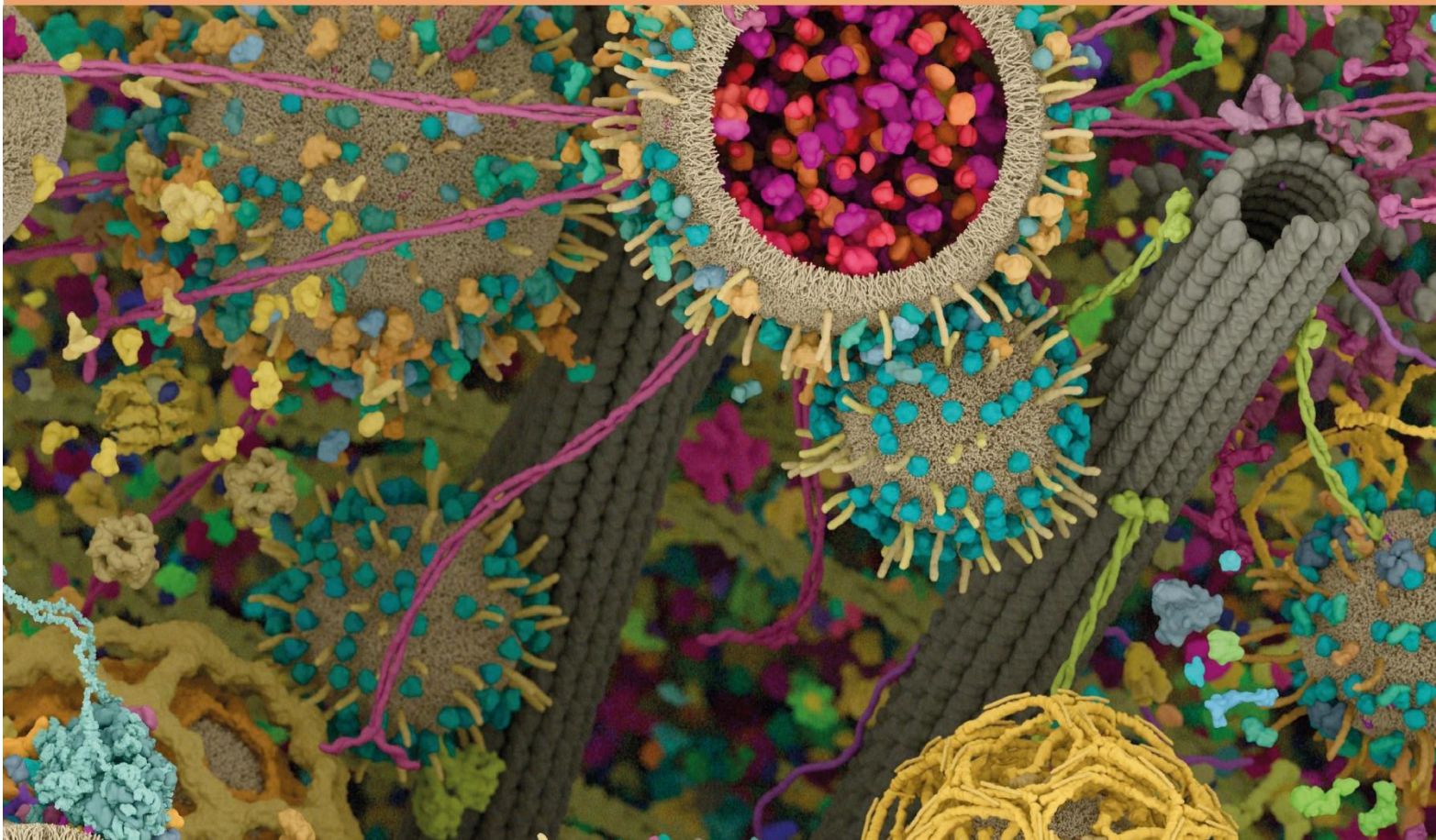
biomembranes '24



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And others … TO BE UPDATED

CONTENTS

Satellite event:
Advanced school on structural biology and drug discovery

06 October 2024 – Sunday	
9:00-10:00	Registration
10:00-10:15	Organizing Committee **Introduction: the Pipeline**
10:15-11:00	Andrei Gilep, *Institute of bioorganic chemistry, NASB, Belarus* **Drug target discovery and validation**
11:00-11:45	Konstantin Usachev, *Centre for Integrative Structural Biology, National Research Centre Kurchatov Institute, Russia* **Structural studies of translation regulation in prokaryotic and eukaryotic pathogenic microorganisms**
11:45-12:05	Coffee Break
12:05-12:50	TBA (Speaker confirmed) **Advanced applied crystallography on modern synchrotron sources**
12:50-13:35	Konstantin Usachev, *Centre for Integrative Structural Biology, National Research Centre Kurchatov Institute, Russia* **Structural studies of translation regulation in prokaryotic and eukaryotic pathogenic microorganisms, part 2**
13:35-14:35	Lunch
14:35-15:05	Sergei Evteev, *Dukhov Automatics Research Institute, Russia* **Structure based drug design**
15:05-15:35	Alexander Malyshev, *Dukhov Automatics Research Institute, Russia* **Ligand based drug design**
15:35-16:10	Anton Chugunov, *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Russia* **Computer-aided design of pharmaceutical proteins: 'physical' and 'neural network' approaches**

16:10-16:40	Coffee Break
16:40-17:25	Leonid Kaluzhskiy, *Institute of Biomedical Chemistry, RAS, Russia* **Surface plasmon resonance and drug discovery**
17:25-18:10	Germes Chilov, *JSC "ValentaPharm", Russia* **Case study of innovative Drug Discovery in Russia**

Conference program

7 October 2024 – Monday	
9:30-11:00	Registration and Welcome Coffee, Poster Session 1
Session chair: Ivan Okhrimenko	
11:00-11:40	Keynote lecture: Piotr Bregestovski, *Kazan Medical State University, Russia* **Analysis of nerve cell activity and ion homeostasis using optosensoric approaches**
11:40-12:10	Lihua Song, *Beijing University of Chemical Technology, China* **Research on pangolin coronavirus pathogenesis: implications for COVID prevention and control**
12:10-12:40	Wentong Jiang, *National Institute of Biological Sciences, China* **Structural basis for the ubiquitination of G protein βγ subunits by KCTD5/Cullin3 E3 ligase**
12:30-14:00	Lunch
14:00-14:40	**Conference Opening Ceremony.**
Session chair: Ivan Okhrimenko	
14:40-15:20	Keynote lecture: Sergei Nedospasov, *Engelhardt Institute of Molecular Biology, RAS, Russia* **Cytokines as molecular mediators of immunity and targets for therapy**
15:20-15:50	Plamena Angelova, *UCL Queen Square Institute of Neurology, England* **Protein misfolding, aggregation and seeding- the trigger for neuronal death in Parkinson's disease?**
15:50-16:35	Coffee Break and Poster Session 1

Session chair: Piotr Bregestovski	
16:35-17:05	Michael Firsov, *Sechenov Institute for Evolutionary Physiology and Biochemistry, RAS, Russia* **Advances in retinal prosthetisation**
17:05-17:45	Keynote lecture: Mikhail Ostrovsky, *Lomonosov Moscow State University, Russia* **Molecular physiology and pathology of vision: rhodopsin**

8 October 2024 – Tuesday	
Session chair: Alexey Mishin	
9:30-10:10	Keynote lecture: Zhi-Jie (James) Liu, *iHuman Institute, China* (remote) **Structural biology investigation on human bitter taste receptors**
10:10-10:40	Jianjun Cheng, *iHuman Institute, China* (remote) **GPCR drug discovery: Rational design of polypharmacology**
10:40-11:30	Coffee Break and Poster Session 2
Session chair: Aleksandra Luginina	
11:30-12:00	Suwen Zhao, *iHuman Institute, China* (remote) **The annotation, classification, structure and database building of chordate olfactory receptors**
12:00-12:40	Keynote lecture: Konstantin Anokhin, *Lomonosov Moscow State University, Russia* **Memory self-degradation in Alzheimer's disease and new opportunities for cognitive neuroprotection**
12:40-13:10	Sergey Kozin, *Engelhardt Institute of molecular biology, RAS, Russia* **Pathogenic agent of Alzheimer's disease and molecular tools for its neutralization**
13:10-14:30	Lunch
Session chair: Konstantin Boyko	
14:30-15:10	Keynote lecture: Alexey Amunts, *Westlake University, China* **Structure of the II2-III2-IV2 mitochondrial supercomplex from a parasite reveals a protein inhibitor of complex III**
15:10-15:25	Nikolay Ilyinsky, *Moscow Institute of Physics and Technology* **Optogenetics of lysosomes: monitoring differences in the type and stage of disorders and possible interventions**

15:25-15:40	Ivan Okhrimenko, *Moscow Institute of Physics and Technology* **Molecular interactions of promising drugs based on D-enantiomeric peptides with the β-amyloid precursor protein**
15:40-16:30	Coffee Break and Poster Session 2 **Masterclass from the Helicon Company at the foyer**
16:30-19:00	**Translational Research, Development and Innovation in Biotechnology and Bioeconomics (in Russian)** Andrey Rogachev, *MIPT Center for molecular mechanisms/LPR* Valentin Borshchevskiy, *MIPT Center for molecular mechanisms* Maxim Ostras, *LIFT (Life Improvement by Future Technologies)* Nikita Trofimov, *MIPT Business School* Sergey Vakhterov, *MIPT Startup Studio* Philipp Maximov, *MIPT Biotechnology Center* Maxim Magruk, *Geropharm* Mikhail Selyanin, *Martinex*

9 October 2024 – Wednesday	
Session chair: Valentin Borshchevskiy	
9:30-10:10	Keynote lecture: Vsevolod Belousov, *Federal Center of Brain Research and Neurotechnologies, FMBA , Russia* **Chemogenetic technologies for metabolism control**
10:10-10:40	Yan Zubavichus, *Synchrotron Radiation Facility 'SKIF', Russia* **Synchrotron Radiation Facility 'SKIF': New Experimental Capabilities for Structural Biology**
10:40-11:30	Coffee Break and Poster Session 3
Session chair: Tatiana Murugova	
11:30-12:10	Keynote lecture: Norbert Kučerka, *Joint Institute for Nuclear Research, Russia* **Arrangements of pre-AD membranes due to Aβ(25-35) peptide**
12:10-12:40	Na Li, *Shanghai Advanced Research Institute, CAS, China* **Structural characterization of biomembrane using synchrotron small-angle X-ray scattering**
12:40-13:10	Eugene Vysotski, *Institute of Biophysics, SB RAS, Russia* **Crystal structures of obelin with coelenterazine-v before and after light emission confirm coelenterazine dioxyethanone decomposition through 'light' and 'dark' pathways**
13:10-14:30	Lunch
Session chair: Kiryl Piatkevich	
14:30-15:10	Keynote lecture: Chunfu Xu, *National Institute of Biological Sciences, China* **Computational Design of Transmembrane Channels and Beyond**
15:10-15:40	Dmitri Dormeshkin, *Institute of Bioorganic Chemistry, NASB, Belarus* **Generation of Synthetic Antibodies for CAR T-cell Therapy Development**

15:40-16:10	Nikolai Sluchanko, *Federal Research Centre 'Fundamentals of Biotechnology', RAS, Russia* **Novel nanobodies targeting receptor-binding domains of some relevant viral and bacterial human pathogens**
16:10-17:00	Coffee Break and Poster Session 3
Session chair: Ivan Okhrimenko	
17.00-17:30	Alexis Ivanov, *Institute of Biomedical Chemistry, RAS, Russia* **SPR analysis of proteins and ligands interaction with target membrane protein embedded in a lipid bilayer**
17:30-18:00	Alexander Molochkov, *Pacific Quantum Center, Far East Federal University, Russia* **Protein dynamics within the Lattice Abelian Higgs model**

10 October 2024 – Thursday	
Session chair: Ivan Gushchin	
9:30-10:10	Keynote lecture: Kiryl Piatkevich, *Westlake University, China* **New molecular tools for scalable mapping and recording of neuronal activity in vivo**
10:10-10:40	Georgii Nosov, *Federal Center of Brain Research and Neurotechnologies, FMBA, Russia* **Nano-architecture of the presynaptic active zone in different physiological conditions**
10:40-11:10	Oleg Podgorny, *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Russia* **Thermogenetic control of neuronal circuits in vivo**
11:10-11:40	Zakhar Shenkarev, *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Russia* **Structural basis of TRPA1 channel modulation by Cys-knot spider toxins**
11:40-12:20	Coffee Break
Session chair: Valentin Ivanovich	
12:20-13:00	Keynote lecture: Roman Efremov, *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Russia* **Computational structural biophysics of TRPV ion channels**
13:00-13:30	Georgy Guria, *Medical Research Center for Hematology, Russia* **CAR nucleation as a driving force of biomembrane antigen pattern recognition**
13:30-13:45	Anatoliy Belousov, *Moscow Institute of Physics and Technology* **Monitoring GPCR conformation with GFP-inspired dyes**
13:45-14:00	Andrey Nikolaev, *Moscow Institute of Physics and Technology* **Engineering of soluble bacteriorhodopsin**
14:00-14:30	Ekaterina Lyukmanova, *Shenzhen MSU-BIT University, China* **Role of Ly6/uPAR proteins in the brain pathology**

11 October 2024 – Friday	
Session chair: Alexey Mishin	
9:30-10:00	Yurii Krupyanskii, *Semenov Federal Research Center for Chemical Physics, RAS, Russia* **DNA architecture in bacteria subjected to various types of stress**
10:00-10:40	Keynote lecture: Arun Shukla, *Indian Institute of Technology, India* (remote) **Structure, function, and regulation of G protein-coupled receptors**
10:40-12:00	Coffee Break and Poster Session 4 (extended)
Session chair: Nikolay Ilyinsky	
12:00-12:40	Keynote lecture: Claudio Franceschi, *University of Bologna, Italy* (remote) **Inflammaging and longevity within the framework of personalized aging**
12:40-13:10	Alexey Golubev, *N.N. Petrov National Medical Research Center of Oncology, Russia* (remote) **Consistency of non-linear dynamics of human aging with a generalized Gompertz-Makeham law**
13:10-14:30	Lunch
Session chair: Valentin Ivanovich	
14:30-15:10	Keynote lecture: Elizaveta Bonch-Osmolovskaya, *Lomonosov State University, Russia* **Metabolic diversity of extremophilic prokaryotes: results of genomic and metagenomic analysis**
15:10-17:00	Early career researcher presentations Best poster awards Concluding remarks
17:00-18:30	Coffee Break

LECTURES

Advances in retinal prosthetisation

Firsov M.L.

Sechenov Institute for Evolutionary Physiology and Biochemistry RAS, St.-Petersburg, Russia
E-mail: Michael.Firsov@gmail.com

Severe neurodegenerative diseases of the retina often result in partial or complete loss of vision. These diseases may be genetic, age-related or result from other co-morbidities. While there are a variety of causes of vision loss, in the late stages of degenerative processes, the retina usually loses its photoreceptor layer and becomes insensitive to light. The architecture of the retina is significantly disrupted, but the descending neuronal pathways that transmit visual information to the brain via the optic nerve are partially preserved. This circumstance makes it possible to develop and apply technologies that, on the one hand, restore the function of light sensitivity in the retina and, on the other hand, use retinal neurons that have survived neurodegenerative processes to transmit information about light stimulation to the visual cortex of the brain using partially preserved pathways for the normal passage of visual information.

These technologies include implantation of stimulating electrode matrices and optogenetic retinal prosthetics. Optogenetic retinal prosthetics ideally approach the resolution of natural vision and the prosthetic procedure is reduced to a single intraocular injection. The technology relies on the use of photosensitive proteins - ionotropic channelrhodopsins or opsin-based metabotropic proteins - to transduce and photosensitise remaining retinal neurons. This presentation will discuss the potential and limitations of different approaches to retinal prosthetics. The advantages and disadvantages of the use of channelrhodopsins and opsins for the prosthesis of different types of retinal neurons will be discussed. The main achievements in this field and the main problems that remain to be solved will be outlined.

Analysis of nerve cell activity and ion homeostasis using optosensoric approaches

Bregestovski P.D.

Institute of Neurosciences, Kazan State Medical University, Kazan, Russia
E-mail: pbreges@gmail.com

Optosensing is a research area that enables noninvasive monitoring of intracellular ion concentrations, enzyme activity, lipids, and other cellular components using special optical sensors. In recent years, various types of genetically encoded sensors have been created for these purposes. These probes have fluorophore groups that can change fluorescence when interacting with certain ions or molecules. The use of modern optical and fluorescent technologies in combination with molecular genetic methods has made it possible to monitor activity of reactive oxygen species, cAMP, glucose, glutamate, pyruvate, lactate, various ions, and neural activity.

The development of many pathologies of the nervous system is based on an imbalance of excitation and inhibition in neural networks, as well as changes in intracellular ion concentrations. For non-invasive analysis of intracellular concentrations of chloride ([Cl-]i) and hydrogen ([H+]i), we created a line of transgenic mice expressing a genetically encoded biosensor (ClopHensor) for simultaneous registration of changes in these ions in neurons and obtained a map of its expression in various brain regions [1,2] and developed the program for analysis [3]. In the talk will be presented results on the analysis of [Cl-]i and [H+]i in brain slices under various experimental conditions, including changes in external ion concentrations (Ca2+, Cl−, K+, Na+), synaptic stimulation, as well as in the in vitro model of epilepsy.

1.Diuba, A.V., Samigullin, D.V., Kaszas, A., Zonfrillo, F., Malkov, A., Petukhova, E., Casini, A., Arosio, D., Esclapez, M., Gross, C.T. and Bregestovski, P.. CLARITY analysis of the Cl/pH sensor expression in the brain of transgenic mice. *Neuroscience* (2020). DOI: 10.1016/j.neuroscience.2019.07.010

2.Ponomareva, D., Petukhova, E. and Bregestovski, P. Simultaneous monitoring of pH and chloride (Cl−) in brain slices of transgenic mice. *International Journal of Molecular Sciences* (2021). DOI: 10.3390/ijms222413601

3.Zakharov, A., Ponomareva, D., Petukhova, E. and Bregestovski, P.. DriveLEDs: Software for synchronous control and video acquisition of fluorescent signals. *Frontiers in Physics* (2023). DOI: 10.3389/fphy.2023.1283684

Arrangements of pre-AD membranes due to Aβ(25-35) peptide

Kučerka N.[1,2], Arzumanyan G.[1], Badreeva D.R.[1], Dushanov E.B.[1], Ermakova E.V.[1], Ivankov O.I.[1], Kholmurodov Kh.[1], Klochkov V.[3], Kondela T.[2], Kuklin A.I.[1,4], Kurakin S.A.[1,3], Murugova T.N.[1], Rogachev A.V.[1,4], Skoi V.V.[1]

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[2]Comenius University Bratislava, Bratislava, Slovakia
[3]Kazan Federal University, Kazan, Russia
[4]Moscow Institute of Physics and Technology, Dolgoprudny, Russia
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The amyloid-beta (Aβ) peptide is considered a key factor in Alzheimer's disease (AD) ever since the discovery of the disease. The understanding of its damaging influence has however shifted recently from large fibrils observed in the inter-cellular environment to the small oligomers interacting with a cell membrane. By means of small angle neutron scattering (SANS), we have observed for the first time a spontaneous reformation of extruded unilamellar vesicles (EULVs) to discoidal bicelle-like structures (BLSs) and small unilamellar vesicles (SULVs). These changes in the membrane self-organization happen during the thermodynamic phase transitions of lipids and only in the presence of Aβ [1]. The complementary experimental data and molecular dynamics (MD) simulations results revealed further the structural arrangements of the observed morphologies. We show by using solid-state 31P nuclear magnetic resonance (NMR) spectroscopy that lipids are found located not only in the flat bilayered part of BLS but also around its perimeter, which is corroborated by the results of coarse-grained (CG) MD simulations. Peptides, on the other hand, appear to mix randomly with lipids in ULVs, while colocalizing with the lipids at the perimeter of BLS and aiding thus a formation of stable bicelle-like object consisting of a single type of lipids and Aβ peptides [2]. Importantly, despite Aβ peptides affecting the elasto-mechanical properties of membrane itself, the observed morphological transformations caused by their disruptive effect were not prevented by the additional presence of cholesterol or melatonin [3], nor by the presence of charged lipids in the membrane [4] or calcium ions in solution [5].

This work has been supported by the Russian Science Foundation grant 19-72-20186.

1. Ivankov: Amyloid-beta peptide (25-35) triggers a reorganization of lipid membranes driven by temperature changes. Sci Rep 11 (2021). DOI: 10.1038/s41598-021-01347-7.
2. Kurakin: Arrangement of lipid vesicles and bicelle-like structures formed in the presence of Aβ(25-35) peptide. BBA-Biomembranes (2024.) DOI: 10.1016/j.bbamem.2023.184237.
3. Ivankov: Cholesterol and melatonin regulated membrane fluidity does not affect membrane's breakage triggered by amyloid-beta peptide. Biophys Chem (2023). DOI: 10.1016/j.bpc.2023.107023.
4. Ivankov: Anionic lipids modulate little the reorganization effect of amyloid-beta peptide on membranes. General Physiology and Biophysics (2023). DOI: 10.4149/gpb_2022052.
5. Kurakin: Calcium ions do not influence the Aβ(25-35) triggered morphological changes of lipid membranes. Biophys Chem (accepted for publication).

CAR nucleation as a driving force of biomembrane antigen pattern recognition

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Chimeric antigen receptors (CAR) therapy is currently used for cancer treatment [1]. Treatment efficiency greatly depends on the accuracy of molecular pattern recognition. An ability for antigen pattern recognition is strictly determined by CAR clustering on a membrane surface [2]. Moreover, our recent survey suggests to consider CAR nucleation as a driving force of biomembrane pattern recognition [3]. As a result, the difference between target and non-target tissues manifests itself as relevant critical cluster sizes. The mathematical expressions for the probabilities of CAR therapies side effects are derived.

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Computational Design of Transmembrane Channels and Beyond

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Protein pores play key roles in fundamental biological processes and biotechnological applications, such as DNA nanopore sequencing, making the design of pore-containing proteins a topic of significant scientific and biotechnological interest. The *de novo* design of stable, well-defined transmembrane protein pores, capable of selectively conducting ions or large enough to allow the passage of small-molecule fluorophores, remains a significant challenge. In this talk, I will present the computational design of protein pores formed by two concentric rings of α-helices that are stable and monodisperse in both water-soluble and transmembrane forms. Patch-clamp electrophysiology experiments demonstrate that the transmembrane form of the 12-helix pore, expressed in insect cells, allows the passage of ions across the membrane with high selectivity for potassium over sodium. The transmembrane form of the 16-helix pore allows the passage of biotinylated AlexaFluor 488 when incorporated into liposomes using in vitro protein synthesis. Additionally, I will discuss our ongoing efforts in designing fluorescent proteins in the far-red and shortwave infrared regions, as well as recent advancements in computational protein design and their potential impact on basic science, biotechnology, and biomedicine.

Computational structural biophysics of TRPV ion channels.

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Understanding at the molecular level the structure, dynamics, and mechanisms of functioning 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. Structural biology is solving an ever-increasing number of snapshots of ion channel conformational ensembles. Deciphering functional mechanisms of these membrane proteins (MPs), however, requires understanding the ensemble dynamics beyond the static structures. Computer modeling provides important information about the features of MPs, allowing, on the basis of experimentally obtained models, to evaluate the fine details of their conformational dynamics, analyze the evolution of their physicochemical properties, assess the integral membrane effects and the role of individual bound lipids, etc. Here, we present a molecular modeling-based approach characterizing the structural intermediates ion channels by assessing water and ions conductivity along with the detail evaluation of pore hydrophobicity and residue packing – their so-called "dynamic molecular portraits". We illustrate the power of this approach by analyzing structures of several proteins belonging to the vanilloid-subfamily of transient receptor potential (TRPV) channels. Based on pore architecture, three major states that are common for each of these proteins were delineated: the so-called α-closed, π-closed, and π-open [1]. It was shown that the pore hydrophobicity and residue packing for the open state is most favorable for the pore conductance. On the contrary, the closed state with extremely hydrophobic gate is the most stable and always non-conducting. Other conformational states of TRPV channels appear to be intermediates between the three major states. The results of calculations contribute to the identification at the atomic level of the relationship of these characteristics of MPs with the parameters of their action in the cell. The results obtained significantly supplement the information obtained on the basis of structural models deciphered in experiments. Our approach can be used for structural and functional classification of a wide variety of ion channels and may foster in channel-targeting drug design.

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Consistency of non-linear dynamics of human aging with a generalized Gompertz-Makeham law

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According to the Gompertz law, the age-dependent change in the logarithm of mortality (life-table aging rate, LAR) is equal to the population-averaged biological aging rate (γ), and LAR would be constant if aging occurring at a constant rate were the only cause of mortality increase. However, LAR is influenced by population exposures to the external hazards. If they were constant, according to the Gompertz-Makeham law (GML), LAR would be below γ at lower ages and asymptotically and monotonically approach γ with increasing age. Actually, LAR trajectories derived from data on mortality in different countries and historical periods feature systematic undulations. In [1], mortality-vs.-age trajectories were modeled based on a generalized GML (gGML). Unlike the canonical GML terms, which are population-specific constants, the respective terms of the gGML are represented with some population-specific functions of age. Invariant in gGML are the modes of translation of these functions into the dependency of mortality on age: (i) linear for population exposure to the irresistible external hazards or (ii) exponential for population-averaged ability to withstand the resistible external and internal hazards. Modeling suggests that, at earlier ages, LAR undulations are attributable to changes in population exposures to the former hazards. However, only unrealistically high levels of such hazards can produce the transient increase in LAR, which starts at about 65 years. This pervasive undulation of LAR-vs.-age trajectory is rather caused by an increment in γ. A recent addition to the physiological confirmations of such non-linearity of human aging that were cited in [1] was presented in [2] where it was shown that the most significant adult age-related changes in multi-omics patterns occur in humans at around 60 years of age. This is another case consistent with the belief that gGML is a genuine natural law, which defines relations between mortality, aging and environment.

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Crystal structures of obelin with coelenterazine-v before and after light emission confirm coelenterazine dioxyethanone decomposition through 'light' and 'dark' pathways

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The structures of Ca^{2+}-regulated photoprotein obelin activated with coelenterazine analogue (CTZ-v) before and after bioluminescence reaction were respectively determined at 1.80 Å [1] and 2.1 Å [2] resolution. The structures of obelin activated with CTZ-v and native CTZ revealed almost no differences. In contrast, the crystal structure of Ca^{2+}-discharged obelin-v showed the drastic changes in CTZ-binding cavity as compared to that with native CTZ caused by the completely different reaction product, coelenteramine-v (CTM-v), that is considered to be a product of the "dark" pathway of dioxetanone intermediate decomposition [3]. To test whether significant formation of coelenteramine occurs in the reaction of photoproteins activated by native CTZ, we re-examined the ratio of coelenteramide to coelenteramine after Ca^{2+}-induced bioluminescence of obelin and aequorin considering their extinction coefficients. In addition, we report the ratio of "dark" and "light" reaction products for some mutants with substitution of the residues situated near N1-atom of 2-hydroperoxyCTZ and discuss a possible role of some residues in dioxetanone decomposition through "dark" pathway.

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Cytokines as molecular mediators of immunity and targets for therapy

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Cytokines are produced by various cell types and deliver signals through high-affinity receptors present on the membranes of many immune cells. The constellation of cytokines (and of their genes) comprises about 100 species that can be subdivided into about dozen groups based on the type of biochemical signal delivered into the target cell. The physiological consequences of such signaling include activation, differentiation, proliferation, survival or – alternatively - a regulated cell death. Additionally, there are about 50 chemotactic cytokines (chemokines) that can signal through evolutionary ancient rhodopsin-like receptors and induce polarization and cell migration.

In spite of the fact that each cytokine has multiple “relatives” that can transmit very similar signals to the target cell (especially, in vitro), there is accumulating evidence that most cytokines possess non-redundant functions in vivo. Although cytokine genes were selected in evolution for certain protective and homeostatic functions, some of them may become pathogenic when overexpressed (ex. “cytokine storm”). Therefore, anti-cytokine therapy is now widely used for treatment of a number of autoimmune diseases, in particular, with an inflammatory component. Such therapy is often very effective but is not free from unwanted side effects. Our laboratory focuses on two prominent pro-inflammatory cytokines, TNF and IL-6, and promotes a novel concept of a safer and more specific anti-cytokine neutralization in disease. This concept is based on the experiments with a unique panel of gene-modified mice and its verification utilizes bioengineering of a new type of bi-specific antibodies.

