicine and Biology;*
[2]*Kazan E. K. ZavoiskyPhysical -Technical Institute (KPhTI);*
* ratno1992@mail.ru

In the conditions of the industrial and urban environment life of modern man offers the organism a form of vital activity of its organs and systems far from the level of motor activity and safety from the necessary requirements inherent in evolution. There is extensive and convincing experimental and clinical evidence about the destructive effect that the lack of movement has on the body's organs and systems. There is a decrease in the load on the muscular apparatus, which leads to changes in functional and morphological changes to pathological conditions depending on the duration and degree of hypokinesia. It is known that during movement deficit, the hind limbs of rats are under-loaded and significant structural changes in the muscles in the form of dystrophy are detected. NO is an important modulator of cellular activity in many tissues in vertebrates and invertebrates. NO is able to interact with a variety of substances - thiols, proteins, sugars, metal ions, heme proteins, etc., localized in a variety of tissues and organelles, which suggests the presence of NO and its complexes in various tissues. The nitric oxide system, which plays a role in the activation of antioxidant enzymes, limits the stress response. The electron paramagnetic resonance method was used to investigate the intensity of nitric oxide production by analyzing the amount of NO-containing paramagnetic complexes in the tissues of calf muscle of rats growing under conditions of movement deficit. Restriction of motor activity in penile cells was 30 days. Rats of the control group were kept under conditions of natural motor activity, 4-5 animals per cage. Due to the short lifetime of NO, which manifests itself in its low concentration in tissues, the method of electron paramagnetic resonance (EPR) is the most expedient to detect and quantify NO. The method is based on the reaction of a radical (in this case NO) with a spin trap - we applied the Fe2+ complex with diethyldithiocarbamate (DETC), which allows to capture NO and form a stable triple complex (DETC)2-Fe2+-NO in animal tissues. Nitric oxide production was evaluated by the intensity of the EPR signal belonging to the (DETC)2-Fe2+-NO complex. The signals were compared by the integral intensity value, since the integral intensity of the EPR signal is directly proportional to the concentration of paramagnetic complexes. Tissues of animal calf muscle were taken for the study. The EPR spectra of the prepared samples were recorded on an ER-200E-SRC Bruker EMX/plus X-band EPR spectrometer with an ER 4112HV temperature attachment at 77 K. The following parameters were kept constant in all experiments: microwave power of 30 mW, modulation of 5 G, amplification of 4 × 104 , time constant of 100 ms, spectrum recording time of 50 s, and number of accumulations of 8. It was found that the amount of NO in the skeletal muscles of rats growing under hypokinesia conditions did not differ from those of the control group.

### S9.731. The study of morphometric parameters of platelets using electron microscopy

Obydennyi S.I.[1,2]*, Kuznetsova A.A.[3], Kireev I.I.[3], Panteleev M.A.[1,2,3]
[1]*Centre for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russian Federation ;*
[2]*National Scientific and Practical Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation;*
[3]*Moscow State University, Moscow, Russian Federation;*
* obydennyj@physics.msu.ru

Platelets are non-nuclear blood cells measuring 2-4 micrometers. Their main function is to assemble a hemostatic thrombus at the site of vessel injury. There are a number of inherited diseases in which morphological changes in the structure of platelets are observed: Wiskott-Aldrich syndrome, MYH9-associated syndromes, Gray Platelet syndrome, Germansky-Pudlak syndrome, Paris-Trousseau syndrome, Chediak-Higashi syndrome. Morphological abnormalities can be divided into abnormalities of the platelet cytoskeleton, alpha and dense granules, and membrane abnormalities. Diseases affecting the structure or number of granules usually require confirmation by transmission electron microscopy.
The purpose of this work is to develop a method for analyzing the morphometric parameters of platelets using electron microscopy.
Results. The methods of sample preparation of platelets for electron transmission microscopy were debugged and the parameters for the morphometric evaluation of platelets were selected. The following indicators were selected: the ratio of platelet sizes, the number of alpha granules per platelet and their size, platelet area and the ratio of the number of granules to this area. A set of norms was produced for comparison with the results of patient analyzes for selected indicators.
Conclusion. Electron microscopy in the clinical diagnosis of platelets plays an important role in disorders associated with dense and alpha granules, as this is the only method that allows you to visualize and reliably determine their condition and number. To a lesser extent, electron microscopy is used to confirm cytoskeletal or membrane

abnormalities. The uniqueness of this method is that it allows us to consider the structure of such small structures.
This work was supported by the Russian Science Foundation grant 21-75-00109.

**S9.732. Tissue engineering as a means to investigate fundamental processes in cardiology and biophysical features of cardiac tissue formation**

Tsvelaya V.A.[1,2]*, Slotvitsky M.M.[1,2], Berezhnoy A.K.[1,2], Shcherbina S.A.[1], Aitova A.A.[1], Nizamieva A.A.[1], Kovalenko S.G.[1,2]
[1]*Moscow Institute of Physics and Technology National Research University;*
[2]*Moscow Regional Research and Clinical Institute ("MONIKI");*
* vts93@yandex.ru

At the moment there is a lot of controversy about the dependence of cardiovascular diseases on the phenotype of cardiac cells obtained in the stage of heart formation and in its development throughout the life of the patient. There are examples of cardiac arrhythmias acquired from taking medications, from stressors, as a consequence of other diseases [1,2,3]. In this case, external factors can affect cardiac tissue irreversibly, changing their phenotype and functionality. One of the tasks of modern medicine, therefore, can be considered a comparison of the factors leading to a pathological phenotype depending on the genotype and regardless of it. The present work is a study of cardiac tissue formation and electrophysiological properties of cardiomyocytes under different conditions that set the phenotypic manifestations of functional differences in cardiomyocytes during maturation of cardiac tissue in vitro.
The most important results of the presented study can be considered:
1) Identification of stages and correlations with cardiac embryogenesis in the development, maturation, and functionality of patient cardiomyocytes obtained in vitro during differentiation [4].
2) Creation of a test detecting the risk of arrhythmias both under the influence of external factors and congenital patient-specific pathologies [5,6]
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**S9.733. Transcranial neuromorphic stimulation (tNMS) as the most universal way to activate brain neuroplasticity of the brain and devices for its implementation**

Ponomarenko A.[1]*
[1]*Energotex;*
* alex.ponomarenko@mail.ru

Methods of transcranial brain stimulation have been developed for more than 100 years. At the same time there is a constant expansion of parameters of applied electrical signals. Before 70s of the last century mainly electrical influence of stun character was applied: electronarcosis, electrosleep, electroshock therapy.
The method of micropolarisation (hereinafter referred to as tDCS) suggested by N.P.Bekhtereva initiated a series of techniques of transcranial stimulation differing in the form and frequency of electrical influence. At the same time, the principle of weak influence remains unchanged, the one that does not cause effects on sensory organs. For transcranial stimulation it became the norm to limit the average current to 2 mA, and 5 mA in the amplitude of the pulse.
The task of development was to find optimal parameters of exposure. A simplified model of the electrical circuit during transcranial stimulation was created in order to find the optimal form theoretically. It included the following elements: electrode, skin, bone tissue, dura mater, gray matter, white matter, brain ventricles. More than 200 cells connected with neighboring cells were used in the model. The software package TINA V12 by DesignSoft was used for modeling.
Current in the forms of the most known transcranial stimulation methods ( tPCS, tACS, tVNS, tRNS ) was passed through the conditional circuit "forehead"-"occiput" of the model. The shape and amplitude of the signals on the model cells were analyzed.
The greatest waveform distortion is expectedly recorded in the most common group of methods - tPCS ( TES, meso-diencephalic modulation, tVNS, etc.). All these methods are united by a rectangular pulse shape, characterized by a high rate of signal rise and fall. Unipolar and bipolar pulse waveform signals receive the same level of distortion.
Distortion of the form leads to critical values of currents and voltages in some tissues. Thus the highest voltage is formed in the skin, and the highest current in the brain ventricles. These distortions that can explain the side effects of the used signals.
As a result of experiments on the model, an electric pulse of the optimal shape was selected. The basis of the pulse shape is a harmonic oscillation with an exponential decay. Such pulse is practically not distorted on living tissue models. The signal shape turned out to be very similar to a single nerve impulse. The name for the group of methods using such signal form was suggested - transcranial neuromorphic stimulation (tNMS).
To verify the results an electronic generator capable of forming all known forms of transcranial stimulation signals, including a constant component, was created. Output cascade is fully coordinated for direct connection of electrodes. For safety purposes, a battery supply is used and current limitation in a pulse at the level of 5 mA is structurally ensured.
The first version of the generator "Zybbio" had dimensions of 20x30x6 mm, the last version of full-featured generator "Neuravin" occupies an area of 2 sq.cm and is powered from a 1.5V cell.
Comparative experiments with pulses of known form and newly designed showed that exposure to the proposed pulses in a wide range of repetition frequencies (from 7Hz to 500Hz) at maximum exposure (5mA in amplitude) does not cause side effects in the form of irritation under the electrodes, stun and others.
The device has been successfully applied in the following cases:
1. Female, 35 years old, complete loss of smell (anosmia) within 7 months (after Covid19). Received 10 sessions of 30 minutes each day. The pulse repetition rate was 60 - 100Hz. Appearance of odor perception fact without any classification - after the first session. Partial recovery within 14 days.
2. Male, 37 years old. Distorted perception of smels (hyposmia) for more than 3 months. Improvement of correct perception during a course of stimulation, 10 days of 30 minutes daily. The pulse repetition rate is 60 - 90 Hz. Recovery of the complete classification of odors within a month.
3. Female, 82 years old. Hip fracture 7 years ago, unstable gait, spontaneous falls. The innervation of the legs is not disturbed. Two courses of therapy were conducted with an interval of 1 month. Pulse repetition rates are 11 Hz and 60 - 90 Hz. Spontaneous falls stopped, the shape

of the foot was corrected, the gait became more stable. Support tool (walking stick) it began to be used extremely rarely.
The circuit designs and software developed make it possible to create devices for a wide range of applications, from scientific research (including on small animals) to medical devices, as well as for people wishing to learn the capabilities of the brain or improve athletic performance.
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### S9.734. Transcranial pulsed current stimulation normalizes the concentration of fructosamine in rats on a fructose and fat enriched diet

Chabanetc E.A.[1], Zanin S.A.[1], Kade A.Kh.[1], Trofimenko A.I.[1,2,3]*
[1]*Kuban State Medical University;*
[2]*Scientific Research Institute – Ochapovsky Regional Clinical Hospital no. 1;*
[3]*Kuban State Technological University;*
* artemtrofimenko@mail.ru

The diet of a modern person includes a large number of affordable high-calorie foods that are enriched with saturated animal fats, sugar and fructose. The problem is exacerbated by the fact that the consumption of this category of products causes "food addiction". Such a diet significantly increases the risk of developing insulin resistance, impaired glucose tolerance and type II diabetes. Currently, methods of non-invasive modulation of brain regions responsible for decision-making and food reward mechanisms by electric current are being actively studied. Of particular interest among them are the methods of transcranial pulsed current stimulation, in particular, TES-therapy, in which there is a selective effect of bipolar pulsed current on the structures of the antinociceptive, stress-limiting system of the brainstem.
Objective: to study in dynamics the effect of TES-therapy on the serum concentration of fructosamine in an experiment in rats on the background of fructose and fat enriched diet.
Materials and methods: The study protocol was approved by an independent ethics committee. All painful interventions were performed under zoletyl-xylazine anesthesia. Two weeks before the start of the study, all animals were implanted with subcutaneous electrodes for electrical stimulation (on the forehead and back of the head), with a magnetic system for non-contact connection of the electrical stimulator outputs.
Characteristics of animal groups: group 1 (n = 60, control) - standard diet (3000 kcal/kg); group 2 (n = 60, comparison) - diet enriched with fructose and animal fat (4400 kcal/kg); group 3 (n = 60, main) - a diet enriched with fructose and animal fat (4400 kcal/kg) and TES therapy. The diet of rats was modified by adding fructose and rendered pork fat to the standard feed in the proportion (66:17:17), 10% fructose (by weight) was added to the drinking water.
At the control points of the study: on the 30th, 60th and 90th day, 20 animals from each group were euthanized. The determination of fructosamine in blood serum was carried out by a set based on the reduction of nitro blue tetrazolium.
TES-therapy was performed in animals of group No. 3 using the TRANSAIR-03 device (Centre for Transcranial Electrical Stimulation, Russia) with the following parameters: pulsed bipolar mode, pulses with a duration of 3.75 ± 0.25 ms, current strength 0.6 mA , current frequency 70±2 Hz, session duration 30 min., frequency 1 time per day, throughout the entire study period. Thus, in the main group: 20 rats received 30 sessions, 20 rats received 60 sessions and 20 rats received 90 sessions.
Statistical processing of the results was carried out using GraphPadPrism 7 (GraphPad Company, USA). Checking the normality of the distribution of quantitative traits in the study groups was carried out using the Shapiro-Wilk criteria and distribution histograms. The median and interquartile interval were used to describe the data. The dynamics of the studied parameters was expressed as a percentage change, intergroup differences as a percentage. When comparing the three groups, the Kruskell-Wallis test was used, further pairwise comparisons were performed using the Dunn post-hoc test.
Results: On the 30th day from the start of the study, there were no differences in the serum concentration of fructosamine between the study groups (pkw = 0.86). On the 60th day, intergroup differences were noted (pkw = 0.0010), with subsequent post-hoc analysis in group No. 2 vs. No. 1, the content of fructosamine was 15.3% higher (pd = 0.0006). When comparing group No. 1 vs No. 3 (pd = 0.27) and group No. 2 vs No. 3 (pd = 0.14), there were no statistically significant differences. On the 90th day, intergroup differences were also noted (pkw < 0.0001), with subsequent post-hoc analysis, it was found that the content of fructosamine in group No. 2 vs No. 1 was 31.2% higher (pd < 0.0001) and in group No. 2 vs No. 3 it was 17.1% higher (pd = 0.0089). When comparing group No. 1 vs No. 3, there were no statistically significant differences (pd = 0.18). In an intragroup analysis of the concentration of fructosamine in dynamics according to the control points of the study: in group No. 1, a decrease in the indicator by 18.1% (pkw = 0.0051) was revealed, in group No. 2 an increase by 9.1% (pkw = 0.0278) and in group 3 without statistically significant differences (pkw = 0.38).
Conclusion: It was shown that in rats with a diet enriched with fructose and animal fat on the 60th and 90th days of the study, the serum concentration of fructosamine increased, which indicates the development of a carbohydrate metabolism disorder in them. The concomitant use of TES-therapy was accompanied by a statistically significant normalization of the concentration of fructosamine, which was most clearly manifested by the 90th day of the study.

### S9.735. Transcriptomic analysis of DNA repair pathways in radioresistant sublines of human non-small cell lung cancer

Pustovalova M.[1]*, Guryanova A.A.[1], Sorokin M.I.[1,3], Suntsova M.V.[3], Alhaddad L.[1], Buzdin A.A.[1,3], Osipov A.N.[2], Leonov S.V.[1]
[1]*School of Biological and Medical Physics, Moscow Institute of Physics and Technology;*
[2]*State Research Center-Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency ;*
[3]*Sechenov First Moscow State Medical University;*
* pu.margo@mail.ru

Lung cancer is now the leading cause of cancer death worldwide, with 85% of diagnosed cases being non-small cell cancer (NSCLC). Radiotherapy is one of the main methods of treatment for patients with NSCLC. However, during therapy, tumor cells may be selectively selected for clones that have an advantage in DNA repair in a given microenvironment or therapeutic context, and thus acquire resistance, leading to metastasis and cancer recurrence. The curative potential of radiotherapy depends on its ability to cause a reproductive death of tumor cells via accumulation of non-repairable DNA lesions, thereby removing cancer cells from the clonogenic pool. Therefore, identifying

key components of tumor cell DNA repair signaling pathways and then targeting them is an attractive strategy to counteract radioresistance. The aim of this work was a transcriptomic analysis of DNA repair pathways in NSCLC cells that survived after fractionated exposure to IR. Radioresistant sublines of non-small cell lung cancer cells differing in the p53 status, A549 (p53 wild type) and H1299 (p53 deficient) were obtained. Exposure to ionizing radiation was carried out using a standard protocol consisting of exposure at a dose of 2 Gy once a day, 5 days a week until a total dose of 60 Gy. Irradiation survived cells showed a decrease in radiosensitivity, as well as the increased ability to anchorage-independent growth. Transcriptome analysis revealed 322 differentially expressed genes (log10(control)>1, |log2FC|>1) between irradiation survived and control A549 cells and 1628 differentially expressed genes from irradiated and control H1299 cells. A549HR and H1299HR cells showed activation of survival signaling pathways and G2/M cell cycle progression involving ATM kinase. In A549HR cells, activation of the BRCA1 pathway was found, which causes large-scale chromatin decondensation. Activation of the ATR kinase to repair spontaneous DNA DSBs by homologous recombination was also found in these cells. In H1299HR cells, homologous recombination is activated via the Fanconi anemia pathway, which includes the RAD51 recombinase and the tumor suppressors BRCA1 and BRCA2. The data obtained are extremely important for the development of antitumor therapy, since the simultaneous inhibition of ATM and components of homologous recombination and non-homologous joining of the ends of DNA repair pathways can contribute to the successful therapy of patients with NSCLC.

**S9.736. Type 1 K channel and Epilepsy**

Kodirov S.A.[1,2,3]*
[1]*Institute for Physiology and Pathophysiology, Johannes Kepler University, Linz, Austria;*
[2]*Pavlov Institute of Physiology, Russian Academy of Sciences, Saint Petersburg, Russia;*
[3]*University of Texas at Brownsville, Department of Biological Sciences, Texas 78520, USA;*
* skodirov@gmail.com

Voltage-dependent K+ (Kv) channels are diverse, comprising the classical Shab - Kv2, Shaker - Kv1, Shal - Kv4, and Shaw - Kv3 families. The Shaker family alone consists of Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv1.6, and Kv1.7. Moreover, the Shab family comprises two functional (Kv2.1 and Kv2.2) and several 'silent' alpha subunits (Kv2.3, Kv5, Kv6, Kv8, and Kv9), which do not generate K current. However, e.g., Kv8.1, via heteromerization, inhibits outward currents of the same family or even that of Shaw. This property of Kv8.1 is similar to those of designated beta subunits or non-selective auxiliary elements, including ADAM or AMIGO. Kv channels and, in turn, ADAM may modulate LTP. Prevailingly, Kv1.1 and Kv1.5 are attributed to respective brain and heart pathologies. The aforementioned channel proteins are apparently involved in several brain pathologies, including schizophrenia and seizures.

**S9.737. Validation of mathematical models for the assessment of vasculature thrombogenicity**

Salikhova T.[1,2]*, Ponomarev I.A.[1], Pushin D.M.[1], Ivlev D.A.[1], Uzlova S.G.[1], Guria G.Th.[1,2]
[1]*National Medical Research Center for Hematology, Moscow, Russia;*
[2]*Moscow Institute of Physics and Technology, Dolgoprugny, Russia;*
* salikhova.ty@gmail.com

The analysis of rapidly developing biological processes with the help of physical methods is the task of modern biophysics. Blood aggregate state transition is a prominent example. The mechanisms responsible for the change in blood aggregate state underlie a number of serious pathologies, such as stroke, heart attack, pulmonary embolism.
Evaluation of the potential thrombogenicity of a certain vessel remains an urgent problem. To date, several mathematical approaches have been developed for investigation of blood flow features in vessels of various geometries: stenotic vessels, aneurysms, arteriovenous fistulae for haemodialysis [Carroll et al. 2020, Vassilevsky et al. 2020]. These approaches are used for the analysis of blood coagulation in the so-called "normal" person. However, the results obtained with described approaches do not take into account the personalized features of the anatomy and physiology of patients. Due to the development of modern physical methods of medical imaging (MRI, CT, dopplerography and angiography), it becomes possible to obtain detailed information about the geometric structure of any vasculature elements and the properties of blood flow. The question is to what extent these personalized data can be used for the assessment of intravascular thrombotic risks triggered by the stability loss of blood liquid state [Erdemir et al. 2020].
One of the methods for assessing the risks of thrombus formation in intense blood flow was published in the recent paper [Pushin et al. 2021]. The aim of the present work is to develop an experimental in vitro method for validating the results of numerical modeling of platelet activation in personalized vascular configurations.
The technique that allows creating 3D silicone castings accurately reproducing geometric features of patients' vessels was developed. For this purpose, ultrasound and MRI diagnostics data were used. The developed technique was applied for the investigation of platelet activation in arteriovenous fistulas for hemodialysis. The latter type of vascular configuration is characterized by intensive blood flow and a high degree of thrombogenic danger.
As part of the developed approach, 3D printing was used to create master models that reproduce the geometry of real vessels. Then, on the basis of master models, hermetic castings were made from biologically neutral silicone. The castings were included in the experimental circuit [Ivlev et al. 2019], through which blood was pumped. The processes of blood clotting were detected optically, by means of digital video filming, and also acoustically, using ultrasonic Doppler methods. In addition, the state of the blood coagulation system in some cases was analyzed using standard methods of aggregometry and thromboelastography.
Conducted experiments showed that the manufactured hermetic silicone castings reproduce the geometry of the patient's vessels with an accuracy of micrometers. The casting material itself did not cause contact activation of blood coagulation. At the same time, aggregometry and thromboelastography showed that the degree of general thrombogenicity of the casting significantly depends on the intensity of intrinsic blood flow. When the casting was perfused with blood plasma (or whole blood), it was possible to detect the appearance of fibrin and platelet microclots in the flow. The experiments have shown that the main assumptions of derived mathematical models could be controlled. The authors believe that the developed experimental technique allows validating mathematical models for the assessment of vascular configurations thrombogenicity degree (stents, fistulas, etc.).
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**S9.738. What is human consciousness**

Ivatitsky G.R.[1]*
[1]*Institute for Theoretical and Experimental Biophysics RAS. 142290, Moscow region Pushchino, Institutskaya st. 3, Russian Federation;*
* ivanitsky@iteb.ru

It is shown that one of the significant results of human creativity in the 21st century was the creation of android robots equipped with artificial intelligence. The level of perfection of these robots is becoming so high that it will soon be impossible to establish their difference from living people by their external features and behavior. This leads to the logical fallacy of equating humans and creative android robots, assuming they are conscious. The existing tests by A. Turing and J. Searle are untenable. They expand the understanding of the phenomenon of consciousness, but the problems with its definition do not disappear. The report provides evidence that the application of the term consciousness to robots can lead to serious consequences: to the substitution of the real world of the external environment by the virtual world.
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**S9.739. professional antigen-presenting cells maturation after co-cultivating with murine glioma cells photoinduced by tetra(aryl) tetracyanporphyrasine**

Redkin T.S.[3]*, Saviuk M.O.[3,1], Sleptsova E.E.[3], Turubanova V.D.[3], Vedunova M.V.[3], Krysko D.V.[3,1,2]
[1]*Cell Death Investigation and Therapy (CDIT) Laboratory, Department of Human Structure and Repair, Ghent University, Ghent, Belgium;*
[2]*Cancer Research Institute Ghent, Ghent, Belgium.;*
[3]*National Research Lobachevsky State University of Nizhny Novgorod.;*
* big.t.nsdav@outlook.com

Our previous studies demonstrated the immunogenic potential of dead/dying GL261 glioma cells induced by pz II-based photodynamic therapy (PDT). Photoactivated cells emit damage-associated molecular patterns (DAMPs) such as ATP and HMGB1, and exhibit calreticulin (CRT) on the cell membrane. DAMPs recognition activates dendritic cells maturation.
The aim of this study was to evaluate the maturation of dendritic cells co-cultured with PDT-activated glioma cells in vitro.
To confirm the maturation of dendritic cells, we assessed the level of exposure of CD86 cell receptors on the surface of dendritic cells. First, GL261 glioma cells were activated by pz II-PDT. Subsequently, dead glioma cells were co-cultured with naive dendritic cells isolated from the C75/Bl7 mouse bone marrow for 24 h. Dendritic cell maturation was assessed by flow cytometry using anti-CD86-eFluor 450 dye (eBioscience), which binds to CD86 on the surface of dendritic cells (CD11c+).
CD86 receptors were actively exposed on the surface of dendritic cells indicating their maturation. However, to verify the complete antigen presentation process, it is necessary to consider the phagocytic activity of dendritic cells, and analyze the levels of the main signaling molecules IL-12p70 and IL-6 produced by activated dendritic cells.
The study was supported by the Russian Science Foundation grant No. 22-25-00716, https://rscf.ru/project/22-25-00716/

## S10. Biophysical education

**S10.740. Approaches to teaching mathematical disciplines for Biophysicists at the Siberian Federal University**

Sukovataia I.E.[1]*, Shishov V.V.[1], Sutormin O.S.[1,2], Sukovatyi L.A.[1], Samoylova A.A.[1]
[1]*Siberian Federal University;*
[2]*Surgut State University;*
* ISukovataya@sfu-kras.ru

The main problems of teaching and learning mathematics and computer science in the educational programs of biophysicists are such as: lack of motivation and misunderstanding by students of the need for knowledge and skills in the field of mathematical sciences in their future professional activities, standardized "simplified" processing of experimental results using applied software packages, the ability to use open on-line resources for calculations, it often leads to the denial of the need for mathematical skills, teaching "pure" mathematics without the specifics of biological sciences, poor school preparation, etc. In addition, the digital transformation of the economy and all spheres of life, including teaching, learning and research activities, the transition to a hybrid (mixed) model of learning and academic communications in scientific and innovative activities, the need for Life Long Learning (LLL), interdisciplinary competencies for new professions: hard-skills + IT-skills + soft-skills, requirements of the Federal State Educational Standard, etc. modern challenges determine the development of new approaches to teaching and learning mathematical sciences for biophysicists. In particular, interdisciplinary competencies for new professions, for example, in the field of bioinformatics, genomics, proteomics and other omics sciences are mentioned in various documents of the Ministry of Education and Science, in particular as an list of basic knowledge and skills in the development and application of genetic technologies, including genomic editing technologies, in order to update educational programs based on them higher education and additional professional education. In this regard, to attract talents and build a globally competitive human resource potential for the research and development sector by ensuring the formation of a set of key interdisciplinary competencies for research, information and educational activities in the field of bioengineering and biotechnology, biophysics, bioinformatics, biochemistry and medical biology, bioecology, genetics, genomics and proteomics, etc. the Siberian Federal University (SibFU) has developed and is implementing a target graduate model in the Biophysics profile, which includes professional interdisciplinary competencies (biotechnology, biophysics, bioinformatics, biochemistry and medical biology, bioecology, genetics, genomics and proteomics), "digital" skills (data analytics, machine learning, artificial intelligence, programming, etc.) and "soft" skills (project activities, teamwork, etc.). For general professional competencies aimed at the application by graduates of methods

of mathematical analysis and modeling, theoretical and experimental research, the acquisition of new mathematical and natural science knowledge using modern educational and information technologies and to understand the principles of modern information technologies and their use to solve problems of professional activity, as well as understanding the principles of modern information technologies and using them to solve the tasks of professional activity, indicators for achieving these competencies have been developed. A professional competence has been developed aimed at developing the ability of biophysics graduates to collect, process and analyze scientific and technical information to solve the tasks of professional activity in the field of biological sciences using modern information technologies, with indicators, for example, such as "uses basic knowledge of fundamental sections of mathematics and bioinformatics to the extent necessary for information processing and analysis of biological data, including in accordance with the tasks of genetics, genomics and genetic technologies", "applies modern programming languages to adapt machine learning algorithms to tasks formed by the subject of scientific research", etc.

For the formation of competencies in these interdisciplinary areas, the module "Mathematical methods and computer technologies in biology" has been developed, the content of which includes, among other topics, the following sections: Mathematics and Fundamentals of Statistics for Biologists, Applied biological statistics and programming Elements, Elements of classification theory, Biometrics, Mathematical modeling of biological processes, Data Science in Biology, Bioinformatics, Omix data analysis.

New Master's degree programs in the field of applying machine learning and artificial intelligence methods to solve problems in biology, ecology and medicine: "Genomics and Bioinformatics" and "Biomedical Data Science" (in English, completely online) are open and implemented at the university for the development of new professional interdisciplinary competencies of biophysics graduates.

### S10.741. Computer Reconstruction of Networks of Protein-Protein Interactions: Educational and Research Aspects

Orlov Y.L.[1,2,3]*, Turkina V.A.[1], Orlova N.G.[5], Anashkina A.A.[1,4], Savina E.A.[1,4]

[1]*I.M.Sechenov First Moscow State Medical University (Sechenov University);*

[2]*Peoples' Friendship University of Russia;*

[3]*Institute of Cytology and Genetics SB RAS;*

[4]*V.A.Engelhardt Institute of Molecular Biology RAS;*

[5]*Financial University under the Government of the RF;*

* y.orlov@sechenov.ru

Biophysical education requires the use of modern computer tools for modeling protein-protein interactions. The use of online bioinformatics tools makes it possible to reconstruct both protein and gene networks, and develop modeling skills for students. We consider the issues of computer reconstruction of gene networks - complexes of interacting macromolecules - using a list of genes associated with a particular disease, or a complex disorder based on public online bioinformatics tools - STRING-DB, GeneMANIA, Metascape, Cytoscape applications. Examples of computer construction and visualization of gene networks of oncological diseases - glioma, breast cancer, as well as complex mental disorders such as Parkinson's disease, schizophrenia, which were published in co-authorship with students in Russian and international journals in recent years [1, 2 ,3].

The use of only online bioinformatics tools is educational in nature, focused on students, both in mathematics and in natural sciences and medical disciplines, who do not have enough skills in computer science, programming, and writing their own code. Automatic construction of lists of genes associated with a disease using open databases (OMIM, GeneCards.org, MalaCard.org), computer reconstruction of gene networks, calculations of enrichment statistics for gene ontology categories have been successfully mastered by students and presented in a series of theses and scientific publications. The problems of mastering educational materials by students on the basis of teaching at NSU, FEFU, Peoples' Friendship University of Russia, the Financial University under the Government of the Russian Federation, the First Moscow State Medical University, I.M. Sechenov of the Ministry of Health of Russia.

The tasks of digitalization of medicine, the development of IT technologies are a priority in Russia. The epidemic situation that has existed in recent years and the forced transition to distance learning had accelerated the adoption of measures to change the formats of education, the emergence of new learning platforms [4]. Of particular interest is the computerization and automation of teaching the disciplines themselves related to informatics, including in medicine - in the fields of telemedicine, e-health [5].

The issue of developing training courses in biophysics and bioinformatics is related to the need to adapt training to the profile of education of students and trainees. According to the experience of teaching bioinformatics, mathematics students require not only a different presentation of the material, but also the methodology itself, in contrast to medical students and students of natural sciences. Let us note a number of qualitatively new tasks of education in the field of digital healthcare, such as the use of blockchain technologies, the use of Artificial Intelligence (AI) methods in support of medical decision-making [5]. An educational course has been developed that includes a theoretical part (listening to the course in the form of lectures, video lessons) and a practical part - performing tasks on the use of computer programs and databases that have found a number of applications for medical problems in the reconstruction and analysis of networks of interactions of macromolecules [1,6,7].

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### S10.742. Department of Biophysics and Biotechnology of Voronezh State University: scientific and educational processes, their organizers and partners

Artyukhov V.G.[1]*, Antipov S.S.[1], Kalaeva E.A.[1], Nakvasina M.A.[1]
[1]*Department of Biophysics and Biotechnology;*
* artyukhov@bio.vsu.ru

Currently, the Department of Biophysics and Biotechnology of Voronezh State University is a graduating department for students in the areas: 06.03.01 Biology, profile Biophysics (full-time and part-time education), 06.04.01 Biology, profile Biophysics, 06.06.01 Biological sciences, profile Biophysics and specialties 30.05.02 Medical Biophysics and 30.05.03 Medical Cybernetics.
Bachelors of the profile Biophysics are preparing for researching in biotechnology, medicine, agriculture, ecology and nature conservation, molecular cell biology, immunology. The plan of profile training of bachelors (2-4 years) includes disciplines: "Modern methods of biophysical research", "Biophysics of membrane and cellular processes", "Physics of enzymes", "Computer research and modeling of bioprocesses", "Special practicum", "Structure and functions of biomacromolecules and their complexes", "Radiation and photobiophysics", "Biomedical nanotechnologies". The arsenal of various practices includes an educational training practice on the department of biophysics and academic institutes of the Russian Academy of Sciences in Pushchino-on-Oka.
Graduates of the master's program "Biophysics" are prepared for research and pedagogical activities in the field of general and medical biophysics, bioinformatics, molecular biology, biotechnology. The master's training plan includes special disciplines: "Molecular biology and biophysics", "Regulation of intercellular processes and interactions", "Photophysics, photochemistry and photoimmunology of blood components", "Intracellular signaling pathways and methods of their regulation", "Fundamentals of commercialization of biophysical research". During the period of study, research work is carried out continuously.
Education in an enlarged group of specialties 30.00.00 Fundamental medicine began at the Faculty of Medicine and Biology of Voronezh State University in 2016, in 2022 the first graduation of biochemists and cybernetics doctors took place, in 2023 the first biophysicists will graduate.
The organization of the educational process for students of the medical branch of Voronezh State University differs from that in medical universities, since students are more research-oriented than graduates of "classical" medical institutes and universities. It means that the teaching of fundamental disciplines (biology, chemistry, physics, computer science, etc.) are not only and not so much a basis for higher education, but their close integration with classical clinical disciplines, the presence of synthetic courses ("Medical Biophysics", "Mathematical statistics in medicine", "Physiological cybernetics", "Clinical cybernetics", "Biophysical foundations of functional diagnostics", "Information medical systems", "Computer analysis of medical data and images", etc.).
The implementation of high-quality biophysical education at the University is currently impossible without the participation of external partners: municipal schools, potential employers, academic research institutes.
Thus, employees of the Department of Biophysics and Biotechnology of the Voronezh State University conduct lectures and practical classes with school students, participate in the preparation of individual research projects with them on the basis of the department. This allows not only to expand and deepen the knowledge of students in the field of physical and chemical biology, but also to form an individual scientific and educational trajectory of the future applicant, as well as adapt it to research work at the university. School students present individual research projects at university conferences in the natural science cycle (in particular, the League of Innovations at VSU), winning which gives additional points for admission.
Attracting potential employers to the implementation of the educational process in the bachelor's degree can significantly increase the level of professional competence of graduates. An example of mutually beneficial cooperation is the interaction of the Department of Biophysics and Biotechnology of the Voronezh State University with the companies "EFKO", "BioCad" and other biotechnological and pharmaceutical enterprises in the real sector of the economy. Students of the department get the opportunity to undergo an internship on the basis of these enterprises without interrupting the educational process during the holidays with compensation for living expenses and wages.
At the stage of preparing masters and graduate students, in addition to attracting enterprises from the real sector of the economy, academic research institutes, it is advisable to involve students in the activities of professional scientific communities, participation in competitions for the implementation of research and development work.
The results of such cooperation are the employment of qualified graduates of the department in leading academic research institutes and enterprises of the real sector of the economy and effective promotion along the "career ladder", the integration of the scientific activities of the department into solving applied problems of enterprises and major fundamental research of academic organizations in Russia and abroad. Cooperation with external partners, intensive communications with scientific institutions, research and production enterprises, medical organizations, multi-level training of graduates, continuous improvement of the scientific and pedagogical qualifications of employees, strengthening the material and technical base of the department and increasing the level of funding for work - the basis of modern scientific and educational Process of the Department of Biophysics and Biotechnology of the Voronezh State University.