DNA architecture in bacteria subjected to various types of stress

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DNA is organized in an actively growing cell hierarchically with three levels of DNA compaction. Actively growing cells maintain a dynamic, far from equilibrium order through metabolism. Under starvation stress, cells go into a dormant state (almost complete lack of metabolism), the usual biochemical ways to protect DNA stop working, and cells are forced to use the physical mechanisms of DNA protection. The architecture of DNA in the nucleoid of dormant cells was studied by synchrotron radiation diffraction and transmission electron microscopy (TEM). Intracellular nanocrystalline, liquid-crystalline and folded nucleosome-like structures of DNA have been found [1]. Next, we studied changes in DNA architecture under stress of exposure to a chemical analogue of the anabiosis autoinducer (4-hexylresorcinol, 4HR). An increase in the 4HR concentration induces the transition of a part of the cells into an anabiotic dormant state, and then into a mummified state. Studies of the DNA architecture in the anabiotic and mummified states show the identity of the DNA structure in the anabiotic state and in the dormant state under starvation stress [2]. The architecture of DNA in a mummified state is very different from that of DNA in an anabiotic state [2].

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Engineering of soluble bacteriorhodopsin

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Engineering of soluble analogues of membrane proteins has long captivated researchers aiming to unlock their full potential. At the same time, protein design and engineering approaches are undergoing rapid transformation due to advancements in machine learning and computer sciences. Neural network-based methods were previously developed that can predict amino acid sequences folding into user-defined backbone structures. In this study, we employed ProteinMPNN, a machine learning-based protein design approach, to engineer soluble analogues of bacteriorhodopsin. We generated three artificial sequences, with two resulting in stable, soluble, and monomeric proteins upon expression in E. coli. These engineered analogues were able to covalently bind retinal and exhibited absorption spectra similar to that of the wild type bacteriorhodopsin. Furthermore, they displayed a characteristic photocycle featuring retinal Schiff base deprotonation and reprotonation. Our work provides a promising new avenue for development of microbial rhodopsin-based soluble photoactive proteins and highlights the great potential of advanced protein engineering approaches in general.

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Generation of Synthetic Antibodies for CAR T-cell Therapy Development

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In the past decade, CAR T-cell-based immunotherapy has made significant progress in the treatment of malignant hematological diseases and has become one of the major frontiers in the crowded field of immune-oncology, with complete remission rates exceeding 50%, even for relapsed/refractory patients [1]. However, most approved CAR T-cell therapies are based on murine-derived scFv antibodies, which limit the efficacy of the therapy due to several drawbacks of these modules, including immunogenicity, tonic signaling, and chain mispairing [2]. Since single-domain antibodies (VHH) offer greater flexibility for drugs and cell therapy development (smaller size, higher stability, lower immunogenicity, and ability to bind "cryptic" epitopes) compared to conventional scFvs, we developed a universal synthetic humanized VHH library for binder isolation in a controlled enviroment without immunization. We compared the amino acid distribution in the CDRs of matured *Camelid* antibodies with their germline sequences. "Hot spot" positions were identified and randomized to mimic the natural amino acid diversity on the structure-optimized humanized scaffold. Additionally, we aimed to expand the paratope's conformational space by introducing a pair of mutations into the VHH scaffold, which significantly shifted the long CDR3 away from the VHH core, increasing the flexibility of the originally “twisted” CDR3 loop. A synthetic nanobody library with over 10^{11} diversity was generated via Kunkel mutagenesis. A panel of anti-CD30 and anti-CD19 antibodies (both potent and clinically relevant targets for targeted therapy of classical Hodgkin lymphoma and multiple B-cell leukemias) was then generated via phage display and affinity-matured to achieve sub-nanomolar affinity. Investigational CAR T-cell products showed promise in vitro, demonstrating specific activation against tumor cell lines. This approach could significantly enhance the clinical efficacy of CAR T-cell therapy while reducing the risk of relapses related to insufficient sensitivity of CAR and immune mediated CAR T-cell rejection

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GPCR drug discovery: Rational design of polypharmacology

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The etiology of neuropsychiatric diseases is complicated and effective treatments for these complex disorders require drugs with polypharmacological profiles. The modulation of many dopaminergic and serotonergic G protein-coupled receptors (GPCRs) have been demonstrated to be useful for alleviating mental disorder symptoms, but the rational design of polypharmacology against a selection of specific targets among these receptors is a great challenge due to their homologous nature. Through the in-depth analysis of the drug binding pockets between the dopamine D2R and the serotonin 5-HT_{2A}R receptor, we designed IHCH-7041, a D2R+D3R+5-HT_{1A}R partial agonist, with negligible binding at other aminergic GPCRs such as the 5-HT_{2A}R. Compound IHCH-7041 displayed superior antipsychotic-like activity than the third-generation drug aripiprazole. Inspired by this, we also developed a flexible scaffold-based cheminformatics approach (FSCA), which features the employment of a flexible scaffold to fit into different receptors using different binding poses, for the rational design of polypharmacological drugs targeting aminergic GPCRs. Using this approach, we designed compound IHCH-7179, which adopted a ‘‘bending-down’’ binding pose at 5-HT_{2A}R to act as an antagonist and a ‘‘stretching-up’’ binding pose at 5-HT_{1A}R to function as an agonist. IHCH-7179 demonstrated promising results in alleviating cognitive deficits and psychoactive symptoms in mice by blocking 5-HT_{2A}R for psychoactive symptoms and activating 5-HT_{1A}R to alleviate cognitive deficits. Taken together, our results show that rational design of GPCR polypharmacology can be achieved through in-depth structural analysis of homologous GPCRs.

Inflammaging and longevity within the framework of personalized aging

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Human aging is characterized by a chronic, low-grade inflammation, a phenomenon that I suggested to term "INFLAMMAGING [1]." Inflammaging is a highly significant risk factor for both morbidity and mortality in the elderly people, as most if not all age-related diseases (ARDs) and geriatric syndromes (GSs) share an inflammatory pathogenesis [2].

I will illustrate the last development of this inflammatory theory of aging ("GARBAGING") which suggests that the most important/causal inflammatory stimuli fueling inflammaging are to be identified in the lifelong, persistent exposure to exogenous (non-self) agents/pollutants, to the age-related dysregulation of the production of endogenous [self and quasi-self (gut microbiota, GM)] "molecular garbage" [3]. Such garbage is continuously/physiologically produced as a consequence of cell death (necroptosis; altered/misplaced molecules), metabolism [4] and GM function [5], but also continuously neutralized by the remodeling and adaptive capability of the body (degradation of inflammatory molecules/molecular fragments; production of anti-inflammatory molecules) which quickly and efficiently down-regulate inflammatory responses in young subjects [6] but fail to do so in older bodies. Self, non-self and quasi-self garbage/stimuli are sensed by and converge on a limited number of DAMAGE SENSORS (PRRs Pattern-Recognition Receptors, including TLRs cGAS-STING, NOD, DAI, RIG-I, AIM2, RAGE AHR) which are highly "promiscuous" being characterized by a high degree of "DEGENERACY" and are activated to mount an innate inflammatory response. Accordingly, aging and INFLAMMAGING can be conceptualized as AN EVOLUTIONARY-UNPREDICTED BYPRODUCT OF THE DEGENERACY OF PRRs. Inflammaging is accelerated by persistent infections, lifestyle habits such as nutrient excess (overweight/obesity and metaflammation), low socio-economic status, emotional stressors and environmental pollutants [4].

The new perspective of GEROSCIENCE [7] suggests that aging is the most important risk factor for ARDs and GSs, and that aging and ARDs/GSs share the same basic molecular mechanisms, including inflammaging [2]. Accordingly, I will argue that:

- ARDs and GSs, including obesity and metabolic diseases can be conceptualized as manifestations of accelerated aging [8], and clinically different ARDs/GSs are the result of peculiar combinations of alterations regarding the same, limited set of basic mechanisms shared with the aging process. Whether an individual will follow a trajectory of accelerated or decelerated aging will depend on his/her genetic background interacting lifelong with environmental and lifestyle factors (nutrition, physical and mental activity) [4].

- According to this integrated view, aging and ARDs/GSs become part of A CONTINUUM8 where precise boundaries do not exist, and the two extremes are represented by centenarians [9] and their offspring [10, 11] who largely avoided or postponed most ARDs/GSs and are characterized by decelerated aging [11], and patients who suffered one or more severe ARDs/GSs in their 60s, 70s, and 80s and show signs of accelerated aging, respectively.

- If ARDs and GSs are MANIFESTATIONS OF ACCELERATED AGING, it is urgent to identify markers capable of distinguishing between BIOLOGICAL AND CHRONOLOGICAL AGE in order to identify subjects at higher risk of developing ARDs and GSs. To this aim, I will propose the use of DNA methylation [12], N-glycans [13] profiling, GM composition [5] and circulating cell-free DNA [14] to complement the available disease-specific markers4.

Finally, I will argue that human aging/inflammaging as well as human longevity, including their genetic [9,15-17] and metabolomics [18] basis, are highly context-dependent, dynamic processes/phenomena both historically and individually ("IMMUNOBIOGRAPHY" [19], "liquid immune self" [20]), which necessitate a new integrated (nature/nurture) demographic [21], ecological and evolutionary perspective [9,15] to be fully appreciated and investigated.

Within this scenario, particular attention will be devoted to: i) centenarians as calorie restricted-like persons [22] characterized by a peculiar/mild inflammaging [23]; ii) GM and its changes with age5; iii) Mediterranean diet [24] and food timing [22], in turn related to the maintenance of circadian rhythms, including sleep; iv) inflammaging in between a digitalized/big data and a personalized approach [25].

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Lysosomal anti-aging paradigm: proof of concept via optogenetic monitoring of metformin and rapamycin effects

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Some of the most important drugs for life extension are metformin [1], rapamycin [2] and their derivatives. They have been shown to be effective in model organisms, and large-scale trials (MET-PREVENT, TAME) are underway to test their efficacy in humans. By acting on AMPK and mTOR, respectively, metformin and rapamycin slow down cell growth, activate autophagy, and don't increase the risk of cancer. Acting on specific molecular regulators of metabolism gives an advantage in the selectivity, but may not be as effective as the polypharmacological approach. In order to have a high impact on the lifespan of the cell and the organism, we propose an organelle targeting, namely, managing the work of lysosomes. Optogenetic control of lysosomal pH allows activation or inhibition of autophagy and the identification of the activity of vATPase (natural lysosomal acidifier). We have shown that metformin has a two-step mechanism of action. First, there is vATPase inhibition, which is manifested as a slowing of lysosomal acidification after optogenetic alkalisation. Then, after two days of metformin treatment, autophagy activation accelerates vATPase activity. This cell behaviour in response to metformin is consistent with recent findings and demonstrates the important role of the lysosome in adjusting metabolism to the "centenarian mode". In the future, we plan to investigate how other longevity interventions affect the lysosome in order to confirm the lysosomal anti-aging concept. Optogenetic experiments were done with support of Russian Science Foundation (RSF) Project 21-64-00018. Rhodopsins expression was done with support of the Ministry of Science and Higher Education of the Russian Federation (agreement # 075-03-2024-117, project FSMG-2024-0012).

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Memory self-degradation in Alzheimer's disease and new opportunities for cognitive neuroprotection

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Alzheimer's disease (AD) is characterized by dementia - a dramatic damage of already existing old memory. I propose a new hypothesis that this loss is to a large extent due to gradual self-degradation of old memory when it is retrieved in a brain whose ability for reconsolidating is impaired by neurodegeneration. Testing this hypothesis in transgenic 5xFAD mice we found that their contextual memory was impaired following retrieval compared to control animals. To protect memory during reconsolidation, we administered the NMDA receptor antagonist MK-801 which is known to inhibit memory labilization. MK-801 protected memory from deterioration, which confirms our hypothesis. Interestingly, MK-801 had no effect on memory reconsolidation in 2-year-old normal mice, indicating that its effect is specific to amyloid pathology. We then used c-Fos expression mapping of brain activity during contextual memory retrieval following reconsolidation in 5xFAD mice, in normally aged mice, and after MK-801 administration. 5xFAD mice showed increased activation in the frontal cortex and hippocampus compared to control C57 Bl/6 mice. MK-801 increased activity in prelimbic cortex during memory retrieval in all groups. Its protective effect on memory in 5xFAD mice, manifested by increased freezing, was accompanied by increased activation of the amygdala and CA1. This was in contrast to the effect of MK-801 in control mice, where it reduced amygdala activity and impaired memory, as evidenced by reduced freezing. Using the model of retrieval-induce memory impairment we also tested possible protective properties of drugs currently used in the clinic or in clinical trials that have NMDA antagonist properties - riluzole, amantadine andneramexane. We found that each of the drugs tested, when administered systemically before memory reactivation, was able to prevent memory impairment caused by the intracerebral administration of the protein synthesis inhibitor anisomycin. Our work demonstrates a potential new mechanism for an impairment of old memory in Alzheimer's disease and highlights potential strategies to protect this pathology.

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Metabolic diversity of extremophilic prokaryotes: results of genomic and metagenomic analysis

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The ability to thrive under extreme physicochemical conditions (extremes of temperature, pH, salinity) is a unique property of prokaryotes. Interest in the study of extremophiles is primarily related to the expansion of our knowledge of the physicochemical limits of life and mechanisms of biomolecules stabilization. Various properties of extremophilic microorganisms find application in biotechnology and medicine. Besides, extremophilic microorganisms often represent deep phylogenetic lineages and possess unusual activities and metabolic pathways.

Our genomic studies of hyperthermophilic archaea of the genus *Thermococcus* leaded to a discovery of a new energy-generating process - anaerobic formate oxidation coupled with hydrogen formation [1]. In another member of genus *Thermococcus*, a unique superstable multidomain cellulase was discovered and characterized [2].

Sulfate reduction is a crucial biospheric process, and until recently it was found mostly in bacteria, with archaea merely able to acquire a bacterial set of essential genes through horizontal transfer. We discovered an alternative, 'archaeal' mechanism of sulfate reduction in the hyperthermophilic archaeon *'Vulcanisaeta moutnovskia*', suggesting a much earlier origin of this process than it was supposed [3].

The most important mechanism of inorganic carbon assimilation, the Calvin cycle, is known for its extraordinary conservatism following a uniform pattern in all plants, algae, and most bacteria on Earth. Using genomic, biochemical, and proteomic studies of the thermophilic bacterium *Thermodesulfobium acidiphilum*, we were able to describe the first alternative variant of the Calvin cycle, the possibility of which was predicted 20 years ago [4].

In the Western Aral Sea, as a result of its desiccation during recent six decades, water salinity increased from 2 to 22%. Metagenomic analysis of its water showed that halophilic archaea of the genus *Haloquadratum* represent from 40 to 50% of microbial population [5]. Metagenome-assembled genomes (MAGs) of archaea and bacteria inhabiting Aral water and sediments contained genes of xenobiotic degradation including those of halogenated compounds utilization. It demonstrated the ability of microbial population to fight the pollution of Aral with pesticides coming from surrounding fields during tens of years.

The above results show how diverse the world of extremophilic prokaryotes is and how much we can learn about it by combining different investigation approaches.

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Molecular physiology and pathology of vision: rhodopsin

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For almost 150 years, visual rhodopsin has remained a 'hot spot' of biology, and first of all of visual sciences. Rhodopsin provides key role in phototransduction, spectral sensitivity, light and dark adaptation. The pathogenesis of numerous forms of retinal degenerative diseases is related to the retinoid cycle of rhodopsin. Recently, visual rhodopsin has been considered as a promising optogenetics 'tool' for degenerative retina prosthetics.

The presentation reviews the most relevant problems in molecular physiology and pathology of vision related to rhodopsin, including:

- supramolecular organization of rhodopsin in the photoreceptor membrane of the visual cell outer segment;
- photochemistry of rhodopsin on femto- and picosecond time scales;
- retinoid cycle and the fate of free all-*trans* retinal released by rhodopsin photolysis;
- formation of bisretinoids-containing lipofuscin granules and their light-induced generation of reactive oxygen species;
- search for antioxidant and optogenetic ways to protect the retinal pigment epithelium cell against the damaging effect of lipofuscin granules;
- prospects for optogenetics prosthetics of the degenerative ("blind") retina.

Monitoring GPCR conformation with GFP-inspired dyes

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GPCRs constitute the largest class of membrane proteins in humans that regulate critical physiological processes. More than one third of drugs approved by FDA have GPCRs as their primary targets [1]. Therefore, it is very important to understand the mechanism of functioning and activation of proteins. There are several methods that allow to track the conformational dynamics of GPCR (for example, smFRET), but they are quite complex. Here we wanted to create a simple and fast method for tracking protein conformational dynamics.

We chose GFP-inspired solvatochromic fluorophores, which should sense changes in the local environment when the state of the protein changes. From in vivo imaging to single-molecule tracking, the GFP has become an indispensable tool for many biological studies [2]. Structurally modified synthetic analogues of the GFP chromophore represent a class of benzylidene imidazolones that found many applications as versatile labels due to their exceptional fluorescent properties, small size, and easy synthesis [3].

Here we assessed four GFP-inspired fluorophores for their potential to serve as environmentally sensitive labels to report on conformational changes in proteins. For this, we attached a maleimide group to them for cysteine labeling and studied their spectral properties in solvents with varying polarity and viscosity. The best of them were then employed to label two proteins: bovine recoverin and $A_{2A}AR$. Recoverin is a convenient model protein to evaluate the performance of environmentally sensitive labels. This is attributed to the presence of a cysteine residue and the considerable conformational alterations upon activation by Ca^{2+}. Using the best performing dye attached to $A_{2A}AR$, we investigated effects of various ligands on its fluorescence and observed reliable and distinctive changes. Finally, we conducted molecular dynamic simulations of the $A_{2A}AR$ complexes with the dyes to obtain structural insights into the observed changes.

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New molecular tools for scalable mapping and recording of neuronal activity in vivo

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Our brain mediates everything we think, feel, sense, and do. The brain is an incredibly complex organ - while the brain computes events on a millisecond scale, it spans years of a lifetime, and at the same time, while the brain is organized at the nanoscale level, it spans meters in size. Therefore, a holistic understanding of the brain, from intracellular processes to cell-cell interactions across the whole organ, requires scalable integrated technologies that can simultaneously map neuronal computations on both functional and structural levels within the intact brain. In the talk, Kiryl Piatkevich will present the development and validation of novel scalable molecular, imaging, and multi-omics technologies that enable recording neural activity with ultrahigh temporal resolution and mapping the nanoscale cellular structures on the same subset of neurons within intact brain circuits. In particular, the talk will cover the development and validation of the novel genetically encoded indicators for voltage, calcium, and potassium. In addition, technology for recording long-term translational histories in single cells will be presented and discussed. The talk will introduce a novel method called microProteomEx, which combines spatially resolved visual proteomics with small-cell population proteomics analysis and super-resolution imaging. microProteomEx enables the quantitative profiling of spatial proteome variability in mammalian tissues with an impressive lateral resolution of approximately 57 µm without a need of any special setup or equipment. In the final part of the talk, Kiryl Piatkevich will provide perspectives on the development of advanced molecular technologies to study the brain. All plasmids presented in the talk are available from WeKwikGene (https://wekwikgene.wllsb.edu.cn/) for free.

Novel nanobodies targeting receptor-binding domains of some relevant viral and bacterial human pathogens

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The development of antibodies targeting receptor-binding domains (RBDs) of viral glycoproteins or bacterial exotoxins is a common strategy for prophylaxis and containment of a range of infectious diseases. Our recent research has focused on two pathogens: the SARS-CoV-2 coronavirus, which has resulted in a pandemic with millions of deaths worldwide, and the Gram-positive bacterium *Clostridioides difficile*, which causes severe nosocomial colon infections with high mortality rates. We have isolated and characterized two single-domain camelid antibodies (VHH), P2C5 and B10, which exhibit remarkable potent neutralizing capabilities against SARS-CoV-2. We have determined the crystal structures of these antibodies in complex with the RBD, providing insights into their mechanism of neutralization through the occlusion of the Spike protein interaction with its natural ACE2 receptor. Crystallography also elucidated why the recent SARS-CoV-2 variants, Delta and Omicron XBB, gained the ability to evade from the action of P2C5-VHH, which was undergoing clinical trials as a "GamCoviMab" component, but not from the action of B10-VHH. Furthermore, two camelid VHHs, C4.2 and H5.2, targeting RBD and thereby neutralizing toxin A from *C. difficile*, have been isolated and their complexes with the corresponding antigen regions have been crystallized. Unexpectedly, these VHHs exhibited a common C-terminal epitope involving a QTIN motif, yet differed in their affinity and capacity to recognize multiple QTIN-like repeats distributed throughout the RBD of toxin A. The C4.2 and H5.2 nanobodies target the unique epitope, which makes them a valuable addition to the limited number of structurally characterized anti-toxin A antibodies. This provides new modalities for the prevention of *C. difficile* intoxication. Partially supported by RSF grant 23-74-30004.

Pathogenic agent of Alzheimer's disease and molecular tools for its neutralization

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A hallmark of Alzheimer's disease (AD) are the proteinaceous aggregates formed by the amyloid-beta peptide (Aβ) that is deposited inside the brain as amyloid plaques. It has been known for the past twenty years that the formation of amyloid plaques in Alzheimer's disease is impossible without the participation of chemically modified isoforms of Aβ, as well as in the absence of zinc ions. However, it was not until 2023 that animal model experiments revealed that the pathogenic agent that causes endogenous Aβ molecules to aggregate on the α4β2 nicotinic acetylcholine receptors (α4β2-nAChRs) is a non-covalent complex between Asp7 isomerized beta-amyloid (isoD7-Aβ) and a zinc ion [1]. These data also showed that the most effective way to destroy amyloid plaques, as well as prevent their formation, is to target zinc-dependent interactions in which the 11-EVHH-14 and 35-HAEE-38 regions of Aβ and the α4 subunits of the α4β2-nAChR receptor, respectively, play a critical role [2]. Both specific monoclonal antibodies to isoD7-Aβ [3] and a synthetic analogue of the 35-HAEE-38 region of α4 subunits of the α4β2-nAChR receptor [4] can act as molecular tools aimed at neutralizing the pathogenic isoD7-Aβ / zinc complex.

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Protein dynamics within the Lattice Abelian Higgs model

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Protein structure dynamics still remains unsolved problem. The recently developed machine learning approaches can provide predictions only for static protein structure within the conditions similar to the fixed one in the RCSB Protein Data Bank, and cannot provide any information about the protein dynamics at different thermodynamical parameters, in macromolecular interactions and external fields. All atom calculations within molecular dynamics cannot solve the problem of protein dynamics due to the enormous computational complexity.

In the present talks I will review the structure-based model of protein dynamics based on the principles of gauge symmetry, topology and universality. Within the model the large-scale protein structures are defined by scalar field condensates and gauge field theory topological structure such as vortices and sphalerons. The protein dynamics is defined by the Abelian Higgs model Hamiltonian. As a result, the model provides the link between protein topology its thermodynamical phases and dynamics.

Protein misfolding, aggregation and seeding- the trigger for neuronal death in Parkinson's disease?

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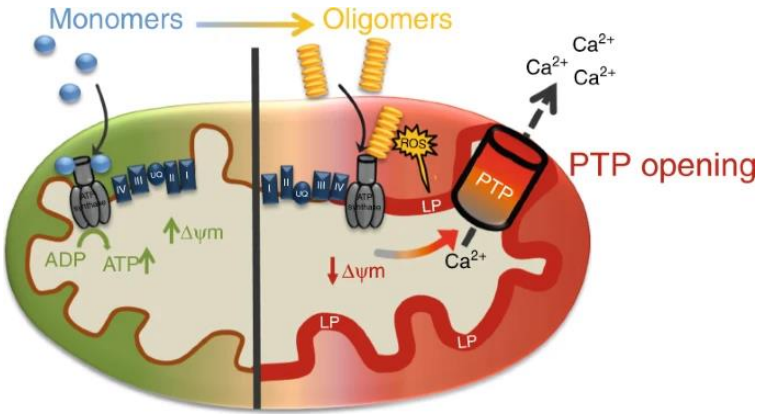
Aging-related neurodegenerative diseases are characterized by the occurrence of misfolded proteins. α-Synuclein is a protein, which is linked genetically and neuropathologically to Parkinson's disease (PD). Parkinson's disease (PD) is characterized by the formation of Lewi bodies, formed by aggregated alpha-synuclein. α-Synuclein may contribute to PD pathogenesis in a number of ways, but it is generally thought that its aberrant soluble oligomeric conformations, known as protofibrils, are the toxic species that mediate the disruption of cellular homeostasis and neuronal death, through effects on various intracellular targets compromising cellular function. Aggregation of alpha-synuclein affects neuronal calcium homeostasis, mitochondrial function and leads to transition metal-dependent ROS production and lipid peroxidation [1], [2], [3]. Furthermore, secreted α-synuclein may exert deleterious effects on neighboring cells, including seeding of aggregation, thus possibly contributing to disease propagation.

Alpha-synuclein monomers are constantly formed throughout life and play important role in physiological regulation of mitochondrial function [4]. When aggregated, alpha-synuclein that is directly interacting with the ATP synthase in physiology, switches to pathology via the adoption of a toxic gain of function. This results in the opening of the mitochondrial permeability transition pore and ultimately leads to neuronal loss [5]. Suppression of the rates of lipid peroxidation by different strategies restores physiological calcium signalling in human cellular PD models. Furthermore, prevention of ferroptosis by iron chelators, lipid soluble antioxidants, deuterated PUFAs or by ferrostatin-1: each is a promising approach towards alpha-synuclein- or dopamine-induced neuronal toxicity [6], [7],[8], [9].

This talk will unravel the interaction between protein misfolding, mitochondrial function [10], metal homeostasis and oxidative status and will highlight the rational basis for the modern therapeutic strategy in PD.



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Research on pangolin coronavirus pathogenesis: implications for COVID prevention and control

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Prior to the outbreak of the COVID-19 pandemic, researchers identified two SARS-CoV-1-related coronaviruses, GuangXi/2017 and GuangDong/2019, in smuggled pangolins in GuangXi and GuangDong provinces of southern China. The receptor-binding domains (RBDs) of their Spike proteins share 86.8% and 97.4% amino acid identify with the RBD of wild-type SARS-CoV-2, respectively. In our previous studies, we reported the cultivation and characterization of GuangXi/2017, and that its cell-adapted strain GX_P2V(short_3UTR) could cause 100% lethality in CAG-hACE2 mice due to late brain infections. This animal infection study, combined with the relatively low homology between the RBDs of GuangXi/2017 and SARS-CoV-2, sparked concerns on social media and among some professionals about the potential of this virus to spill over into human and to cause an even worse SARS-3 outbreak. Here, we conducted a comprehensive analysis of the research advances made by our team and others on this GuangXi/2017 virus, focusing on its in vitro and in vivo infectivity, pathogenicity, host range, adaptive mutations, and cross-immunity. Our findings indicate that GuangXi/2017 exhibits low pathogenicity and a minimal spillover risk, with evidence of cross-immunity with SARS-CoV-2. Therefore, concerns about GuangXi/2017 leading to a SARS-3 pandemic are unwarranted. Notably, our research suggests that GuangXi/2017 is a natural attenuated live vaccine candidate with broad-spectrum protection against pan-SARS-CoVs.

Role of Ly6/uPAR proteins in the brain pathology

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The Ly6/uPAR protein family gets its name from its two members, lymphocyte antigen-6 and urokinase-type plasminogen activator receptor. Members of the Ly6/uPAR family are characterized by the β-structured LU-domain (60-90 amino acids) stabilized by the system of highly conserved 4 disulfide bonds and containing three loops (“fingers”). This conservative spatial structure emphasizes the evolutionary importance of the Ly6/uPAR three-finger fold. Ly6/uPAR proteins were discovered in the wide diversity of species, and in humans they were found in the immune system, skin, brain, and reproductive system. Ly6/uPAR proteins are involved in regulation of many essential processes and influence the development of various pathologies.

Several Ly6/uPAR proteins are expressed in the CNS in the different brain regions. Little is known about their function, although, some of them target neuronal membrane receptors. Thus, Lynx1 potentiates nicotinic acetylcholine receptors in the brain, while others like Lypd6 and Lypd6b suppress it. Expression of the Ly6/uPAR proteins in the brain can change in various neurodegenerative pathologies. For example, Lynx1 and PSCA expression decreased and increased in Alzheimer disease, respectively, while up-regulation of Lypd6 and Lypd6b expression associates with autism. The lecture will discuss new advancesin understanding the role and function of some human Ly6/uPAR proteins in the brain with a particular focus on the cognitive processes in which they may be involved.

SPR analysis of proteins and ligands interaction with target membrane protein embedded in a lipid bilayer

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Membrane proteins and their intermolecular interactions play an important role in the processes of signal transmission, transport and synthesis of metabolites, as well as the functioning of electron transport chains. Cytochromes P450 are membrane proteins involved in the metabolites biosynthesis and xenobiotics biotransformation. Previously, we investigated protein-ligand interactions (PLI) of the CYP51A1 (cytochrome P450 51A1) embedded in the lipid bilayer membrane [1, 2]. In this research we investigated the protein-protein interactions (PPI) of CYP11B1 (mitochondrial cytochrome P450 11B1) with its redox partner adrenodoxin. We used surface plasmon resonance (SPR) with the formation of a planar bilayer lipid membrane on the surface of an optical chip, followed by the incorporation of the target membrane protein and analysis of the kinetics and affinity of molecular interactions [3]. The presence of cholesterol, sphingomyelin and cardiolipin can significantly alter the affinity and kinetics of cytochromes P450 interactions with ligands and proteins. PPI are more dependent on the lipid composition of the membrane than PLI. The obtained data indicate the importance of the lipid composition of membranes in which membrane proteins are embedded to study their intermolecular interactions. We believe that SPR with the formation of a bilayer lipid membrane is a promising technology for studying membrane proteins and molecular interactions involving them.