### S10.743. Design of interdisciplinary competencies in the new master's program "Medical and Biological Physics"

Sukovataia I.E.[1]*, Kratasyuk V.A.[1,2], Salmina A.B.[3,4], Sukovatyi L.A.[1], Deeva A.A.[1], Sutormin O.S.[1]
[1]*Siberian Federal University;*
[2]*Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Federal Research Center 'Krasnoyarsk Science Center SB RAS;*
[3]*Laboratory of Experimental Brain Cytology, Department of Brain Sciences, Research Center of Neurology, Moscow, Russia;*
[4]*Research Institute of Molecular Medicine & Pathobiochemistry, the Krasnoyarsk State Medical University named after Professor V. F. Voyno-Yasenetsky;*
* ISukovataya@sfu-kras.ru

Biophysical education is no longer limited to any one professional field or sphere of activity. Currently, biophysicists are increasingly working in interdisciplinary fields, including biomedical physics.
The development and further implementation of the interdisciplinary English-taught master's degree program "Medical and Biological Physics" meets global challenges and their interdisciplinary nature, changes in the landscape of higher education and the transition to a hybrid model of teaching and learning [1, 2], and also aims to train specialists of a new generation with new interdisciplinary competencies in the field of biomedical physics, with an understanding of the role of science to solve health problems, who possess modern information technologies (big data analytics, machine learning, artificial intelligence, programming, etc.), as well as "soft" skills (intercultural and linguistic competencies, teamwork, empathy and emotional intelligence, systemic and critical thinking, adaptability, the ability to self-study in the mode of Life Long Learning, etc.),

which can effectively compete at the regional, national and global labor markets.
The aim of the program Medical and Biological Physics is to train specialists capable of utilizing the existing tools and developing new approaches for handling the contemporary problems in medical and life sciences. The participants will become familiar with the concepts and methods of physics that are applied in research and diagnostics. The program includes general courses aimed at training professional, digital and soft skills, as well as two separate educational trajectories that students can master to pursue their future career: Basic and Applied Neurobiology or Bioluminescent Biotechnologies in Life Sciences.
Participation in the development of the program and its further implementation by partner organizations, including international ones, such as the Laboratory of Experimental Neurocytology of the Brain Research Department of the Scientific Center of Neurology (Moscow), Krasnoyarsk State Medical University named after Prof. Voino-Yasenetsky, University of Cadiz (Spain), Department of Medical Elementology and Toxicology of Hamdard Jamai University (India), Institute of Biophysics (Siberian Branch, the Russian Academy of Sciences), affirms to the relevance and demand for the training of unique specialists in such interdisciplinary and practice-oriented areas of medical and biological physics for the regional, national and international market.
Features and competitive advantages of the program include the following aspects:
- design of the program in the paradigm of intended learning outcomes, the design of which is based on reverse design technology
- modular program structure and increased project and practical training
- blended learning model and the use of distance learning technologies, including for the opportunity of implementing the program by foreign and Russian partner organizations
- formation of a learning system through the integration of science and education, updating content and learning technologies in the context of digital transformation, improving the quality of learning through integration into the international space
- practical orientation of the program, when students' scientific research will take place in modern equipped laboratories in research groups of partner universities too
- cross-cultural environment of the teaching staff and students
- the opportunity of implementing English-language academic mobility modules with partner universities, including foreign ones, including virtual and individual mobility
- student-oriented approach to learning through flexible personalization of educational tracks, selection of topics of scientific directions, joint management of research by undergraduates of teachers of their partner organizations, etc.

The implementation of the program expands the list of English-language programs of the university in addition to the Master's degree program "Biological Engineering" implemented in English, the semester module "Bioluminescent Biotechnologies" and the higher qualification training programs "Biophysics" / PhD's Program "Biophysics" and meets the goals of developing biomedical education in SibFU from international comparability to international competitiveness [3].
The interdisciplinary and practice-oriented program will contribute to the development of fundamental applied problems of medical and biological physics and the training of unique specialists whose competencies meet the priority directions of the development of science and technology in medical and biological physics and the problems of health-saving technologies.
The project "New International Master's Program "Medical and Biological Physics" in English is implemented by the winner of the Competition for grants for teachers of the master's program of the charitable program "Vladimir Potanin Scholarship Program" of the Vladimir Potanin Charitable Foundation (grant agreement No. GSGC-0028/21)
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## S10.744. Methodological support for remote lessons in medical and biological physics

Digurova I.I..[1]*, Machneva T.V..[1], Digurova A.I..[1]
[1]*Pirogov Russian National Research Medical University;*
* digurova56@mail.ru

The education system has experienced the most restrictions and the need to comply with quarantine requirements during the COVID - 19 pandemic [1]. The forced transition to remote learning has made the issue of methodological support for distance learning relevant. At the Department of Physics and Mathematics of the Pirogov Russian National Research Medical University, when studying the discipline "Medical and Biological Physics", both in traditional classroom and remote learning, students can use, in addition to textbooks, video lectures and methodological developments on the topics of classes. Video presentations or presentations with an audio "track" and cards with assignments for the practical part of the laboratory work were prepared for the remote conduct of a laboratory workshop at the department. However, such a set, in our opinion, is not sufficient. When studying physics and biophysics at a medical university, one often has to deal with reduced motivation, which is especially noticeable when working remotely. When conducting classes online, the role of visibility increases. Another problem is the implementation of remote modular control. In this regard, we have developed and tested sets of methodological materials for remote laboratory and practical classes in medical and biological physics, conducted in real time.
The increase in students' interest is facilitated, first of all, by the selection of specialized tasks related to the diagnostic and therapeutic use of physical factors, the study of the body from the point of view of physics, and the operation of medical devices. As in traditional classes, the materials were selected taking into account the direction of the students' training (general medicine, pediatrics or dentistry). For each topic, presentations were made on solving professionally oriented problems in medical physics and biophysics. Taking into account the training of students of non-physical specialties, simple tasks of the training type with a detailed analysis of the solution were used. Also, to enhance motivation, it seemed useful to draw up diagrams demonstrating interdisciplinary connections and the possibility of practical application of the studied phenomena in medicine [2]. To repeat the main theoretical questions, presentations with a small number of slides were used, not duplicating lectures, but focusing students' attention on the most important issues. In the future, when preparing distance learning, you can add other forms of knowledge consolidation, for example, a glossary. Also, if time permits, presentations on additional material not included in the lectures can be used in class. Small messages with text, graphics or animation illustrations can be made by the students themselves. This will increase their activity, independence and interest in the material being studied, thereby contributing to the formation of professional competencies.

When conducting modular control, a combined knowledge test was justified: a written survey and an interview via video conference using such a didactic tool as a blitz survey [3, 4]. For the written part, at least 15 ticket options were compiled for each topic. With their help, knowledge of the formulations of laws, schemes, graphs, formulas, skills in solving graphic or calculation problems were tested. When interviewing in the blitz mode - a survey, not only the quality, but also the speed of the answers made it possible to assess the level of the student's preparation. To conduct such surveys, a bank of tasks for each topic was prepared.

The approach used to organize remote classes and its methodological support made it possible to make learning more effective and improve interaction between the teacher and students. Preparation for a distance lesson is time-consuming, so it would be optimal to distribute the topics studied among teachers and create a single methodological complex. Considering remote classes as a temporary phenomenon, attention should be paid to the possibilities of integrating distance and traditional classroom learning formats.

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**S10.745. Model of integrated academic MSc-PhD program**

Evstigneev M.P.[1]*
[1]*Sevastopol State University;*
* max_evstigneev@mail.ru

The current situation with PhD education.

Research postgraduate studies currently form the basis of the system for the reproduction of scientific and pedagogical personnel in the developed countries of the world. Moreover, the effectiveness of the institution of postgraduate studies correlates with the technological development of the state and, in this regard, has an economic aspect.

There is a strong opinion that in the Russian Federation the institution of postgraduate studies is relatively ineffective: statistics indicate that only 30% of PhD graduates receive a Ph.D. degree, and no more than a third of them subsequently engage in scientific activities. One of the components of this problem is the disunity of university and academic science that is characteristic of the modern Russian system.

Let us consider the problem of elevating the efficiency of the institute of integrated academic master's-PhD studies (IAMP) on the basis of the world-class scientific and educational center "Marine Sciences, Technologies and Regional Ecosystems" (SEC), created with the participation of Sevastopol State University (SevSU) in cooperation with five institutes of the Academy of Sciences.

General principles of IAMP

1. "Product" of IAMP is a candidate of sciences in the field of natural sciences, possessing research competence, as well as the knowledge and skills necessary for organizing independent research activities.

2. The performance indicators of IAMP are: 1) the fraction of undergraduates who continued their research trajectory in graduate school, or as a full-time researcher, 2) the fraction of graduate students who submitted their scientific work to the dissertation council on time (i.e. during the period of postgraduate study).

3. Continuity of master's and postgraduate curricula. It assumes the formation of a conditionally "single" curriculum "2+4"-six-year educational process, a feature of which is its design, taking into account the scientific direction and the qualifying scientific task that will be solved by the future graduate of the master's program - a graduate student - at the stage of preparing a PhD thesis.

4. Continuity of scientific management of the student's research program. Assumes that in the ideal case, the supervisor of the educational program at the stage of master's and postgraduate studies is the same leading scientist.

5. The priority of designing IAMP as "a research program that includes an educational component, and not as an educational program that includes a research component." This approach primarily reflects the principle of deep integration and continuity of research and educational processes.

Within the framework of the general approaches to the formation of IAMP, described above, the institute of integrated master's and PhD studies, implemented on the basis of cooperation between universities and research institutes of the Sevastopol SEC, has the following focuses:

1. The main customer and consumer of the IAMP graduate are the research institutes participating in the SEC.

2. The instrument for the implementation of IAMP is the so-called "department of research type": this is the basic department of the research institute, created at the university, in which there is a research laboratory that conducts research on jointly coordinated thematic research plans of the university and the research institute. The supervisors of the main educational programs of master's and/or postgraduate studies, as a rule, are staff members of research institutes.

3. The model of the educational process at the stage of the magistracy is problem-centered, i.e. focus on solving a scientific problem by students within the framework of an actual scientific problem (research topic). Within the framework of the subject, a set of specific research tasks is formed. The group of undergraduates is divided into subgroups, each of which is assigned one task from the pool. The solution of this problem forms the basis of the future master's graduate qualification work and the basis of the candidate's dissertation of the most talented graduates.

Experience in implementing the IAMP model

The prototype of the IAMP model has been implemented by us on a regular basis for a relatively long time, since 2005, in the direction of preparing the master's program "Physics" profile "Biophysics" and postgraduate studies in the specialty 03.01.02-biophysics (physical and mathematical sciences). The topics of scientific research and the curriculum were built in a single logic starting from the 3rd year of undergraduate studies up to postgraduate studies under a single subject of research - the complexation of biomolecules - imposing certain specifics on the content and list of training courses. From 2005 to 2014, according to this curriculum, 8 Ph.D. theses in the specialty "Biophysics" were defended strictly on time for the completion of postgraduate studies by applicants who were graduates of this master's program. The disadvantage of this experience was the lack of partners from the Academy of Sciences as "customers" of young researchers in this area, as a result of which most of the defended graduate students "settled" at SevSU as lecturers with partial degradation of research competence, or immigrated abroad.

Starting from 2021, on the basis of the Institute for Advanced Studies of SevSU, four full-fledged IAMP cases are already being implemented with admission to the graduate school of academic partner institutions:

- "Physics" profile "Biophysics",
- "Physics" profile "Satellite Oceanology",
- "Biology" profile "Hydrobiology" and "Botany and functional genomics".

- "Ecology and nature management" profile "Natural and technical systems".
In these cases, it was possible to achieve a level of cooperation between the university and academic institutions, which has not yet been in the Sevastopol region, which indicates the prospect of increasing the efficiency of academic postgraduate studies.

**S10.746. Problems of teaching medical technology as a component of the discipline "Medical and biological physics" and ways to solve them**

Machneva T.V.[1]*, Digurova I.I.[1]
[1]*Pirogov Russian National Research Medical University;*
* machneva_tv@mail.ru

Modern medical technology is used for diagnosis, treatment, rehabilitation and prevention. In accordance with the federal law of November 21, 2011 N 323FZ "On the fundamentals of protecting the health of citizens in the Russian Federation" [1], patients can be provided with high-tech medical care, including, among other things, assistance using robotic equipment, information technology and genetic engineering methods. Thus, physicians must use sophisticated and often expensive equipment in their daily practice.
In the Pirogov Russian National Research Medical University according to the Federal State Educational Standard 3++ for the professional training of doctors, for example, in the specialty 31.05.01 "General Medicine" [2], the mathematical and natural science cycle includes the disciplines "Physics, Mathematics", "Medical and Biological Physics" with a total labor intensity of 3 credits (108 academic hours) each. Such a volume is not sufficient for obtaining theoretical knowledge, acquiring the necessary skills and abilities, and, consequently, mastering professional competencies in the use of high-tech equipment. Another problem is the weak basic knowledge of students in physics. In connection with the above, the introduction of a new discipline "Medical equipment"could contribute to the engineering training of future doctors, taking into account the modern development of technologies for the creation and operation of medical equipment.
Practical classes in the discipline "Medical equipment" should include the mandatory solution of problems in physics and biophysics, combining fundamental and special knowledge, as well as a laboratory workshop using modern medical devices and devices [3]. At present, the integration of distance and traditional classroom learning allows the use of new forms of work. Interaction with teachers of clinical departments is also important.
A serious issue is also the level of knowledge of teachers of the departments of physics and mathematics, medical and biological physics regarding the creation and use of modern medical equipment. The inclusion of classes on the design and operation of complex medical equipment is a topical issue.
The proposed ways of solving the problems of teaching medical equipment will contribute to a more complete mastery of future doctors with the knowledge that allows them to work with modern medical and technical equipment.

**S10.747. Solving problems in physics and biophysics in different types of classes at medical university**

Digurov R.V.[1]*, Digurova I.I.[2]
[1]*Technological Institute for Superhard and Novel Carbon Materials;*
[2]*Pirogov Russian National Research Medical University;*
* roman.digurov@yandex.ru

The study of life phenomena using physical concepts and methods cannot be overestimated from the standpoint of the formation of medical competencies among students of a medical university. Professionally-oriented tasks in physics and biophysics integrate fundamental and special knowledge, create skills and abilities necessary in the future activities of a doctor, and an emphasis on interdisciplinary connections enhances students' motivation to study these disciplines, increases their activity, develops the ability for logical and reasoned analysis. It is desirable that the solution of problems in the learning process at the departments of physics and biophysics be systematic. However, the use of this element of educational work in a practical lesson can be difficult. Here one should take into account, firstly, the insufficient level of school preparation of first-year students in physics. This factor affects the level of mastering physics and biophysics by students in non-core universities. And secondly, it can be difficult to eliminate the gap in the inability to solve problems in the first year because of the lack of time due to the small number of study hours. To find ways to solve this problem, we studied the possibility of using tasks in different types of classes in physics, mathematics, medical and biological physics with first-year students of a medical university: laboratory-practical, seminary and control.
At the seminar-type classes, tasks were offered related to the study of the body from the point of view of physics, the use of physical processes, phenomena and devices for diagnosis and treatment. It seemed important to use problems when considering questions on the biophysics of tissues and organs, cell biophysics, modeling of biophysical processes, the impact on a person of external physical fields and fluxes of radioactive radiation. It is useful to include tasks in homework to consolidate the acquired knowledge and skills. Taking into account the fact that classes are held with students of non-physical specialties, the proposed tasks were not difficult. When discussing theoretical material in laboratory classes, tasks related to a specific laboratory experiment were solved, including graphical ones that most clearly reflect functional dependencies [1]. The use of such tasks in a laboratory workshop removed the issue of insufficient time for solving problems, increased interest in conducting an experiment, and improved the quality of reports on laboratory work. The experimental part of the laboratory-practical lesson in this case becomes the development of the theoretical and computational parts [2]. If the duration of the lesson allows, then simple tasks can also be used in the defense of laboratory work for a more complete assessment of the acquired knowledge and skills. An important criterion for the assimilation of the material is the quality of solving profile problems during control classes [3]. This method of control stimulates the cognitive activity of students. It turned out to be especially effective during the forced transition to distance learning. The tasks included in the tickets for modular control made it possible to effectively check the assimilation of the professionally oriented content of physics and biophysics by future doctors. Profile physical tasks can also be used in the work of a student scientific circle at the departments of physics and biophysics. Research situational tasks model professional activity to a greater extent. The practice of compiling tasks by students with the help of a teacher on the topic of scientific research seems interesting.
Thus, the use of specialized tasks in different types of classes largely eliminates the problem of lack of time to solve them, increases students' interest in studying physics and biophysics, and contributes to the formation of doctor's competencies.

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### S10.748. The course "History of Biology and Biophysics" as a tool for self-determination of a biophysicist student in scientific activity

Samoylova A.A.[1,2]*, Kratasyuk V.A.[1,2]
[1]*Siberian Federal University;*
[2] *Federal Research Center "Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences";*
* samalice@mail.ru

Currently, it is in the interests of the state to attract talented young people to the research field, as evidenced, for example, by the initiation of a new federal project "Popularization of science and technology" and other programs.
Success in educating a young scientist consists in building a chain of a schoolboy - a junior student - a senior student and/or a graduate of a bachelor's degree - a master's student - a postgraduate student - a young scientist. This approach is implemented by the Department of Biophysics of the Institute of Fundamental Biology and Biotechnology of the Siberian Federal University.
It is possible and necessary to work with each of the listed categories of researchers in a certain specificity.
In this connection, in order to solve one of the tasks - the task of involving first-year students in research activities - the Department of Biophysics of the Siberian Federal University has developed courses "History of Biology" and "History of Biology and Biophysics". The first one studied by students of Biology (whose distribution to the specialty of Biophysics occurs after the 2nd year) and students of Physics, specialty Biochemical physics (set from the 1st year).
The course for both specialties takes place in 1st semester in the 1st year of study and amounts to 108 credits. It is implemented in a blended learning model using the SFU electronic information educational environment based on LMS Moodle, which allows you to combine work in a digital environment with classical lectures, as well as seminar classes, such as discussions, game and creative tasks in full-time mode, presentations, etc.
The discipline includes three main blocks:
• from proto-knowledge to natural history (from primitive society to the Renaissance);
• from natural history to modern biology (biology of Modern times to the middle of the XIX century);
• establishment and development of modern biology (from the middle of the XIX century to the beginning of the XXI century).
The course examines as historical facts - great discoveries that influenced the development of biology and biophysics, also the emergence and transformation of scientific knowledge and concepts, and philosophical problems of the methodology of science. In the classroom, one talk about the criteria of scientific knowledge, the role of errors and scientific misconceptions in subsequent research, ethical aspects in science, science as a social institution and many other.
For a better assimilation of the course, the student is invited to present himself as a great scientist or compare him and his character traits when preparing a report on a scientist.
In the third module of the course, students get acquainted with the activities of modern scientists. These can be lectures, meetings, seminars on topics, scientific and popular science events. This approach allows students to meet with advanced developments and discoveries of our time, to learn the success stories of contemporaries. Separately, in practice, the scientific method, project activities, grant applications, analysis of scientific articles, principles of presentation of scientific work - reports and presentations are worked out with each student.
At the end of the semester, students are invited to take a survey, the purpose of which is to assess the student's adaptation to the institute and readiness to engage in scientific activity.
181 students were interviewed from 2021 to 2023 academic years. Answers to the question "Do you have a desire to become a scientist?" they were distributed as follows:
yes - 128 (71%)
no - 53 (23%)
It should be noted that some students have previously expressed a desire to be a scientist, but often in the process of learning they strengthened their choice.
Thus, new principles and methodological approaches for attracting young people to engage in scientific research in biophysics, mechanisms for transferring biophysical knowledge into the educational process are proposed. The proposed approaches are implemented in the framework of the courses "History of Biology" and "History of Biology and Biophysics" to solve the problems of self-determination of the younger generation in the field of biophysics.

### S10.749. Use of information technologies in the physical workshop of a medical university

Lysenko E.P.[1]*, Reznikov I.I.[1]
[1]*Federal State Autonomous Educational Institution of Higher Education N.I. Pirogov Russian National Research Medical University., Moscow, Russia;*
* elysenko1@mai.ru

In the course of medical and biological physics at the Department of Physics and Mathematics of the Russian National Research Medical University. N.I. Pirogov in the section "Medical equipment" students get acquainted with the classification of medical equipment; a general scheme for the collection, transmission and registration of medical and biological information; devices for collecting and converting biomedical information (electrodes and sensors); classification of sensors and their characteristics. The principle of operation of sensors and their characteristics (transformation function, sensitivity) are studied by students doing laboratory work using models of two parametric sensors: strain-resistive and inductive. In the laboratory work "Studying the operation of an electrocardiograph", students get acquainted with the structural diagram of an electrocardiograph; record an ECG in three standard leads from an ECG simulator; calculate the characteristics of the ECG (height of the teeth; the duration of the intervals, the angle of inclination of the dipole moment of the "heart"), and also take the frequency response of the electrocardiograph amplifier and get acquainted with the possible frequency and amplitude distortions of the signals in the amplifiers.
To acquaint students with modern methods of collecting, presenting and processing medical and biological information at the Department of Physics and Mathematics, laboratory work was developed using MacLab and LabVIEW systems.
In the work "Using the MacLab - Macintosh system for automating cardio-hemodynamic studies", registration and standard analysis of ECG were carried out, as well as spectral analysis of the ECG was performed and the variability of R-R intervals was assessed; the speed of propagation of the pulse wave was determined using the methods of electrocardiography and sphygmography. The variability of R-R intervals was estimated by constructing Poincaré plots, which express the dependence of the values of the current R-R interval on the R-R interval immediately preceding it. From these plots, histograms of R-R intervals and delta R-R intervals were obtained. Analysis of Poincaré plots is a clinical method for quantifying heart rate variability and allows for the evaluation of the complex effects of different branches of the nervous system on cardiac activity.

The use of the LabVIEW system made it possible to register and process various physical quantities on a computer using special sensors: biopotentials, blood pressure, temperature, etc. Any combination of various electronic devices, such as generators, oscilloscopes, voltmeters, frequency meters, etc., was created on one computer. At the same time, the visualization of the results obtained on the computer display was significantly improved, and it was possible to register the obtained data in an electronic journal. Several laboratory works using the LabVIEW computer system were devoted to the study of electrical methods for measuring muscle effort; body temperature monitoring at various points with a thermistor sensor; studying the method of ultrasonic echolocation; registration and analysis of the spectral characteristics of the ear at the threshold of hearing; the study of the physical foundations of obtaining and processing electrocardiograms.

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## S11. New methods in biophysics

### S11.750. Distant Non-contact Fungicidal Effect of Electromagnetic Waves on the Development of Bipolaris sorokiniana Fungi

Vorobyov N.I.[1]*, Kovalenko N.M.[2], Popova E.V.[2], Tolmachev S.Yu.[1]
[1]*All-Russian Institute for Agricultural Microbiology;*
[2]*All-Russian Institute for Plant Protection;*
* Nik.IvanVorobyov@yandex.ru

The electromagnetic effect on phytopathogenic fungi and their development has been little studied. Currently, only chemical fungicides are being developed and applied. Chemical fungicides require the development of a technology for their large-scale production and the development of an effective technology for spraying these preparations in agricultural areas. In this situation, non-contact exposure of plants to electromagnetic waves that can transmit signals about the presence of fungicides in the environment and prevent the development of phytopathogenic fungi can be preferable.

The objective of this study was to find a way to modulate electromagnetic waves with a fungicidal signal. To solve this problem, an experiment was conducted with 6 Petri dishes.

The experiment's description.

To solve the problem, a nutrient medium was placed on all 6 Petri dishes, and disks (d=0.6 mm) with the pathogenic fungus Bipolaris sorokiniana were placed in the center of these dishes. On dishes No. 1, 2, the fungicide "Titl" was additionally added at a concentration of 0.005%, and on dish No. 3 - at a concentration of 0.01%. After that, dish No. 1 was installed on one generator of electromagnetic waves (100 Hz; RF patent No. 2297392) (dish No. 4 on top of it), and dish No. 3 on the other generator (dish No. 5 on top of it). In the experiment, dishes No. 1(4), 3(5) were continuously irradiated with electromagnetic waves for seven days, and dishes No. 2 and 6 were located far from the generators of electromagnetic waves and separately from each other.

The discussion of the experimental results.

After seven days of continuous irradiation on dishes No. 1(4), 3(5), the mycelium of the fungus on dishes No. 1, 3 and 2 did not develop, since the fungicide was present in the nutrient medium on these dishes. At the same time, on dishes No. 4 and 5 the phytopathogenic fungus formed a colony constituting 40% and 10% of the dish's area, respectively, although fungicide was also absent on these dishes. We believe that electromagnetic waves, passing through dishes No. 1 and 3, received a modulation that transmits a false signal to the phytopathogen wich located on dishes No. 4 and 5. Therefore, the result of a false signal was a slow growth of fungi mycelium on dishes No. 4 and 5. At the same time, the fungus colony grew more slowly on dish No. 5 than on dish No. 4. Perhaps this effect is associated with different concentrations of the fungicide on the corresponding dishes No. 3 and 1 (0.01% and 0.005%).

Thus, it can be argued that electromagnetic waves are able to transfer quantitative and qualitative information about the presence of a fungicide in the environment and, by transferring this information, slow down the development of a phytopathogenic fungus on any nutrient media. Consequently, biophysical studies of the impact of electromagnetic waves on biological objects will make it possible to create the least expensive protective agricultural technologies for plants.

### S11.751. Efficacy of cell penetration by boron-containing aptamers - novel potential boron delivery agents for boron neutron capture therapy

Novopashina D.S.[1], Dymova M.A.[1], Davydova A.S.[1], Meschaninova M.I.[1], Malysheva D.O.[1]*, Kuligina E.V.[1], Richter V.A.[1], Kolesnikov Y.A.[1], Taskaev S.Y.[2], Vorobyeva M.A.[1]
[1]*Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia;*
[2]*Budker Institute of Nuclear Physics SB RAS, Novosibirsk, Russia;*
* d.malysheva@g.nsu.ru

Glioblastoma multiforme is a common aggressive type of brain tumor. The heterogeneity and high invasiveness of this tumor make it resistant to traditional radio- and chemotherapeutic treatments. The two-year survival rate with the current approach to treatment is 26.5% [1], and the rate of long-term survival (>10 years) is 0.71% [2]. A promising approach to the treatment of this type of tumor is boron neutron capture therapy (BNCT), which is based on the decay of the isotope boron-10 inside tumor cells upon irradiation with a stream of epithermal neutrons. The main obstacle to the introduction of BNCT into mainstream clinical practice is the issue of targeted delivery of the boron-10 isotope into cancer cells. The boron-containing drug should have minimal toxicity, provide highly efficient delivery to cancer cells and have minimal uptake by healthy cells.

Boron-modified aptamers (DNA or RNA oligonucleotides, conjugated to clusters of clozo-dodecaborate) that are able to selectively bind to tumor cells are promising drug candidates for BNCT. The possibility of using aptamers for targeted boron delivery to tumor cells for BNCT was previously demonstrated by researchers at ICHBFM SB RAS [3].

The objectives of this study were to: (1) qualitatively evaluate the efficiency of boron-containing aptamer penetration into human glioblastoma U-87 MG cells; (2) quantitatively assess boron content in human glioblastoma cells after their incubation with boron-containing aptamers.

Materials and methods. In this study, human glioblastoma U-87 MG cell cultures and normal human hFF8 fibroblasts were used. Aptamer penetration into human U-87 MG glioblastoma cells was assessed by confocal microscopy; boron accumulation was assessed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Results. According to microscopy, the 2'-fluorine-modified RNA aptamer GL44 penetrated cells most efficiently. According to ICP-AES analysis, the cellular uptake of boron by U-87 MG cells following their incubation with the aptamer 2-F-GL44-5'-B12 and subsequent washing was equal to 1.5*109 B atoms per cell.

Conclusion. The principle of using aptamers for boron delivery has been demonstrated: the prospective delivery agent was shown to penetrate into tumor cells and achieve the required intracellular concentration of boron needed for BNCT. We plan to further analyse the effectiveness of modified aptamers as boron delivery agents for BNCT in experimental animals in future studies. This work was supported by the Russian Science Foundation (grant no. 19-74- 20127).

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### S11.752. Elastic properties of structural layers of the intestinal wall studied with compression optical coherence elastography

Kiseleva E.B.[1]*, Sovetsky A.A.[2], Ryabkov M.G.[1], Gubarkova E.V.[1], Bederina E.L.[1], Bogomolova A.Yu.[1], Gladkova N.D.[1], Zaitsev V.Yu.[2]
[1]*Privolzhsky Research Medical University;*
[2]*Institute of Applied Physics of the RAS;*
* kiseleva84@gmail.com

Compression optical coherent elastography (C-OCE) enabling high resolution (~40-50 μm) tissue elasticity mapping is currently actively used for such applications as differential diagnosis of tumor and non-tumor lesions [1], as well as for assessing the response of tissues to treatment (identifying foci of edema and necrosis) [2]. The literature data indicate that the main field of C-OCE is related to characterization of oncological processes [3]. In this study, for the first time we use C-OCE to evaluate the elastic properties of the small intestine wall in norm. Quantitative intraoperative evaluation of stiffness of the intestine layers is needed for differential diagnosis of acute and chronic inflammation in the intestinal wall [4]; the introduction of microsurgery techniques into reconstructive surgery of the digestive tract, involving precise comparison of the layers with different stiffness parameters [5]; the long term elasticity characterization can be used to control destruction enterogenesis in short bowel syndrome [6].
The aim of this study was to develop a C-OCE-based methodology and apply it for measuring stiffness of all layers of the small intestine wall in norm. The measurements were performed on 16 samples of small intestine taken from 6 minipigs (males, weighing 28-34 kg). Elastograms were obtained using own C-OCE algorithms and an optical coherence tomograph (OCT) developed at the IAP RAS (Nizhny Novgorod). OCT operates at a wavelength of 1310 nm, enabling a depth resolution of 10 μm, and a lateral resolution of 15 μm. For tissue deformation mapping a vector approach was used to assess the gradient of interframe phase variations of the OCT signal [7]. For stiffness quantification, a calibration silicone layer with a known stiffness (40 kPa for the small intestine) was placed on the tissue surface to evaluate the absolute values of the tissue stiffness (Young's modulus - E, kPa) for a chosen standardized level of pressure applied to the tissue [8]. C-OCE data were obtained both from the side of the serous membrane (such that the stiffness of the serous and muscle layers was measured), as well as from the side of the mucous membrane (such that the stiffness of the mucous and submucosal layers was measured). Stiffness mapping was performed with varying degrees of OCT probe pressure on the tissue, using both one-time and repeated loading, as well as using tissue compression followed by unloading. Thus, dependences of stress on strain, stiffness on strain, and stiffness on stress were obtained for all four layers.
It was established that under a single loading, the highest stiffness values for the applied uniaxial stress $\sigma$=2 kPa were found for the serous membrane (E≃40 kPa). The muscularis externa was less stiff (E≃30 kPa). The lowest values were obtained for the mucosal (E≃20 kPa) and submucosal (E≃10 kPa) membranes. With uniform compression of the serous membrane and muscularis, the dependences of Young's modulus on stress had less pronounced nonlinearity than the curves for the mucous and submucosal layers. C-OCE can visualize some morphological features within the intestine wall: follicles and large vessels in the submucosal layer, and nerve ganglia between the muscle layers. In addition, the method allows for detecting changes in the thickness of the layers, for example, atrophy of the serous membrane, a decrease in the height of the villi, thickening of the submucosal layer.
Tissue compression with subsequent unloading after reaching a compressive stress of more than 30 kPa showed the appearance of a significant hysteresis in the nonlinear stress-strain dependences with a partial loss of the ability of the tissue to return to its original shape. Repeated loading of the tissue led to an increase in the initial stiffness values.
To conclude, the performed C-OCE-based study indicated that the mechanical properties of each layer of the intestine wall demonstrated both quantitative and pronounced qualitative differences in the nonlinearity of the Young's modulus dependences on stress. The presented study is the initial stage of the application of C-OCE to assess the elastic properties of individual layers of the small intestine wall. The results obtained are of considerable interest in the perspective of intraoperative use of C-OCE in a wide range of surgical manipulations with the small intestine, involving its compression and controlled stretching.
The study was supported by Russian Science Foundation, grant No.19-75-10096.
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### S11.753. Estimating of water transport through plasmodesmata in plant roots using gradient NMR with paramagnetic doping

Suslov M.A.[1]*, Anisimov A.V.[1], Ahtyamova G.A.[1], Aglyamova A.R.[1]
[1]*Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences;*
* makscom87@mail.ru

Crop production and crop management systems that maintain high yields under water limited conditions require a clear understanding of the processes that control root uptake of water for its subsequent use by plants. In his time, Steudle proposed a composite transport model of radial water

transfer in plant roots. According to this model, there are three parallel pathways of water and solute transport in the roots: apoplast, symplast and transmembrane. New experimental data of the last decade have led to the need to improve the classical model of water transport in the root with the addition of sequentially connected in the root resistances of epidermis, cortex, endodermis and other tissues. To date, the latest step in improving the composite transport model in the root has been the emergence of the MECHA (model of explicit cross-sectional hydraulic anatomy) model. This model allows the calculation of water flow across the root, taking into account the anatomic structure of the root and the hydraulic parameters of membranes, cell walls and plasmodesmata (intercellular channels) on the scale of individual cells throughout the root cross-section. This model predicts high sensitivity of root hydraulic conductivity to changes in root symplast permeability and water flow rate through plasmodesmata. However, a weakness of this model is the lack of experimental data on the hydraulic conductivity of the plasmodesmata due to the lack of necessary methodological and technical approaches for measuring the rate of water flow through the plasmodesmata.

In this study, we measured translational water diffusion selectively along symplast pathway through plasmodesmata in maize roots, and the effective plasmodesmata permeability coefficient (P) was determined using a nuclear magnetic resonance (NMR) spin echo method. Measuring of water transport selectively along the plant root plasmodesmata was achieved with paramagnetic complexes (PCs) of high relaxation efficiency. PCs penetrate into the intercellular space of root tissue, but not into cells, and accelerate the magnetic relaxation processes of intercellular water, thereby excluding the contribution of intercellular water to the registered NMR diffusion echo attenuation. In result, NMR control of translational diffusion can be applied to the signal of the water moving along the symplast pathway through plasmodesmata, where the PCs do not penetrate. Diethylenetriaminepentaacetic acid (GdDTPA), Mn2+-trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (MnDCTA), and GdCl3 were used as PCs. An increase in the PCs concentration led to a side effect in the form of a varying decrease in diffusive water transport in the roots. The P was determined by extrapolating the concentration dependence to zero concentration of PCs. Among the PCs studied, MnDCTA had the least side effects on the water transport when the concentration dependence was linear. When MnDCTA was used, the P accounted for 30–35% of the total cell water permeability (by transmembrane and symplast pathways). The rate of water flow along the plasmodesmata in the approximation of the piston mode of flow along the linear cell chain was estimated to range from $4.5 \times 10–7$ to $8.8 \times 10–7$ m/s. The results obtained in this study on estimating the permeability of the symplast system of maize root and the rate of water flow through the plasmodesmata can be used in improving existing mathematical models of radial water transport in the roots, including the MECHA model, and can also be useful in studying the mechanisms of coordination of plant hydraulic system components.

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### S11.754. FLIM of NAD(P)H immune cell autofluorescence in lymph nodes as a marker of immunotherapy efficacy

Yuzhakova D.V.[1]*, Izosimova A.V.[1,2], Sachkova D.A.[2,1], Shcheslavskiy V.I.[1], Mozherov A.M.[1], Zagainova E.V.[2], Sharonov G.S.[3,1], Shirmanova M.V.[1]
[1]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia;*
[2]*Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;*
[3]*Pirogov Russian National Research Medical University, Moscow, Russia;*
* yuzhakova-diana@mail.ru

Over the past decade, therapies that promote antitumor immune responses have revolutionized the treatment of cancer, resulting in marked and durable responses in subsets of patients across many different tumor types. Despite this, only a subset of patients responds to the therapy, and smaller portions achieve maximum clinical benefit. Reliable markers for the treatment efficiency is required for improvement of cancer immunotherapy. The promising strategy can be the evaluation of the metabolic status of the lymphocytes that reflects the key changes in response to tumor and therapy. An innovative technology for the metabolic assessment is fluorescent lifetime imaging (FLIM) of metabolic coenzymes. Unlike standard methods, FLIM does not require the cell staining, tissue destruction, and allows real-time imaging. However, studies on metabolic FLIM imaging of immune cells are still rare.