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Structural basis for the ubiquitination of G protein βγ subunits by KCTD5/Cullin3 E3 ligase

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G protein–coupled receptor (GPCR) signaling is precisely controlled to avoid overstimulation that results in detrimental consequences. Various mechanisms have evolved to fine-tune GPCR signaling across almost all stages of signal transduction. One such mechanism involves the negative regulation of Gβγ signaling by a Cullin3 (Cul3)–dependent E3 ligase, KCTD5, which triggers ubiquitination and degradation of free Gβγ. We report the cryo–electron microscopy structures of the KCTD5-Gβγ fusion complex and the KCTD7-Cul3 complex. KCTD5 in pentameric form engages symmetrically with five copies of Gβγ through its C-terminal domain. The unique pentameric assembly of the KCTD5/Cul3 E3 ligase places the ubiquitin-conjugating enzyme (E2) and the modification sites of Gβγ in close proximity and allows simultaneous transfer of ubiquitin from E2 to five Gβγ subunits. Our studies provide insights into mechanisms of substrate recognition by these unusual pentameric E3 ligases and highlight the KCTD family as emerging regulators of GPCR signaling.

Structural basis of TRPA1 channel modulation by Cys-knot spider toxins

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P-loop channels have a modular structure and consist of four homologous voltage-sensitive like domains (VSLDs), formed by four transmembrane helices (S1-S4), and a central pore domain. VSLDs of voltage-gated channels are targets for gating-modifying spider toxins, which have the Inhibitory Cystine Knot (ICK) fold, demonstrate membrane affinity, and block or promote the channel activation by binding to the membrane-embedded domain regions. TRPA1 is the voltage unsensitive P-loop channel involved in nociception. This channel is a sensor for various electrophilic ligands, including external chemicals and endogenous inflammatory mediators. Activation of TRPA1 leads to inflammation, causing pain or itch, making it a potential target for new analgesics. Several promising TRPA1 ligands have been found in spider venoms. Protoxin-1 (ProTx-I) of Thrixopelma pruriens inhibits the channel, while Pha1β (PnTx3-6) of Phoneutria nigriventer acts as a positive modulator, but demonstrates analgetic effect in vivo. Both toxins contain the ICK motif and exhibit membrane affinities similar to gating modifiers specific to the voltage-gated channels. The binding site of spider toxins at the voltage unsensitive TRPA1 channel remain unknown.

To study the mechanism of action of spider toxins on TRPA1, we examined the complexes of ProTx-I and Pha1β with the human channel using various structural biology methods. Cryo-EM revealed binding of ProTx-I to the S1-S2 and S3-S4 loops of the VSLD from the membrane bound state in a manner similar to the gating-modifying spider toxins. Similarly, NMR revealed binding of Pha1β to isolated TRPA1 VSLD in micellar medium. The complex of Phα1β with membrane-embedded full-length TRPA1 was modelled using NMR data and a combined docking/molecular dynamics protocol. Simultaneous toxin interaction with the extracellular interface of VSLD and pore domain was proposed. The mechanisms of TRPA1 gating modulation by spider toxins are discussed.

Structural biology investigation on human bitter taste receptors

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The human bitter taste modality is perceived through bitter taste receptors and bitter taste perception is mediated by taste type 2 receptors (TAS2Rs), or class T G protein-coupled receptors. Understanding the detailed molecular mechanisms behind taste sensation is hindered by the lack of experimental receptor structures. There are 25 TAS2Rs which are divided into 4 groups: broad, intermediate, narrow and group specific, based on the spectrum of bitter substances. The cryo-electron microscopy method has been implored in the structural biology elucidation of the TAS2Rs, including human TAS2R46 and TAS2R14. Several unique features are disclosed, including distinct receptor structures comparing with known GPCRs, new “toggle switch”, activation-related motifs and pre-coupling of G protein gustducin. Furthermore, the dynamic extracellular and more static intracellular portions of receptor suggest possible diverse ligand recognition, yet similar activation process. This study provides a basis for further exploration of more bitter taste receptors and their therapeutic applications.

Structural characterization of biomembrane using synchrotron small-angle X-ray scattering

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Membrane proteins and lipids play essential roles in many cellular processes, such as signal transduction, cell-to-cell communication, membrane transport, and lipid metabolism. However, only 1-2% of the known protein structures in the Protein Data Bank (PDB) are from membrane proteins, which is limited by the lack of proper structural characterization techniques. In the past 5 year, we built a biomembrane macromolecules structural characterization platform based on the synchrotron small-angle X-ray scattering (SAXS) beamline [1,2]. The high brightness of synchrotron X-ray beam together with optimized sample preparation methods render SAXS a powerful complementary technique even when high-resolution information is available from other biophysical techniques [3]. Here in this talk, I will review the progress of national facility construction and PDB establishment in People's Republic of China. Recent advances in solutions SAXS in biomembrane studies will also be discussed [4]. Given the potential offered by the artificial intelligence (AI), further SAXS applications in the field of biomembrane are expected.

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Structure of the II2-III2-IV2 mitochondrial supercomplex from a parasite reveals a protein inhibitor of complex III

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We report a new type of II2-III2-IV2 supercomplex in Apicomplexa-related parasites. The 1.8 megadalton supercomplex comprises 104 proteins and 114 lipids. The 2.1-Å resolution structure represents the first observation of CII in this type of supercomplex, and activity assays confirmed the presence of a complete electron transfer from succinate to molecular oxygen utilising CII. The distinctive feature is achieved via an apicomplexan subunit, which bridges two copies of CII with CIII dimer and CIV. The second finding is the identification of a negative regulator at the conserved site on CIII. We identified a heterodimer that locks the Rieske Iron-Sulfur Protein head in the c1 state, effectively inactivating electron transfer. Its C-terminus stabilizes the mobile head, while the N-terminus associates the heterodimer in the binding site of the universal electron carrier Cyt c. From an evolutionary perspective, the structure reveals a programmed +2 frameshifts, which illuminate evolutionary adaptations at the gene expression level.

Structure, function, and regulation of G protein-coupled receptors

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Our research program is focused on understanding the largest class of cell surface proteins in our body which are referred to as G protein-coupled receptors (GPCRs). These receptors are intricately involved in almost every physiological process and approximately half of the currently prescribed medicines exert their therapeutic effects through these receptors. The overarching theme of our research is to understand the structure, function, and regulation of GPCRs, and leverage this information to design and discover novel therapeutics with minimal side-effects. Our research has elucidated the details of how clinically prescribed drugs for a range of human disorders interact with and regulate the function of their cognate receptors in human body. We have also discovered previously unappreciated mechanisms that GPCRs utilize to receive the information outside the cells and relay the message across the cell membrane. More recently, we have identified synthetic antibody fragments that can be utilized to monitor GPCR activation and trafficking, and to rewire GPCR signaling in cellular context.

Synchrotron Radiation Facility ‘SKIF’: New Experimental Capabilities for Structural Biology

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Synchrotron Radiation Facility ‘SKIF’ is a novel large-scale research facility being currently constructed in the Science City of Koltsovo, 15 km apart from Novosibirsk. The 3 GeV electron storage ring designed by specialists from the Budker Institute of Nuclear Physics SB RAS (Novosibirsk) augmented with advanced superconducting undulators will provide the record low emittance of 75 pm·rad to serve the beamlines with ultrabright photon beams. Facility commissioning and first beam experiments are scheduled for the second half of 2025.

Figure 1. Aerial view of the ‘SKIF’ construction site in Koltsovo as of August 2024 (left), MX diffractometer during Factory Acceptance Tests (right).

Structural biology is envisaged as one of the major scientific directions of the ‘SKIF’. Macromolecular (MX) diffractometer will be installed as a part of the 1-2 “Structural Diagnostics” beamline supervised by the Institute of High-Current Electronics SB RAS (Tomsk). Specialized auxiliary equipment for the protein purification, quality control, and automated crystallization will be available for users in the support Labs building.

The present lecture briefly outlines the history and current status of the ‘SKIF’ project, its major research directions and plans for the infrastructure development with the emphasis placed on structural biology-related instruments.

The annotation, classification, structure and database building of chordate olfactory receptors

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Olfactory receptors are poorly annotated for most genome-sequenced chordates. To address this deficiency, we developed a nhmmer-based olfactory receptor annotation tool Genome2OR (https://github.com/ToHanwei/Genome2OR.git), and used it to process 2800+ sequenced chordate genomes in the NCBI Assembly database. In total, over one million olfactory receptor genes were annotated. Based on the annotation data, we built a database called Chordata Olfactory Receptor Database (CORD, https://cord.ihuman.shanghaitech.edu.cn) for archiving, analyzing and disseminating the data. The current state-of-the-art nomenclature system of olfactory receptors is based on protein sequence identity which causes many wrong classifications. We developed a new classification method that based on DNA alignment and phylogenetic tree. The new method can greatly improve the classification quality. We use the new method to classify all the OR sequences we annotated in CORD.

Thermogenetic control of neuronal circuits *in vivo*

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In recent years, opto- and chemogenetic tools provided the great advances in studying how the brain works. However, their translation into medicine remains complicated. Optogeneics uses non-human opsins able to trigger immune response, and chemogenetics does not provide enough spatiotemporal resolution of neuromodulation. Thermogenetics based on the use of the human thermosensitive channel TRPV1 is a highly promising tool for therapeutic neuromodulation. Here, by a combination of adenoassociated viral vector delivery, advanced infrared fiber optics, and all-optical thermometry, we reveal that human TRPV1 can be used for thermogenetic control of neuronal circuits *in vivo*. Thermal stimulation of neurons in the locomotor area of the midbrain by treatments with the infrared laser induced evident behavioral effects with simultaneous elevation of neuronal calcium followed by the increase of c-Fos expression. Thermometry provided evidence that the observed neuromodulation effects were induced by heating brain tissue within non-harmful temperature range. In sum, our data confirm robustness of the human TRPV1-based thermogenetic tool for neuromodulation *in vivo*.

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POSTER SESSION

A model for the generation and spread of limbic seizures

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Limbic epilepsy is one of the most common types of epilepsy, which is accompanied by convulsive activity. Epileptogenesis is most often associated with injury to hippocampal cells [1], however, at the moment the mechanism of seizure propagation is not clear and the area responsible for its generation is not localized. The authors adhere to the hypothesis of the existence of a small epileptic focus (generator) in the hippocampus [2], which is able to spread the main pathological rhythm to all higher structures of the limbic system. A mathematical model of the limbic system was built, which consisted of 101 neurons, including: 1) a neuron connectivity matrix constructed according to anatomical rules; 2) different types of neurons, which are described by specialized equations in the frames of the Hodgkin-Huxley formalism; 3) two types of synapses with AMPA and GABA receptors respectively. Our results demonstrate spread of the pathological frequency (40 Hz) generated in a small hippocampal circuit (epileptic focus) throughout the limbic system providing a reasonable mathematical explanation of discharge genegalization.

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A novel additional function of dUTPases in T5-like phages

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dUTPases are enzymes that facilitate the conversion of dUTP into dUMP and pyrophosphate (PPi) through hydrolysis. By managing the concentration of dUTP and, as a result, the dUTP/dTTP ratio in the cell, they avert the accidental incorporation of uracil during DNA replication. Although mutant organisms encoding inactive dUTPase (e.g., *E. coli* [dut-1]) remain viable, gene knockout of *dut* is shown to be not feasible, suggesting an additional function performed by these enzymes.

In this study, we demonstrate that dUTPases of T5-like bacteriophages, in addition to their canonical (enzymatic) function, perform an additional one necessary for the normal progression of the bacteriophage lytic cycle. We determined the crystal structures of dUTPases of two representatives of T5-like phages, T5 and phi3, with resolutions of 2.0 Å and 1.95 Å, respectively. Comparative analysis of the host (*E. coli*) and phages dUTPases spatial structures revealed an additional structural component (a short loop) in the latter. This short loop in the N-terminal region of the protein appears to be a characteristic structural feature of T5-like bacteriophage dUTPases and one of the elements responsible for their ability to perform the additional function in the phage life cycle. Deletion of the loop did not affect either the spatial structure or the enzymatic activity of the mutant T5 phage dUTPase but negatively impacted phage development in *E. coli* cells. Moreover, our results indicate that the enzymatic and novel functions of the T5 phage dUTPase are structurally and functionally unrelated and independent of each other.

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A structural approach to identify the first protein encoded by the Haseki tick virus genome

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Haseki tick virus (HSTV) is a recently identified, potential human pathogenic virus, discovered in the Russian Federation. However, there is currently a lack of precise information about the structure and function of its proteins. One method for protein identification of novel viruses may be the annotation of protein functions based on comparison of their structures by similarity coefficient. In the first step of this research, it was predicted the location of transmembrane domains in the HSTV polyprotein. Thus, it was possible to determine that the first protein (SP1) encoded in the HSTV genome has a length of 168 amino acids (a.a.).

Then, a model of the SP1 HSTV structure was constructed using the AlphaFold3 algorithm with a pLDDT=92. SP1 models of structure have topological similarities to the solved structures of the N-terminal protease (Npro) of Bovine diarrhea virus (PDB: 3ZFN) and Classical swine fever virus (PDB: 4H9J). The presence of Npro is a distinctive feature of viruses of the genus *Pestivirus*. The model of SP1 HSTV is highly structured and consists of two domains connected by a 9 a.a. long non-structural region. The domains form a clam shell-like structure that is unique to pestiviral Npro. The C-terminal substrate-binding domain is formed by one α-helix and 5 β-sheets forming a Greek key structure. The C-terminal 10 a.a., are the site of proteolysis, that don't form secondary structures and are represented by unbranched amino acids. The active center of Npro is represented by the catalytic dyad of His49 and Cys69. In the obtained model of SP1 HSTV, Cys69 is present, spatially corresponding to the position of Cys69 in the structure of Npro, but His49 is absent. Pairwise structure alignment also showed the similarity of the SP1 HSTV model with the structure of the Erns glycoprotein precursor of bovine diarrhea virus (PDB: 4DVK). However, the obtained level of similarity TM-score = 0.6 does not allow us to unambiguously determine the relationships of these proteins. In addition, Erns may not be encoded first in the viral genome.

Research is currently underway to obtain recombinant SP1 HSTV, confirm its proteolytic properties and resolve its structure by X-ray crystallography.

Amyloid Aβ-42-induced mitochondria dysfunction in yeast *Yarrowia lipolytica*

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The progression of Alzheimer's disease is increasingly being linked to mitochondria dysfunction and oxidative damage in neurons. The complexity of Alzheimer's disease requires the development of simplified models and direct research methods [1]. Cryo-CLEM is a promising technique to study changes in mitochondrial ultrastructure because the sample preparation methods allow samples to be kept in a near-native state. In situ expressed constructs containing genes of fluorescent proteins are the most suitable way to obtain information about the localization of individual proteins. The simplest eukaryotic cell is yeast; however, baker's yeast *Saccharomyces cerevisiae* is a poor model for studying mitochondria. In this study, we generated mutants of the aerobic yeast *Yarrowia lipolytica* [2] expressing a genetic construct for CLEM containing a green fluorescent protein (eGFP) fused to the Aβ-42 protein. Expression of the eGFP-Aβ-42 construct was shown to cause the appearance of fluorescent intracellular aggregates located primarily at the periphery of the cell, leading to mitochondrial dysfunction. Partial colocalization between mitochondria and eGFP-Aβ-42 aggregates was observed, possibly indicating contact of the aggregates with mitochondria. Potential contact sites between mitochondria and protein aggregates were then examined using cryo-CLEM. Mitochondrial dysfunction in the presence of Aβ-42 was shown to be induced indirectly, as amyloid aggregates do not interact directly with the mitochondrial membrane [3].

The work was carried out within the state assignment of NRC "Kurchatov institute".

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Analysis of the effect of hydrogen sulfide donor (NaHS) administration on the stability of mast cells in prenatal hyperhomocysteinemia

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Migraine is a neurological disease, one of the main mechanisms is an increase in the excitability of trigeminovascular system, including nerve endings, vessels and mast cells. Mast cell degranulation can be activated by a number of factors and is accompanied by the release of neuroinflammatory agents having effect on TG nerve. Homocysteine is a sulfur–containing compound formed from methionine coming from food. Disorders of methionine metabolism lead to an increase in the level of homocysteine in plasma and cerebrospinal fluid –hyperhomocysteinemia (hHCY). According to clinical data, hHCY is a factor in the development of migraine, the analysis of the mechanisms of migraine, including hyperexcitability at hHCY, is an urgent problem. The aim of the work is to study the effect of hydrogen sulfide donor (NaHS) administration on the stability of mast cells in prenatal hyperhomocysteinemia.

Mast cell degranulation was studied by staining rat meninges with Toluidine Blue, after which the number of degranulated mast cells was calculated under visual control (P 35-45). Rats with prenatal hHCY were obtained from females fed a methionine-rich diet before and during pregnancy. There was also a group of hHCY animals that received a peritoneal injection of a hydrogen sulfide donor (NaHS).

In the dura mater of intact rats, 24.4±1.8% (n=7) of mast cells were degranulated. In the group with hHCY, the number of degranulated mast cells increased to 49±3.5% (n=6; p=0.01). At the same time, preliminary peritoneal administration of a hydrogen sulfide donor (NaHS) prevented degranulation of mast cells in animals with hHCY and amounted to 0.9±0.5% (n=5).

In conditions of prenatal hyperhomocysteinemia, there is a significant increase in the number of degranulated mast cells. However, the injection of a hydrogen sulfide donor prevented the degranulation of mast cells. Thus, the hydrogen sulfide donor exhibits protective properties and can be used as a new method for the treatment and prevention of migraine.

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Analysis of the influence of heavy water on the dynamics of an ensemble of ion channels by entropy methods

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Bilayer lipid membranes (BLM) modified with gramicidin channels can serve as sensors for assessing the interactions of physicochemical factors with BLM components [1]. The nature of the interactions is assessed by changes in the conductivity of ion channels or by analyzing conductivity fluctuations, i.e., analyzing the time series of ion currents. Often, standard statistical methods for time series analysis are insensitive to changes in system parameters, so other approaches are used, among which entropy methods are promising. In this work, the effect of low concentrations of heavy water (D_2O) on the characteristics of an ensemble of ion channels (gramicidin D) in BLM was determined using the patch-clamp method. BLM from a solution of diphytanoylphosphatidylcholine in n-decane, 15 mg/ml, was formed on the opening of a micropipette with a diameter of 10 μm. Modification of BLM with ion channels was performed by adding an ethanol (95%) solution of gramicidin D (30 nM) to the external medium, which was an aqueous solution of HCl (0.1 M, pH 1.1, 21 °C). D_2O was added to the bathing solution to a final concentration of 1% (v/v). Ion currents were measured using silver chloride electrodes connected to a Keithly-427 amplifier and digitized. The mean values, variances, and spectra of ion currents did not change significantly in the presence of D_2O. Entropy calculations were performed using programs [2] in the Python environment. The dependencies of the permutation (PE), dispersion (DE), and bubble (BE) types of entropy on the transmembrane potential difference (TMP) changed significantly with the addition of D_2O. The minima of PE, DE, and BE in the absence of D_2O were observed at TMP values (mV) of –90, –70; –60, +5; 0; and in the presence of D_2O: 0; –10; –80, 0 (with an accuracy of ±15 mV). Thus, the values of entropy of different types of ion currents change significantly with changes in the operating conditions of ion channels. This indicates the sensitivity of the methods used in comparison with generally accepted ones.

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Analysis of whole-genome maps of chromatin folding in SIRT6-KO neurons

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Aging is characterized by various molecular alterations, such as genomic instability, telomere shortening, and mitochondrial dysfunction. SIRT6 deacetylase plays critical roles in these processes, particularly in DNA repair and telomere maintenance. Overexpression of SIRT6 has been shown to extend lifespan, while its deficiency leads to premature aging symptoms [1,2]. Recent research has focused on the effects of SIRT6 depletion in the brain, revealing molecular changes reminiscent of aging and neurodegenerative diseases [3]. However, the precise mechanisms by which SIRT6 influences chromatin dynamics during aging remain unclear. To address this problem, we analyzed Hi-C data obtained from wild-type, SIRT6-knockout (SIRT6-KO), and aged cortical mouse neurons. Our findings showed that both aging and the absence of SIRT6 lead to more compact local chromatin interactions, increased interchromosomal interactions, and stronger compartmentalization, accompanied by interaction strength gain in inactive (B) and loss in active (A) chromatin compartments. In addition, SIRT6-KO neurons also demonstrated more intermingling between A and B compartments compared to wild-type neurons. Functional analysis highlighted the involvement of immunity-related genes, particularly immunoglobulin heavy chain variable region (Ighv) genes, in these changes, suggesting their role in aging and SIRT6 deficiency. While both aging and SIRT6-knockout affect long-range chromatin interactions, they differ in their impact on topologically-associated domains (TADs) and loops: aging increases their prominence, while SIRT6-knockout decreases them. Overall, our results provide insights into SIRT6 role in regulating chromatin architecture during aging, with implications for understanding age-related neurological disorders.

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Analytical systems for studying affinity of peptide blockers to K_v1 channels in open and closed state

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Voltage-gated potassium channels Kv1 are promising therapeutic targets for treatment of autoimmune, neurological and oncological diseases [1]. Peptide blockers, found in animal venoms, possess ability to bind Kv1 channels and inhibit their activity with high affinity and specificity. Therefore, such peptide blockers have potential to be templates for creating therapeutic agents.

Radioligand analysis and patch-clamp technique are comprehensive methods for studying peptide blockers affinity to Kv1 channels, but the channel state remains either unknown or strictly open in conditions, required for mentioned techniques. Here we report on the development of analytical system aimed to study the binding affinity of the peptide blockers to Kv1 channels by the means of fluorescent microscopy. The system, which is based on living eukaryotic cells with high-level expression of the fluorescently labeled Kv1 channels allows one to switch the channels from the open conductive state to the closed state [2], and, thus, to determine state-dependent binding of peptide blockers to the target channels.

Our research demonstrates no alterations in the affinity of the studied peptide blockers to Kv1.1 and Kv1.3 channels in open and closed states. This suggests that structural rearrangements during channel transition to the open state hardly influence the conformation of the P-loop (including the selectivity filter) of Kv1 channel involved in the formation of the binding site of peptide blockers.

The developed method may promote studies of ligand-channel interaction interface and search for state-dependent channel blockers with distinct affinity to open and closed Kv1 channels, which can find a wide clinical application.

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Antitumor drug based on the chimeric protein L-methionine-γ-lyase and VHH antibody against tumors with overexpression of the epidermal growth factor receptor

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Methionine dependence is the inability of tumor cells to proliferate in a medium where methionine is replaced by homocysteine. The fact that tumor cells are methionine auxotrophs is known in science as the Hoffman effect, which was first shown back in the 1970s [1]. Dietary methionine elimination has been tested *in vivo* and *in vitro*, causing apoptosis and inhibiting the cell cycle in the S/G2 phase.

In clinical models, methionine starvation caused a decrease in tumor growth and inhibited the growth of metastases. However, dietary elemination of methionine is quite difficult to achieve therapeutically.

The most effective method for reducing methionine concentrations is irreversible enzymatic degradation. For these purposes, L-methionine-γ-lyase (MGL) is used, a pyridoxal-5'-phosphate-dependent enzyme that catalyzes the γ-elimination reaction of L-methionine with the formation of methyl mercaptan, α-ketobutyric acid and ammonia. The ability of MGL to reduce methionine concentrations *in vivo* and exhibit therapeutic activity against various types of tumors was previously demonstrated [1, 2]. However, as a drug, the enzyme has several disadvantages. Due to the fact that MGL has a general biochemical effect, it deprives not only cancer cells, but also healthy cells of methionine. In addition, it causes liver inflammation and immune response.

Overexpression of the epidermal growth factor receptor EGFR is observed in many types of tumor cells (breast cancer, ovarian cancer, etc.) [3]. Thus, targeting protein drugs to the epidermal growth factor receptor is a promising strategy for the development of new drugs.

In our work we created a chimeric MGL fused with the VHH antibody 7D12 [4], which binds to the epidermal growth factor receptor (EGFR). It is assumed that the use of the developed chimeric protein MGL-7D12 should increase specificity and reduce therapeutic doses of the drug as a result of a local decrease in methionine in the tumor area.

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Anxiety, photophobia, mechanical allodynia and oxidative stress in rats with hyperhomocysteinemia in chronic migraine model

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Migraine is a common chronic neurovascular disease, accompanied by nausea, photophobia, allodynia and other discomforts [1, 2]. Elevated homocysteine levels cause endothelial dysfunction and oxidative stress, which may be involved in migraine pathogenesis [3]. Cortical spreading depression (CSD) is a slowly propagating wave of depolarization of neurons and glial cells, that is a potential contributor to headache [3]. The study was conducted on male Wistar rats (5-7 months) born from females on a control or methionine diets. Chronic migraine was induced by CSD evoked by repeated application of KCl (1 M) to the dura mater every other day for 9 days. The light-dark box test was used to measure anxiety and photophobia. Mechanical sensitivity was assessed with Von Frey filaments. To analyze oxidative stress in rat brain tissues, the content of malondialdehyde and the activity of glutathione peroxidase were measured by spectrophotometric method. Our results demonstrated that prenatal hyperhomocysteinemia causes anxiety and increased mechanical sensitivity in rats, and also contributes to the development of photophobia and allodynia in modeling chronic migraine. Moreover, in rats with prenatal hyperhomocysteinemia, the activity of glutathione peroxidase in brain tissues was reduced after migraine modeling.

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Application of the optogenetic tool lyso-Rhodopsin to determine the cause of pathological lysosome alkalization

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Pathological alkalization of lysosomes induced by various causes often precedes certain pathologies, such as Alzheimer's disease [1], and therefore the development of methods for early detection of pathological alkalization of lysosomes and establishing its cause is a relevant purpose. Here, we demonstrate the effectiveness of the optogenetic tool lyso-Rhodopsin as a means of identifying the cause of lysosomes pathological alkalization.

Three main causes of pathological alkalization of lysosomes modeled and studied: 1) inhibition of vATPase activity (modeled by the influence of bafilomycin A1), 2) accumulation of proton sponges in the lumen of lysosomes (modeled by the influence of hydroxychloroquine), 3) permeabilization of the lysosomal membrane (modeled by the influence of LLOMe). The experimental groups compared by two parameters - the amplitude of the change in the lysosome lumen pH during optogenetic exposure and the time of lumen re-alkalinization after the end of this exposure. It found that relatively to other comparison groups (including untreated control) the amplitude of the pH change during optogenetic exposure is greater with the inhibition of vATPase activity and less with permeabilization of the lysosomal membrane. In addition, it also found that the time of lumen re-alkalinization relative to other comparison groups is greater with the inhibition of vATPase activity.

Based on the results of the work, it can be concluded that probably two parameters of the lysosome response to optogenetic exposure assessed in this work can be used to determine the cause of pathological alkalization of lysosomes, and the optogenetic approach proposed here can be a promising method for early detection of lysosomal function disorders.

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Approaching molecular mechanisms involved in differentiation of iPSCs to cardiomyocytes

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According to the World Health Organization, cardiovascular diseases are the leading cause of death worldwide [1]. In 2021, cardiovascular diseases caused the death of 20.5 million people, and this figure accounted for about a third of all deaths worldwide. To understand the pathogenesis of cardiovascular diseases, as well as to develop new treatments, it is necessary to create realistic disease models [2]. The use of induced pluripotent stem cell (iPSC) technology has great promise in the field of both fundamental and translational medicine. The developing technology of using cardiomyocyte iPSCs can significantly facilitate the study of hereditary and acquired cardiovascular diseases, infectious diseases, research into the development of the cardiovascular system, drug development, toxicological screening and personalized cell therapy [2]. But there is a problem of cellular phenotyping and maturation of cardiomyocytes during differentiation from iPSCs, so it is necessary to study the differentiation process itself to obtain an adult patient-specific phenotype in vitro.

In the previous study [3] it was revealed that iPSC-derived cardiomyocytes seeded before day 20 of differentiation can effectively form a functional syncytium, while cells seeded after day 20 cannot. To find out what causes changes in electrophysiological parameters after day 20, we performed transcriptomic analysis.

Samples from three different differentiation days (0, 17, and 25) were frozen and submitted for transcriptome analysis. Total RNA was isolated, mRNA libraries were enriched, and sequencing (Illumina NovaSeq) was performed. We obtained paired-end reads for each sample and performed differential expression analysis (salmon, DESeq) for pairs of days 0-17, 0-25, and 17-25. Comparisons of days 0-17 and 0-25 confirmed that day 17 and 25 samples were cardiomyocytes and obtained lists of differentially expressed genes. The lists show that the expression of a number of contractile apparatus proteins (MYH7, TNNC1) and EMC proteins (SPOCK2, MGP, ITLN1) increases from day 17 to day 25.

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Arrangements of pre-AD membrane mimetics due to the presence of Aβ(25-35) peptide

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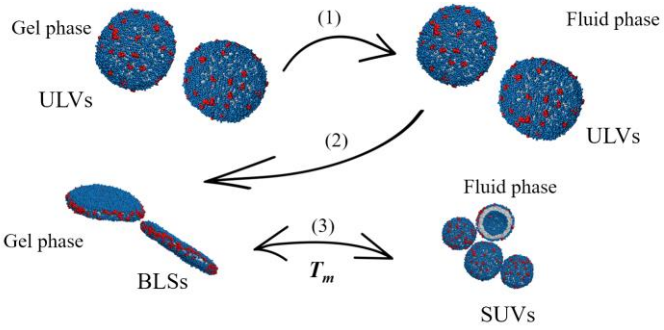
The complementary experimental data and molecular dynamics (MD) simulations results reveal the structure of previously observed lipid bicelle-like structures (BLSs) formed in the presence of amyloid-beta peptide Aβ(25-35) below the main phase transition temperature (T_m) of saturated phosphatidylcholine lipids and small unilamellar vesicles (SUVs) above this temperature. First, we show by using solid-state ^{31}P nuclear magnetic resonance (NMR) spectroscopy that our BLSs being in the lipid gel phase demonstrate magnetic alignment along the magnetic field of NMR spectrometer and undergo a transition to SUVs in the lipid fluid phase when heated through the T_m. Secondly, thanks to the BLS alignment we present their lipid structure. Lipids are found located not only in the flat bilayered part but also around its perimeter, which is corroborated by the results of coarse-grained (CG) MD simulations. Finally, peptides appear to mix randomly with lipids in SUVs while assuming predominantly unordered secondary structures revealed by circular dichroism (CD), Raman spectroscopy, and all-atom MD simulations. Importantly, the former is changing little when the system undergoes morphological transitions between BLSs and SUVs. Our structural results then offer a platform for studying and understanding mechanisms of morphological transformations caused by the disruptive effect of amyloid-beta peptides on the lipid bilayer.



Biofilms of MDR *K. pneumoniae* (hvKp) strains can be destroyed *via* Lewis acid–base/van der Waals interactions of fatty acids with bacterial wall membranes

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Hypervirulent mucoviscus *Klebsiella pneumoniae* (hvKp) is rapidly emerging as opportunistic pathogens that have a global impact leading to a significant increase in mortality rates among clinical patients. Anti-virulence strategies that target bacterial behavior, such as adhesion and biofilm formation, proposed as alternatives to biocidal antibiotic treatments to reduce the rapid emergence of bacterial resistance [1]. The main objective of this study was to examine the efficacy of fatty acid-enriched extract (AWME3) derived from the fat of Black Soldier Fly larvae (*Hermetia illucens*) in fighting against biofilms of multi-drug resistant (MDR) and highly virulent hvKp pathogens [2]. The study also aimed to investigate the potential mechanisms underlying this effect. To reach these goals we used crystal violet (CV) and ethidium bromide (EtBr) assays to show how free fatty acids (FFAs) from AWME3 affects the formation of mixed and mature biofilms by the KP ATCC BAA-2473,KPi1627, and KPM9 strains. We used light, fluorescence, and scanning electron microscopy (SEM) as direct microscopic methods to gather evident information about the effect of FFAs on treated by AWME3 biofilms [3]. Our results demonstrated the exceptional efficacy in combating the hypermucoviscosity (HMV) virulent factors of KPi1627 and KPM9 strains after treatment by AWME3. The rudimentary motility of MDR KPM9 and KP ATCC BAA- 2473 strains detected through swimming, swarming, and twitching assays. The cell wall membrane disturbances induced by AWME3 validated by microscopy methods and by an increase in the bacterial cell wall permeability and Lewis acid-base/van der Waals characteristics of *K. pneumoniae* strains tested by MATS (microbial adhesion to solvents) method [4]. In conclusion, our study demonstrates the exceptional capability of the natural AWME3 extract enriched with a unique combination of FFAs to eliminate the biofilms formed by the highly drug-resistant and highly virulent pathogens. Our results highlight the opportunity to control and minimize the rapid emergence of bacterial resistance through the treatment using AWME3 of biofilm-associated infections caused by hvKp pathogens.

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Biological activity of polysaccharide from seeds *Brassica rapa*

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For isolation of polysaccharides, Brassica rapa turnip seeds cultivated in Uzbekistan were used. The seeds were pre-cleaned and crushed in a laboratory grinder. For defatting, pre-crushed seeds of Brassica rapa were extracted with petroleum ether in a Soxhlet apparatus for 72 hours. To remove low-molecular impurities and coloring substances, the raw material was extracted in a Soxhlet apparatus with a mixture of chloroform and ethyl alcohol 96% (1:2). To isolate water-soluble polysaccharides, defatted seeds were extracted three times with water in a water bath at 95°C under reflux (the ratio of raw material and extractant was 1:20, 1:15, 1:15). The duration of each extraction was 2 h. The resulting aqueous extracts were combined and evaporated on a rotary evaporator at a temperature of 50 °C to 1/5 of the volume. From the resulting concentrate, water-soluble polysaccharides were precipitated by adding a fourfold volume of 96% ethanol and left at 4 °C overnight. The precipitate was separated by centrifugation, washed with ethanol, and freeze-dried. Deproteinization of the sum of polysaccharides was performed according to the Savage method. The IR spectra of the sample were recorded on an IRTracer-100 SHIMADZU IR-Fourier spectrometer (Japan), system 2000 in the frequency range 400–4000 cm-1. The spectra of the studied sample were recorded by the method of Attenuated Total Internal Reflection (ATR) spectroscopy in the infrared region with Fourier transform spectroscopy. In the IR spectrum absorption bands corresponding to polysaccharides were observed. The antidiabetic, hypoglycemic and hepatoprotective activity of *Brassica rapa* polysaccharide as a promising source of medicines and prophylactic agents has been studied. The study of the effect of *Brassica rapa* polysaccharides in comparison with C-4 (development of the Institute) and Phosphogliv (Russia) on the course of experimental acute toxic hepatitis caused in mice by carbon tetrachloride (CCl4) showed that *Brassica rapa* polysaccharides at a dose of 20 mg /kg in comparison with C-4 and Phosphogliv preparations has. It has hepatoprotective activity and in the future can be used to create a biologically active supplement (dietary supplement) for the prevention and treatment of diseases of the hepatobiliary system.

Biomembrane as an important intermediate interface for aging signals arising under inadequate external conditions

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It is known that aging is a regulated and partially reversible process [1]. It depends on the interaction of multiple regulatory signals, both extracellular and intracellular. Biomembranes are interfaces between extracellular ligands (hormones, growth factors, etc.) and the network of intracellular signal transduction pathways. They carry out an important function in processes of signal transmission from extracellular regulatory ligands via respective receptors and subsequent cascades of intracellular biochemical reactions up to regulation of activity of transcription factors, which control gene expression. A detailed study at the macromolecular level of the entire diversity of reactions of the body during aging was very difficult. Therefore, we developed a holistic approach to origin of aging and black box phenomenology of macro-systems behaviour in different environments [2-5].

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Biosensor based on graphene transistor for detecting heart failure marker

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For the diagnosis of heart failure, it is recommended to measure the level of the NT-proBNP protein in the blood. This method allows for the identification of patients both at an early stage of disease development and those in need of medical assistance. Currently, there is interest in a non-invasive method for determining the concentration of NT-proBNP in human saliva [1]. Due to the fact that the concentration of the protein in saliva is lower than in blood [2], there is a need to develop highly sensitive sensors. We conducted extensive work on the development of biological aptasensors based on field-effect transistors with graphene and graphene oxide channels. We found that materials with a large bandgap help to increase the sensitivity of the device by reducing noise [3]. Detection limits for sensors with graphene and graphene oxide channels were 1 and 0.1 pg/ml, respectively. We propose correlation analysis of independent sensor parameters to improve the reliability of the results [4]. Sensor selectivity studies were conducted using another marker of heart failure and showed results at noise levels.

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Capabilities of SEC-SAXS method in research of polydisperse systems: case of apoferritin

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Membrane proteins (MPs) play many important roles in organisms, so structural studies of MPs are of great value for science and medicine. There are many difficulties to obtain high-resolution structures of MPs. In this regard, results from using low resolution methods, as small-angle X-ray scattering (SAXS), are also acceptable. Oligomerization polydispersity is a common effect for both MPs and soluble proteins; it may lead to difficulties in SAXS data interpretation. In some cases, SAXS allows to define preferred type of oligomer in mixture with varied possible types of particles with same weight [1]. Coupling of SAXS with size-exclusion chromatography (SEC) seems to be a universal way to defeat the problem of polydispersity, however, it also has a set of limitations [2]. Here we explore such capabilities of SEC-SAXS method in case of horse spleen apoferritin solution as an example of polydisperse system [3].

For interpretation of the data collected during SEC-SAXS experiments on the ESRF (beamline BM29), we suggest a model of polydisperse solution containing monomers, dimers and different trimers of apoferritin globules. Minimum loss values were obtained from sets containing one type of trimer with values of effective apical corner $120\text{-}130^0$. Suggested model was validated using a set of methods, it corresponds to experimental data starting from ~1.2 mL from the beginning of the observed peak fractions; deviation of calculated values of validation parameters Rg and Vp from experimental data with decrease of elution volume indicates the presence of considerable amount of aggregates in this fraction.

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Carbon monoxide prevents on ATP-mediated nociceptive activity of rat trigeminal afferents

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Migraine is a prevalent neurological disorder with unilateral headache as a main symptom. Pain in migraine mediated by activation of meningeal afferents of trigeminal nerve. Carbon monoxide (CO) is a toxic gas that can cause headache, but it is also an endogenous neurotransmitter that plays a role in blood flow regulation and nociception. There is evidence of pro- and anti-nociceptive properties of CO. Purinergic system is involved in generation of nociceptive signaling and in neuron-glia-mast cell signaling. The aim of our study was to analyze the effects of exogenous CO on the ATP mediated activity of peripheral afferents of rat trigeminal nerve (TG).

Action potentials (APs) of the TG were recorded using an extracellular electrode in a rat hemi-skull preparation. To analyze effects of CO solution was bubbled with CO (98.5 %) for 30 min before application or CO donor (CORM-2 (30 mM) was used.

In control conditions, trigeminal nerve afferents demonstrated spontaneous regular activity. Application of the ATP (100 mM) resulted in a significant increase in the frequency of APs from 225.5±59.49 to 671.7±71.76 ($n=6$, $p<0.05$) due to activation of ionotropic P2X3 receptors [1].

Incubation of hemiscull preparation in solution with CORM-2 or bubbled with CO gas did not alter activity of trigeminal afferents significantly (before 425.8±70.58, $n=4$ and 333.1±115.1, $n=11$; to 312.5±84.17 and 340.6±78, correspondingly). Further application of ATP did not increase frequency of APs (359.3±81.38 and 319.6±63.57). The obtained data indicate that CO prevented nociceptive activity of TG nerve mediated by P2X3 receptors which suggest antinociceptive properties of CO and further experiments will elucidate cellular mechanisms of CO action in TG afferents.

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Changes of the structure and permeability of lipid membranes caused by nanoparticles and pulsed electromagnetic effects

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Currently, a relevant and important interdisciplinary area of science is the development of new highly effective methods of drug therapy aimed at solving the problem of controlled delivery of drugs and genes directly to target areas and cells of the body [1].

Our work is devoted to the development of new biocompatible means and methods of encapsulation, targeted delivery and controlled release of drugs in aqueous media including living systems. New nanostructured biomimetic lipid vesicles (nanocomposite liposomes) have been developed, the membranes of which are functionalized with magnetite and gold nanoparticles [2]. As the safe external control physical effects that provide selective remote control of the structure and permeability of the membranes of nanocomposite lipid vesicles, we use non-thermal effects of powerful ultrashort electrical pulses with a duration of less than 10 ns, providing the effect of selective electroporation of lipid membranes containing conductive nanoparticles polarized in an external electric field.

The anticancer antibiotic doxorubicin and the fluorescent dye carboxyfluorescein were used as model molecular compounds encapsulated within nanocomposite liposomal carriers and released by the electric pulses.

The obtained experimental data and theoretical estimates indicate a controlled activation and increase in the permeability of nanocomposite lipid membranes and an effective release of the encapsulated compound from nanocomposite lipid vesicles as a result of external pulsed electrical action.

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Changes in electrical properties of bilayer lipid membranes induced by colloidal magnetite (Fe3O4) nanoparticles in a static inhomogeneous magnetic field

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Colloidal magnetic nanoparticles under the influence of an external magnetic field are a very promising tool for targeted delivery of genes and drugs through lipid membranes.

In this study we present changes of electrical parameters of azolectin membranes in a static inhomogeneous magnetic field at the one-sided addition of positively charged quasi-spherical superparamagnetic magnetite nanoparticles with a diameter of about 4 nm. The magnet was located at different distances from the membrane, and the magnetic field attracted the nanoparticles to the membrane surface with different strengths. The membranes were prepaired using the method, decribed by [1]. After the addition of MNPs, a decrease in capacitance, polarization and further depolarization of the membranes was observed. Zero current appeared on the BLMs. However, there is no unambiguous dependence of the zero current on the membrane depolarization and no dependence of the zero current on the strength of the magnetic field and on the concentration of added MNPs. The membrane conductivity increased on average with an increase in the external magnetic field and depended on the ionic strength: the membrane conductivity at 100 mM is 14 ± 11 times greater than at 5 mM.

It follows from the results that the magnetic field causes a rapid accumulation of MNPs on the surface of the BLMs. As a result, the membrane is polarized. Depolarization of the membranes can occur when MNPs pass through the BLMs. Moreover, it was shown by our team that MNPs penetrated the BLMs and reached the target in the opposite chamber near a magnet using Energy-dispersive X-ray (EDX) spectroscopy and spectrophotometry. The increase in conductivity reflects the processes of lipid bilayer disordering.

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Characterization of a proton pumping rhodopsin MAR mutant by time-resolved visual spectroscopy

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Rhodopsins are light-driven membrane proteins, commonly consisting of seven transmembrane alpha-helices. One of the functions of these proteins, including recently discovered marine actinobacterial rhodopsin (MAR), is pumping protons through the membrane [1,2]. On the basis of previous studies, we were able to draw some conclusions about the structure of this protein. Our predictions show some key amino acids in MAR structure, playing an important role in proton pumping through the membrane and this assumption was tested by point mutation one of this residues.

We have studied the photocycles of mutant MAR protein by time-resolved visual spectroscopy. The photocycles were measured at acidic, neutral and alkali pH values. The time-constants of the decays of the intermediate states in the mutant rhodopsin were different from wild type MAR [3].

Our results contribute to better understanding of rhodopsin proton pumping and engineering of enhanced optogenetic tools. The expressed and purified MAR mutant will be used to determine high-resolution crystal structure.

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Characterization of primary and subsequent waves of cortical spreading depolarization caused by photothrombotic stroke in awake mice

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Introduction. Spreading depolarization (SD) is a massive change of the intercellular ionic composition spreading in a wave-like manner from injury site through the gray matter.

Materials and methods. Male (n=6) and female (n=8) C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J mice (Jackson Laboratory) six to nine months old were used. These mice express GCaMP6f (a fluorescent Ca^{2+} sensor) in neurons. All animals underwent a "cortex-wide cranial window" operation (70% of a dorsal skull). Cortical activity was recorded by the wide-field optical imaging (WFOM) method in the fluorescence mode ($[Ca^{2+}]_i$ measurement: Ex/Em 470/520 nm 20 Hz) and in the scattered light mode ([Hb]: 530 nm 10 Hz and 656 nm 10 Hz). Photothrombotic stroke was induced by laser (λ=532 nm, Ø=1 mm, 10 min) after injection of Bengal Rose (40 mg/kg, i.v.).

Results. It has been shown that the occurrence of a stroke injury is always coupled to the occurrence of SD. The primary SD peak amplitude was 302±11% (mean±SEM, F/F0 GCaMP fluorescence). Primary SD provoked vasospasm in all observations. Subsequent SDs is associated with an abrupt expansion of the stroke core by 12,9±2,4%. The subsequent SDs peak amplitude was 194±36% (F/F0 GCaMP), and the various vascular responses was caused (vasospasm, vasodilation, biphasic response). The subsequent SDs provokes a massive edema of an ischemic hemisphere in 28% of mice. No sex differences were observed.

Taking into account the obtained data, it can be assumed that SD is a promising target for the treatment of ischemic stroke.

Comparative analysis of high-resolution structures of membrane proteins

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Membrane proteins are important in cell life and its communication with the environment: they serve as transporters, receptors, enzymes, etc. Obtaining high-resolution structures of membrane proteins plays a significant role in studying their functions and interactions with ligands (including drugs). There are several databases used to index membrane proteins: MemProtMD [1], mpstruc [2], OPM [3], PDBTM [4], SCOP [5]. Due to the different criteria by which membrane proteins are entered into the databases, these databases do not completely overlap with each other, making it difficult to fully analyze the entire set of membrane proteins. The aim of this work is to investigate the efficiency of different methods of crystallization and obtaining structures of membrane proteins, and to evaluate the dynamics of crystallization methods and structure solutions over time.

We created a merged database of membrane proteins. From the merged array of membrane proteins, diameters of the molecules and sizes of the water-soluble parts were calculated. The dependence of the efficiency of crystallization and structure solution methods on protein size and molecular weight was analyzed. Our results show that the character of resolution dependence on protein size is the same (within error) for the two most common methods of protein crystallization: in lipid cubic phases (*in meso*) and in detergent micelles (*in surfo*). We also show that the electron microscopy method, which is rapidly gaining popularity, performs better in resolving structures of larger molecular weight compared to X-ray diffraction.

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Comparative analysis of the effect of human STARD1 and STARD3 proteins on the activity of the steroidogenic cytochrome P450 system reconstructed in *E. coli* cells

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The construction of microorganisms capable of carrying out processes that occur in the steroidogenic organs of mammals is an important area of scientific research. Such microorganisms are increasingly being used in biotechnological processes for the synthesis of steroid drugs. Cytochrome P450scc (CYP11A1), as part of the mammalian enzyme system (including P450scc, AdR and Adx), catalyses the initial reactions of the steroidogenic cascade, ensuring the conversion of cholesterol to pregnenolone. This research was carried out to optimise the function of the bovine P450scc system reconstituted in *E. coli* cells.

It has been shown that the synthesis of the human cholesterol transfer proteins STARD1 or STARD3 in BL21(DE3) cells leads to an increase in the efficiency of sterol assimilation by the bacteria. Using heterologous expression model systems based on *E. coli* BL21(DE3), it has been shown that cells expressing the gene encoding STARD1 or STARD3 with the pelB sequence and genes encoding P450scc system proteins are more efficient at bioconversion of cholesterol to pregnenolone than cells expressing only P450scc system protein genes. As a result of the expression of the STARD1 or STARD3 gene, the level of cholesterol bioconversion by the P450scc system is increased by 1.8 and 4.8 times, respectively (determined by ELISA and HPLC).

A search was made for the *E. coli* host strain that would provide the most optimal conditions for STARD3 to function in conjunction with cytochrome P450scc. Polycistronic plasmids were used to generate a panel of *E. coli* strains that synthesise proteins of the P450 system and the STARD3 protein. The *E. coli* Rosetta(DE3)pLysS strain was found to be optimal for the function of the reconstructed P450 system. The use of this strain allowed to increase the pregnenolone yield by 6.5 times compared to the BL21(DE3) strain.

Comparative analysis of weighted amino acid networks of the sodium-dependent phosphate transporter NaPi2b

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The active roles of individual amino acids within the protein are determined by various factors, including nearby amino acids, secondary structure, and post-translational modifications. Comparison of Weighted Amino Acid Networks (WAANs) of proteins with different molecular characteristics allows us to explore topological information and capture the global connectivity in proteins.

Sodium-dependent phosphate transporter NaPi2b is a membrane protein that belongs to the SLC34 family, it is a marker of ovarian and other cancers [1]. We assume that the MX35 epitope that located in the large extracellular domain (ECD) and is recognized by the MX35 antibody in tumor cells has tumor-specific conformation due to the presense of disulfide bonds within ECD [2].

The aim of this work was to develop robust algorithm for comparative analysis of WAANs based on amino acid residue characteristics of NaPi2b with the absence and the presence of potential disulfide bonds within ECD.

The NaPi2b structure was predicted and thermodynamically stabilized by Molecular Dynamics. Two potential disulfide bonds (aa 303-328, aa 328-350) were introduced into ECD by Controlled Molecular Dynamics. WAANs were constructed, the weights were determined by several parameters of amino acid residues. Clustering of the WAANs was performed using proximity propagation of adjacency matrices, and the similarity score was calculated for each cluster. The overall similarity score was calculated as the median of the cluster scores.

This algorithm that takes into account the amino acid residue interactions and weights, as well as the topology of the WAANs was utilized to compare the WAANs of NaPi2b with and without disulfide bonds. The similarity scores of WAANs were as follows: no bond vs 303-328 − 0.85, no bond vs 328-350 − 0.83, 303-328 vs 328-350 − 0.87. The scores of WAANs showed the difference not only in the ECD where two potential disulfide bonds (aa 303-328, aa 328-350) were introduced but in several other extra- and transmembrane domains of NaPi2b.

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Complementary studies of biological and model membranes using small-angle neutron and X-ray scattering methods

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The small-angle scattering (SAS) method, implemented at the "YuMO" spectrometer (JINR, Dubna, Russia) and the Rigaku X-ray system (MIPT, Dolgoprudny, Russia), is widely used for studying biological and model membranes [1-3]. The capabilities of this method are further enhanced by X-ray scattering using a synchrotron source (SEC-SAXS). This paper presents the results of recent studies in the area of biological membranes [4], model membranes [5], and proteins [6].

Neutrons offer the complementary advantages of contrast variation, high transparency, and a distinctive sensitivity to heavy and light water. On the other hand, X-rays require only a small amount of sample and provide high flux at the sample, especially when using synchrotron sources. The application of additional experimental conditions, such as light, temperature, pressure, and magnetic or electric fields, opens new avenues for research in soft matter [7].

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Complex of Kindia Tick Virus untranslated RNA regions with phage MS2 coat protein in structural RNA research

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Kindia tick virus (KITV) is a recently discovered flavi–like virus that still unclassified. This virus has an unusual genome architecture. The structure and role of the 5′ and 3′ UTR RNA KITV in the implementation of its genetic information remains virtually completely unexplored to date. Previously, we carried out modeling of the secondary structure of the 5′ and 3′ UTR RNA KITV and identified important its structural and functional elements. 5′ UTRs of all KITV genome segments have length from 90 to 150 bp. They are highly structured and have conservative regions. 3′ UTRs are more extended and their length ranges from 130 to 350 bp. They are polyadenylated and high variable that is atypical for the *Flaviviridae* family. We designed the UTR complex with a model MS2 phage coat protein to obtain experimental data of the structure of 5′ and 3′ UTR RNA KITV Because the RNA becomes more stable in complex with the protein. This RNA-binding protein has a small size that facilitates crystallization, and the conditions for its crystallization have already been described. In the first stage, we constructed full-length synthetic DNA copies of the 5′–3′ UTR RNA KITV of all four KITV genome segments. This synthetic DNA contained a T7 promoter region for subsequent transcription and an 18-nucleotide hairpin region (MS2-tag) that interacts with the phage MS2 coat protein to produce RNA-protein complexes. For each 5′ and 3′ UTR RNA KITV, the position of the MS2-tag was selected separately. We controlled that the original secondary structure of 5′ and 3′ UTR RNA KITV was not changed by addition of the MS2-tag. Also, the phage MS2 coat protein containing a hexahistidine affinity tag (6xHis-MS2) was obtained recombinantly in the KRX *E.coli* cell. Next, we plan to crystallize and X-ray diffraction analyze of complexes of the phage MS2 coat protein with the chimeric 5′ and 3′ UTR RNA KITV. This will allow us to experimentally confirm the secondary structure of the 5′ and 3′ UTR RNA KITV and establish the role of the viral RNA untranslated regions in the implementation of the genetic information of novel flavi-like viruses with a segmented genome.

Computer Analysis of the Inhibition of influenza hemagglutinin by polyene antibiotics and Identification of Their Potential Antiviral Pharmacophore Site

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Influenza remains one of the most widespread infections, causing an annual illness in children and adults [1]. Therefore, the search for new antiviral drugs is one of the priorities of practical health care. There has been much supposition that polyene antibiotics may have antiviral activity against several viruses including influenza. Eight polyene antibiotics were evaluated as potential agents against influenza virus. The goal was to predict the binding sites and energies of the derivatives with respect to the receptor amino acids. Antiviral activity of polyene antibiotics was evaluated for the first time based on POM (Petra/Osiris/Molispiration) theory and docking analysis [2]. POM calculation was used to analyze the atomic charge and geometric characteristics. The drug similarities, side effects, and drug scores were also assumed for the stable structure of each compound. The POM study confirmed the predominant antiviral profile of most compounds in polyene antibiotics. This is highly encouraging to screen the polyene antibiotics as potential antiviral candidates.

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Conductive properties of endogenous mechanosensitive Piezo1 channels in human myeloid leukemia cells K562

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Piezo1 is a calcium-permeable mechanosensitive channel that participates in many key cellular reactions and biological functions physiologically and pathologically. In 2015, a novel synthetic specific activator of Piezo1 channel, Yoda1, which can stimulate Piezo1's activity in the absence of mechanical stimuli was discovered. Using Yoda1 as a convenient tool allows us to study the fundamental mechanisms of functioning and the role of Piezo1 channels in cellular processes. In this research human myeloid leukemia cell line K562 was used to study conductive properties of native Piezo1 as an adequate model for recording and analyzing single currents in the whole-cell configuration of patch-clamp technique. We determined the concentration of Yoda1 (1 μM) sufficient to evoke stable single Piezo1 channel activity which was used to solve experimental tasks. By varying the ionic conditions of experimental solutions, the unitary permeability of endogenous Piezo1 channels for the main physiologically significant cations (Na^+, Ca^{2+}, Mg^{2+}) was studied. By single-channel recording and fluorescent measurements of the intracellular Mg^{2+} concentration, the fundamental possibility of Piezo1 participation in Mg^{2+} transport and Mg^{2+}-dependent intracellular processes was demonstrated. It was shown for the first time that the antibiotic gentamicin effectively blocks the activity of Yoda1-induced Piezo1 by the open channel block mechanism. This approach can be used for rapid suppression of Piezo1 activity and has an obvious advantage over other non-selective Piezo1 blockers acting on the lipid microenvironment of channels. The obtained results indicates the promise of using K562 cells as an experimental model for testing the effect of various compounds as potential modulators of endogenous Piezo1 channels in the plasma membrane.

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Copper ions influence thiocyanate dehydrogenase crystal packing and subunit conformation

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Homodimeric thiocyanate dehydrogenase (TcDH) catalyzes oxidation of thiocyanate to cyanate and elemental sulfur and contains copper ions, which form a unique trinuclear active center [1]. Moreover, TcDH from *Pelomicrobium methylotrophicum* has a peculiar structural feature – its subunits have different conformations in a crystal: open and closed [2]. To address a putative reason for the asymmetry of the homodimer, we demonstrated that crystals of the apo-enzyme (without copper ions) possessed an alternative packing of dimers, where both subunits had the closed conformation. Soaking of the crystals of the apo-enzyme with copper ions led to the restoration of the trinuclear copper active center together with the transition of subunits between the open and closed conformations. Thus, copper ions in the pmTcDH active center mainly influence the packing of the enzyme in the crystal as well as affect subunit conformation.

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Correlation Between Biological Indicators and MSC Cultivation with iPSC-Cardiomyocyte Conditioned Media for Cardiac Differentiation

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Stem cell therapies for cardiac diseases hold great promise and gained attention, particularly due to the capacity of mesenchymal stem cells (MSCs) to differentiate into cardiomyocytes. MSCs, which are known for their accessibility, availability and high proliferative potential[1], offer a viable approach for cardiac repair. This study investigates the potential of MSC differentiation into cardiomyocytes using conditioned media from induced pluripotent stem cell (iPSC)-derived cardiomyocytes and explores the correlation between MSC proliferation and various clinical parameters. Patient-specific MSCs were cultured in the presence of iPSC-derived cardiomyocytes -conditioned media, which provided a rich source of growth factors. The conditioned media caused MSC differentiation into cardiomyocytes, as confirmed by functional calcium imaging and alpha-actinin expression, highlighting the potential of this approach for cardiac regeneration. Additionally, we assessed how clinical biological parameters influence MSC proliferation. A strong correlation was observed between lymphocyte count and MSC proliferation, suggesting that the immune microenvironment significantly affects MSC growth. Ischemic disease moderately impacted MSC proliferation, while other factors such as atherosclerosis, hypertension, and age showed no significant effect. These findings emphasize the dual importance of optimizing both the differentiation conditions and the immune context when developing MSC-based therapies for cardiac repair, which will further develop the efficacy of MSC-based regenerative strategies in clinical settings.