The studies were carried out on C57Bl/6 FoxP3-EGFP mice with subcutaneous B16F0 melanoma implanted near the inguinal lymphatic node (LN). The mice were treated with anti-CTLA-4 antibodies (Bio X Cell, USA) (250 μg per mouse, intraperitoneal injection at 7, 8, 11 and 12 days of tumor growth). Nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) fluorescence lifetime images were acquired using an LSM 880 (Carl Zeiss, Germany) fluorescence confocal laser-scanning microscope equipped with an FLIM module Simple Tau 152 TCSPC (Becker & Hickl GmbH, Germany) (ex. 375 nm, em. 435–485 nm). The flow cytometry was performed using a FACSAria III cell sorter.

For the first time, a protocol for studying the autofluorescence of immune cells from fresh LN fragments has been developed. NAD(P)H lifetime parameters show sensitivity to tumor development. The most pronounced changes during tumor growth were an increase in the relative amplitude of free NADH α1 and an increase in the contribution of the phosphorylated form of NADPH α3. A likely reason for the increase in α1 and α3 may be, respectively, a shift towards glycolysis and an increase in the pentose phosphate pathway to provide the increased demand of antigen-activated T cells. Flow cytometry data confirmed an increase in the expression of surface activation markers CD25 and CD69 and production of interferon-gamma (IFNγ), as well as an increase in the proliferation index Ki67 in CD4+Th and CD8+ T cell subpopulations. Concerning the anti-CTLA-4 therapy, mice with tumor growth inhibition (responders) showed an increase in the relative amplitude of glycolysis-associated free NADH α1 compared to advanced tumor mice as well as untreated control mice with similar tumor size. At the same time, non-responder mice show a pronounced decrease in α1 even compared to the control group. FLIM data correlate with the level of expression of activation (CD25, CD69 and IFNγ) and proliferative (Ki67) markers.

Therefore, FLIM microscopy of NAD(P)H coenzyme autofluorescence in immune cells can be a powerful tool for assessing the immune response to a tumor and predicting the effectiveness of immunotherapy.

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### S11.755. Gradient NMR technique for monitoring water transport in plants in a one-dimensional pressure field

Anisimov A.V.*, Suslov M.A.
[1]*Kazan Institute of Biochemistry and Biophysics of the Federal Research Center of the Russian Academy of Sciences, Russia;*
* anisimov@kibb.knc.ru

Previously, studies conducted by gradient NMR on the effect of bulk pressure (hyperbaria) on translational water transport in plant tissues showed a relatively wide range of plant responses to the pressure factor (Anisimov, Suslov, Abdrakhimov, et al.2013,2014,2016, 2019) In particular, the effect of hyperbaria on the formation of cluster aggregates from elements of the endomembrane system, local destruction of the tonoplast effect of oxygen doping, due to an increase under pressure in the concentration in dissolved oxygen was demonstrated. These and other results, have motivated the tasks of detailing the targets of pressure on translational water transport by different pathways (symplast,

transcellular transport), the reaction of water transport through aquaporins with the prospect of evaluating the hypothesis of the possibility of pumping function of aquaporins. and the study of the water transport polarity phenomenon. In this connection, studies of transport under one-dimensional pressure seem promising, which can be generated by centrifugal force. In its turn, there is a methodical problem of combining the technique of controlling water transfer with the technique of generating centrifugal force. As a transfer control technique informative and technically adequate to the task is the spin-echo NMR method with a pulsed magnetic field gradient - gradient NMR (Anisimov 2021).
The work describes a complex: gradient NMR on a centrifuge, consisting of three portable self-sufficient units:1. portable unit of gradient NMR, with the possibility of working in the classical version of a stationary spin-echo NMR relaxometer-diffusion meter; 2. specialized centrifuge; 3. computer for control and information gathering.
A feature of gradient NMR is: 1.fully autonomous sample thermostat system, for the temperature range -5-+80C, built on Peltier thermomodules, with cooling of the latter with water circulating through a closed loop; 2.possibility of applying the vector of centrifugal force at any angle to the longitudinal axis of the sample, thanks to the movable suspension of the diffuser magnet, 3.autonomous digital control system of pulse sequences and magnetization signal registration with transfer of experimental data to the computer via a radio link. Block of pulse gradient is made two-channel with gradients, respectively, along the Z and Y directions of up to 4 T/m.
The centrifuge is designed as a five-supported symmetric cantilever, with a fixed rotation axis for the rotor - the NMR unit itself. The drive pulley rotates on the axis, which is also a drive element of the cam coupling. Responsive part of the coupling is made in the form of a tray for installation of gradient NMR unit. In turn, the tray is mounted on the carriage, which rolls on a flat support ring attached to the perimeter of the support console. The ring serves to prevent the possibility of accidental overturning of the NMR unit during rotation. Safety of experimenter's work is provided by protective cylinder made of high pressure polyethylene with external bandage made of steel mesh and limitation of centrifuge rotation speed by value g not more than one.
The complex can be also used for investigations of hypergravity and acceleration influence on biological objects within the frames of problems of space biology and medicine.
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**S11.756. Immobilized enzymes in molecular biophysics: computational and instrumental methods for assessing their state**

Holyavka M.G.[1]*, Artyukhov V.G.[1]
[1]*Voronezh State University;*
* marinaholyavka@yahoo.com

One of the promising areas of modern molecular biophysics is the study of immobilized biological systems – enzymes and their complexes. It is generally recognized that in the industrial scale-up of catalytic processes, the heterogeneous mode of their implementation (the biosystem is in a state immobilized on an insoluble carrier) is more economically advantageous compared to homogeneous technologies (biological objects are evenly distributed in the solvent phase), since this greatly simplifies and reduces the cost of the entire production production cycle. The addition of experimental empirical approaches to the selection of successful enzyme-carrier combinations in various microenvironments with modern methods of computer and mathematical modeling allows not only saving material resources for testing immobilization agents and revealing the mechanisms of the immobilization process, but also creating fundamentally new complexes based on the use of directed materials design (ligands, substrates, crosslinks) [1]. To obtain an immobilized biological object that is in demand on the modern market, a researcher should be well versed not only in the field of classical biophysics, but also in modeling and analyzing the structural and functional features of a wide range of molecules, be able to identify the fundamental mechanisms that control conformational rearrangements in biopolymers, determine the most probable ways of various nature complexes formation and the course of chemical reactions. To do this, it is necessary to be able to actively manipulate theoretical approaches – modern methods of quantum chemistry: methods of molecular dynamics in the full-atom approximation, flexible molecular docking, methods for predicting the spectra of biological activity and high-performance virtual screening of compounds.
To date, our research team has revealed the features of the physicochemical, kinetic, structural and functional properties of inulinases and some cysteine proteases (bromelain, papain, ficin) under conditions of various microenvironments from the point of view of fundamental and applied science [2]. In a comparative aspect, methods for regulating the activity of these hydrolases are described, heterogeneous preparations based on immobilized enzymes are characterized, and ways of their application are proposed [3, 4].
The physicochemical and kinetic properties of heterogeneous biocatalysts are analyzed. Particular attention was paid to describing the functional properties of enzymes under different microenvironment conditions, identifying optimal system parameters for their functioning, characterizing the stability of protein macromolecules, their resistance to temperature effects and extreme pH values [5].
It has been shown that one of the effective ways to regulate and stabilize the activity of hydrolases studied by us is their immobilization. It has been suggested that the mechanisms of the immobilized enzyme stabilization under conditions of extreme pH values, temperature, and other denaturing agents largely coincide and are primarily due to a change in the degree of the protein tertiary structure mobility responsible for the formation of the enzyme-substrate complex. It has been established that there is currently no universal method for the immobilization of hydrolytic enzymes; each of the methods has its own advantages and disadvantages [6]. In general, the choice of a biocatalyst immobilization method depends on the objectives of the study and the direction of the resulting preparation use in a particular area of science and production. The scientific positions we are discussing will be substantiated by the demonstration of the necessary illustrative material.
The study was supported by a grant from the Russian Science Foundation (project No. 21-74-20053)
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### S11.757. Intracellular microplastic detection and identification using enhanced dark-field microscopy with hyperspectral imaging

Ishmukhametov I.R.[1], Akhatova F.S.[1], Fakhrullina G.I.[1], Khaertdinov N.N.[1]*
[1]*Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia;*
* Xaertdinov@yandex.ru

The ubiquity of microplastics is one of the major environmental issues. Even though most habitats are already contaminated with microplastic, the aspects involved in their cellular uptake and response remain incompletely disclosed [1]. One of the valuable resources in studying microplastic toxicity is polystyrene microbeads due to their consistency and ease of access. Despite the prevalence in nature of secondary microplastic with irregular size and morphology, polystyrene microspheres are widely used for general establishing the cellular and molecular effects of microbeads [2]. Optical microscopy techniques, such as enhanced dark-field microscopy amended with hyperspectral imaging (EDFM-HSI), have found many applications in nanotechnology research, including the detection and identification of various nanomaterials in cells and organisms. The present study shows the potential of EDFM-HSI for the rapid and accurate uptake detection of microplastics of various diameters in primary cell culture [3].
The viability of human skin fibroblasts after the 24-h incubation with polystyrene with a diameter of 100-1000 nm was measured using a colourimetric MTT-assay. The morphology of microparticles and their cellular uptake after co-incubation were investigated using CytoViva's enhanced dark-field microscopy system. Hyperspectral images of pristine particles and cell samples in the 400-1000 nm wavelength range were captured to verify microbeads in solution and cells using the spectral angle mapper algorithm. To optimize the method for high-throughput analysis, the collected imaging data from differently sized microplastic were used as a dataset for the neural network training. The obtained model was then tested on the classification of living cell samples to assess the possibility of dynamic cellular uptake analysis.
The results showed that polystyrene samples at concentrations up to 5 μg/mL did not have a significant impact on cell viability. However, at 10 μg/mL, the viability of cells slightly decreased when exposed to 200 and 1000 nm particles, while exposure to 100 nm particles stimulated cell viability. Imaging revealed that microplastic particles were present both inside and outside the cell membrane, but not within the cell nuclei. The hyperspectral analysis allowed us to classify particles of different sizes based on their reflectance with a small portion of misclassified objects, which was caused by their identical chemical composition. Involving the neural network in the process of microplastic analysis made it possible to maintain high detection accuracy with the increased data processing rate. Further research using this technique may give insights into the process of microplastic uptake and improve the understanding of its impacts on human health and the environment.
The study was funded by the Russian Science Foundation grant 21–73-00097.
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### S11.758. Investigation of conformational changes in peptide Aβ(1-42) by Raman spectroscopy

Shutikov A.A.[1]*, Arzumanyan G.M.[1], Arynbek Y.[1,2], Demina E.M.[1], Zakrytnaya D.S.[1], Mamatkulov K.Z.[1]
[1]*Joint Institute for Nuclear Research, Dubna, Moscow Region, Russia;*
[2]*Institute of Nuclear Physics, Almaty, Kazakhtan;*
* artyom.shutikov@nf.jinr.ru

The aggregation of β-amyloid peptide (Aβ) is known to contribute to the accumulation of amyloid plaques in the human brain, leading to the formation of a variety of diseases, including Alzheimer's disease. The neurotoxicity of the major components of Aβ peptides, may be mediated by a direct interaction between proteins and lipid membranes.
This work focuses on the study of conformational changes of Aβ(1-42) peptide in the presence of a phospholipid system - in mimetics such as liposomes and lipodiscs. The study was performed by Raman spectroscopy which is non-invasive, fast and one of the most powerful diagnostic tools that does not require complex preparation and a large volume of material. In this work we present a comparative analysis of conformational changes of pure peptide Aβ(1-42) during the time, including in the phospholipid membrane system.
The aim of our study was to clarify the pattern of structural changes in the amide I region in the Raman spectra of peptides that contribute to the formation of amyloid plaques. The fully saturated phospholipid – DMPC, were chosen in our study.
The results showed that the peptide dissolved in water, outside the lipid system, was prone to aggregation. When Aβ(1-42) peptide is added during liposome formation, the probability of protein incorporation into the lipid bilayer is increased In such a system, the protein passes to a more stable structure – the α-helix conformation prevails. When Aβ peptide is added to the formed liposomes, the probability of peptide incorporation into the bilayer structure decreases, which also leads to the formation of amyloid fibrils. In the case of lipodiscs, with an embedded Aβ peptide, its structure changes to a β-turn conformation. The obtained spectral data are confirmed by simulation calculations – molecular dynamics (MD), as well as by the density functional theory (DFT) calculation method.
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### S11.759. Laser tweezers and their applications in biophysical studies

Priezzhev A.V.[1]*
[1]*Lomonosov Moscow State University;*
* avp2@mail.ru

Laser tweezers (LT), also referred to as optical traps (OT), are a new technology that emerged in the mid-1980s. The emergence of LT has opened up new possibilities for manipulating without mechanical contact microparticles of various nature, in particular live cells, and measuring the forces of interaction between them and the environment. Great impact of LT on fundamental and applied research was marked by awarding the 2018 Nobel Prize in physics to Arthur Ashkin, the inventor of LT.

The operation principle of the LT is the change in the momentum of forces acting on the cell during refraction on it of a sharply focused or otherwise formed beam of light with a strongly pronounced spatial intensity gradient. Near the beam focus, the OT behaves as a linear spring generating forces on the cell proportional to its displacement from the center of the trap. If the beam waist position is spatially manipulated, so is the position of the cell. The displacement of the cell from the equilibrium position by external forces can be calibrated so that these forces can be precisely measured in the range 0.1 – 100 pN. This is the range of forces of elastic deformations of live cells during mitosis, interaction with each other and environment, etc.

LT are used to study a wide range of phenomena in cell biophysics, e.g., the mechanisms of functioning of molecular motors responsible for cell motility, changing the cells shape and intracellular transport. With the ability to apply forces on individual molecules and measure the forces generated in their chemical reactions, analytical LT are ideally suited to investigate the mechanisms of mechanochemical transformations.

This paper presents the latest results obtained using LT in the study of biophysics of the interaction of living cells during reversible red blood cells (RBC) aggregation - a fundamental process, the mechanisms of which remain not fully understood. Alterations of RBC aggregation are related to the development of such diseases like arterial hypertension (AH), diabetes mellitus (DM), etc. We measured the forces of RBC interaction during aggregation and disaggregation, depending on the composition of the environment, area and duration of the initial contact, characteristic of the blood of healthy donors and patients suffering from various diseases. We found that the measured forces are sensitive to various alternations typical of the blood of AH and DM patients.

We conclude that using LT allows for performing the research on single cells level that was impossible earlier.

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### S11.760. Manifestation of the action of the alternating voltage electric field on barley seeds

Yudaev I.V.[1]*, Kazakova A.S.[2], Tatyanchenko I.S.[2], Dontsova V.Yu.[2]
[1]*Kuban State Agrarian University named after I.T. Trubilin;*
[2]*Azov-Black Sea Engineering Institute - branch of FSBEI HE "Don State Agrarian University";*
* etsh1965@mail.ru

According to the UN FAO, already in the 80s of the last century in the agricultural sector, it was recommended to use more than 700 types of various electrotechnological operations, among which the direct effect of electric current on materials and raw materials was also considered. The essential advantage of this electrotechnological operation lies in its environmental and food safety, which allows talking about the possibility of its inclusion in the implementation of the organic farming program and the "Agriculture 4.0" concept of re-equipment of the agricultural sector. From a practical point of view, the result of the influence of the physical factor on the biological object, for example, seeds of cereal crops, is considered as an increase in their germination and, ultimately, productivity. At the same time, the modes of seed treatment and the mechanisms for implementing the resulting impact, leading to the increase in yield, remain insufficiently studied. In this regard, the purpose of this work was to study the effect of pre-sowing treatment of spring barley seeds in the electric field of alternating voltage (AVEF) in order to increase germination and accelerate their germination, intensify plant growth and development, and increase yields.

The object of the study was the seeds of Sokol, Vakula, Gris and Priazovsky 9 spring barley varieties of local selection for three years of reproduction, which were processed in AVEF with the voltage of 0.5 kV/cm on the laboratory unit for 20, 40 and 60 seconds. Seed germination was determined within 10 days after treatment. Water absorption was determined by the control and treated seeds by the weight method during the day after soaking; seedling power was determined during seed germination in filter paper rolls; amylase activity was determined by the standard method according to the microphenological phases of seed germination (MPPSG); under the conditions of the field experiment, the yield was determined. It has been established that all tested modes of pre-sowing seed treatment have a positive effect on all the studied traits, however, the highest and most stable effect was obtained when seeds were treated in AVEF for 40 seconds and then aged for 4 days; this mode of seed treatment was used in further work.

Pre-sowing treatment of seeds in AVEF under the optimal mode leads to the increase in germination energy by 18-34%, and germination by 4-6% on average for all studied varieties for different years of their reproduction. Electrical stimulation of seeds has a positive effect on water absorption. The increase in the mass of seeds is of a general nature in the control and in the experiment, however, the difference in the values of their mass in the process of water absorption gradually increases: the difference in the mass of the seeds after 30 minutes of swelling was 4.6%, and after 12 hours it increased by 3.9 times and amounted to 17.9%. A higher moisture content of the treated germinating seeds is also maintained for all MFFPS until their germination.

The stimulating effect of presowing seed treatment in AVEF is also manifested in the formation of a more powerful primary root system of 7-day-old seedlings: the total length of roots increases due to an increase in the length of each. The greatest effect was noted along the length of 1-4 seedling roots, their length increases by 25-30%. A strong correlation was found between the values of the increase in the total length of the seedling roots and the increase in the length of each root after seed treatment in AVEF. When evaluating the results of the impact of presowing treatment of barley seeds with physical factors, it is necessary to use not the absolute values of the length of the first germinal root, as is often done, but the amount of increase in its length in the experiment compared to the control.

Presowing seed treatment leads to an increase in the activity of amylases: the activity of β-amylase increases by 30%, and the activity of α-amylase - by 40-50% on average for all varieties and MPPSG. There are also varietal differences: for example, in the seeds of the Priazovsky 9 variety, the total activity of amylases increases 5 times, while in the Gris variety, only by 10%.

Pre-sowing treatment of seeds of Vakula spring barley variety led to an excess of grain yield compared to the control in an acutely dry year by 20.2%, and in an optimal year by 10.5%. The increase in yield occurred due to such elements of its structure as the number of grains per ear, the weight of 1000 grains, the number of plants per 1 m2 and productive tillering.

Thus, the manifestation of the action of an electric field of alternating voltage on barley seeds manifests itself immediately after the contact of seeds with moisture, a cascade of physiological and biochemical processes is launched at a higher level, which leads to an increase in yield.

### S11.761. Mobility of upconversion nanosensors NaF4Yb,Er in the body of a terrestrial snail

Andrianov V.V.[1,2]*, Gainutdinov Kh.L.[1,2], Shmelev A.G.[2], Nikiforov V.G.[2], Zharkov D.K.[2], Leontyev A.V.[2], Mitushkin E.O.[2], Arslanov A.I.[1], Muranova L.N.[1]
[1]*Kazan Federal University;*
[2]*Zavoisky Physical-Technical Institute, FRC Kazan Scientific Center of RAS;*
* vvandrianov@kpfu.ru

We report the results of a study focused on the mobility of NaYF4: Yb, Er nanoparticles (NPs) injected into a snail as a colloidal solution (0.2 ml, dosage 600 mg/kg). Hydro- and solvothermal methods allowed us to synthesize HPs in the form of nanorods up to 1 micron in length. They exhibited bright upconversion luminescence upon laser excitation at a wavelength of 980 nm. We analyzed the distribution of NPs in the organs of the snail, as well as the rate of their natural excretion. The idea of using various nanosensors for research, control and therapy in biology and medicine is now rapidly developing. One of the promising field is fluorescent nanosensorics, when the fluorescence response is excited by an external light source. UV radiation, which is commonly used for excitation, negatively effects on biological objects. Its strong absorption leads to photodestruction of biomolecules and heating of tissues. In addition, there is an intense scattering of UV radiation by tissues and autofluorescence of proteins, which adversely affects the accuracy of the method. In this work, we are testing the possibility of using upconversion nanoparticles (NPs) NaYF4: Yb, Er as fluorescent nanoprobes. Such NPs exhibit bright green luminescence upon excitation by a laser at a wavelength of 980 nm, which is in the "transparency window" of biological tissues. The use of such upconversion excitation makes it possible to completely avoid the problems with UV radiation described above.
Hydro- and solvothermal methods described in [1–3] were used in the synthesis. Then, the synthesized NPs were purified from by-products and coated with a silicone shell to protect the NPs from the undesirable effects of surface luminescence quenchers in the biomedium and impart hydrophilic properties to the NPs. We studied of the mobility of NPs injected into the body of the snail. Aqueous colloidal solutions of NPs injected into the internal cavity through the region of the sinus node of the snail (with no pain receptors). Then the behavior of the snail had been monitored for seven days together with the collection of excreted excrements. After that, we prepared the samples containing several organs of the snail and collected excrement according to the following method. The organic components of the preparations annealed at a temperature of 500°C for several minutes. The obtained ashes dissolved in 0.06 N hydrochloric acid and washed twice with water. These procedures yielded an unburned and insoluble precipitates. These studies provided information on the mobility of injected NPs into the body of the snail, the rate of their natural excretion from the body of the snail, and residual localization in organs seven days after injection. The report raises questions about the future prospects of using this type of upconversion NPs in biological applications (bioimaging, remote temperature measurement, etc.). Dependences of the mobility of NPs on their shape and size, as well as issues of toxicity, are shortly discussed.
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### S11.762. Noise suppression in electronic absorption spectra of proteins by non-uniform rational B-splines

Lavrinenko I.A.[1]*, Barysheva V.E.[1], Karpova O.V.[1], Cherniaev P.S.[1], Shatskiy I.V.[1], Boyko A.A.[1], Marchukova K.N.[1], Podgornaya A.A.[1], Vashanov G.A.[1]
[1]*Voronezh State University;*
* lavrinenko_ia@bio.vsu.ru

Molecular absorption spectra of proteins are characterized, as a rule, by wide poorly resolved and often overlapping bands of absorption. This is because besides electronic levels of potential energy, molecules, unlike individual atoms, are characterized by the presence of additional vibrational and rotational levels with lower quantization values relative to electronic ones. Unquantized shifts in potential energy levels due to thermal and Doppler effects lead to the broadening of these bands and the formation of an almost continuous absorption spectrum. This circumstance makes it very difficult to correlate the peaks of the light absorption bands with the electronic transitions that form them.
The use of the technique of quasilinear Shpol'skii spectra, matrix isolation of molecules in inert gases, and low-temperature selective laser spectroscopy, although partially solving this problem, also has several methodological limitations. Another way to increase the resolving power in molecular absorption spectra was the use of derivative spectrophotometry [1]. There are also limitations here: numerical differentiation artifacts, false satellite peaks, and deterioration of the signal-to-noise ratio with increasing order of the derivative of the analyzed spectrum.
The best-known ways to reduce noise in light absorption spectra are increasing the integration time when measuring the signal, accumulation of absorption spectra and their subsequent averaging, use of smoothing algorithms based on a moving average, various linear, nonlinear, recursive and nonrecursive filters, as well as filters using Fourier and wavelet transforms, etc. [2-3]. Among smoothing filters with minimization of quadratic error in spectroscopy the Savitzky-Golay polynomial filter which in essence is an evolution of a moving average method is most often applied.
We propose to perform noise filtering using non-uniform rational B-splines, also known as NURBS curves. Being, in fact, a smoothing approximation, this spline can be used for low-frequency filtering of initial experimental data with their subsequent differentiation. If the Savitzky-Golay filter is set by parameters of the smoothing window width, the approximating polynomial order, and the number of successive passes of this filter, then NURBS-smoothing is determined by the number of control points, the order of the spline as well as by the number of successive approximations of the spectrum. Controlling the number of control points, which are the absorption spectrum measurement data, the order of the approximating spline function and the number of successive approximations, we can find the solution that best meets the signal/noise ratio (S/N).
On the absorption spectra of hemoglobin and albumin, we have demonstrated the possibility of using NURBS as a method of noise suppression in the light absorption spectra of proteins. It was shown that the method of smoothing using NURBS curves successfully competes with the Savitzky-Golay window smoothing method. However, it should be noted that the selection of optimal parameter values for one or another smoothing method requires some a priori knowledge about the spectral properties of the chromophores under study, in particular proteins. This makes it possible to minimize the interpretation errors of poorly resolved absorption bands. At the same time, the proposed method of noise filtering using NURBS requires further investigation to evaluate its advantages and disadvantages with respect to already used methods in terms of spectral data analysis [4-5].
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### S11.763. Raman Spectroscopy Enables Non-Invasive Identification of Mycotoxins p. Fusarium of Winter Wheat Seeds

Moskovskiy M.N.[1]*
[1]*Federal Scientific Agroengineering Center "VIM", Moscow, Russia;*
* maxmoskovsky74@yandex.ru

Identification of specific mycotoxins p. Fusarium contained in infected winter wheat seeds can be achieved by visually recognizing their distinctive phenotypic species. The visual identification (ID) of species is subjective and usually requires significant taxonomic knowledge. Methods for the determination of various types of mycotoxins of the p. Fusarium are laborious and require the use of chemical invasive research methods. In this research, we investigate the possibility of using Raman spectroscopy (RS) as a tag-free, non-invasive and non-destructive analytical method for the rapid and accurate identification of p. Fusarium. Varieties of the r. Fusarium can produce mycotoxins that directly affect the DNA, RNA and chemical structure of infected seeds. Analysis of spectra by RS methods and chemometric analysis allows the identification of healthy, infected and contaminated seeds of winter wheat with varieties of mycotoxins p. Fusarium. Raman seed analysis provides accurate identification of p. Fusarium in 96% of samples. In addition, we present data on the identification of carbohydrates, proteins, fiber and other nutrients contaminated with p. Fusarium seeds obtained using spectroscopic signatures. These results demonstrate that RS enables rapid, accurate and non-invasive screening of seed phytosanitary status.

### S11.764. Role of fibrinogen in the interaction of red blood cell and endothelium at single cell level measured in vitro using laser tweezers in healthy conditions

Ermolinskiy P.B.[1]*, Maksimov M.K.[1], Scheglovitova O.N.[2], Lugovtsov A.E.[1], Priezzhev A.V.[1]
[1]*Faculty of Physics, Lomonosov Moscow State University, Moscow, Russia;*
[2]*N.F. Gamaleya National Research Center for Epidemiology and Microbiology, Russia;*
* ermolinskiy.pb15@physics.msu.ru

Blood microrheology is conditioned by the properties of blood plasma and the interaction between blood cells, i.e., red blood cell (RBC) aggregation, interaction between blood cells and endothelium, etc. [1]. Endothelial cells play a major role in the blood flux regulating blood vessels and capillaries. Moreover, these cells act as the insulating layer between blood and tissues, and they impact the properties of blood cells. In this work, only the interaction between endothelium and RBCs was considered. It is well known that RBCs can reversibly interact with each other under low shear stress forces producing coin-shaped structures. These structures are called RBC aggregates and the degree of RBC aggregation determine the viscosity of blood. Fibrinogen is the main inducer of RBC aggregation, as well as platelets aggregation, may somehow influence the interaction between RBC and endothelium. The main objective of this study was to explore at single cell level the interaction of healthy endothelium and RBC of healthy volunteers under different concentrations of fibrinogen in vitro. Laser tweezers were used for experiments to manipulate single cells without mechanical contact, as well as to measure the interaction forces [2].
Blood for the study was drawn from the cubital veins of healthy donors. Endothelial cells were forming a monolayer of cells grown on a round glass plates [3]. They were conserved in a desiccator (CO2 environment) placed in a preheated thermostat at 37°C before each measurement. The blood sample consisted of autologous serum, different fibrinogen concentrations and a small amount of blood (1:1000). The following concentrations of fibrinogen in serum were applied: 0, 2, 4, 6, 8 mg/ml. The blood sample was placed into the air isolated cuvette with endothelium monolayer and the forces of interactions were measured. As the result, it was shown that the interaction force between RBC and endothelium leads to the saturation for the fibrinogen concentration of 4 mg/ml. The interaction forces between RBC and endothelium at healthy conditions are about 4 pN which is comparable with the interaction forces between RBCs. These results are important for better understanding of RBC and endothelium interaction.
This work was supported by the Russian Science Foundation (Grant No. 23-45-00027) and performed according to the Development program of the Interdisciplinary Scientific and Educational School of Lomonosov Moscow State University «Photonic and Quantum Technologies. Digital medicine».
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### S11.765. Study of the cellular reactions peculiarities using the anomalous viscosity time dependence method

Ivanov K.Yu.[1]*
[1]*Russian Federal Nuclear Center — All-Russian Scientific Research Institute of Experimental Physics, Sarov, Russia;*
* gane@orb2.vniief.ru

The state of the body and its reactivity determine individual peculiarities of responses to various influences. Cell reactions are associated with DNA spatial structure changes. The initial chromatin conformational state can determine individual peculiarities of the cell reactions. The method of the anomalous viscosity time dependence (AVTD) based on the research of DNA-protein complexes allows studying into the chromatin conformational state and evaluating its reactivity by the reaction to heat shock.

The goal of the research is the analysis of individual peculiarities of chromatin conformation modification in case of hyperthermia, irradiation, and magnetic field effect dependently on its initial conformational state and reactivity.
Material and methods. The experimental research was performed using human blood cells and white outbred rats blood cells in vitro. The human blood samples (n=18) and rat blood samples (n=18) were subjected to hyperthermia at 46°C for 30 min, the human blood samples (n=18) were exposed to 60Co γ-irradiation of in 3.0 Gy at the dose rate of 1.0 Gy/min, and the animal blood samples (n=32) were affected by low-intensity low-frequency bipolar magnetic field with induction of 11.8 mT for 30 min.
Determination of chromatin conformational state was performed after a four-hour cell lysis. Parameters of the AVTD-curve were recorded with help of cylindrical Zimm-Crothers viscosimeter. The evaluation criterion for chromatin conformational state was the viscosity value, which was proportional to the maximum the rotor`s rotation period of viscosimeter. The chromatin reactivity evaluation was based on its conformational state change after heat shock.
The comparison groups were formed according to initial chromatin conformational state and reactivity.The groups were formed based on the ratio of individual AVTD indicative values and group-averaged value. The donors and animals with indicative values from M-0.67s to M+0.67s got into middle group, where M was the group-averaged value, and s - the standard deviation. The indicative values of lower or higher aforementioned range served as the criteria for formation of the extreme groups.
The statistical significance of studied factor effects was assessed using the Wilcoxon test. To analyze the differences between selective comparative groups, the Kruskal-Wallis test was used.
Results. The heat shock caused chromatin decondensation in human blood cells (p=3.27E-04) and animal blood cells (p=0.001). Between the groups of hypo-, normo-, and hyper-reactive donors, a statistically significant difference in response of chromatin to heat shock was found (p=0.001). A pronounced difference in chromatin reactivity to heat shock was also revealed with a similar division of animals into groups (p=0.001). So, the initial conformational state determined chromatin reactivity to heat shock. Between donor groups different in terms of the initial chromatin conformational state (p=0.001), the difference in response of chromatin to heat shock was found (p=0.003). Chromatin reactivity in animal groups formed according to the initial conformational state (p=0.001) also differed (p=0.031). The heat shock caused maximum chromatin decondensation in the donors and animals cells with its maximum condensation state.
Using human blood cells it was found that irradiation at a dose of 3.0 Gy caused chromatin decondensation (p=0.001). The initial chromatin conformational state and reactivity to heat shock determined the degree of chromatin radiation-induced decondensation. The maximum conformational state changing occurred in donor groups with chromatin maximum condensation state (p=0.022) and hyper-reactive donors (p=0.014).
Using animal blood cells, it was found that magnetic field causes weak chromatin decondensation in the group in general. The significant effect was found credibly in animals with maximum chromatin condensation state (p=0.022).
Conclusion. The initial chromatin conformational state determined chromatin decondensation degree after heat shock, exposure to ionizing radiation and magnetic field action, and the initial chromatin reactivity, defined conformational state change after irradiation. The AVTD-method allows researching chromatin conformational state, evaluating its reactivity in response to heat shock and, on the basis of this, predicting the features of cellular responses to various influences.
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**S11.766. Study of the influence of the paracrine effect on the differentiation of mesenchymal stem cells into cardiomyocytes and their delivery to the heart tissue on microcarriers**

Aitova A.A.[1]*
[1]*MIPT, Laboratory of Experimental and Cell Medicine;*
* alika_aitova@mail.ru

In the stroma of the bone marrow, there is a subset of non-hematopoietic cells called mesenchymal stem cells (MSCs). These cells can be cultured and further differentiated into other cell types, making them a unique tool in cell therapy.
Cell therapy is one of the most promising strategies in modern medicine. Cardiac tissue engineering and cell technologies, including direct cell injection, are key approaches for the treatment of heart diseases, however, such methods are associated with a number of difficulties: the functional activity of cardiomyocytes after transplantation and the possibility of an immune response from host tissues. The solution to these problems is stem cells obtained from the patient, for example: induced pluripotent stem cells, mesenchymal stem cells, and others. The ability of such cells to differentiate into a variety of cell types makes them suitable for use in cell therapy. The problem of functional integrations of cells in the patient's tissue still remains.
The present work is devoted to the study of the paracrine effect during stem cell differentiation and the development of a new method for delivering cells to the heart tissue of an animal, followed by an assessment of the functional integration of cells. The paracrine effect makes it possible to differentiate cells directly into tissues up to the terminal stage, which makes it possible to create intercellular connections. The identification of stages of differentiation using the paracrine effect and the possibility of transplanting differentiable cells into the tissue at different stages were the objectives of this work. To solve the posed problems, human MSCs were used in the work. Protocols for MSC isolation from human bone marrow biopsy were developed and modified. MSC cell lines have been obtained. The optimal substrate was chosen, on which the required culture confluency was achieved in the optimal time. One protocol for differentiation from MSCs to cardiomyocytes was obtained using factors derived from the simultaneous differentiation of iPScs to cardiomyocytes with a result of about 10%. MSC-derived cardiomyocytes were characterized by optical mapping and immunostaining. The resulting culture was delivered to the rat heart tissue on nanofiber structures that provide more efficient functional cell integration. In the future, it is planned to improve the protocol for the differentiation of MSCs into cardiomyocytes and the method of cell delivery to achieve better functional integration.