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Cross-reactivity analysis of dominant T cell epitopes of *Bordetella pertussis*

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Whooping cough is a serious illness affecting young children. There are two main types of vaccines for whooping cough: cellular and acellular. Research indicates that the rise in cases of pertussis infection is due to the shift towards acellular vaccines [1]. Thus, it is crucial to estimate the immune response to *B. pertussis* based on the type of vaccine to uncover factors that affect its effectiveness.

T cells are vital for the immune response, yet there is limited information regarding the systemic T cell response to *B. pertussis* in humans [2]. This study demonstrates that memory T cells react to components of the *B. pertussis* bacterium, and the strength of this response is independent of the vaccine type. In most donors, the T cell response to the pertussis component was more robust than to pertussis toxin (PTx), a key ingredient in acellular vaccines. This indicates the existence of dominant epitopes beyond PTx that elicit an immune response.

Additionally, a bioinformatic analysis was conducted on the epitopes of various *B. pertussis* proteins, predicting their dominance based on HLA alleles and assessing the potential for cross-reactive T cell responses to other *Bordetella* species and human microbiota.

These findings could aid in the development of new vaccines against *B. pertussis* or in modifying current vaccines to enhance their efficiency and minimize side effects.

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Cryo-EM structures of the light-harvesting complexes from the purple bacterium *Ectothiorhodospira haloalkaliphila*

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The main structural components of the photosynthetic apparatus of bacteria are light-harvesting complexes (LH1 and LH2) and photochemically active reaction center (RC). In contrast to non-sulfur purple bacteria, there are only one LH2 [1] and four LH1-RC structures [2-5] for sulfur bacteria. In the present work, structures of LH2 and LH1-RC complexes from the purple sulfur bacterium E. haloalkaliphila were obtained by cryo-electron microscopy at 1.7 Å and 2.5 Å resolution, respectively. LH2 is an octamer, each subunit containing α- and β-polypeptides, three BChl and one carotenoid molecules. The LH1 complex from E. haloalkaliphila consists of 16 identical subunits arranged in a similar manner to LH2. RC is composed of cytochrome c and three polypeptide chains that bind various cofactors. Comparative analysis of the obtained structural data was done.

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Design of the helicase of the novel Haseki tick virus and its structural and functional analysis in AlphaFold 3

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Haseki tick virus (HSTV) is a novel tick-borne virus, that was first detected in patient after a tick bite in the Russian Federation in 2020. Currently, there is no information about HSTV proteins, thus making it challenging to study the HSTV biological properties.

In this study, AlphaFold 3 was used to identify of HSTV helicase protein (HasHel) in polyprotein and determine of their structure and functions.

We obtained the HasHel location in the genome (5661-7131 bp). The HasHel size is 490 a.a. The HasHel topology structure is similar to helicases of *Flaviviridae* family viruses by more than 60%. TM-score between HasHel and helicase of *Hepacivirus hominis* (PDB ID: 1HEI) is 0.67. Furthermore, the modelling results show that HasHel consists of three globular domains (D1, D2 and D3) that are similar in size. The HasHel D1 and D3 are located head-to-tail like helicases of *Flaviviridae* family viruses. The HasHel D1 contains the conserved motifs Walker A (I) and Walker B (II). These motifs are associated with ATP hydrolysis. Motif I forms a loop that allows binding of the β-phosphate of a ATP. Motif III acts as an ATP-binding domain and coordinates with motifs I, II, VI and D1-D2 to create an RNA-binding pocket. HasHel D2 has motif IV (QRRGRGRxGR), that is involved in ATP processing. Motif VI is an arginine finger that stabilises interactions in the ATPase active site. HasHel D3 is highly variable within the *Flaviviridae* family and consists predominantly of α-helices. In addition, D2 contains a β-hairpin (β14-β16) that is structurally conserved in members of the *Flaviviridae* family and interacts with D3. D3 together with the conserved D2 β-hairpin plays a key role in viral nucleic acids unwinding.

This work discovered the Haseki tick virus helicase and proposed a model of its structure. The results show that HSTV is similar to *Flaviviridae* members, confirming their possible evolutionary relationship.

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Design, synthesis and biological evaluation of colchicine-based lipid prodrugs as potential treatment of oncological diseases and liver fibrosis

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Natural alkaloid (*aR*,7*S*)-colchicine represents strong antimitotic and anti-inflammatory activity. It reversibly binds to β-tubulin that prevents microtubule assembly and furter leads to disruption of microtubule equilibrium, intracellular transport and chemotaxis blockage, cell cycle arrest and apoptosis[1]. An application of colchicine in medicine in their parent form is limited due to severe side effects and unpredictable general toxicity[2]. Therefore, the development of prodrug form of colchicine and its congeners becomes a significant issue.

Here we describe synthesis, biophysical and biological properties of colchicinoid-lipid conjugates and therapeutic liposomes bearing abovementioned colchicine derivatives[3–5]. The potent biomedical application of these liposomes for oncology and liver fibrosis therapy is discussed.

This research was supported by the federal academic leadership program "Priority 2030" of the Ministry of Science and Higher education of the Russian Federation.

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Development and characterization of a new cellular model of Huntington's disease based on direct reprogramming techniques

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Huntington's disease (HD) is an inherited incurable neurodegenerative disorder that predominantly affects striatal neurons. In the present study, we successfully applied an optimized *in vitro* model of HD based on direct reprogramming of dermal fibroblasts from patients with this neuropathology into induced striatal neurons. We showed that striatal neurons obtained by direct reprogramming of dermal fibroblasts from HD patients (HD iSN) contain mutant huntingtin aggregates, but the number of cells containing aggregates is less than 5% of the total number. We also showed that the level of mitochondrial membrane potential detectable by TMRM in HD iSN lower than in induced striatal neurons obtained from healthy donor fibroblasts (HC iSN). We also showed that HD iSN has a depleted dendritic tree compared to HC iSN. In particular, a decrease in the number of primary dendrites, their branching, the total number of processes, and the total length of dendrites is observed. HD iSNs are also characterized by more pronounced cell death upon removal of neurotrophic factors from the culture medium and are more vulnerable to the toxic effect of glutamate. Thus, the developed cellular model reflects the characteristic pathological phenotype of HD at the cellular level and can be used for personalized replication of therapeutic agents.

Development of a cell-based system for searching and studying ligands of the calcium-activated potassium channel KCa3.1

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Potassium channels play an important role in pulmonary physiology, functioning of the nervous, immune and cardiovascular systems. The family of calcium-activated potassium channels is involved in the regulation of cell potential and in membrane transport of potassium and calcium. A representative of this family, the KCa3.1 intermediate-conductance channel, is a potential-independent K^+ channel that mediates Ca^{2+}-induced membrane hyperpolarisation. The development of a cell system, in which fluorescently labelled KCa3.1 channels are expressed, is of great interest for studying KCa3.1 channels and searching for high-affinity peptide blockers of this channel.

To create such a cell-based system, we designed and produced plasmids encoding the α-subunit of the KCa3.1 channel fused at the C- or N-terminus to the fluorescent protein mKate2, and obtained fluorescent ligands of this channel in a recombinant expression system in *E.coli* cells.

The study of the KCa3.1 channel was performed using Neuro2a cells transfected with the obtained plasmid. We report on the features of the channel expression, as well as its localization in the plasma membrane and in cellular organelles: lysosomes, mitochondria, endoplasmic reticulum and Golgi apparatus. Results of the studies of the properties of the created fluorescent ligands are presented.

Development of new protocols for chemical transdifferentiation of fibroblasts into cardiomyocytes using bioinformatics prediction of signaling pathways

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Electrical waves are transmitted in cardiac tissue by excitable cells – cardiomyocytes. Excessive fibroblast content can interfere with the propagation of electrical signals. The presented work is devoted to the development of protocols for the chemical transdifferentiation of fibroblasts into cardiomyocytes [1-4]. To solve this problem, rat and mouse embryonic fibroblasts, neonatal rat fibroblasts and immortalized human lung fibroblasts were used in the work. A control transdifferentiation protocol [5] was established for mouse embryonic fibroblasts and transferred to rat embryonic fibroblasts. Four different protocols for transdifferentiation of rat embryonic fibroblasts were performed with different concentrations of the molecular cocktail based on CHIR as well as growth factors. The resulting cardiomyocytes analyzed from the point of view of functional and electrophysiological maturity. This work represents a new potential application of regenerative medicine in the field of cardiovascular disease.

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Development of optogenetic tools for cell physiology research

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Optogenetics is based on the expression of light-sensitive proteins in specific cell populations, which allows the use of light to control cell physiology [1]. For a long time, optogenetics focused on the light control of brain function through the expression of rhodopsins on the plasma membrane surface of neurons [2]. In recent years, a new direction has been developing in the field of optogenetics. It is based on the delivery of rhodopsins into cell organelles.

This work demonstrates the use of optogenetic tools developed by us to apply them to neuronal cultures. Using fluorescence microscopy, we evaluated protein delivery into cellular compartments and demonstrated some physiological effects.

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Dimerization of the ATP-binding protein EttA from the pathogenic bacterium *Staphylococcus aureus*.

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Staphylococcus aureus remains one of the main pathogenic bacteria with multiple resistance to a wide class of antibiotics. This bacterium can cause complex skin infections, bloodstream infections, pneumonia and lead to many other negative consequences [1]. One of the most effective mechanisms for protecting bacterial cells in response to stress is the transfer of the protein synthesis apparatus to an inactivated state, which is called ribosome hibernation.

The EttA (Energy-dependent translational throttle protein) is the most common eubacterial protein ABC-F, which blocks the entry of the ribosome into the translation elongation cycle through a nucleotide-dependent interaction sensitive to the ATP/ADP ratio. The EttA is able to regulate protein synthesis in energy-depleted cells that are having a low ATP/ADP ratio [2]. It has been suggested that the dimer of EttA may be capable of co-binding four ATP molecules, although experimental evidence for such co-binding has not yet been obtained. This dimer may represent an inactive form that buffers the active monomer pool at high EttA concentrations, but further investigation will be required to understand the significance of the dimer [2].

In this work, we have demonstrated the dimerization mechanism of EttA from the pathogenic bacterium *S. aureus*. The dimerization was analyzed by dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS). We showed that the EttA dimer is stable and does not degrade over time. We also obtained a model of the EttA dimer using SAXS methods.

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DNA-mimic ArdA protein imitates a specific DNA site

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ArdA is a DNA mimic protein, whose antirestriction activity is due to competitive inhibition of type I restriction-modification complex (RMI) [1]. In this work we tested the antirestriction activity of three structurally similar, but not identical ArdA proteins against three RMI systems. It was shown that despite the similarity of predicted structures of the studied ArdA proteins, they have significant specificity for three RMI systems [2]. The results obtained may indicate the ability of DNA-mimetics to imitate specific DNA sites. In the present study, we also confirmed the antirestriction function of the *ardA* gene from the *Bifidobacterium bifidum* chromosome. Transcriptome analysis in *Escherichia coli* showed that the range of regulated genes varies significantly for *ardA* from conjugative plasmid pKM101 and from the *B. bifidum* chromosome. Moreover, if the targets for both *ardA* genes match, they often show an opposite effect on regulated gene expression[3]. Compiling our results, we can assume the use of DNA mimic proteins in targeted therapy.

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Dynamics of the photochromic reactions of *Halobacterium salinarum* bacteriorhodopsin on femto- and picosecond time scales

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Bacteriorhodopsin is a transmembrane protein of the halophilic archaeon *Halobacterium salinarum*, the first to be discovered [1] and the best-studied type 1 (microbial) rhodopsin, acting as a light-dependent proton pump. Bacteriorhodopsin function is based on the photochemical isomerization reaction of the chromophore group, the retinal protonated Schiff base [2]. Rhodopsins are known to have photochromic properties [3]. The ultrafast dynamics of the reverse photoreaction of bacteriorhodopsin was studied using femtosecond laser absorption spectroscopy in comparison with the forward photoreaction. The reverse photoreaction was initiated by photoexcitation of the product K_{590} at an early stage of its formation. It has been demonstrated that conversion of the excited K_{590} to the ground state proceeds at times of 0.19, 1.1 and 16 ps with the relative contributions of ~20/60/20, respectively. All these decay channels lead to the formation of the initial state of bacteriorhodopsin as a product with a quantum yield of ~1. Probably, the heterogeneity of the excited state of K_{590} is determined by the heterogeneity of its chromophore center. The forward photoreaction includes two components – 0.52 and 3.5 ps, with the relative contributions of 96/4, respectively. The reverse photoreaction initiated from K_{590} proceeds more efficiently in the conical intersection region, but overall at a lower rate compared to the forward photoreaction, due to significant heterogeneity of the potential energy surface.

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Effect of chemical activation of Piezo1 channels and electrical stimulation on the intracellular anabolic signaling in cultured myotubes

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Mechanically activated Piezo1 channels have recently emerged as key membrane mechanosensors involved in the regulation of postnatal myogenesis, muscle regeneration after injury and immobilization-induced atrophy in skeletal muscle cells/fibers. However, the role of Piezo1 in the propagation of mechanical stimuli to the intracellular anabolic processes is not well understood. The aim of the study was to find out if chemical activation of Piezo1 channels during electrical pulse stimulation (EPS), an in vitro exercise model, is able to enhance anabolic signaling in cultured murine myotubes. C2C12 myotubes were subjected to EPS alone (45 Hz, 21 V, 2 ms for 3h) or EPS in combination with Yoda1 (a selective activator of Piezo1) or Yoda1+gadolinium (a non-specific inhibitor of mechanosensitive channels). Myotubes were collected for WB or RT-PCR analysis 4h after completion of EPS. Thirty minutes before collection, myotubes were incubated with puromycin to determine the rate of protein synthesis using a SUnSET method. EPS alone elicited a small but statistically significant increase in phosphorylation levels of protein kinase B (Ser473), p70S6K (Thr389) and rpS6 (Ser 240/244) and the rate of protein synthesis compared to non-stimulated myotubes. Phosphorylation levels of GSK-3beta (Ser9) and nNOS (Ser1417) also tended to increase in response to EPS alone. We also observed a significant increase in c-Myc and 45S pre-rRNA gene expression following EPS. Incubation of myotubes with Yoda1 during EPS resulted in accelerated protein synthesis rates and enhanced phosphorylation status of protein kinase B (Ser473), p70S6K (Thr389), GSK-3beta (Ser9) and nNOS (Ser1417). Moreover, Yoda1+EPS induced a significant decrease in elongation factor 2 (eEF2) Thr56 phosphorylation but did not affect c-Myc and 45S pre-rRNA expression in comparison with EPS alone. Incubation of myotubes with Yoda1+gadolinium during EPS abolished the Yoda1+EPS-induced effects on phosphorylation levels of p70S6K (Thr389), GSK-3beta (Ser9), nNOS (Ser1417), eEF2 (Thr56) and the rates of protein synthesis but did not affect protein kinase B (Ser473) phosphorylation as well as c-Myc and 45S pre-rRNA expression. In conclusion, our findings suggest that Yoda1-induced activation of Piezo1 channels during EPS-induced contractions significantly enhances anabolic signaling and the rate of protein synthesis in C2C12 myotubes. The study was funded by the Russian Science Foundation (RSF) grant # 22-75-10046.

Effect of DHPR inhibition on energy and Ca^{2+} metabolism of rat *m. soleus* during unloading

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Dihydropyridine receptors (DHPR) participate in changes of the membrane potential during muscle unloading [1, 2, 3]. We used nifedipine, a dihydropyridine derivative and Ca^{2+} antagonist to study the role of L-type Ca^{2+} channels (Cav1.1, DHPR) in Ca^{2+} and energy metabolism of *musculus soleus* during unloading. Male Wistar rats were divided into 3 groups (n=8 in each): vivarium control with placebo (C), 3-day hindlimb suspension with placebo (HS) and 3-day hindlimb suspension with intraperitoneal administration of the nifedipine (N). It was found that the nifedipine administration during himdlimb suspension prevents: 1) the ATP accumulation; 2) the reduction of the maximum force of a single contraction and the time of contraction; 3) the increase in the content of intramitochondrial and myoplasmic calcium. Thus, DHPR participates in the energy and Ca^{2+} metabolism and affects the functional properties of *m. soleus* during unloading.

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Effect of respirasome destruction on slowing down the rate of oxidized cytochrome C reduction by the III respiratory complex in Brownian dynamics model

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Cytochrome C (Cc) is crucial in electron transports between III and IV respiratory complexes. Individual complexes can be organized into large assemblies, respiratory supercomplexes (SCs). The physiological roles of SCs remain controversial. Cristae organization is disturbed in many diseases and aging. It was believed that supercomplex assembly is important in Cc diffusion between the respiratory complexes and in electron transfer efficiency [1]. There are debates on whether SCs confer catalytic advantages compared with the non-bound individual complexes [2].

To study the role of SCs in mitochondrial electron transfer, we used the Brownian dynamics simulation in ProKSim software [3]. We designed model of oxidized Cc molecule (pdb ID 3O1Y) diffusion and binding with reduced dimeric III complex (PDB ID 1BGY) in presence of reduced IV respiratory complex (pdb ID 6JY4). The respiratory complexes were as in respirasome 5LUF on the surface simulating the membrane. The second surface of cristae at 12 nm limited Cc diffusion. Initial position of Cc was as in 5IY5 complex.

We obtained 2-exponential kinetics of Cc reduction by III2. With a membrane width 160 nm, time of the slow phase was 129 mks. This time exceeds the time of electron transport in Cc-III2, Cc-IV complexes and and may limit the rate of electron transport in mitochondria. With increasing distance between respiratory complexes impact of slow faze increased. We got limitation of mitochondrial electron transport in Brownian Dynamics model at III2, IV concentration less than 5.4 mkM.

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Effects of dopamine and tyramine on electrical activity of trigeminal nerve afferents in DAT-KO (dopamine trasporter-knock out) rats

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Migraine is a common neurological disorder characterized by severe headaches. The trigeminal-vascular system, containing nociceptive fibers that innervate dural vessels, is thought to be a key source of pain signals. Endogenous molecules like ATP and serotonin play roles in this nociceptive signaling. The DAT-KO (dopamine transporter-knock out) rat model is used for studying dopaminergic disorders but has not been explored in the context of headaches. Trace amines, which act as endogenous ligands for TAAR, interact with dopamine, but their role in migraine pathogenesis remains unclear.

Methods: The electrical activity of trigeminal ganglion (TG) afferents in the dura mater was recorded using isolated rat hemi-skull preparations (male rats, P 40-45, Wistar and DAT-KO groups). Tyramine (tyr, 10 μM) and dopamine (DA, 100 μM) were applied to assess their influence on electrical activity. Data presented as action potentials (APs) per 5 minutes±SE.

Results: In the control group, DA significantly increased AP frequency from 149.5 ± 27 to 217.1 ± 43.9 APs (n=12, p=0.03) after 20 minutes of application. In DAT-KO rats, DA also raised APs frequency from 321.5 ± 70 to 354.4 ± 78.3 APs (n=6, p=0.036) after 15 minutes, but not significantly after 20 minutes (368 ± 64.8 APs, n=6, p=0.059). Comparison between two series showed significant difference in DA effect between groups, after 15 minutes (n=8, p=0.007) and 20 minutes of application (n=10, p=0.01). Tyr significantly increased AP frequency after 5-minute application similarly in both groups (in control group: from 458.2±130.2 APs up to 520.8±130.2 (n=10, p=0.01); DAT-KO group: from 529.1±106.5 up to 682±107.1 APs (n=10, p=0.009)).

Conclusion: These findings enhance understanding of the molecular mechanisms involving trace amines and dopamine in migraine pathophysiology, highlighting differences in dopamine's effects between control and DAT-KO groups, while showing no significant difference in tyramine effects. Further research is needed to explore the interplay between trace amines and dopaminergic signaling.

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Endogenous ANO6 channels are activated downstream to calcium store depletion in HEK293T cells.

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Proteins of anoctamines (TMEM16) family form chloride calcium activated channels and scramblases. ANO6 combines properties of ion channel and of calcium activated phospholipid scramblase on cellular membrane. ANO6 channels are almost ubiquitously present in various cell types, where they participate in numerous cellular processes, including setting membrane potential, exocytosis, regulation of fluid transport and secretion, cell volume regulation and etc. Their activation depends on intracellular calcium elevation [1]

Store-operated calcium entry, which depends on STIM calcium sensors and Orai channels is one of the main pathways of calcium entry in non-excitable cells [2]. Its implication in regulation of endogenous ANO6 channels activity in non-excitable cells is studied insufficiently.

We have recorded endogenous store-operated calcium currents and endogenous calcium activated chloride currents in HEK293T cells in whole cell, cell attached and inside-out patch clamp experiments. We have shown that passive store depletion with SERCA inhibitor and direct activation with recombinant STIM1 fragments induces ion flux through endogenous ANO6 channels in HEK293 cells. Experiments with disruption of store-operated calcium entry showed that endogenous ANO6 activity in HEK293T cells depends on calcium entry through Orai channels.

Overall, our data provide new insight into ANO6 and Orai endogenous channels interplay in the calcium entry pathway in non-excitable cells.

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Enhancing i-motif pH Sensors: how labeling patterns affect intracellular localization

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Intercalated DNA motifs (iMs) provide a convenient scaffold for the design of biocompatible pH sensors. Among unimolecular iM-based sensors, only those labeled with conventional fluorophores or fluorophore/quencher pairs at the 3' and 5' termini for FRET/quenching upon pH-dependent iM folding have been tested in cells and tended to accumulate in the nuclei. Here, we used cytosine mimics as internal iM labels and synthesized a new phenoxazine-based non-fluorescent nucleoside analog, $tC°_{Azo}$, which quenches the fluorescence of a known cyto- sine mimic, tC°. Incorporation of the tC°/$tC°_{Azo}$ pair into a genomic iM-forming sequence CST resulted in a high-contrast pH sensor with an increased pH transition point and a working range compatible with physiological conditions. Importantly, unlike the known nuclei-specific C5T-based sensors with conventional labels that provide a fluorescent signal in the green/red channels, the new sensor localized mainly in the cytoplasm and allowed pH monitoring based on the tC° signal in the blue channel. As the labeling scheme was the only unique feature of the new sensor, it must account for the unique distribution pattern, i.e., the accumulation of the sensor in the cytoplasm. These findings highlight the importance of the labeling scheme of unimolecular iM-based pH sensors and open the way for multiplexed pH monitoring in different cellular compartments.

Evaluation of the activation level of neutrophils using atomic force microscopy

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Atomic force microscopy (AFM) is a technique that enables the visualization of surfaces at the nanoscale level. Beyond its primary use for imaging, AFM can assess the mechanical properties of samples, making it especially useful for studying biological specimens. The advancement of AFM modes has demonstrated that the AFM can also be utilized to study the complex biological objects such as DNA, RNA, biomembranes, proteins, and even cells [1].

Neutrophils are the most prevalent type of white blood cell and play a vital role in the human body's natural immune defense. Their activation, often triggered by reactive oxygen species at infection sites, is crucial for an effective immune defense [2]. Understanding the extent of neutrophil activation is vital for gaining insights into immune responses.

In this study [3], topographic and adhesion atomic force microscopy maps were analyzed for neutrophils with varying levels of activation. Three data sets were collected, comprising topography, restored, and viscoelastic adhesion images. A set of 37 cell surface parameters was examined for each data type to ascertain whether a correlation existed between the activation time and the cell surface changes. It was demonstrated that AFM adhesion maps can be correlated with alterations in the plasma membrane and the pericellular layer of cells, and thus can be employed to evaluate the level of neutrophil activation.

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Examining Transfer of TERT to Mitochondria under Oxidative Stress

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The protein subunit of the telomerase, TERT, has been shown to have multiple functions in the cell. In particular, when the cell is affected by oxidative stress, TERT is redistributed from the nucleus to the mitochondria, where it protects mitochondrial DNA from damage [1-3]. However, the exact redistribution mechanism remains unclear: is mature TERT transported to the mitochondria, presumably going through a refolding procedure to pass through the TIM/TOM complex on the mitochondrial membranes? Or is it synthesized *de novo*, with the transport regulation mechanism signaling the intermembrane import on the nascent protein stage?

The main challenge for answering this question comes from the fact that labeling the TERT protein with a usual fluorescent GFP fluorescent protein interferes with the mitochondrial transmembrane transport system. We proved that a SNAP-tag label can be transported through the mitochondrial membrane in its mature state, and employed this it to track TERT in living cells. Our results demonstrate the absence of mature TERT transport into mitochondria under oxidative stress.

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Exploring the dynamics of eu- and prokaryotic microtubules through molecular simulations

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Microtubules perform various functions in living organisms, ranging from maintaining cell structure and forming pathways for intracellular transport to capturing and moving chromosomes during cell division. The complex dynamic behavior of microtubules, which enables their multifunctionality, has been the focus of intense study for many years. However, much remains unclear about the specific mechanisms driving this behavior, often referred to as "dynamic instability." Microtubules can stochastically switch between phases of slow growth and rapid depolymerization. Eukaryotic microtubules typically consist of 13 protofilaments, and it was long believed that only eukaryotic organisms possessed the hollow cylindrical structures formed by heterodimers of globular tubulin proteins from the α and β families, which have GTPase activity. Recently, studies [1,2] have shown that bacterial tubulins BtubA and BtubB, found in *Prosthecobacter* bacteria, can form structures similar to eukaryotic microtubules. These structures exhibit dynamic behavior characteristic of eukaryotic microtubules and demonstrate dynamic instability *in vitro*. However, these bacterial microtubules are significantly smaller in size, consisting of only 4-5 protofilaments, which makes them convenient model systems for molecular dynamics studies. Using previously developed approaches [3,4], we examine bacterial microtubules as a new minimal system exhibiting dynamic instability. Due to their small size, entire bacterial mini-microtubules can be simulated, which may provide important insights into how the molecular properties of tubulins contribute to spontaneous assembly and disassembly.

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Expression and purification of DML family DNA glycosylases from Staphylococcus aureus

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Staphylococcus aureus is a Gram-positive, spherical-shaped bacteria that causes a large number of hospital-acquired and nosocomial infections and is highly resistant to most antibiotics and antiseptics [1]. Understanding the principles of bacterial DNA function will allow us to understand the process of pathogen adaptation when external conditions change.

Environmental factors can have an effect not only on DNA structure, but also on changes in gene expression that don't affect DNA structure. It is an epigenetic regulation with DNA methylation/demethylation as a prime example. DNA methylation involves the addition of a methyl group to cytosine in CpG dinucleotides to form 5-methylcytosine (5mC). Methylated CpG dinucleotides are chemically stable, but the methylation pattern can change over the lifetime of an individual. Similarly, DNA demethylation is a programmed process at certain stages of organismal development, which can also occur spontaneously in response to environmental factors, leading to abnormalities in the genome [2]. The initiators of the DNA demethylation process are specific enzymes - proteins of the DML family, which in *S. aureus* includes AtDML1, AtDML2, AtDML3 and AtDME.

In this work, we have developed and optimized protocols for the expression and purification of DML family proteins for further structural studies. This will allow us to understand the role of these enzyme proteins in the process of bacterial genome regulation and their influence on the activation or repression of specific DNA regions.

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Expression and stabilization of recombinant human C3aR receptor for structural studies

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G protein-coupled receptors (GPCRs) are the main targets for a significant portion of modern pharmaceuticals [1], making them important objects of research. C3aR receptor is a GPCR expressed in most tissues of the human body. The natural ligand of the receptor is anaphylatoxin C3a, which is formed in the cascade of proteolytic reactions of the complement system. Recent studies have shown the therapeutic effect of C3aR inhibition in animal models of neuromyelitis optica, Alzheimer's disease, systemic lupus erythematosus, and others. This makes the receptor a promising target for the development of drug compounds. A structure in the inactive state of the receptor will complement the existing knowledge [2,3] about the mechanisms of receptor functioning and will increase the potential for the rational development of therapeutic agents for this target.

In the present work, various approaches were used to obtain a purified and stable preparation of the C3aR receptor, including the introduction of point mutations and partner proteins. The monomeric receptor C3aR-BRIL suitable for further structural and functional studies expressed in Sf9 insect cell line was obtained in detergent micelles with the yield of 0.6 mg per 1 liter of cell culture. The introduction of BRIL into the receptor sequence makes it possible to perform both further X-ray structural analysis of the receptor due to an increased probability of crystallization contacts during crystal formation and Cryo-EM of the complex C3aR-BRIL with fab fragment Bag2 [4] and anti-Fab nanobody.