**S11.767. The fusion of somatic cells using the Biotracker 400 Blue Cytoplasmic Membrane fluorescent dye by femtosecond laser nanosurgery**

Zalessky A.D.[1,2]*, Osychenko A.A.[1], Martirosyan D.Yu.[1], Tochilo U.A.[1], Fedotov Yu.A.[1,3]
[1]*N.N. Semenov Federal Research Center for Chemical Physics Russian Academy of Sciences;*
[2]*MIPT;*
[3]*Burnasyan SRC-FMBC FMBA;*
* aleksandr.zalesskij@phystech.edu

The team of authors develops the original method of combining (fusion) of cells using femtosecond laser radiation. Traditional alternative cells of cells are electric science and chemical fusion (using polyethylene glycol). There is also a method of fusion of cells using

hemagglutinating viruses, which is used in the works of both animal embryos and humans. All these methods are significantly invasive, since they inevitably affect the united cells entirely.
The most important feature of the developed methodology is the ability to direct the laser effect strictly to the cell contact area. With this approach, the cells themselves remain almost intact. In addition, the technique has a wide variability of dose parameters and the intensity of laser exposure, which allows the use of a laser to merge both very small and delicate objects (for example, somatic cells in a suspension) and sufficiently large (mouse oocytes).
Earlier, we showed that using femtosecond laser radiation, you can enucleate of mouse oocytes with high accuracy and low invasiveness (Osychenko A.A. et.al, Biomedical Optics Express, 2022). Within the framework of this work, it is proposed to supplement the methodology of the femo -section laser fusion of cells with the use of fluorescent dye Biotracker 400 Blue Cytoplasmic Membrane Dye, which is built into lipids, including The cytoplasmic membrane to increase the efficiency of the absorption of femtosecond laser radiation. Two model systems were used in the work: a suspension of cells of the A549 line and two -cell mouse embryos. The effectiveness of the procedure of laser fusion of cells was studied depending on the presence of fluorescent dye, wavelength of femtosecond laser radiation, pulse energy, and pulse frequency. The ability to control the shares of absorbed femtosecond laser radiation due to the spectral features of the used fluorescent dye was demonstrated. The result of this work is general in nature for the use of femtosecond laser radiation as nanoscalpel and can be used in other works associated with femtosecond laser nanosurgery.
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### S11.768. The mode of laser irradiation of the PHA cell scaffold determines the cellular response

Dudaev A.E.[2,1]*, Ryltseva G.A.[2,1]
[1]*Institute of biophysics SB RAS;*
[2]*Siberian federal university;*
* alex15-96@mail.ru

The active development of science, technology and production leads to an ever wider introduction of high-molecular polymer compounds of synthetic and natural origin in various spheres of human life. Requirements for the structure and physical and mechanical properties of polymers and products made from them are different and are determined based on specific areas of application (construction, agriculture and utilities, medicine, etc.).
To improve the properties of polymeric materials, 3 main approaches are used - biological, chemical or physical. By physical means, the use of various physical technologies (plasma treatment, ion implantation, etc.)
Relatively recently, laser processing has been used to improve the properties of plastics, which has an undeniable advantage over other methods, since it allows selective modification of the surface without destroying the material and forming toxic products.
The purpose of this work was to study the effect of two modes (continuous and quasi-pulsed) exposure to a carbon dioxide (CO2) laser on the microstructure and surface properties of polymer films of four types of PHA - poly-3-hydroxybutyrate and three copolymers. : 3-hydroxybutyrate with 4-hydroxybutyrate, 3-hydroxyvalerate or 3-hydroxyhexanoate.
The biocompatibility of cell matrices is largely determined by the physicochemical reactivity of the surface. The main factors regulating cell growth on a matrix are surface topography, roughness, chemical and phase compositions. The initial behavior of the cell on the surface largely determines the subsequent processes of cell differentiation and proliferation.
To confirm the above thesis, a study was made of the functional state of cells after cultivation on the test samples by flow cytometry after staining with fluorescent dyes. The stages of cell apoptosis were determined by flow cytometry on the 3rd day of cultivation, measuring the signals of annexin V and propidium iodide (PI) (Thermo Fisher Scientific, USA). The stages of apoptosis were studied on a CytoFLEX S cytofluorimeter (Beckman Coulter, USA). Annexin V-positive/PI-negative and annexin V-positive/PI-positive cells were considered to be in the early and late phases of apoptosis, respectively. Annexin V-negative/PI-positive cells were considered necrotic. Cells cultured on cultural polystyrene were used as a positive control.
Native untreated P(3HB) films are characterized by the active work of the process of programmed cell death, the number of viable cells is 34.16%, while for copolymers the percentage of living active cells is 60.58-72.77%. Processing in a continuous mode is accompanied by an increase in the rate of the apoptosis process for the P(3HB/3HHx) homopolymer and copolymer, the number of cells in the late stage of apoptosis for them is 77.54 and 59.94%, respectively; for copolymers with 3HB and 4HB, the ratio of living and apoptotic cells relative to untreated films increases towards the latter. Quasi-pulse irradiation, on the contrary, is accompanied by an increase in the number of viable cells - up to 44.61% for a homopolymer, up to 69.88-87.15% for copolymers, while the proportion of apoptotic cells decreases.
In this paper, for the first time, we present the results of a comparative study of the biological properties of films of 4 types of PHA treated with a laser in the modes of constant and quasi-pulsed radiation. The constant mode of laser radiation was accompanied by a decrease in the number of viable fibroblasts compared to their percentage on untreated films by 7–36%; in contrast, the number of viable cells increased on films treated in the quasi-pulse mode. : by 4-15% and 15-40%, respectively, compared with the original films and those treated with constant irradiation. This is an important result that opens up the possibility of stimulating the development of cell cultures when films are used as matrices in cell technologies.

### S11.769. The use of non-uniform rational B-splines in the resolution of overlapping protein absorption bands

Lavrinenko I.A.[1]*, Boyko A.A.[1], Marchukova K.N.[1], Podgornaya A.A.[1], Barysheva V.E.[1], Karpova O.V.[1], Cherniaev P.S.[1], Shatskiy I.V.[1], Vashanov G.A.[1]
[1]*Voronezh State University;*
* lavrinenko_ia@bio.vsu.ru

Analysis of the electronic absorption spectra of proteins and their complexes is one of the most accessible, universal, practically inertial free, and non-destructive ways to study their structural and functional properties. However, unlike atomic line spectra, molecular absorption spectra are a superposition of poorly resolved overlapping bands whose origin is due to quantum-mechanical transitions of different chromophore properties. The splitting of a molecule's system of electronic transitions to finer energy states due to the appearance of vibrational and rotational energy levels of atomic nuclei, together with the unquantized thermal energy levels and Doppler broadening, makes such absorption spectra virtually continuous. In addition, in condensed media, including aqueous solutions of proteins, intermolecular interactions additionally arise, leading to a significant smoothing of the absorption band peaks in such a way that the observed peak can eventually represent a superposition whose maximum does not correspond to any of the main electronic transitions in the molecule.
In this regard, the identification of peaks in poorly resolved absorption bands is a problem that to some extent can be solved both by minimizing the intermolecular interaction by isolating individual molecules

and reducing the kinetic energy of the system, and mathematically, by minimizing the absorption constant component in the spectrum. The best-known solutions were the baseline, differential (i.e., difference), and derivative spectrophotometry methods. The general principle of these methods consists in decreasing the absorption constant component due to its subtraction, which leads to an increase in the ratio of values along the Oy axis of the analyzed spectrum and, therefore, to an increase in the resolution [1-3].

In addition to these methods, we proposed a method combining difference and derivative spectrophotometry. In this case, an approximating spline from the absorption spectrum of the sample acts as a baseline (or a subtracted spectrum). Subtracting this spline from the absorption spectrum implemented similarly to the baseline or difference spectrophotometry method yields a function which is similar in form to the second derivative of the absorption spectrum and mirrors it with respect to the Ox axis.

As an approximating function we used non-uniform rational B-splines (Non-uniform rational B-spline, NURBS), which are widely used in solving problems of 2 and 3-dimensional graphics, and in particular in automatic design systems. Construction of the baseline to the studied absorption spectrum was carried out by varying the number of control points (grid nodes) and the order of the spline. As a demonstration of the possibility of applying this solution, we used the absorption spectrum of hemoglobin solution measured at a step of 0.2 nm in the 240-320 nm wavelength range (the absorption range of chromophores of side groups of amino acid residues). This spectrum was "sliced" (decimated) using a cubic spline into spectra with a fixed interpolation step: 0.2, 0.5, 1.0, 2.0, and 5.0 nm. These spectra were then approximated by second to eighth order NURBS curves, which were subsequently resampled to an initial measurement step value of 0.2 nm. Subtracting these NURBS curves from the original hemoglobin absorption spectrum yielded difference spectra from which poorly resolved absorption band peaks can be identified. Decreasing the order of the NURBS-curve and the interpolation step leads to an increase in the resolution of the absorption peaks. However, this resolution is limited by the signal-to-noise ratio (S/N) in the original absorption spectrum of the sample, which depends on the spectrum measurement conditions. Already for the case when the eighth order NURBS curve is obtained from the decimated absorption spectrum with a step of 2.0 nm, it becomes possible to identify 10 poorly resolved absorption band peaks without additional suppression of the noise of this spectrum.

Thus, in general form, the separation of analytically significant signal in the absorption spectra is achieved by minimizing the constant component, where the NURBS-curves can be considered as a high-pass filter. It should also be noted that the possibilities of NURBS-curves, the peculiarities of their application, as well as the method of absorption spectra analysis proposed in the present work are not fully disclosed and require further study [4-5].

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## S11.770. Ultralocal thermodynamic control of biological processes using a nanodiamond heater-thermometer

Romshin A.M.[1]*, Zeeb V.E.[2], Osypov A.A.[2,3], Popova I.Yu.[2], Radenovic A.[4], Glushkov E.[4], Sedov V.S.[1], Bagramov R.K.[5], Filonenko V.P.[5], Vlasov I.I.[1]

[1]*Prokhorov General Physics Institute of the Russian Academy of Sciences;*

[2]*ITEB RAS;*

[3]*IHNA&NPh RAS;*

[4]*Laboratory of Nanoscale Biology, Institute of Bioengineering, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland;*

[5]*Vereshchagin Institute of High Pressure Physics RAS, Moscow, Russia;*

* alex_31r@mail.ru

The study of the thermodynamics of intracellular processes is a novel direction in science, the progress of which critically depends on the creation of tools capable of accurately inducing and measuring temperature gradients in micro-/nanoscopic volumes inside and near living cells. Such an ultralocal heater-thermometer(HT) based on a luminescent nanodiamond localized at the end of a glass microcapillary was recently developed by us [1]. The thermal sensitivity of HT is provided by an ensemble of “silicon vacancy” color centers (SiV center) embedded in the crystal during synthesis. The position of the maximum of the SiV fluorescence zero phonon line depends on temperature and allows recording the temperature of any micro-/nanosystem after preliminary calibration. As the first example of the use of diamond HT in ultralocal thermal stimulation, we demonstrated changes in the level of free intracellular calcium in individual HeLa cancer cells and primary neuron culture [2], as well as neuroblastoma and H9C2 cardiomyocytes. It was found that a local temperature increase of 12 °C relative to the ambient level (22 °C) in a volume of ~1-3 μm^3 near the plasma membrane initiates the release of calcium from intracellular compartments into the cytoplasm and triggers a cascade of physiological processes.

The effectiveness of using diamond HT in ultralocal thermometry is demonstrated by the example of detecting a temperature burst near micron clusters of mitochondria in an aqueous medium under uncoupling of the electron transport chain [3]. Experiments have shown that during total uncoupling of transmembrane potential by CCCP application the temperature near mitochondria rises by 4-22 °C above the ambient temperature, with an absolute maximum of 45 °C. Spontaneous temperature bursts with the comparable amplitude were also detected prior to CCCP application that can reflect involvement of some mitochondria to ATP synthesis or membrane potential leaking to avoid hyperproduction of reactive oxygen species.

The proposed novel approach opens up unprecedented opportunities for micro- and nanoscale thermal initiation of physiological processes in living cells, as well as modulation of their rate in various intracellular compartments.

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## S12. Discussions

### S12.771. Methylation-Sensitive High Resolution Melting Application for disease diagnosis

Belov D.A.[1]*
[1]*Institute for Analytical Instrumentation of the Russian Academy of Sciences;*
* belov.da@list.ru

DNA methylation status is an epigenetic trait widely used as biomarkers for disease diagnosis and treatment planning[1-3]. One of the most promising methods for detecting such biomarkers is methylation-sensitive high-resolution melting (MS-HRM), which is implemented on detection amplifiers after qPCR using fluorescent dyes. Bisulfite treatment of DNA prior to performing MS-HRM provides a different base composition between methylated and unmethylated DNA, which is used to separate the resulting amplicons by high resolution melting. Convenience, low cost, high sensitivity and specificity make MS-HRM a popular approach to solving applied problems. Commercially available kits for the diagnosis of autoimmune and mental diseases, oncological diseases: cancer of the breast, colon, stomach, liver, lungs, prostate, melanoma, leukemia, lymphoma, etc. [4-6].
The main parameter for evaluating and classifying melting curves is the melting temperature; known methods are based on the comparison of this parameter of the curves with each other and with reference samples. These methods often do not allow one to identify differences between samples and adequately interpret the results of the analysis due to high errors and a limited number of determined parameters of melting signals.
It is planned to create new methods for processing MS-HRM signals, which make it possible to interpret the results of analyzes more reliably. New methods for processing DNA melt signals will reduce analysis time, provide high resolution and automate the processing of DNA melt analysis results.
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### S12.772. Mitochondrial-arginine theory of aging according to the mechano-chemiosmotic mechanism

Kasumov E.A.[1]*, Kasumov R.E.[1], Kasumova I.V.[1]
[1]*Research and Production Center «KORVET»;*
* kasumov_eldar@mail.ru

Harman's mitochondrial theory of aging [1], which is an "extended version" of the free radical hypothesis, is based on the assumption that aging occurs due to the cumulative effect of free radicals on mitochondrial DNA and its function. According to this theory, mitochondria are the main source of destructive free radicals that attack various components of the cell, and the leakage of free radicals from the respiratory chains of mitochondria occurs almost uncontrollably and constantly. This means that animals with a high metabolic rate generate free radicals quickly and have a short lifespan, while animals with a low metabolic rate do the opposite. Despite the fact that this point of view was not justified, the mitochondrial theory of aging takes into account the exceptional importance of the role of mitochondria in the vital activity of living organisms and aging, and therefore is of great interest. In our opinion, this theory is unable to fully explain the mechanism of aging due to insufficient knowledge of the mechanisms of mitochondrial functioning. It is known that the reactive oxygen species (ROS) is also formed in young organisms and plays an important physiological role in cellular processes and development of the organism. For example, a low level of ROS is involved in the selective removal of mitochondria in mitophagy, while a high level is involved in nonselective macroautophagy [2]. It is believed that the reason for the formation of an increased level of ROS is the delay of an electron for more than the optimal time required in the electron transport chain (ETC) in sites up to cytochrome c of the inner mitochondrial membrane, where the formed ROS causes chain reactions of lipid peroxidation, damage to mitochondrial DNA, mitochondrial dysfunction, apoptosis and cell death. However, the reason for the electron delay in the ETC still remains unclear. According to the mechano-chemiosmotic model proposed by us, the electron transfer in the ETC, a cyclic low-amplitude swelling-shrinkage of mitochondria, and ATP synthesis are coupled (https://www.youtube.com/watch?v=48jScej4dl0) [3]. According to this model, when the intracristal space of mitochondria shrinks, an electron is transferred from the [2Fe-2S] cluster of one dimer to the heme c1 of another dimer of the cytochrome bc1 complex located on the opposite side of the cristae membrane, and when the intracristal space swells, electron transfer stops, and this performs the regulatory role. Hyperosmotic conditions, incl. caused by water deficiency in the cytosol of old organisms, increase the time of cyclic swelling-shrinkage of mitochondria, which causes a delay in electron transfer in the ETC, a decrease in the rate of ATP synthesis and the formation of ROS. In turn, an increased amount of ROS causes strong depolarization (mild depolarization-repolarization is an integral part of the functioning of mitochondria), opening of the mPTP, and apoptosis. According to the mechano-chemiosmotic mechanism, cyclic low-amplitude swelling-shrinkage of mitochondria is accompanied by rotation of the γ-subunit and twisting-unwinding of the b2 subunits of ATP synthase, where arginine and lysine residues play a key role. Lysine and arginine residues are involved in energy transformation, both in the synthesis of ATP in mitochondria and in the hydrolysis of ATP in muscles. Due to the fact that the synthesis of arginine decreases in the human body after 28 years, the deficiency of arginine and lysine leads not only to a lack of energy in the body, but also other important functions associated with these amino acids are disrupted. Lysine and arginine affect the length of telomeres, hormonal regulation, epigenetic regulation of histones and other proteins. High glucose concentrations cause swelling of mitochondria [4], which reduces the effect of hypoxia in cancer cells [5] and causes glycation of protein lysine and arginine residues during aging [6].

Thus, we propose a mitochondrial-arginine theory of aging based on a decrease in arginine synthesis and mitochondrial dysfunction with age. Mitochondrial dysfunction, accompanied by a decrease in the frequency of a low-amplitude swelling-shrinkage cycle, is caused by cascade processes occurring genetically determined by a decrease in the amount of water in the body, associated, incl. with an increase in glucose concentration with age; by a deficiency of arginine and lysine; by a decrease in physical activity, leading to an increase in the level of sugars and a decrease in the level of ADP (ADP in the cell is a trigger for the functioning of mitochondria). In turn, due to mitochondrial dysfunction, protein glycation occurs with the increasing the concentration of sugars and ROS. So, to achieve healthy longevity, it is necessary: regular physical activity, intake of sufficient water, a balanced diet, taking into account the elimination of arginine and lysine deficiency, sleep and the absence of distress [7-8].

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### S12.773. Nonlinear dynamics of cells in oncological transformations and microgravity conditions

Naimark O.B.[1]*
[1]*Institute of Continuous Media Mechanics UB RAS;*
* naimark@icmm.ru

Field theory of defects is used to study the mechanobiology of cells in the case of oncological transformations and in microgravity conditions. After Schrödinger's definition of the structure of DNA as a biological crystal, the mechanisms of functioning of DNA, cells can be associated with the nonlinear dynamics of defects (open states). Statistical thermodynamics of the open states in the DNA ensemble allowed the definition of out-of-equilibrium free energy in term of open-states induced strain as the "order parameter". New type of critical phenomena (structural-scaling transition) was established linking the nonlinear dynamics of this parameter with "thermalization" conditions" ("effective temperature" caused by open complex interaction) and the types of the collective open states modes providing different scenario of gene expressions [1-4]. Collective modes have the nature of self-similar solutions corresponding to DNA breathing, are observed experimentally and can be considered as triggering mechanisms for gene expression, transcription, and cell division [5, 6]. The variety of structural-scaling transitions scenario and metastability types of out-of-equilibrium free energy, induced by open states allowed the explanation of the Waddington landscape for the cell evolution [7]. The role of open states as defects is associated with manifestations of ductility for normal cells and fragility for cancer cells, which determine corresponding phenotypes due to the subjection of cell dynamics to collective open states modes Regularities of "criticality", caused by the collective behavior of defects (open states), are studied using original experimental data on in vivo cell dynamics as fluctuations of "phase thicknesses" obtained by laser (interference) microscopy [8]. Analysis of these data made it possible to establish multifractal dynamics characteristic of "normal" cells ductility and monofractal dynamics for cancer cell reflecting cell fragility [9]. Conclusion is substantiated that monofractal dynamics, caused by development of collective "blow-up modes" in an ensemble of open states, corresponds to modes of spontaneous cell division.The analysis of external field on the cell dynamics allowed the interpretation of the phenotype changes in the microgravity conditions. Analysis of laser microscopy data in vivo cell dynamics are compared with results of a multifractal analysis of temperature fluctuations field by infrared scanning of «normal tissue» and tissue with oncological pathologies [10].