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Expression of farnesylated GFP in primary cultured cortex neurons leads to impaired spine development

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Genetically encoded fluorescent proteins are a widely used tool in a variety of biological studies, including neurobiological experiments. When using these tools, it is important that the expression of a fluorescent protein does not interfere with naturally occurring physiological processes. The addition of a farnesylation motif to fluorescent proteins results in their anchoring to the plasma membrane, which is often used to visualize specific membrane structures, such as neuronal spines. In our work, we examined the development of spines in primary cultured cortex neurons when cells were transfected with farnesylated and unmodified GFP constructs by electroporation in suspension on the day of seeding. It was found that neurons expressing farnesylated GFP showed pronounced abnormalities in spine development, in particular these cells were characterised by longer spines with more phyllopodia-like structures, which is characteristic of various pathological conditions. Therefore, when using farnesylated fluorescent proteins in experiments, it is necessary to take into account their possible negative effect on the development of various membrane structures of the cell, in particular neuronal spines.

Expression of Kv1.2 potassium channels in eukaryotic cells and study of their interactions with peptide pore blockers.

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Voltage-gated potassium Kv1.2 channels are expressed widely in both the central and peripheral nervous systems. They are involved in mediating the D-type (delay) current, which is a critical controller of neuronal excitability. Some mutations in Kv1.2 channel are linked to epilepsy and severe intellectual disability [1]. Peptide toxins from scorpion venom are able to bind specifically and with high affinity to Kv1.2 channels, suppressing channel conductance, that makes them promising pharmacological agents.

In this work three expression vectors were designed that encode the Kv1.2 channel and its two mutant forms possessing low and enhanced expression on the plasma membrane of Neuro2a cells, respectively. All these variants of channels were fused with fluorescent protein mKate2. Confocal laser scanning microscopy analysis using selective fluorescent labels of cellular organelles showed that wild-type and mutant Kv1.2 channels were predominantly localized in the endoplasmic reticulum and trans-Golgi cisternae. The small number of endosomes and lysosomes containing the studied Kv1.2 variants allows us to conclude that Kv1.2 channels are rarely endocytosed from the plasma membrane and their recirculation is limited. We have not detected localization of Kv1.2 channels in mitochondria.

It has been shown that the analytical system based on Neuro2a cells expressing mKate2-Kv1.2 channels in the membrane and the fluorescent ligand HgTx-L3-GFP is applicable for detecting Kv1.2 channel blockers among peptides of natural and artificial origin. Using the method of competitive binding, the dissociation constants Ki of complexes of the Kv1.2 channel with unlabeled peptide blockers, representatives of various families of toxins from scorpion venom were measured.

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Expression of T-cadherin in adipocyte differentiation in mouse 3T3-L1 preadipocytes

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Adipose tissue is a special type of connective tissue that comprises stromal-vascular cells, committed preadipocytes, and mature adipocytes. It is particularly important to study the mechanisms of adipose tissue cell renewal, induction and regulation of stem/progenitor cell differentiation into mature adipocytes [1]. T-cadherin is a non-canonical representative of cadherins and a receptor for the adipose tissue hormone adiponectin, which is secreted by mature adipocytes. The role of T-cadherin in adipose tissue turnover and function is unknown. This investigation shows the content of T-cadherin by immunoblotting upon adipogenic induction at different stagesw using a 3T3-L1 mouse preadipocyte culture model. The level of T-cadherin changes during the induction of adipogenic differentiation and depends on the differentiation stage (4-7 days): the protein level in 3T3-L1 cells in the adipogenic medium was significantly higher throughout the experiment than in the cells in the control medium, reaching a maximum on the 10th day. The T-cadherin content in the cells in the control medium remained virtually unchanged throughout the study. The obtained data indicate the possible participation of T-cadherin in the processes of adipogenic differentiation both at early and later stages. Supported by the Russian Science Foundation grant no. 23-11-00205.

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Expression temperature influence on adenosine phosphate-bound state of recombinant *Bs*DesK

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Temperature sensing is essential for the survival of living cells. Temperature decrease induces transition of membrane lipids into rigid gel-phase, resulting in unfavorably reduced motility of proteins, lipids, and other constituents within the cell membrane. *Bacillus subtilis* employs DesKR two-component signaling system to respond to cold temperatures. During cold shock histidine kinase DesK adopts active conformation due to increased membrane thickness [1, 2]. DesK then phosphorylates its cognate response regulator, DesR, which acts as a transcription factor of the *des* gene, coding membrane-bound fatty acid Δ5-desaturase.

To phosphorylate its response regulator, DesK utilizes γ-phospate from ATP, bound to ATP-binding domain within the cytoplasmic catalytic core. Thus, the ability to bind ATP is crucial in retaining sensory function and can serve as evaluation of recombinant DesK functionality. We previously observed that a fraction of purified recombinant *Bs*DesK, expressed in *E. coli*, demonstrated spectral properties corresponding to ATP/ADP-bound state [3, 4]. Here we investigated the influence of lowering expression temperature on ATP/ADP binding properties of *Bs*DesK. Our results show that colder expression conditions result in higher yields of ATP/ADP-bound *Bs*DesK.

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Extraction, purification and small angle X-ray scattering analysis of the Era GTPase from *Staphylococcus aureus*

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Ribosome biogenesis is a fundamental and multistage process. It involves the assembly of ribosomal RNA and ribosomal proteins in a highly coordinated way to create functional ribosomes. In this process, even small defects can lead to erroneous RNA conformations, which appear due to degenerate local interactions. Therefore, the assembly process is regulated by a huge number of protein factors known as ribosome maturation factors. One of these proteins is Era, which is involved the maturation of the 30S subunit of the ribosome.

Era is one of the GTPase superfamily proteins that has been well characterized in both prokaryotes and eukaryotes. The protein consists of two domains, the N-terminal domain of Era is a GTP-binding domain, while the C-terminal domain has a KH domain with αββββαβ topology, which is involved in binding to 16S rRNA and 30S ribosomal subunit in a GTP-dependent manner [1]. The KH domain is also required for binding of Era to inner membrane, whereas presence of rRNA prevents its localization to cytoplasmic membrane [2]. Previous studies have shown that the Era GTPase binds to the pre-16S rRNA resulting in the formation of ERA-GTP-pre-30S. Upon pre-16S rRNA binding, the GTP-hydrolyzing activity of Era is stimulated which signals the final processing of 16S rRNA by RNase E, RNase G, and the unknown RNase. Due to increased GTP hydrolysis, the Era is released from the fully assembles 30S ribosomal subunit which subsequently facilitates the assembly of the functional ribosome [3].

In this work, we developed protocols for cloning, expression and purification of the Era protein domains and obtained structural characterization of these domains by small angle X-ray scattering (SAXS). We also obtained a model of the N-terminal domain of the Era.

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Features of binding of antihistamines and tricyclic antidepressants to the H1-histamine receptor

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H1-histamine receptors (H1R) play an important role in triggering allergic reactions. They are a bundle of seven transmembrane helices coupled with a G protein. This work is devoted to the study of the features of H1R binding to antihistamines of different generations, as well as to representatives of tricyclic antidepressants (TCAs) using computer modeling methods. The structure of H1R in the apo form (PDB code: 8X5X) was used as a protein model [1]. The Autodock VINA 1.1.2 flexible docking package was used in the work. Analysis of the obtained results revealed that all drug molecules were localized in the main hydrophobic and secondary binding pockets on the receptor. The Ebastine molecule had the highest affinity – 9.4 kcal/mol. Analysis of the interaction profile (in the PLIP package) showed that most tricyclic drug molecules are characterized by hydrophobic contacts with the residues TYR431 and ILE454. Tricyclic molecules are a good basis for further structural modifications, which was demonstrated by researchers from China [2]. We also considered a molecule representing a modification of Desloratadine. Elongation of the chlorine-containing fragment, as well as the inclusion of an additional COOH group in it, made it possible to increase the affinity value from –9.0 to –9.9 kcal/mol. Importantly, the interactions characteristic of Desloratadine (hydrophobic interactions with TYR431 and ILE454, as well as hydrogen bonding with GLU181) are retained. The results obtained can be used to develop next-generation antihistamines, as well as to better understand the role of H1R amino acid residues in ligand binding mechanisms.

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Fine regulation of translation initiation by structure of Shine-Dagarno sequence on mRNA

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Translation of genetic information encoded in the DNA sequence into proteins occurs at the translation stage. The initiation stage in the bacterial system is the longest and is controlled by three initiator factors IF1, IF2, IF3 [1]. The only variable link in the entire translation process is mRNA, where the efficiency of the process largely depends on its functional regions. The presence of the Shine-Dalgarno sequence (SD), through which mRNA binds to the 30S subunit of the ribosome, is not a strongly necessary condition for the initiation stage to begin [2]. However, its regulatory function has not yet been fully studied. We present a detailed analysis of the efficiency of the formation of 70S initiation complex (70S IC) depending on changes in the SD sequence, its distance from the start codon, and the presence of secondary structures on mRNA. The affinity of ligands to the 30S ribosome subunit decreased many times (up to 25 times) as a result of a decrease in the length of the SD sequence or its distance from the beginning of the coding region. At the same time, the affinity of mRNA binding increased up to 100 times upon binding to the 70S ribosome under conditions of 70S IC formation and did not depend on the studied changes in mRNA, but depended on the presence of IF3. This fact may indicate additional regulation by 70S scanning initiation [3].

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Flexible String Model of Unsaturated Lipid Bilayer

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An analytically solvable model of unsaturated lipid bilayer is derived [1] by making minimal changes to our previously introduced model of semi-flexible strings with mean-field entropic repulsion between lipid chains in saturated lipid bilayer [2]. With this model, bilayer is represented with a single lipid in a mean-field potential. Mean-field parameter is found self-consistently (via membrane and lipid parameters). Lipid chain order parameter, bilayer lateral pressure profile, and area per lipid is computed for POPC and compared with those for DPPC lipid bilayer. The change in order parameter [3-4] and lateral pressure profile [4] matches qualitatively molecular dynamics studies.

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Freezing and storage conditions optimization of GPCR samples

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The quality, stability, and activity of proteins are highly sensitive to environmental conditions during *in vitro* experiments. In certain instances, purified protein samples must be stored for extended periods or transported to experimental facilities, leading to a common challenge among researchers regarding the optimization of freezing and thawing procedures [1]. Membrane proteins, in particular, exhibit heightened sensitivity and instability in aqueous solutions, necessitating the selection of optimal methods for freezing and storage. This study focuses on optimizing freezing conditions for a representative of the G-protein-coupled receptor (GPCR) family. Given the critical role that GPCRs play in human physiology, they represent a compelling subject for various structural and biochemical investigations [2]. Our findings indicate that GPCR samples can be frozen in a detergent-containing buffer without the need for additional cryoprotectants, such as glycerol. Furthermore, the proteins maintain their stability and ligand-binding capabilities over multiple freeze-thaw cycles.

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Functional studies of archaeal cysteine-less LOV domain

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LOV (Light Oxygen Voltage) domains serve as blue light sensors and can be found in different photoactive proteins from bacteria, archaea and eukaryotes. They can be used as genetically encoded molecular tools for optogenetics and fluorescent microscopy [1]. Canonical mechanism of LOV domains signal transduction includes formation of covalent bond between flavin chromophore and conserved cysteine in the active site of the protein that causes protonation of N5 flavin atom and subsequent structural reorganization [2]. However, natural cysteine-less LOV domains are able to signal via generation of flavin photoreduced forms [3]. In this work we successfully cloned and expressed in *E. coli* the gene of previously uncharacterized cysteine-less LOV domain from thermophilic haloarchaea *Halanaeroarchaeum sulfurireducens* and obtained purified chromophore-loaded protein. Finally, we verified that flavin cofactor of this LOV domain undergoes one electron photoreduction to the neutral semiquinone radical state and described the kinetics of this process.

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Functional tests of ATP synthase activity at different ionic strength

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ATP synthase is a membrane protein complex that convert ADP into ATP. The H^+ gradient on the membrane causes protons to flow through the membrane [1], inducing the rotor c-ring rotation and catalyzing ATP synthesis. It has been shown that the change in ionic strength of the solution can change the oligomeric state of ATP synthase [2], however, this phenomenon was shown in non-native system (protein was purified in detergent micelles), so it is unclear whether this has a physiological role. This research has a goal to investigate the effect of ionic strength on ATP synthase.

We used luciferin-luciferase system to analyze the activity of ATP synthase incorporated in phosphatidylcholine liposomes. The tests were carried out for solutions with different concentrations of different salts. For each condition, a graph of ATP synthesis in solution was obtained, and the synthesis rate was calculated.

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Functionally active sodium channel contains delta-ENaC subunit in human chronic leukemia cells

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ENaC is an epithelial sodium channel that forms heteromer from three well-characterized subunits (α, β, and γ). In humans, an additional δ-ENaC subunit exists in non-epithelial tissues, where it may replace the α-subunit to form δβγ-ENaC with properties different from αβγ-ENaC, including the reduced sensitivity to amiloride and activation in response to increasing intracellular $[cAMP]_i$. Expression of δ-ENaC was found in several malignant cell lines but not in normal correlates. The shift of α-ENaC to δ-ENaC subunit composition may be beneficial for cancer development and have an impact on water-salt balance and coupled processes controlling cell cycle, differentiation and proliferation of malignant cells.

Previously, in human myeloid leukemia K562 cells we described amiloride-insensitive ENaC-like channels, but subunit structure of activated channels was poorly understood. Here, using single-channel whole-cell technique we study the action of δ-ENaC activator capsazepine (CPZ) on sodium channel activity in K562 cells. Addition of CPZ (2 μM) to the extracellular solution caused activation of amiloride-insensitive ENaC-like channels (unitary conductance was 15.1 ± 0.8 pS, n=21). Unitary currents and conductance of CPZ-activated channels were higher in Na^+-containing extracellular solution than in Li^+, which is known as the one of distinctive features of δ-ENaC. RT-PCR analysis and immunofluorescence staining confirmed the expression of δ-hENaC. In addition to extracellular δ-ENaC activation, whole-cell experiments with intracellular δ-ENaC stimulation were performed, where $[cAMP]_i$ was elevated by adding of forskolin (1 μM) to the bath solution. After an application of forskolin an activation of single sodium currents with properties similar to CPZ-activated channels was developed in time-dependent manner in 66% of experiments (n=6); unitary conductance was 15.3 ± 0.2 pS.

These findings allow us to speculate that functionally-active amiloride-insensitive ENaC-like channels in leukemia cells contain δ-ENaC. The used agents could be applied as a pharmacological tool to induce sodium permeability in the plasma membrane of transformed blood cells.

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Generating a stable mouse glioma cell line with knockout of the key damage-associated molecular pattern calreticulin

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Mobilization of damage-associated molecular patterns (DAMPs) is a key factor in developing immunogenic cell death to achieve effective anti-cancer therapy. The extracellular release of DAMPs acts as a danger signal, which also activates immune system cells and forms a durable antitumor response. Developing model systems in vitro is a necessary link in studies of various cell death modalities and the contribution of DAMPs to their immunogenic properties.

Using the CRISPR/Cas9 technique, we aimed to obtain a stable mouse glioma cell line with a gene knockdown for one of the key DAMPs, calreticulin (CRT). We selected, designed, and synthesized constructs to make rearrangements/deletions in exon 2 of the CRT gene. The efficiency of construct assembly and their suitability for guide RNA synthesis were confirmed by PCR and sequencing. Mouse glioma GL261 cell cultures were subjected to lipofection with the obtained constructs with subsequent single-cell sorting of transfected cells using a FACS Aria III cell sorter (BD, USA). The monoclones were cultured in a CO_2 incubator and then taken for further PCR analysis and sequencing. The samples showed the presence of deletions (70 and 1 bp) that resulted in reading frame shift of the CRT gene, changes in the amino acid sequence after the 35th amino acid residue, and termination in the protein translation after 73 or 96 amino acid residues out of the 416 amino acid residues constituting a full-length protein. The absence of full-length CRT protein expression in the obtained GL261bCRT cell line was confirmed by western blot. GL261bCRT cells are currently used to investigate the role of CRT in realizing the immunogenic properties of ferroptotic cell death.

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GPR17 receptor preparation for structural and functional studies

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G protein-coupled receptors (GPCR) are a family that includes approximately 800 membrane proteins with 7 transmembrane helices. GPCRs are involved in a wide variety of processes: vision, taste, smell, embryonic development, etc. Our work is focused on the human GPCR receptor GPR17, which regulates the formation of the myelin sheath in the brain. The receptor is expressed in oligodendrocyte precursor cells just before the formation of myelin. Artificial stable expression of GPR17 in mice leads to breakdowns of myelin formation and symptoms similar to those of multiple sclerosis. In addition, recent studies have shown that GPR17 expressed in the gastrointestinal tract is directly involved in appetite regulation and blood glucose control. All of the above makes GPR17 an attractive target for the development of drugs - selective inhibitors of this receptor.

To develop highly specific drugs with minimal side effects, it is necessary to know the high-resolution structure of the receptor in the inactive conformation. Currently, the high-resolution structure of the ligand-binding pocket of GPR17 and, in particular, the structure of its inactive conformation, is unknown. That is why research aimed at determining high resolution structure of this receptor is of great importance.

In this work, we created a number of genetically engineered receptor constructs for expression in Sf9 insect cells and further purification. A set of point mutations, receptor modifications, as well as the isolation and purification protocol were optimized to obtain the highest protein yield and its best stability. As a result, a monomeric sample of this receptor was obtained in detergent micelles, as well as a complex with a monoclonal antibody to the partner protein (anti-BRIL Bag2) and with a nanobody.

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Heterogeneity of Single Platelet Calcium Responses to Activation in pediatric healthy donors is higher, than in adults

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Background: Platelets are blood cell fragments responsible for blood coagulation and thrombus formation. Platelet population of a single donor is highly heterogeneous in morphology, however, whether platelet responses to activation differ within population or between healthy donors remains unclear.

Aims: We aim to develop a method for assessment of the heterogeneity of platelet responses to activation.

Methods: Whole hirudinated blood of 9 healthy pediatric donors and 18 healthy adult donors was collected in compliance with the Declaration of Helsinki. CalBryte 590 loaded platelets were immobilized in parallel-plate flow chambers with protein-coated cover slips. Two types of coating were used, collagen and neutral coating (anti-CD31). Low-angle fluorescence microscopy was used for characterization of platelet calcium responses to ADP or thrombin.

Results: We distinguished four types of platelet calcium response: “no response” (I), “spiking” (II) with calcium oscillations, “clusters” (III), and “sustained high” (IV) with a sustained high calcium level. The distribution of platelets of healthy donors shifted to response types that indicated higher calcium levels upon activation. Using bootstrap method, we obtained confidence intervals for mean fractions of healthy donors’ platelet populations within the groups. Platelet calcium response profiles of pediatric donors have demonstrated weaker response to activation on neutral coating and on collagen.

Conclusions: The distribution of single platelets between response groups could be utilized as a diagnostic technique to determine the fractions of refractory platelets or hyper- active platelets within the platelet population.

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Heterologous expression of trace amine-associated receptors

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The family of trace amine-associated receptors (TAARs) belongs to a large group of G-protein coupled receptor (GPCR) proteins discovered in 2001. TAAR1 is one of the best-studied members of this family and is a promising target for the treatment of schizophrenia. Other 5 human TAARs are believed to serve as a new class of olfactory receptors. However, there is evidence that these TAARs could also play a role in the CNS and periphery. TAARs are known to be poorly expressed on the membrane in heterologous systems *in vitro*. This is because proteins remain in the endoplasmic reticulum and are subsequently degraded in the proteasome. One reason for poor membrane expression may be the lack of glycosylation at the N-terminus, which has been shown for TAAR1. To increase level of plasma expression, various N-terminal tags of other GPCR were used (rhodopsin, beta2-adrenergic, mGlur5) were tested. Also, we evaluated chaperones (RTP1S, RTP2) and G_{olf}, Ric8b cotransfection commonly used in olfactory receptors deorphanization that are also difficult to express in a heterologous system. Within our project, we employed BRET biosensor to monitor cAMP accumulation and NanoBiT complementation assay for TAAR-G protein coupling. As a result, we found that receptors like TAAR9 were active without tags and chaperones although beta2-adrenergic N-terminal tag allowed more robust signal. TAAR5 induced signaling only with N-terminal rhodopsin tag. On the other hand, TAAR6 activation was present only with chaperones cotransfection. Using this approach, we identified two new TAAR6 ligands. Our data suggest that cells expressing TAARs possess a molecular mechanism that facilitates the proper transport and folding of receptors on the cell surface, but the exact components of this mechanism are yet to be understood.

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Hormonal control of synaptic glutamate receptors density

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Synaptic transmission efficiency is mostly maintained via dynamics of glutamate receptor density in synaptic contact zone and controlled by mediator and non-mediator receptor signaling pathways, including peripheral hormones. This ensures functional and structural synaptic plasticity, which in turn underlies basic function of brain neural networks.

We have conducted theoretical analysis of molecular mechanisms behind glutamate receptor density regulation in the zone of synaptic contacts and its connection with the changes in hippocampal synaptic transmission efficiency influenced by peripheral hormones leptin and insulin [1, 2].

In order to construct the model of intramolecular interactions regulatory network in the dendritic spines of mouse pyramidal hippocampal neurons we used GeneNet technology (ROSPATENT No. 990006 dated 15/02/1999). GeneNet enables graphic representation of protein-protein interaction as well as regulatory network visualization. Using open source databases we performed analysis of signaling pathway crosstalk regulation.

We conducted network analysis in the dendritic spine interactome and outlined indications for cross-talk between the receptors for main transmitter signal and receptors for leptin and insulin. Our analysis allowed us to conclude that leptin increases the efficiency of synaptic transmission via glutamate receptors trafficking from the additional pool of ready inputs into the synaptic contact zone. The insulin signaling pathway is activated in response to presence of damaging factors in the system, for example, reactive oxygen species, and decrease in the excitation of the system, in particular through the removal of glutamate receptors from the synaptic contact zone.

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HSP70-NK and target cell interactions: implication to the anticancer effects

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HSP70 and HSP70-based vaccine constructs are applying for anticancer therapy and NK cell activation was shown to be one of the effects of such therapy [1]. Moreover, HSP70-activated NK cells showed effectiveness in preclinical models of glioblastoma and lung cancer [2]. It is thought that expression of HSP70 on the surface of cancer cells stimulates the cytotoxic activity of NK cells [3]; however, the spatial aspects of HSP70-NK-cancer cell interactions are not completely investigated. Recently, using CLSM we have shown that HSP70 covers the surface of dying NK cells, while live NK cells phagocytized the protein [4]. In the present study, we investigate the cytotoxic effect of NK cells on target tumor cell lines such as breast carcinoma BT20 and pancreatic carcinoma PANC1 in the presence or absence of HSP70 or its C- and N-terminal fragments. We trace the interaction of HSP70 with NK and target cancer cells and demonstrate the implication of these interactions in cytotoxic effectiveness.

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Human hyaluronan synthase 2 computed structure models

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Hyaluronan synthases (HASes) synthesize hyaluronic acid from its monomers, and play an important role in wide range of processes and pathologies, including, but not limited to, inflammation process, stress and longevity, joint and cartilage functioning, white matter disease and cancer. Human HAS-2 is the main HAS isoform in human organism. Up-to-date only two HAS homologs structures have been determined experimentally: *Paramecium bursaria Chlorella virus CZ-2* HAS (Cv-HAS) [1] and *Xenopus laevis* (African clawed frog) HAS-1 [2]. Here we introduce three stable computed structure models of human HAS-2: one based on Cv-HAS; another one based on frog HAS-1; and the third one predicted by AlphaFold, using Cv-HAS as a reference. Using these models, we have investigated the following aspects: the mode of action of both known and novel HAS-2 inhibitors; the impact of known [3][4] HAS-2 posttranslational modifications on native ligands and inhibitors binding affinity; the role of the transmembrane domain in ligand binding. We have also proposed four new molecules – potential drug candidates for HAS-2 inhibition. The computational methods utilized in this work include homology modelling, AlphaFold, molecular dynamics and molecular docking. A part of the obtained computational data was verified experimentally, using such methods as cell survival assay, confocal microscopy, FLIM and RT-PCR.

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Human prostatic stem cell antigen: recombinant production, structure and role in the brain

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Prostate stem cell antigen (PSCA) belongs to the Ly6/uPAR protein family. Increased expression of PSCA was found in the cortex of patients with Alzheimer disease (by ~ 70 %) and in samples from patients with glioblastomas. Presently, role of PSCA in the body is unknown.

Herein, we developed the expression system for bacterial production of PSCA in the form of inclusion bodies with subsequent solubilization and refolding and studied its functional and structural properties. The spatial structure of recombinant PSCA was studied by solution NMR using unlabeled and 13C15N-labeled protein samples. In contrast to predicted by Alpha fold structure, NMR analysis revealed that PSCA structure contains large disordered regions at the tips of the loops I and III and an unusual for Ly6/uPAR proteins gap of the β-sheet formed by the central loop II.

We didn't find correlation between PSCA mRNA expression and survival of patients with metastatic melanoma and glioblastomas, however, increased PSCA expression in glioblastomas relative to normal brain tissue corresponds to better survival prognosis. We studied the PSCA effect on proliferation, migration and invasion of U251 MG glioma and metastatic melanoma mel Kor and mel Si cells derived from patients. It was revealed that recombinant PSCA down-regulates growth and invasion of U251 MG cells, shows no effect on mel Kor cells, and slightly increases growth of mel Si cells. Perhaps, PSCA effect depends on the repertoire of target receptors expressed on the surface of tumor cells.

To understand possible effects from increased PSCA expression in the brain, we tested morphological changes of the primary neurons incubated with PSCA. Significant decreased dendritic spine density was revealed upon incubation with PSCA. Data obtained point on PSCA as an important regulatory protein in human body.

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Humoral and cellular immunogenicity of the genetically detoxified S1 subunit of pertussis toxin

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Genetically detoxified pertussis toxin (gdPT) is an attractive candidate for the development of improved pertussis vaccine based on the fact that it retains most of the structural properties of native pertussis toxin (PT) and protective epitopes, compared to chemically detoxified PT.

Based on previously published data [1,2] we obtained a recombinant detoxified PT subunit without a hydrophobic domain (gd15PtxS1). The gd15PtxS1 protein in inclusion bodies was purified using size exclusion chromatography. To test the immunogenicity of the protein, BALB/c and CD-1 mice were immunized three times with 5, 15 and 25 μg doses at 2-3 week intervals. Anti-PT IgG levels were measured in the mice sera using a commercial ELISA kit and by assessing T-cell response by flow cytometry. Main subpopulations of antigen-specific helper T cells (Th) were evaluated by measuring cytokine secretion (IFNγ, IL-4, IL-17) after stimulation of mice splenocytes with whole inactivated Bordetella pertussis. Antigen-specific cytotoxic T cells were detected as IFNγ -producing cells.

According to ELISA, we observed seroconversion starting from the 2nd immunization with IgG titers reaching a plateau after the 3rd immunization in all groups. A pronounced T-cellular response was detected after three doses of gd15PtxS1, with a shift to Th1 phenotype in the presence of Th2 and Th17 responses. The control mice immunized with whole inactivated B. pertussis (wP) had a more robust shift to Th1/Th17 phenotype, while Th2 and Th17 responses did not differ. Cytotoxic T-cellular response to the antigen was as pronounced as in the wP group.

Our results show that gd15PtxS1 is immunogenic in mice and is a promising vaccine candidate to be assessed in further protectivity studies.

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Impact of single mutations on the properties of Fatty Acid Photodecarboxylase from *Chlorella variabilis*

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Currently, the chemical industry and the exploitation of natural resources have a significant negative impact on the environment, threatening its health. One potential solution to this problem is the development of biotechnological methods that replace fossil resources with renewable ones. Photobiocatalysis is a promising approach in this regard, as it enables environmentally friendly and highly selective transformations. To date, three truly photocatalytic enzymes (excluding those involved in photosynthesis) are known, with fatty acid photodecarboxylases (FAPs) being of particular interest for industrial applications [1]. The fatty acid photodecarboxylase from *Chlorella variabilis* (CvFAP), discovered in 2017, demonstrates activity towards a broad range of substrates without the need to consume additional cofactors. However, limitations such as low thermo- and photo-stability restrict its application and functional activity [2]. Although some progress has been achieved in improving enzyme stability and repurposing it for other substrates [3], there remains potential for further enhancements. In this study five constructs with single mutations in the fatty acid photodecarboxylase from *Chlorella variabilis* (CvFAP) were produced, and their expression levels, thermostability, and optical properties were analyzed in comparison with the wild type.

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In search of visual opsin in two species of gastropods

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Opsins represent a large group of transmembrane proteins that belong to G-protein-coupled proteins family. Being bound to the chromophore, visual opsins represent a light-sensitive chromoprotein.

Nowadays, despite progress in determination of structure and photochemistry of visual opsins in some animal, the overall evolutionary picture remains incomplete. A significant gap is the lack of such information for Gastropoda. Among them the Lissachatina fulica [1] and Pomacea canaliculata [2] are examples of promising model organism for visual opsin studies because of its capability for eye regeneration including light sensitivity. However, data on the visual opsins of these molluscs (their structure, spectroscopic characteristics, etc.) are lacking.