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### S12.774. Prolonged impact of mm waves on the plant genome

Minasbekyan L.A..[1]*
[1]*Yerevan State University;*
* minlia@ysu.am

With the development of telecommunications and modern digital technologies, covering all spheres of human living space, the study of the subtle mechanisms of the impact of mm-waves on biological organisms is an essential component of the security problem. Plants are the most convenient model for studying the effects of mm-waves on the genome of organisms since they retain a fixed orientation relative to the source of radiation, and it is also possible to carry out effects in vivo and study its prolonged effect on DNA.

The studies were carried out in the range of 45GHz-51.8 GHz frequency. The results of our studies indicate a decrease in the melting temperature of DNA by 2-30C under the action of mm-waves on DNA. At the same time, there is an increase in the width of the transition,

and the identification of peaks in both low-melting and high-melting regions of DNA. This indicates the melting of blocks of different sizes, which melt cooperatively, which can be explained by an increase in the activity of DNA replication and a change in the level of DNA methylation. In all cases, under the influence of mm-waves, conformational changes in DNA are observed, which are supposed to be of an epigenetic nature. To confirm this fact, we also determined the levels of DNA methylation for several frequencies in the first and second generations. Possible mechanisms of such changes under the influence of mm waves are discussed.

**S12.775. Quantum bioenergetics of living cells and organisms**

Gall L.N.[1]*
[1]*Institute of Analatical Instumentation Russian Academy of sciences;*
* lngall@yandex.ru

In the report, with references to primary sources, the author outlines basic principles of quantum bioenergetics. Bioenergetics, based on the laws of quantum physics and understood as the movement of energy flows through molecular systems of a living cell (organism), is the result of the unity of physical mechanisms of three systems: the molecular system of biopolymers of a given organism, structured systems of water molecules that bind its biopolymers, and flows of electromagnetic quanta fields in the energy range that does not destroy the single molecular-water structure of the organism[1]. Biopolymers of living organisms, once victorious in prebiological evolution, form a structure saturated with oscillators capable of converting external, non-polymer-specific[2], quantum or mechanical energy into quantum-specific energy, moreover, in the energy range that is non-destructive for living water-molecular systems. The number of oscillators increases significantly with biopolymer hydration[3]. This makes biopolymers unique "energy machines" of a living system that create quantum energy flows. During hydration of a biopolymer, water molecules form core quasi-fractal energy-intensive structures on its hydrophilic centers[4] that bind all biopolymers and small bio-molecules both within cells and between them, and serve as highways for transporting quantum flows, both biochemical (non-specific) and resonant (controlling), without their absorption by the aquatic environment of the cell cytoplasm. This provides a unified system of bioenergetics in a living organism of any complexity, both in providing intermolecular reactions and in controlling their sequence and synchrony. All of the above described processes already have reliable experimental confirmation: re-emission of energy by a biopolymer — in the works of Careri[5], Kokaya[6] and others; the formation of solitons during hydration — in the works of Alexander, two states of water in living systems — in the works of Khokhlova[7], Gall et al[8]. The conditions for the experiments and their results will be presented in the report.

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**S12.776. Revealing specific effects of high concentrations of cesium or plutonium-americium-strontium on the generative sphere of plants (SEM-palynoteratical data on the surface soil samples from Chernobyl exclusion zone)**

Levkovskaya G.M.[1]*, Tarasevich V.F.[2], Shamal N.V.[3], Kasparov A.K.[1], Bogolubova A.N.[2]
[1]*Institute for the History of Material Culture RAS;*
[2]*Komarov Botanical Institute RAS;*
[3]*Institute of Radiobilogy of NAS of Belarus;*
* ggstepanova@yandex.ru

The authors have previously developed a statistical palynoteratical method for distinguishing geobotanical optima and catastrophes based on quantitative data on pollen morphology variations in pollution-free deposits of many regions of the former USSR, surface soil samples from South Arabia, all subzones of Western Siberia, and archaeological profiles with clear ecology confirmed by paleozoological data (see references in [1, 2]).

The first stage of the study of the surface soil samples from the 30-km Chernobyl zone was aimed at confirming the suitability of the palynoteratical method for identification of environmental disasters, not only of natural, but also anthropogenic origin. The samples were collected from three locations in 1988 by colleagues from the Institute of Radiobiology of NAS of Belarus. The palynoteratic indicators of extreme environmental conditions caused by natural factors or radiation pollution were determined: 1. almost complete absence of morphologically typical palynomorphs; 2. single determinable forms; 3. dominance of sterile forms; 4. for natural extremes - the dominance of the underdeveloped due to the immaturity pollen, and in the Chernobyl complexes - the underdevelopment of most morphological features; 5. only in the Chernobyl complexes - the dominance of ugly forms caused by mutations; 6. in some types of natural complexes - the dominance of dwarf forms, and in the Chernobyl complexes - the size variability. In this article, for the first time palynoteratic Chernobyl materials were used to identify the degree of the impact on the reproductive sphere of all plants of radionuclide complexes with maximum concentrations of plutonium-americium-strontium (239/240Pu, 241Am, 90Sr) on the one hand, or cesium (137Cs) on the other. Their half-lives are 24360, 433.2, 28.8, 30.17 years respectively.

Maximums of 239/240Pu=99, 241Am=150, 90Sr=14,000 Bq/kg were recorded at Masany site (12 km from the ChNPP). A significant amount of 137Cs=52,000 Bq/kg was also found there. Surface soil exposure dose rate (SSEDR) is 530 μR/h.

Using SEM micrography, the most extreme palynoteratical complex was documented for Masany. It looks like a "cemetery of empty forms", as in almost all palynomorphs all morphological characters are absent, except for thickened exines without sculpture that outline empty grains. All forms are sterile. In the complex only one morphologically typical grain with a normally developed ornamentation, cf. Asteraceae (?) was found. Two malformed grains of Pinaceae were identified: one is atypically small with asymmetrical sacs, and the second - with a giant body and dwarf sacs.

At Kryuki site (16 km from the ChNPP), the maximum of 137Cs=270,000 Bq/kg contamination was detected (compared to 52,000 Bq/kg at Masany). SSEDR is 2200 μR/h. Also significant concentrations of 239/240Pu=48, 241Am=81, 90Sr=4500 Bq/kg (compared to 99, 150 and 14000, respectively at Masany) were found.

Traditional palynological and SEM palynoteratical studies of the complex showed the absence of morphologically typical pollen grains in it. The Kryuky complex does not look like a "cemetery of empty forms" anymore, since in all grains most of the features are developed. But, due to the mutations of each feature, the complex looks like a mass of monstrous palynomorphs with variability of: 1. pollen grain contours; 2. asymmetries; 3. shape or size of the same feature; 4. exine thickness from “tumor-like” growths to erosions, 5. pollen grain size, that range from almost normal (two Pinaceae pollen grains) to ultra-dwarf (pollen grain of Alnus sp. ~ 10 µm, while the minimum size of Alnus glutinosa and A. incana – 21.3-21.6 µm [3: 62]). All grains are lacking sculpture, protoplast, and sterile.

Lesok site (22 km from the ChNPP) is partially protected by forest from the spread of radioactive particles. The studied sample is contaminated with 239/240Pu=83, 241Am=140, 90Sr=9 100 and 137Cs=84 100 Bq/kg. SSEDR is 890 µR/h. Regardless of the significant contamination of this sample with 239/240Pu, 241Am, 90Sr (levels are comparable with the Masany sample), the completely underdeveloped forms are scarce in the pollen complex. It is dominated by the forms with various mutations, similar to the Kryuky complex with maximum 137Cs. Single normally developed pollen grains were found. Presumably, this is due to the protective role of the forest.

Studies have shown that the most extreme conditions for the reproductive sphere of all plants have been identified for the Masany site characterized by the highest contamination with 239/240Pu, 241Am, 90Sr. This type of pollution leads not only to the complete sterility of the complex, but also to the complete underdevelopment of most morphological features of pollen grains - male gametophytes (a sort of spermatozoa) of seed plants.

The complex with the maximum 137Cs contamination (Kryuki) is also characterized by pollen sterility and absence of sculpture in most forms, though already developed morphological features have monstrous deviations from palynomorphological norms due to mutations. Data on Lesok site show that the forest acts as a kind of "umbrella" and mitigates the impact of radiation on the reproductive sphere of plants.

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### S12.777. Stages of the origin of life recorded in the sequence of a bacterial cell exit from anabiosis

Kompanichenko V.N.[1]*
[1]*Institute for Complex Analysis of Regional Problems RAS, Birobidzhan;*
* kompanv@yandex.ru

According to the inversion concept of the origin of life (TI concept), the intermediate position of the prebiotic system between the inanimate and living states maintains in an oscillatory mode [1]. Thermodynamically, this situation corresponds to the approximate equality of the contributions of entropy and free energy in the system. Within the framework of the theory of anabiosis in microbiology, a resting (sleeping) bacterial cell occupies a similar intermediate position between non-living and living: on the one hand, it is no longer able to counteract the growth of entropy, and on the other hand, it retains the structural memory of the previous living state. The sequence of metabolism formation in primary living cells has not yet been reliably established by researchers, while the sequence of changes in metabolic processes in the simplest bacterial cell entering and exiting the state of anabiosis has been well studied both experimentally and theoretically. This presentation substantiates the general sequence of the formation of metabolism in the process of the emergence of life, based on the correlation of these two intermediate states between non-life and life (prebiotic and bacterial). According to the inversion concept, life originated in a pulsating updraft of hydrothermal fluid. In general, this process included the following steps. 1) Accumulation of dispersed organic matter in the geospheres through its synthesis and inflow from space at the pre-biological stage of the Earth’s evolution. 2) Self-assembly of three-dimensional prebiotic microsystems of predominantly lipid-protein composition in hydrothermal fluid. 3) Formation of protocells: the transition of microsystems to an intermediate state between non-life and life by actively responding to fluctuations in physical and chemical parameters in the environment (i.e. to periodic stress - [2]), including the appearance in them of a weak energy-giving process of respiration for due to redox reactions and local watering of the membrane. 4) The formation of living subcells in the process of formation of a non-enzymatic antioxidant system and the emergence of a protein-synthesizing apparatus. 5) The formation of living cells (correlates with progenotes according to C. Woese [3]) with arising of the growth cell cycle and the formation of a genetic apparatus. Within the framework of this reconstruction, the sequence of metabolism formation in stages 3–5 correlates with the sequence of metabolism recovery when a bacterial cell exits from anabiosis [4].

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### S12.778. The adaptation mechanism of action of hypoxia according to the mitochondrial- arginine theory of aging

Kasumov E.A.[1]*, Kasumov R.E.[1], Kasumova I.V.[1]
[1]*Research and Production Center «KORVET»;*
* kasumov_eldar@mail.ru

Hypoxia causes regularly developing changes in the ultrastructure of cells, and damage to mitochondria plays a decisive role in them. Pathology of mitochondria is expressed in the appearance of several typical forms of changes depending on the duration and severity of hypoxic exposure and is a complex multi-stage process [1]: stage 1, short-term activation of complex I of the electron transport chain and an increase in the content of cytochrome bc1 complexes subunits; stage 2, with increased hypoxia, suppression of complex I and compensatory activation of complex II occur; stage 3 (depletion) develops at very low pO2 values or prolonged hypoxic exposure and is accompanied by suppression of complex III (cytochrome bc1 complex), and then complex IV, which leads to deenergization of the cell.

At the same time, this hypoxic effect in stage 1 led to an increase in matrix density, an increase in the number of organelles with densely and parallel packed cristae, which reflects an increase in oxidative phosphorylation and a decrease in the level of reactive oxygen species (ROS). A similar effect of hypoxia occurs on plant mitochondria [2].

Prolonged hypoxia (stage 3) causes swelling of mitochondria with a decrease in cristae, condensation of the mitochondrial matrix, and an increase in the level of ROS.
The acquisition of parallel packed cristae of mitochondrial ultrastructure under the influence of the initial stages of hypoxia cannot be explained in terms of the classical mechanism of mitochondrial functioning, but can be easily explained using the mitochondrial-arginine theory of aging. This theory is based on a mechano-chemiosmotic coupling mechanism, where electron transfer, low-amplitude swelling-shrinkage of mitochondria, and ATP synthesis are coupled (https://www.youtube.com/watch?v=48jScej4dl0) [3]. According to this mechanism, when the intracristal space of mitochondria shrinks, an electron is transferred from the [2Fe-2S] cluster of one dimer to the heme c1 of another dimer of the cytochrome bc1 complex located on the opposite side of the cristal membrane, and when the intracristal space swells, electron transfer stops. This mechanism plays an important regulatory role. Under hypoxic conditions, for the most efficient consumption of deficient oxygen, mitochondria acquire a parallel packing of cristae, which creates the possibility of simultaneous contacts between dimers of cytochrome bc1 complexes and reduces the level of ROS.
Thus, episodic hypoxia can provide protection against cellular stress and apoptosis by reducing ROS [4], which is the most important contribution to the anti-aging program to prolong active longevity.
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### S12.779. The role of stress factors in the formation of magnetic properties in living systems

Khalilov R.I.[1,2], Nasibova A.N.[1,2]*, Fridunbeyov I.Y.[2]
[1]*Azerbaijan National Academy of Sciences, Institute of Radiation Problems;*
[2]*Baku State University;*
* aygun.nasibova@mail.ru

Using the method of Electron Paramagnetic Resonance (EPR), we studied the influence of stress factors on the living systems of the Apsheron Peninsula (Azerbaijan). The paramagnetic centers of the research objects were studied [1,2,3]. For the first time, we found that under the influence of stress factors in living systems, broad EPR signals (g=2.32; ΔH=320 Gs) are formed that characterize iron oxides magnetic nanoparticles [1, 4].
In the course of primary research on the territory of the Iodine Plant in the village. Raman of the Absheron Peninsula, where the radiation background ranges from 4-400 μR/hour, it was found that the impact of radioactive contamination on plants leads to the formation of a broad EPR signal characterizing nanophase particles of iron oxide, both in leaves and in plant seeds. It was found that the intensity of this signal is higher in leaves than in plant seeds [1].
The results obtained made it possible to assume that the main key role in stimulating the formation of magnetic nanoparticles in plant leaves belongs to an increased radiation background with a high content of radionuclides (primarily this applies to the 226Ra and 238U isotopes). Such radionuclides accumulate in large quantities in the leaves.
It can be assumed that the generation of magnetic nanoparticles that create a broad EPR signal is associated with the operation of the photosynthetic apparatus in plant leaves [1,5,6].
Indeed, the intensities of broad EPR signals that we observed in plant seeds were significantly lower than in leaves. It can be said that the phenomenon of stimulation of the formation of magnetic nanoparticles in plants growing in radioactively contaminated areas is associated with a partial violation of the integrity of chloroplasts due to high gamma radiation.
In this case, the proximity of exogenous sources of nanoparticle formation (for example, iron ions) to the electron transport chain (ETC) of chloroplasts will increase, which will stimulate the formation of nanoparticles.
In addition to plants, we also studied some animal organisms. The behavior of paramagnetic centers under gamma radiation was also studied in the organisms of grape snails (Helix pomatia) and laboratory rats (Wistar albino).
When studying the effect of ionizing gamma radiation on grape snails, it was found that with an increase in the radiation dose, the intensity of the signal of free radicals in the body and shells of snails increases linearly.
It has been found that the intensity of the EPR signal characterizing magnetic iron oxide nanoparticles increases with an increase in the irradiation dose up to approximately 250–350 Gy and decreases linearly with a subsequent increase.
Thus, the parameters of the EPR spectra of the body and shells of snails can be used in the study and biomonitoring of the ecological state of the environment.
In the course of EPR studies on laboratory rats, we have shown for the first time that ionizing gamma radiation causes the formation of magnetic iron oxide nanoparticles in the organs of their liver.
Summarizing the results obtained in studies with living systems, we can say that the stress factor causes anomalous magnetic properties in living systems.
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**S12.780. The role of the gamma subunit in the molecular mechanism of ATP synthesis by the ATP-synthase complex**

Kasumov E.A.[1]*, Kasumov R.E.[1], Kasumova I.V.[1]
[1]*Research and Production Center «KORVET»;*
* kasumov_eldar@mail.ru

The synthesis of ATP by the ATP synthase complex plays a fundamental role in oxidative phosphorylation and photophosphorylation in the formation of cellular energy. The synthesis of one ATP molecule requires one ADP molecule, a phosphate ion, and a proton in the active site of the enzyme, although proton and phosphate delivery routes are not considered in the classical model. At the same time, it is well known that phosphate ion binding and ATP release are energy dependent processes. It is assumed that the rotation of the gamma subunit is associated with the formation and disappearance of the phosphate-binding site [1]. In the synthesis and hydrolysis of ATP, an important role is played by the Pi-binding positively charged P-loop pocket formed from the residues βLys155, βArg182, and αArg376, as well as the residues βThr156, βGlu181, βGlu185, βAsp242 [2,3].

According to our mechano-chemiosmotic mechanism [4](https://www.youtube.com/watch?v=48jScej4dl0), protons and phosphate ions are delivered in a bound state with the gamma subunit to the active sites of ATP synthase against the energy barrier of the DELSEED loop formed from acidic amino acid residues. During ATP synthesis, the phosphorylated lysine or arginine residue at the C-terminus of the γ-subunit will interact with βGlu181 (during ATP hydrolysis, it binds a water molecule) of the Pi-binding site, where, at a neutral pH region, βGlu181 will act as a proton donor for βArg182.

In the catalytic center of the β-subunit, apparently, the protonated lysine or arginine of the gamma subunit protonates βGlu181, and the residues βLys155, αArg376 and βArg182 loosen the structure of the phosphate ion delivered along with the proton by the gamma subunit. Finally, βGlu181 removes the OH- group from HPO42- as a result of nucleophilic substitution located in the P pocket. The high-energy PO3-radical (phosphoryl group) binds to MgADP-, resulting in the formation of MgATP2- and a water molecule (H2O). It is possible that, in order to avoid hydrolysis of the synthesized ATP in the catalytic center, there is an exchange of MgATP2- in the β-subunit and MgADP- in the α-subunit.

Thus, the energy-dependent delivery of protons and phosphate ions to the active sites of ATP synthase occurs with the help of the gamma subunit against the energy barrier. The report also discusses the role of b2-subunits in the delivery of protons and phosphate ions to the active sites of the enzyme during rotation of the gamma subunit.

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