Here we report a possible visual opsins of L. fulica. Eight sequences were considered [3] but only one (Afu005002) was confirmed as candidate for visual opsins after structural (AlphaFold2) and BLAST analyses. In order to evaluate spectroscopic properties, we calculated excitation energy and intensity of the main absorption band using hybrid Quantum Mechanics/Molecular Mechanics methodology. The theoretically predicted position of the main absorption maxima is in excellent agreement with the available experimental data [4]. The structure of Afu005002 show high similarity with the structure of squid rhodopsin (PDB:2ZIY).

Simultaneously, the structural analysis of 10 opsins of P. canaliculata (AlphaFold2) was performed. The resulting structures were compared with the structure of squid rhodopsin using RMSD value, based on which it was found that rhodopsin-like (XP_025114680.1) had the highest similarity of structure with squid protein.

So, Afu005002 and XP_025114680.1 are the most probable visual opsins of L. fulica and P. canaliculata respectively.

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In vivo and *in vitro* study of fuzzy Dsup-DNA complex

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Dsup protein is one of the tardigrade-unique proteins discovered in the most stress-resistant animal, *Ramazzottius varieornatus* [1]. This DNA-protective protein is extremely valuable from fundamental and practical points [2,3]. Within our recent study, the resistance to oxidative stress and ionizing radiation in *Dsup*-expressing complex model organisms were induced and the specific interaction pattern (transcriptional repression) for chromatin and Dsup was established by the transcripromic approach [3]. The combination of techniques (SAXS, CD, bioinformatics) was used to validate intrinsically disordered nature of Dsup and to approve fuzzy properties Dsup-DNA complex [4]. Hypotheticaly, the ability to induce large scale effects in chromatin predisposes the role of Dsup as genome architectural protein and may be important for DNA-protection. To challenge this, we perform genome-wide chromation accesebility analysis (ATAC-seq) of *Dsup*-experessing organisms and accomplish this by the cryo-EM and SAXS study of Dsup-DNA complex *in vitro*.

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Increase in solubility of recombinant mutant diphtheria toxin crm197 in *E. coli* cells

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The CRM197 protein is a non-toxic variant of diphtheria toxin due to a mutation at position 52 of the amino acid sequence. At the same time, CRM197 retains its immunogenic properties and is safe for use as a carrier protein in conjugate vaccines and immunotherapeutic drugs.

During the biosynthesis of the SUMO-CRM197 hybrid protein in *E. coli*, a significant increase in the expression and solubility of the polypeptide was noted. The outer hydrophilic shell and the inner hydrophobic core of the partner protein could act as detergents, which turned out to be useful for the biosynthesis of CRM197 with a transmembrane region. Conditions for obtaining soluble CRM197 with direct expression are known: secretion into the periplasmic space and the use of Origami B or Shuffle strains. An important factor was the correct formation of disulfide bonds, achieved by folding the protein under conditions of elevated redox potential and correcting incorrect disulfides using DsbC isomerase.

First of all, the effect of sorbitol on the solubility of SUMO-CRM197 was studied. For this purpose, the pET9a(+) and pET39b(+) vectors were used, which differ in the presence of the LacI gene in the latter, providing stricter regulation of expression. As expected, soluble SUMO-CRM197 could not be obtained under the experimental conditions, which makes it difficult to draw conclusions about the effect of sorbitol on solubility, since no clear dependence on the expression level of the target protein was observed.

To test the effect of sorbitol on the solubility of SUMO-CRM197, the pCOLDIII plasmid was used with IPTG concentrations of 0.05 and 0.1 mM. In both cases, the protein was produced in a soluble state, but at 0.05 mM the soluble fraction was close to the maximum, and at 0.1 mM the total yield was observed.

The experimental results confirmed the hypothesis: sorbitol did not affect the solubility of SUMO-CRM197, while DsbC was effective. At an IPTG concentration of 0.05 mM under co-expression conditions, it was possible to obtain a completely soluble product with a yield of more than 1.7 g/L (1.4 g/L for CRM197) during batch cultivation in a flask. These results can significantly affect the development of technologies for the industrial production of CRM197.

The influence of heterologous expression conditions on SUMO-CRM197 biosynthesis was carefully analyzed. The use of genetically modified organisms for the production of high-molecular-weight products is a complex challenge in technology development. However, a systematic approach allowed us to obtain CRM197 in the cytoplasm with a yield of more than 1 g/L.

Further experiments are planned to study the factors that contribute to the increase in the solubility of SUMO-CRM197 without co-expression of DsbC in order to improve the manufacturability, cost-effectiveness and reproducibility of this method.

Insulin-like growth factor (IGF-1) changes the rheological properties of blood in cerebral ischemia

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Cerebrovascular accident remains one of the most significant diseases in the last decade. The ability of red blood cells to transport oxygen depends on many factors, including the ability of the membrane to deform. In various diseases, such as diabetes mellitus, cardiovascular and oncological diseases, as well as in conditions of ischemia, there is a violation of the rheological properties of blood. A decrease in the deformability of red blood cells is a significant risk factor for ischemia. Therefore, it is of particular interest to study the rheological properties of blood and factors affecting them in conditions of impaired cerebral circulation, as a likely mechanism for the progression of acute hypoxia of nervous tissue. The deformation properties of erythrocytes in conditions of forebrain ischemia in rats were studied by osmotic gradient ectacytometry. The effect of insulin-like growth factor (IGF-1) on erythrocytes in ischemia was studied. Insulin-like growth factor (IGF-1) is synthesized and secreted by the liver under the action of hypothalamic growth hormone and plays an important role in protecting cells from hypoxia, ischemia and oxidative stress, as well as being a multifunctional regulator of cell proliferation. A sufficient number of studies indicate that IGF-1 protects against endothelial dysfunction, the development of atherosclerotic plaques, metabolic syndrome, clinical instability and ischemic myocardial damage. We have shown that at high shear stress (30 Pa) in ischemia/reperfusion, the deformation properties of erythrocytes decrease. At the same time, the osmotic resistance of red blood cells decreases. The introduction of IGF-1 increases the deformability of erythrocytes to control values, and also improves their osmotic resistance. At a low shear stress (1 Pa), the administration of IGF-1 against the background of ischemia leads to an increase in the water permeability of erythrocyte membranes. Thus, IGF has a positive complex effect on erythrocytes in ischemia: it increases the deformability and osmotic resistance of erythrocytes.

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Investigating Dual Modulatory Role of Mallotoxin on KCNQ1-KCNE Complexes Using Molecular Modeling

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The voltage-gated potassium ion channel KCNQ1 has physiological importance in modulation of electrical excitability in cardiac and epithelial cells. Its activity is modulated by certain proteins or small molecules and its dysfunction may result in arrhythmia, increasing risk of sudden death[1]. Recent research has revealed that Mallotoxin (MTX), a bioactive compound derived from the plant *Mallotus philippensis*, enhances current generated from KCNQ1-KCNE1 complex while exerting a modest inhibitory effect on the KCNQ1-KCNE3 channel[2]. The molecular mechanisms underlying these contrasting effects remain unclear, given the structural similarities between the KCNE1 and KCNE3 subunits in their transmembrane regions. Therefore, we employed homology modeling to reconstruct a structural model of the KCNQ1-KCNE1 complex based on the cryo-EM structure of the KCNQ1-KCNE3 complex (PDB ID: 6V01). Computational analyses of the electrostatic potential landscapes revealed significant differences between the complexes, with the KCNQ1-KCNE3 complex exhibiting a unique region of positive electrostatic potential located centrally within the channel, a feature absent in the KCNQ1-KCNE1 complex. Considering that MTX displays negative charge at physiological pH, we propose that MTX preferentially interacts with this positively charged domain in the KCNQ1-KCNE3 channel, thereby inhibiting ion flow. This hypothesis aligns with analogous findings in the KCNQ4 channel, where linopirdine, a known inhibitor, occupies a central binding site (PDB ID: 7BYN). Moreover, molecular docking simulations suggest that MTX augments the activity of the KCNQ1-KCNE1 complex by stabilizing its open-state structure. These findings clarify the dual modulatory role of MTX in KCNQ1-KCNEs complex and advance therapeutic design for ion channelopathies.

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Investigating the impact of SkQ1 on NADH and ATP pools in *E. coli* using whole-cell biosensors

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Despite the relatively long history of research on SkQ1, its effects on bacterial cells are still not fully understood. It is known that SkQ1 has uncoupling properties [1] and can act as an antibiotic [2]. We investigated the impact of SkQ1 on the NADH and ATP pools in *E. coli* cells using whole-cell biosensors. CKK1 was used at concentrations below the threshold that affects cell viability. It was demonstrated that SkQ1 affects the cell differently than rotenone and CCCP. Based on the reduction in bacterial luciferase luminescence, it can be concluded that the NADH level in the cell decreases. Surprisingly, SkQ1, which was considered a mild uncoupler, increases the luminescence of cells with firefly luciferase, which might indicate that the ATP concentration in the cell rises. One of the possible reasons for that phenomenon is interaction of SkQ1 with ATP synthase, which leads to a stimulation of ATP synthesis and NADH consumption. To investigate in detail the mechanism by which SkQ1 induces such changes in bacterial cells and to identify specific targets, functional assays on purified cellular components, including bacterial ATPase, are required. Methodology for such *in vitro* experiments was developed and tested on another ATP synthase (from chloroplasts). In the tests with chloroplast ATP synthase, SkQ1 demonstrates exclusively uncoupling properties.

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Investigating the quantitative PALM applicability for studies of protein oligomerization in non-ideal conditions

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Membrane protein oligomerization is frequently connected to protein function, being required for signal transduction (in case of GPCRs) or ion transport (in case of microbial rhodopsins) [1, 2]. One of the methods of fluorescent microscopy to investigate the oligomerization state – quantitative photo-activated localization microscopy (qPALM) – involves labeling the target protein with fluorescent protein and exploiting the photocharasterics of this label – number of photoswitches between fluorescent and dark state ("blinking"). After initial localization of fluorescent molecules, the positional information is separated into single-molecule clusters, the number of blinks in each cluster is obtained, and from overall distribution of the number of blinks across all clusters the oligomerization state is calculated [3].

In this work we aim to investigate *in silico* the extent of the applicability of this method in different conditions such as: protein density in the membrane, presence of additional fluorescent particles and median number of blinks per label. Each of these conditions disturb experimental procedure of qPALM, either by limiting cluster separation or by influencing the observed distribution of the number of blinks per cluster. To achieve this, we synthesize several imageseries with set oligomerization state and varying parameters, analyze the imageseries with localization software, obtain the number of blinks in molecular clusters and explore the relationship between the calculated oligomerization parameter and the real one under different simulated conditions.

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Investigation of azobenzene derivatives for their use as photosensitizers

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Various local anesthetics are widely used in medicine. However, most of them have a disadvantage - their effect cannot be controlled. Therefore, the development of local anesthetics with the ability to control their anesthetic activity is an urgent task of modern biophysics.

In this work, the effect of three different azobenzene derivatives on rat cardiomyocyte culture was analyzed. Making changes to the structure of azobenzene makes it possible to synthesize a substance with photo-controlled anesthetic activity [1]. This property of azobenzene derivatives allows for fairly accurate control of their activity.

The study of tissue excitability was performed using the method of optical mapping of excitation waves. Based on the results of the experiments, measurements were made of the propagation velocity of the excitation wave through the culture at various concentrations and exposure time of the studied azobenzene derivatives.

After the addition of substances to the culture, a complete blockage of conduction was observed, after the action of UV radiation, a partial restoration of conductivity was observed. Measurements of the propagation rate of the action potential were carried out within two hours after the addition of the test substance, thus the long-term effect of azobenzene derivatives on cell culture was clarified. The studied substances can be used to carry out optical control of the excitability of cardiac tissue in in vitro tasks of studying the mechanisms of arrhythmias [2].

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Investigation of ligands stabilizing the chloroplast ATP synthase c-ring during recombinant expression.

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ATP synthase is a crucial enzyme, which plays a central role in cellular energy production by synthesizing adenosine triphosphate. It's membrane domain includes the c-ring rotor, a structure made up of several c-subunit protomers. This c-ring is essential for the translocation of protons across the membrane, which induces the rotational motion necessary for ATP synthesis. The efficiency and regulation of ATP synthase are dependent on the assembly and stability of the c-ring, making its formation critical to the enzyme's function [1].

Despite its importance, the recombinant expression of the c-ring has proven to be a significant challenge in biochemical research. The self-assembly of the c-ring is highly sensitive to environmental conditions. Moreover, the addition of histidine tags, often used to facilitate protein purification, can disrupt the natural assembly process, further complicating the expression of a functional c-ring. Previous attempts to express the chloroplast c-ring recombinantly have been unsuccessful, with researchers only able to isolate the c subunit as separate monomers rather than a fully assembled ring [2].

In our study, we have overcome these challenges by successfully expressing the c-ring of ATP synthase from spinach chloroplasts in *E. coli*. After expression, we isolated and purified the c-ring complex. Interestingly, during purification, we found that the c-ring co-purifies with unknown ligands, which likely play a role in stabilizing the complex. These ligands may interact with the c-ring to enhance its stability. To investigate the properties of the recombinant c-ring and its associated ligands, we employed several analytical techniques. Gel electrophoresis and Western blotting were used to confirm the integrity and purity of the c-ring, while UV-VIS spectroscopy provided insights into the characteristics of the ligands. By analyzing the difference spectrum of the oxidized and reduced states of these ligands, we gained further understanding of their role in the stabilization and function of the c-ring.

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Investigation of regenerative processes in cardiac tissue using tissue engineering complexes from cardiomyocytes

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A feature of the structure of the heart tissue is a pronounced structural and functional anisotropy necessary to maintain normal heart function. The most common model for studying the excitability of cardiac tissue is a monolayer of cardiomyocytes placed on a tissue engineering substrate, structured in such a way as to give the cell culture the required architecture. Cells isolated from neonatal rat hearts or cells of an immortalized cell line are used as cellular material.

In this work, the host–graft interaction was studied during transplantation of cardiomyocytes onto a monolayer using nanofiber microcarriers. Polymer nanofibers were used to form the tissue architecture, excitation waves were recorded using an optical excitation mapping device, three-dimensional structures of the contact spot of the planted subculture and the monolayer of cardiomyocytes were studied using confocal microscopy.

The first and main component is a fragment of polymer fiber with a thickness of approximately 0.85 μm ± 0.18 μm (n = 20) obtained by electroforming a PLLA solution. The second element is the protein coating of the polymer fiber by placing the fibers in an HFN solution for 24 hours. This led to the deposition of protein on the fiber surface, which manifested itself in the ability of cardiomyocytes to adhere to fibers of a given thickness.

Convincing evidence of cell adhesion is the restoration of spontaneous excitability of cells. The average signal (mean) was obtained on the basis of 16 traces of calcium obtained from 16 different cells (4 different samples) with spontaneous excitation frequencies in the range from 0.5 to 1.5 Hz. An important condition was the lack of communication between the cells.

Investigation of the effect of fibrotic lesions in the atrial tissue model in terms of electrophysiological characteristics of the patient's cardiomyocytes

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Atrial fibrillation (AF) is one of the most dangerous cardiovascular diseases. AF therapy with medications often proves ineffective in the long-term course of the disease due to changes in the electrophysiology of atrial cells. Computer modeling is already a convenient predictive tool that allows experiments to search for pathologies of conduction on a virtual copy of the patient's atrium [1]. In this work, the arrhythmogenicity of atrial tissue with fibrous sites was studied depending on the electrophysiological properties of the patient's cardiomyocytes, including under the action of an antiarrhythmic drug (Verapamil). 3D model of the left atrium was obtained by reconstructing the patient's MRI images with gadolinium contrast. Localization of fibrosis varied during the experiments, it was chosen so that the probability of arrhythmia in the area of fibrosis was high. Then the set of images was translated into input data for an OpenCarp – environment for modeling wave dynamics. The electrophysiological model of Contermanche formed the basis for the simulation of the excitation wave [2]. Two models of cardiomyocyte electrophysiology were considered in the work: a normal one [2] and a of remodeled atrial tissue of a patient with a persistent AF [1]. To recreate the effect of Verapamil, model parameters such as ionic currents, pulse stimulation frequency, etc. were varied. As a result, the use of the test medicine (Verapamil) in the presence of prolonged episodes of AF in patients has a positive effect and reduces the risk of arrhythmia in the presence of fibrosis in the tissue. At the same time, in a fibrosis model with normal electrophysiology, Verapamil proved to be ineffective in eliminating atrial fibrillation.

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Investigation of the molecular mechanisms underlying the antitumor action of the human protein SLURP-1

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One of the most common pro-oncogenic receptors is the epidermal growth factor receptor (EGFR), the overexpression and hyperactivation of which are characteristic for many tumors. In addition, there exists a mutant variant, EGFRvIII, which is unable to bind EGF and contributes to tumor cell resistance against EGFR inhibitors. SLURP-1, - a negative modulator of nicotinic acetylcholine receptor of the α7 type (α7-nAChR), is secreted by epithelial cells, regulates epithelial homeostasis and protects them against malignant transformation. Recent studies have shown that SLURP-1 binds to EGFR, although the molecular mechanisms underlying this interaction have not been fully explored.

In this study, using the affinity extraction method, the interaction of SLURP-1 with EGFR and EGFRvIII was confirmed. The negative effect of the R74A mutation of SLURP-1 on the interaction with EGFR and EGFRvIII pointed on the 'head' loop of SLURP-1 as a functional epitope of the modulator. EGFRvIII contains truncated extracellular subdomains I and II, thus the binding site for SLURP-1 locates in the region of the subdomains III and IV of EGFR. This assumption was proved by the binding constants determined by flow cytometry.

The in-cell ELISA method demonstrated that the SLURP-1 binding reduces EGFR autophosphorylation at the Y1173 position, which is important for the receptor activation. This points on at least partial overlapping of the EGF/SLURP-1 binding sites on the EGFR surface. Morever, SLURP-1/R74A mutation enhances the EGF-induced activation of the receptor. This suggests an indirect SLURP-1 related mechanism of EGFR activation.

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Ion channel of alamethicin dimer is capable of independent cationic conductivity

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Antimicrobial pore-forming peptides are considered a good alternative to traditional drugs due to the growing antibiotic resistance of pathogens [1]. Model experiments have shown the potential of using pore-forming peptides as low-toxic antitumor drugs that affect the processes of apoptosis, metastasis and angiogenesis [2]. The pore-forming peptide alamethicin has been shown to have antibacterial, antifungal, antiparasitic and antitumor effects [3]. Despite the numerous studies the pore formation mechanism is not yet clear. An urgent task in preclinical research is the development of simple and effective test systems for studying *in vitro* and *in situ* the possible toxic effects of pore-forming antimicrobial peptides. We have shown that a suspension of tightly coupled rat liver mitochondria or mitoplasts and a sensitive oximetric cell can be used as a biosensor for measuring transmembrane cationic current. The potassium transmembrane current induced in the inner mitochondrial membrane by the studied pore-forming peptides depended linearly on the degree of activation of succinate oxidation. Time-dependent separation of steady-state conductivity in the fraction of lowly and highly oligomeric alamethicin channels was shown. Our results have demonstrated that the ion channel formed by alamethicin dimer in the inner mitochondrial membrane is capable of independent cationic conductivity. Most likely, this pore is toroidal and contains a dimerized peptide and lipid.

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Ion channels and their complexes in 3D culture of human endometrial mesenchymal stem cells

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Three-dimensional (3D) cell aggregates (spheroids) are physiologically closer to the conditions observed in vivo, compared with 2D cell culture. However, significant changes of the cells, including their plasma membrane, occur when spheroids are formed. This could potentially affect the functional properties of plasma membrane proteins, particularly, ion channels. Potentially, the expression profiles, role and mechanisms of regulation of ion channels could be different in 3D compared to 2D cultures. In our previous study to register the activity of ion channels in spheroids, we have developed a specific approach for the formation of a stable superdense electrical contact between the recording pipette and a fragment of the membrane of cells aggregated into spheroids. Using this protocol, we first recorded the activity of single ion channels in the plasma membrane of endometrial mesenchymal stem cells (eMSCs) assembled into spheroids. Based on the biophysical properties, these channels have been identified as mechanosensitive Piezo1 channels and calcium-activated big conductance (BK) channels. Here, the presence of the detected ion channels in the eMSC spheroids was confirmed using a wide range of molecular biological methods (RT-PCR, real-time PCR, immunofluorescence staining). The role of ion channels in the physiology of 3D culture cells was analyzed using functional tests (formation of spheroids in the presence of ion channel modulators, inhibitory analysis, selective pharmacological stimulation of channel activity, fluorescent measurements of intracellular ion concentration, assessment of morphological features, rate of reactivation of spheroids, etc.). The results obtained on eMSC spheroids are compared with the data previously recorded on a 2D stem cell culture as well as to transformed cell lines. Preliminary conclusions have been obtained on the similarities and differences in the ionic mechanisms of cell signaling in 2D and 3D cultures. The work is supported by the Russian Science Foundation, project No. 22-74-10037.

Kinetic characteristics of acetylcholinesterase in ground squirrel erythrocyte membranes during hibernation and awakening

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In winter months, some species of mammals spend most of their time in a torpid state with suppressed metabolism, which provides significant energy savings. Bouts of torpid state last from several days to several weeks and are interrupting by periodic awakening with an increase in body temperature to normothermia. Significant fluctuations in body temperature during the transition of the animal from the torpid state to the euthermic one, leading to a change in the physico-chemical state of the membranes, can affect membrane-bound enzymes. We studied the activity and kinetic parameters of acetylcholinesterase (AChE) in red blood cells of small ground squirrels (Spermophilus pygmaeus Pall.) during deep torpor and in the dynamics of recovery from it.

The activity of AChE in the membranes of red blood cells of summer active (control) ground squirrels in a torpid state with a body temperature (Tb) of ~ 4°C and at different stages of awakening upon reaching Tb of 25°C and 37°C was determined by Ellman's method [1]. The kinetic characteristics of AChE: maximum velocity (Vm), Michaelis constant (Km) and substrate inhibition constant (Ki) were found by the least squares method in accordance with the Haldane model.

The study showed that in the torpid state, neither the activity nor Vm of AChE changes significantly. In this case, Km decreases and Ki increases significantly, which contributes to a significant change in the nature of the concentration dependence of AChE and a decrease in the degree of substrate inhibition. During the awakening of ground squirrels from hibernation at Tb 25°C, Vm increases significantly, which sharply increases the efficiency of AChE catalysis, despite the fact that the Km value also increases. After the final warming of ground squirrels from hibernation (Tb 37°C), the Vm and Km values of AChE and, accordingly, the efficiency of catalysis do not normalize, but remain at the level that was achiev at Tb 25°C. It should be note, that awakening of ground squirrels reduces Ki values, which contributes to an increase in the degree of substrate inhibition and a narrowing of the range of effective concentrations of acetylcholine. The observed sharp changes in the activity of erythrocyte AChE in ground squirrels during awakening may play an important role in the rapid decrease in the level of circulating acetylcholine and improvement of peripheral microcirculation.

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Lipid transmembrane distribution of coral host cells and lipidomic differences from endosymbiotic dinoflagellates

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The profile of lipid molecular species of symbiotic corals is unique and includes both the coral symbiont (dinoflagellates of the Symbiodiniaceae family) and the coral host compartments. The corals *Sinularia heterospiculata* and *Acropora aspera* were cultivated using a flow-through circulation system supplying seawater during cold and warm seasons of the year, then sorted into host cells and symbionts and subjected to phylogenetic, morphological and advanced lipid analyses. Our work shows that plasma membrane of the symbiotic dinoflagellates *Cladocopium* C1/C3 and acroporide-specific *Cladocopium* from the studied corals are constituted mainly by diacyl molecular species of phosphatidylcholines, phosphatidylinositols and diacylglycerylcarboxyhydroxymethylcholines (PC, PI, and DGCC) which built on the basis 20:5*n*-3 and 22:6*n*-3 polyunsaturated fatty acids (PUFAs). The differences in thylakoid lipidome among the Symbiodiniaceae species within the same genus may associate with thermotolerance, where, compeer to thermosensitive *Cladocopium* C1/C3, acroporide-specific *Cladocopium* is characterized by the thermotolerant features of thylakoid lipidome. The thylakoid lipids of the dinoflagellates contained unusual phosphatidylglycerols 16:1/19:2 and 16:2/20:2 which may indicate its special role for coral symbionts. Plasma membrane of coral host cells exhibits aminolipid asymmetry, as most abundant coral aminoglycerophospholipids phosphatidylserines (PS) with unsaturated acyl chains, and saturated aminosphingophosphonolipid ceramideaminoethylphosphonate (CAEP), are localized predominantly on the inner leaflet of the host cell membrane. The difference in saturation of these lipids may drive the formation of lateral heterogeneity (non-raft and raft domains) of coral host membrane. This compositional asymmetry could be actively or passively maintained and intrinsically linked to host cell membrane physicochemical properties (rigidity). It was unexpected to show that the transmembrane distribution of phosphatidylethanolamines (PE) of *S. heterospiculata* host changes during different seasons of the year, possibly contributing to mutualistic nutritional exchange across this membrane complex to provide the host with a secure adaptive mechanism and ecological benefits.

Lipid-polymer levofloxacin delivery system: physico-chemical properties and interaction with pulmonary surfactant

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Today, serious respiratory infections represent a global risk to the healthcare system. The situation is more acute in mycobacteriosis, which includes tuberculosis. Biocompatible inhaling administration strategies can help increase bioavailability. The creation of an inhalation method for administering anti-tuberculosis drugs based on liposome complexes containing various polymers appears to be a promising strategy. The goal of this research is to investigate the physicochemical patterns of interaction between the liposomal form of levofloxacin, functionalized with mannosylated chitosan, and bovine pulmonary surfactant. Liposomal forms of levofloxacin were obtained based on anionic liposomes (DPPC - cardiolipin mass ratio 4 to 1) with a drug inclusion efficiency of 0.2 to 0.5 mg per 1 mg of lipids. The particles were characterized by a zeta potential of -22 mV and a hydrodynamic radius of 100 nm. The resulting complex was characterized by a zeta potential of +13 mV and a hydrodynamic radius 140 nm. Bovine lung surfactant was isolated using a classical extraction technique. According to IR microscopy data the protein and lipid fractions are co-localized. The effect of liposomal forms of levofloxacin (LLEV) on the surface properties of a surfactant monolayer was analyzed using the Langmuir-Wilhelmy method. It was found that when an aliquot of the liposomal form of levofloxacin not coated with a polymer is added, the area per molecule - two-dimensional pressure curves show the appearance of a region responsible for the fusion of the liposome membrane with the surfactant monolayer. On the contrary, when adding an aliquot of polymer-coated vesicles LLEV-Pol stabilization of the surfactant is observed, but fusion does not occur. According to the AFM-microscopy the interaction of surfactant with LLEV occurs over the entire surface area of the monolayer, while for LLEV-Pol binding is observed only at the surfactant-mica interface. The diffusion of the fluorescent label along the surfactant layer was monitored when liposomes or their complex with the polymer were applied. It was found that when free liposomes are added to the surfactant layer, the label is evenly distributed over the entire surface of the layer within an hour, while foci of fluorescence were observed for the liposome-polymer complex, indicating an obstacle to fusion.

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Lipopeptides From Bacillus Probiotics Can Target Transmembrane Receptors NOX4, EGFR, PDGFR, And OCTN2

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The mechanisms of action of probiotics at the molecular level remain an active topic of discussion and study. There is evidence that the bioactive metabolites of probiotic strains exhibit not only antimicrobial and antifungal [1] properties but also more subtle regulatory effects [2]. Of particular interest are the anticancer effects of probiotics, with increasing data accumulating in recent years regarding this area [3]. In this study, we investigate one such potential mechanism. Bioactive peptides characteristic of Bacillus strains, known for their probiotic capabilities in veterinary medicine and aquaculture [4], were tested for their interaction with several key eukaryotic proteins involved in important intracellular signaling pathways. Short peptides such as Bacillomycin, Fengycin, and others derived from Bacillus were selected for docking with the targets NOX4, EGFR, PDGFR, and OCTN2. Iturin D showed high binding energy values with NOX4 and PDGFR (−17.996 and −21.11 kcal/mol, respectively), while Fengycin exhibited even higher free binding energy with these receptors (−28.21 and −24.51 kcal/mol, respectively), indicating its potential binding capability with these key receptors. Furthermore, Fengycin was ranked second in binding energy with EGFR and OCTN2 (−21.97 and −24.35 kcal/mol, respectively), and Plipastatin demonstrated the best results with EGFR (−21.34 kcal/mol), being second-ranked with NOX4 and OCTN2 (−23.20 and −28.95 kcal/mol, respectively). Overall, Plipastatin and Fengycin exhibited significant activity in binding with all receptors, making them promising candidates for further research as modulators of these receptors.

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Local topology and perestroikas in protein structure and folding dynamics

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The folding and unfolding processes of a globular protein are interpreted in terms of conformational bifurcations that change the local topology of the C_α-backbone. The mathematical formulation extends Arnol'd's perestroika [1, 2] to piecewise linear chains using the discrete Frenet frame formalism. We provide modelling of protein dynamics in a framework has been introduced in references [4, 5]. The onset of thermal unfolding begins with perestroikas that change the flattening and branch points, determining the centers of solitons. The cascading of perestroikas, when temperature increases, leads to a progressive disintegration of the modular structures. The folding and unfolding processes are quantitatively described by a correlation function tracking local topology and perestroikas with temperature changes, offering a comprehensive framework for understanding protein folding and unfolding transitions.

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MD simulation of interaction of fullerenes C_{60} with amyloid fibril

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Beta-amyloid peptides can form both toxic soluble oligomers and less toxic insoluble linear fibrils. There are many studies devoted to the investigation of new linear, cyclic or branched peptides and other molecules that can modulate the formation of amyloid oligomers and/or fibrils and disrupt already formed fibrils [1,2]. Recently, Siposova et al. [3] investigated the disassembly of lysozyme- and insulin-based amyloid fibrils by C60 and C70 fullerenes using various experimental methods. In this work, we use a molecular dynamics (MD) modeling approach [4-8] to test whether C60 fullerenes can form a complex with a stack of short Ab peptides and disrupt it. We found that fullerenes indeed disassemble the original linear structure of the amyloid stack and form an irregular complex with the Ab peptides.

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MD study of the interaction of cyclic peptide with amyloid fibril

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It is well known that in patients with Alzheimer's disease, amyloid-beta peptides (Ab1-40 and Ab1-42) and their fragments associate into insoluble linear fibrils. Many studies have shown that some linear and branched peptides and cyclic molecules can slow down fibril formation and destroy already formed fibrils [1-3]. This usually requires that these molecules form a complex with amyloid fibrils or individual amyloid peptides. In this work, we test whether ring peptides can behave in a similar way. For this purpose, we performed molecular dynamics (MD) simulation [4-8] of the interaction of the cyclic peptide with 3 arginine aminoacid residues c-ArgArgTrpPheTrpArg [9] with stack consisting of short fragments of Ab-peptides. We found that the the original linear structure of the stack is disrupted and Ab-peptides forms irregular complex with cyclic peptide.

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Mechanism of proton transport in proteorhodopsin ESR revealed by direct electrometry

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ESR, a retinal protein from *Exiguobacterium sibiricum*, is distinguished by the presence of Lys96 which acts as a proton donor to the Schiff base [1]. The details of proton transport in ESR incorporated into the membrane of the proteoliposomes were studied using the direct time-resolved electrometry. Kinetic analysis of membrane potential generation revealed substantial differences in the electrogenic response of BR and ESR [2]. We have elucidated the significance of Lys96 not only for acceleration of proton delivery to the SB [1] but also for prevention of the reverse transfer reactions from the extracellular side of the protein [3]. It was shown that the possibility for back reactions in the wild type ESR at alkaline pH and especially in the K96A mutant is determined by the His57 residue [4]. Therefore, interaction between His57 and the primary acceptor Asp85 globally affects the kinetics and direction of the proton transfer reactions in ESR.

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Mefenamic acid facilitates the action of the endogenous neurosteroid allopregnanolone as a positive allosteric modulator of GABAA receptors

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The neuroactive steroid allopregnanolone (ALLO) is an endogenous positive allosteric modulator of γ-aminobutyric acid type A ($GABA_A$) receptors. It was shown that neurosteroid effects can be modulated by many clinically used drugs, including anesthetics, sedatives, or anxiolytics [1]. It was demonstrated that the effects of etomidate and neurosteroid pregnanolone on GABA-evoked currents are supra-additive, indicating a synergistic interaction between these two compounds [2]. Mefenamic acid (MFA) is a non-steroidal anti-inflammatory drug which also can modulate $GABA_A$ receptors in the brain. We have proposed that the binding site for MFA which mediates the potentiation of GABA responses coincide or partially overlaps with etomidate binding site. The goal of the present study was to reveal the possible interaction between two allosteric modulators of $GABA_A$ receptors - ALLO and MFA. In this study, we recorded GABA-induced currents in Purkinje cells acutely isolated from young rat cerebellum using whole cell patch-clamp and fast perfusion techniques. It was found that the co-application of ALLO (10-100 nM) and MFA (10-1000 μM) causes potentiation of GABA (0.3 μM)-activated current, which at low concentrations of modulators significantly exceeds the sum of the effects caused by each modulator separately. Co-application of 30 nM ALLO reduced the EC_{50} for potentiation by MFA of currents activated by 0.3 μM of GABA by about 2.5 times. In the presence of 100 nM ALLO, the dose-response curve for currents activated by MFA as an allosteric agonist shifted to the left – EC_{50} decreased from 116 ±18 μM in the control to 8.9 ± 1.8 μM in the presence of ALLO. The findings suggest that the GABAergic effects of ALLO can be enhanced by MFA, which can act as a co-agonist, enhancing the clinical effects of both endogenous potentiating neurosteroids such as ALLO and synthetic steroids currently used in clinical practice.

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Membrane action of echinocandins

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Echinocandins are highly efficient antifungal drugs that inhibit beta-1,3-glucan-synthase leading to disruption of the cell wall synthesis. The exact mechanisms of echinocandins (EC) action are still under debates, in particular the role of target cell membranes in their function. In present study we analyzed the molecular mechanism of the interaction between EC (anidulafungin (AF), caspofungin (CF), and micafungin (MF) and two models of membranes: the ergosterol-enriched (Erg) lipid bilayers mimicking fungal and cholesterol-containing (Chol) mammalian-like membranes by molecular dynamics method (MD).

Model membranes consisted of POPC:Erg (100:50 molecules) and POPC:Chol (100:50) were assembled in CHARMM-GUI. EC topology parameters were generated using CGenFF. 4 molecules of each EC were inserted in the membrane. The size of the simulation box was about 7x7x9 nm. GROMACS 2023.2 was used to perform MD simulation with CHARMM36m all-atom force field. The energy minimization and 6-steps equilibration were followed by 100 ns of production simulation.

The analysis of MD demonstrated that all EC increased area per lipid (APL) in both models of membranes, mostly in Erg-contating ones. AF, CF and MF increased APL by 1.2, 1.8, 2.1 $Å^2$ in Chol-contatinig membrane, and by 1.6, 3.0, 1.2 $Å^2$ in Erg-containing membrane, accordingly. Moreover, the analysis of the bonds between EC and sterols clearly showed the EC preference to Erg over Chol. Thus, we can presume that EC might disorder Erg-containg domains in fungal membrane, where glucan-synthase is located, leading to indirect disruption of its function.

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Membrane-dependent reactions revisited: mechanisms of coagulation complexes formation and effect of blood flow

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Enzymatic complexes which participate in blood coagulation, namely intrinsic/extrinsic tenase and prothrombinase, are structurally and functionally similar. They are localized on the phospholipid membrane surface, are composed of one enzymatic and one cofactor subunit and activate substrate which can reversibly bind to the membrane. However, molecular mechanisms of such complexes formation and substrate delivery to the enzyme and the impact of the blood flow on this system are poorly understood. Experimental investigation gave complex results that are difficult to interpret without detailed computational modeling of the system.

The aim of the current study was to analyze computationally the substrate delivery and enzymatic complex formation pathways and to investigate the impact of blood flow on this system.

Three homogeneous models of intrinsic/extrinsic tenase or prothrombinase were implemented in Matlab or COPASI software. Models were tuned to accurately describe experimental data. Heterogeneous models of complexes functioning in the presence of flow were integrated in Comsol Multiphysics.

The membrane-dependent pathways of substrate delivery were predominant for extrinsic tenase in almost all reaction conditions with exception of high ($> 2.8 \times 10^{-3}$ nmol/cm2) tissue factor surface density. In contrast, for intrinsic tenase and prothrombinase complexes switched to the solution-dependent pathways when phospholipids were deficient and to the membrane-dependent pathways when phospholipids were in excess. For prothrombinase, high factor Va concentration switched the complex formation pathway to the solution-dependent one and significantly inhibited the reaction. In the presence of flow, we observed diffusion-limited layer of the depleted substrate near the membrane surface for very low shear rates (0.2 s^{-1}), but for higher rates diffusion-limited layer became negligible. High arterial shear rates (1000 – 2000 s^{-1}) provided more effective substrate activation when compared to low (0.2 – 100 s^{-1}) rates.

To conclude, the mechanisms of coagulation complexes formation were flexible and could switch from the solution-dependent to the membrane-dependent depending on the reaction conditions. High flow rates effectively removed the active product of the enzymatic reaction and compensated the substrate depletion, thus promoting the reaction.

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Membrane-mediated interaction of Protoxin-1 from *Thrixopelma pruriens* with the TRPA1 ion channel

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Nociception is a distinct somatosensory modality characterized by pain sensation and other responses to noxious stimuli such as inflammation. At the molecular level it is mostly determined by transient receptor potential (TRP) ion channels [1]. One notable member of the TRP family is a TRPA1 [2]. This channel is a sensor of various stimuli including temperature and vast diversity of electrophilic ligands and plays a role of integrator of different signals. Activation of the channel leads to inflammation eliciting pain or itch, which makes it a potential target for novel analgesics and anti-inflammatory drugs. Protoxin-1 (ProTx-I) is a known high-affinity antagonist of the human TRPA1 channel [3]. In this work we determined a 2.8 Å cryo-EM structure of human TRPA1 in complex with ProTx-I in MSP2N2 lipid nanodiscs. We showed that ProTx-I binds to the VSL (S1-S4) domain of the TRPA1 in a region of S1-S2 and S3-S4 extracellular loops. This binding mode is reminiscent of that in complexes of protoxins with voltage dependent channels. Interactions between Arg23 of the toxin with Glu825 of S3-S4 loop and Arg24 with Gln831 probably stabilize the complex, while Arg24, His25 and Trp 27 of ProTx-I displace flexible S1-S2 loop. Additionally, toxin interactions with lipids from a membrane surrounding the VSL domain were characterized, suggesting a membrane-mediated mechanism of the toxin-TRPA1 interaction. Probably upon the channel activation, the *C*-terminal part of the toxin is immersed in the membrane, which creates an additional energy barrier and prevents TRPA1 transition to the open state, explaining the antagonistic action of ProTx-I. This work also characterized the flexibility of the ProTx-I/TRPA1 complex and observed a hydrophobic mismatch between the height of the membrane portion of the channel and the thickness of the lipid bilayer.

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Microbial rhodopsin with DTG motif described as a light-driven Li^+/Na^+-pump is an outward proton pump

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Microbial rhodopsins with the DTG/DTS motif are light-induced transmembrane outward proton pumps, like other members of the proteorhodopsin family [1, 2]. Their pumping activity increases at acidic pH and decreases at alkaline pH, which is opposite to the behavior of typical proteorhodopins. Because of this they have been even called 'mirror proteorhodopsins' [3]. Another representative of the DTG/DTS rhodopsins, MpR from *Methylobacterium populi* was recently described as Li^+/Na^+-pump [4]. Proper and careful determination of the function of rhodopsins is necessary for their further usage as an optogenetic tools.

Here we investigate functional and spectral properties of MpR along with electrophysologial characterization in whole-cell patch-clamp experiments. Our findings show that MpR is an outward proton pump, like other members of the DTG/DTS rhodopsins. We also observed the pH dependency characteristic for this family. These results suggest that DTG/DTS rhodopsins constitute a unified group of proteins that function as pH-sensitive outward proton pumps.

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Model of the recently discovered orthonairoviruses ribonucleoprotein complex formation and future prospects

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In recent years, using NGS technology, many novel tick-borne orthonairoviruses have been detected, such as: Songling virus (SGLV), Yezo virus (YEZV) and Beiji nairovirus (BJNV). The limited data available on these viruses indicate that they are highly likely to cause fever in humans. All orthonairoviruses encode a nucleoprotein (NP) that encapsidates RNA and, with viral RNA polymerase, forms a ribonucleoprotein complex (RNP) that is packaged into virions to serve as a template for RNA synthesis. But how NPs of novel orthonairoviruses oligomerizes to form high-ordered RNP is currently unknown. The SGLV NP, YEZV NP, and BJNV NP proteins haven't structure analogues in the PDB. The identity level of the nucleoproteins of these viruses in the PDB did not exceed 35%. Models of the SGLV NP, YEZV NP, and BJNV NP structures were obtained with pLDDT NP SGLV=86.45, pLDDT NP YEZV=86.85, pLDDT NP BJNV=81.30. All models are highly structured. The YEZV NP, SGLV NP, and BJNV NP have a racket-shaped structure with "head" and "stalk" domains, mainly represented by α-helices and interconnected by free loops. The head domain of YEZV NP, SGLV NP, and BJNV NP shares high structural similarity to the N-terminal domain of CCHFV NP (PDB:3U3I) that exhibits unusual metal-dependent DNA-specific endonuclease activity. The distribution of electrostatic potential on the surface of SGLV NP, YEZV NP, and BJNV NP demonstrates the presence of an RNA-binding site in the central cleft region and in the stalk domain. We reconstructed the three-dimensional structures of the nucleoprotein hexamers using AlphaFold 3. One NP hexamer interacts with adjacent NP hexamer to maintain the oligomeric architecture. The complex of SGLV NP, YEZV NP, and BJNV NP with RNA exhibited a ring-shaped stalk-to-head architecture in a clockwise direction. The relative orientations of the head and stalk domains in SGLV NP, YEZV NP, and BJNV NP hexamers are distinct from the NP monomers. However, the formation of viral RNP is much more complicated than indicated by modeling and is likely highly dependent on the conditions under which the RNP exists. Currently, we obtained full-length recombinant NPs in soluble form and research is underway to select the initial conditions of NPs crystallization.

Modeling of protein-protein interaction within Landau-Ginzburg-Wilson approach using α-synuclein as an example

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Parkinson's disease is a neurodegenerative disease associated with the destruction of motor neurons. One of the biomarkers of the disease is Levy's corpuscles, consisting mainly of α-synuclein protein. The protein forms aggregates, the mechanism of formation of which is still not fully understood [1]. Our work aims to develop a method for modeling protein-protein interaction by combining classical molecular dynamics methods and the Landau-Ginzburg-Wilson approach. A coarse-grained model of the protein chain is used in the simulation, where the spatial shape of the protein is completely described by curvature and torsion angles. Importantly, in the Landau-Ginzburg-Willson approach the local fundamental interactions are integrated in effective dynamical fields – corresponding to these curvature and torsion angles. The energy of the protein chain is represented by the modernized Hamiltonian of the Abelian Higgs model [2]. Multiple chains are taken into account due to the Lennard-Jones potential in the 6-8 form [3]. In this way a van der Waals interaction dependent on the amino acid sequence is introduced. Two structures of α-synuclein were taken for the study: a micelle monomer and one of the tetramer molecules [4]. The modeling is performed according to Glauber's algorithm. The main observable is the radius of gyration, which shows the distribution of atoms relative to a common center of mass. Our results reveal that molecules derived from tetramers show a predisposition to form aggregates, while originally single molecules do not exhibit this tendency.

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Modeling the atrial fibrillation in procedurally generated heart tissue

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New approaches to the treatment of AF are currently being actively developed. First of all, visualization and modeling of cardiac tissue have proven to be extremely valuable and have made a huge contribution to the understanding of electrophysiological disorders of the heart, for example, for atrial fibrillation (AF). According to various estimates, 1 to 2% of the population suffers from AF, and the substrate for the triggering occurrence of AF is considered to be remodeling of cardiac tissue (fibrosis), accumulated throughout life [1]. However, there is no detailed cellular model of diffuse fibrosis, which plays a major role in maintaining persistent forms of AF [2]. We collected and analyzed the morphology and mutual arrangement of cardiomyocytes and fibroblasts in fibrotic atrial tissue. To obtain a three-dimensional structure of cells, a protocol of immunostaining and layer-by-layer visualization of intracellular structures by confocal microscopy was developed. We successfully reproduced the morphology of individual atrial cardiomyocytes for subsequent training of a morphological model based on the formalism of the Cellular Potts model, similar to the previous study of the authors [3]. The resulting model allows simulating excitation wave conduction disturbances based on patient-specific LGE-MRI data, which allows developing the most effective treatment strategies and minimizing the risk of AF. The work performed allowed statistically correct formalization of the spatial arrangement of conductive and non-conductive fragments in atrial tissue with a diffuse distribution of fibrosis, which explains the limited applicability of conventional geometric analysis in solving the percolation problem [3].

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Modeling the distribution kinetics of charged drugs in tumor cells under variable electric field gradients on mitochondrial and nuclear membranes

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Biophysical experiments using fluorescent probes-ions indicate that energy and mitotic processes in cells are associated with certain changes in electrical trans-membrane potentials (TMP), namely: negative TMP in mitochondria and internal nuclear positive TMP [1-3]. On the other hand, the penetration and distribution of charged cytostatic or photosensitizer (PS) in tumor cells is a key factor in the effectiveness of antitumor therapy. The purpose of this work is the modeling the distribution kinetics of charged PS in tumor cells (TC) under constant or variable TMPs. The kinetic model including a system of four differential equations describing the PS accumulation in some model system based on Nernst [4] is suggested.

Numerical solutions of nonlinear equations with given TMP modulation parameters are obtained using a special computer program. In this case, initial PS transfer rates and membrane permeability were determined using curves of fluorescence growth chlorine $E6^-$ and its dimethyl ester (DME^+) in RAJI lymphoid cells, presented in [5]. Comparative data analysis have shown that the effective charged drug accumulation depends on the optimal ratio between TMPs in tumor cells.This ratio can be assessed using potential-sensitive fluorescent probe DSM^+ [1-3].

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Modeling the protein structure with the abelian Higgs model

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The analyzing of multi-atomic systems from the first principles is highly costly, therefore requires finding integral degrees of freedom. In our research we attempt to characterize protein structures using the Frenet framework. The three-dimensional structure of a protein chain in this approach is described by two parameters, curvature and torsion.

$$H = \sum_{x,\mu} \beta\left(1 - cos\tau_{x,\mu\nu}\right) - \sigma\kappa_x\kappa_{x+\mu}cos\left(\tau_{x,\mu} + \alpha_{x,\mu}\right) + \kappa_x^2 + \lambda(\kappa_x^2 - 1)^2$$

In [1] it is shown that the dynamics of a three-dimensional chain can be described within the framework of the Higgs model, in which the absolute value of the complex fields corresponds to the curvature (κ_x) of the chain, and the torsion (τ_x) is determined by the phase. Thus, the 1+1 Abelian Higgs model may be a good candidate for simulating the 3D protein structure using the standard discrete lattice Monte Carlo approach [2]. Thus Hamiltonian illustrates a Higgs model under the external fields ($\alpha_{x,\mu}$) fluctuations, whose source may be the heterogeneous distribution of water molecules around the protein. The heterogeneous susceptibility of the chain itself to external influences is also a consequence of the appearance of $\alpha_{x,\mu}$. In addition, the main task is to find an external field that will fold the structure into the corresponding protein chain. The Hamiltonian shows that τ_x is oriented in the opposite direction of $\alpha_{x,\mu}$ and represents the minimum of the theory. Our modeling is done using the lattice method, for which we initialize $\alpha_{x,\mu}$ as the experimental values of torsion, and then a more accurate field adjustment occurs. As a result, we see that the correct set of $\alpha_{x,\mu}$ allows us to restore torsion and curvature. Thus, we see that the curvature of the protein chain is completely determined by its torsion, and the protein itself is a superposition of a solution of the Ginzburg-Landau equations, known as the Abrikosov-Nielsen-Olesen vortices. We chose the myoglobin 1ABS, found the structure of the external field. As a result, we reproduced the experimental structure of 1ABS with RMSD less than 2A.

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Modelling lysosomal stresses and analysis of response dynamics

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Lysosomes are important organelles in eukaryotic cells. They are necessary for autophagy, endocytosis, protection against pathogens, and maintenance of metabolic balance. The theoretical model of lysosome ion homeostasis allows one to predict responses to external influences and verify experimental data [1]. Currently, there are no models that take into account updated experimental data. Futhermore, there is no consideration of lysosome responces to stresses. In order to solve these problems, we have developed a model based on recent research of the ions composition in lysosomal lumen and cell cytosol, and lysosome membrane ion transporters. Our model was tested using available experimental data, and its applicability was verified in the case of lysosome maturation. Using this new model, we investigated the response of lysosomes to various short-term stresses and the dynamics of their recovery after them. We considered short-term ATP deficiency, lysosome enlagement, vATPase inhibition, accumulation of proton sponges in the lysosome, and calcium release. Functional description of TPC protein has been additionally implemented, and the equations describing vATPase operation have been improved to ATP dependence [2, 3]. In addition to suggestions on the dynamics of response to stress, the model allows us to consider purely selective effects that can be reproduced experimentally by optogenetics possibilities.

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Molecular Dynamics Simulation of Complexation of GED peptide and Lysine Dendrimer with HisArg Spacers

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Molecular dynamics simulation of lysine dendrimers with charged double lysine and arginine (LysLys, ArgArg) spacers as well as hydrophobic (LeuLeu, AlaAla, GlyGly) spacers and pH-dependent (HisHis) spacers was performed in recent years [1-7]. Their complexes with several drug peptide molecules (including AEDG, AED and GED) were studied also [7]. In present paper we performed simulation of complex formation by GED peptide (GlyGluAsp) molecules with a dendrimer containing a histidine-arginine spacers (HisArg) where amino acid residues in spacers are not the same, and the charge of the His residue depends on pH. We performed molecular dynamics simulations [6,7] of complexation of 16 GED molecules with the dendrimer at two different pH values: a) pH > 7 with fully uncharged histidines and b) pH < 5 with fully protonated histidines. It was found that the dendrimer with protonated histidines can carry a larger number of GED tetrapeptide molecules.

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Molecular interactions of promising drugs based on all-D-enantiomeric peptides with the β-amyloid precursor protein

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Alzheimer's disease is the most widespread type of dementia, caused by alterations in the processing of beta-amyloid precursor protein (APP). However, even today, the pathogenesis of the disease is still not fully understood. It is worth noting that a therapeutic approach that could not only cure but also effectively inhibit Alzheimer's disease does not yet exist. According to the amyloid hypothesis, the main event leading to the development of Alzheimer's disease is the formation and accumulation of amyloid aggregates, which are formed by amyloid-beta (Aβ) peptides in neural tissue.

Recently, a modern prospective drug – an *all-D*-enantiomeric peptide (D3) has been developed with the help of mirror phage display. This drug can bind the hydrophilic part of amyloid-beta monomers, thus decreasing the level of toxic oligomers. The aim of our research is to define the protein-protein interactions between APP or its pathogenic mutant forms (Iowa (D694N), Arctic (E693G), and Australian (L723P)) and prospective drugs based on *D*-peptides, as well as to obtain high-resolution structural data for the complexes using NMR studies. A cell-free expression system was chosen to obtain isotopic labeled APP protein. An analytical and preparative-scale reactions were carried out to find optimal conditions for the cell-free reaction. Subsequently, the target protein was synthesized in a cell-free reaction and purified by gel filtration. Further analysis will focus on the protein interactions between APP and prospective drugs based on *D*-peptides using NMR technology.

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Nanosystems consisting on iron oxide and serum albumin as a platform for drug delivery into cells

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Nowadays magnetic nanoparticles (particularly, iron oxide nanoparticles (IONPs)) and human serum albumin (HSA) are common components of hybrid nanosystems for many medical applications. These nanosystems can be used for targeted delivery of biologically active substances (passive targeting due to the effect of increased vascular permeability of the tumor vessels, active targeting using biovectors or magnetic field retention capabilities) and advanced release of these substances at the target location; photodynamic therapy (PDT); photothermal therapy (PTT) and magnetic hyperthermia (heating under the action of alternating fields) to tumors; the contrast modification in magnetic resonance imaging (MRI) of tumor tissues; computed tomography; etc.

In the present study a hybrid nanosystem containing IONPs and HSA was engineered and characterized by composition, thickness of the coating, stability and integrity using new physical-chemical approaches in solution and in cells. Conjugation of HSA and immunoglobulin G (IgG) to Cyanine 5 (Cy5) fluorescent dye was carried out to prepare Cy5-proteins for qualitative and quantitative research. The research included incubation of hybrid nanosystems with cells (HCT116 colon cancer cell line) followed by cell lysis, magnetic separation of IONPs, and quantitative determination of protein in the supernatant and on the surface of the IONPs. Retention of the albumin coating on the surface of the IONPs upon delivery to the cells and the effectiveness of the engineered hybrid particles as a drug (i.e. photosensitizer) delivery platform has been proven.

The work on engineering of the hybrid particles and their physical-chemical analysis was funded by the Russian Science Foundation (RSF) [grant number 22-75-10150] https://rscf.ru/en/project/22-75-10150/.

New approaches to structural investigation of liposomes by SAXS

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Small-angle X-ray Scattering (SAXS) is widely used to study solutions of macromolecules, including those embedded into phospholipid vesicles or liposomes, which serve as potential carrier/delivery systems. Liposomes exhibit heterogeneous electron density; standard *ab initio* algorithms are not directly applicable and the analysis of such systems is a non-trivial task. The parametric modeling using nonlinear minimization methods and quasi-atomistic approaches to quantitatively characterize polydisperse spherical and elliptical liposomes and to restore the electron density profiles across the lipid bilayer have been developed using the scattering data collected over the full angular range of a SAXS experiment [1-3]. In particular, 3D atomistic modeling can be used for structural analysis in lipidomics and for the study of other bilayer systems consisting of different amphiphilic molecules. These approaches have been applied to study the interactions between multicomponent liposomes obtained from synthetic lipid molecules, as well as native proteoliposomes extracted from Influenza A/Puerto Rico/8/34 (H1N1) virions, with the viral M1 matrix protein [4]. In particular, it was shown that the hemagglutinin anchoring peptides stimulate the oligomerization of the M1 protein on the surface of the lipid bilayer, facilitating the process of viral particle assembly. This research was financially supported by the Russian Foundation for Basic Research, grant numbers 20-54-12007 and 20-54-14006.

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New indazole derivatives with antitumor activity

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Nitrogen-containing heterocycles due to their diverse biological potential have attracted great scientific and practical interest for decades, which has not been lost in recent years. Today N-containing heterocyclic drugs are the best-selling drugs in the world [1]. Indazole also called benzpyrazole or isoindazone is a bicyclic condensed ring system of pyrazole and a benzene ring. The indazole fragment is a part of many biologically active compounds with antitumor, antifungal, anti-inflammatory, anti-HIV and other properties [2-3]. Significant success has been achieved in the development of indazole-containing drugs aimed at treating cancer [4].

In this work a series of indazole-containing compounds were designed and a methodology for their synthesis using a cross-coupling reaction was developed. Also, as part of the work, screening of new synthesized compounds as well as similar heterocyclic analogs obtained in our laboratory earlier was carried out. The antitumor activity of a group of indazole derivatives has been studied in relation to a wide panel of tumor cells. For compounds that showed high activity in cellular models, toxicity assessment was carried out in animals.

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Non-canonical amino acids: quantum chemical parameters

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Scientific research aimed at studying the quantum chemical parameters of non-canonical amino acids (NCAs) represents an important step in the field of biochemistry and pharmacology. Non-canonical amino acids differ from regular amino acids in that they are not directly encoded by DNA and have unique chemical and biological properties. In our research, we focused on determining the quantum chemical parameters of these molecules, which could open up new horizons in the creation of drugs and biomaterials.

We performed detailed quantum chemical analysis of a number of NCAs using modern methodologies such as pm7 in the MOPAC package. These approaches allowed us to determine key parameters including electron density, dipole moments, and gaps between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). Despite the lack of experimental confirmation of their biochemical role, the calculations allowed us to obtain valuable data that can serve as a basis for future research.

The main results of our study showed that certain NCAs have unique electronic properties that potentially make them effective for interacting with proteins and enzymes. For example, changes in quantum chemical parameters due to modifications of NCA side chains have been identified. These changes may affect the interaction of NCA with biomolecules, which should be studied further.

Obtaining neuropeptide GPCR structures

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Neuropeptides constitute the most extensive and varied group of neuromediators found in both the central and peripheral nervous systems. The majority of neuropeptides function through G protein-coupled receptors (GPCRs), which are the largest family of receptors located in cell membranes. Neuropeptides and their GPCRs are associated with various medical conditions, including obesity, pain, anxiety, mood and sleep disorders [1,2]. Therefore, medications aimed at neuropeptide GPCRs hold significant promise for the creation of new therapeutic agents. Understanding the structure of neuropeptide receptors would greatly simplify the therapeutics development process by utilizing structure-based drug design (SBDD) approach [3]. The work is focused on obtaining structures of selected neuropeptide GPCRs which involves expression and isolation of a functional and homogeneous receptor sample. In order to increase protein yield and to improve sample monodispersity we employ various strategies, including forming complexes with ligands and G-proteins, as well as introducing thermostabilizing mutations.

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Oligomerization of PEDF in the presence of zinc alters its functions

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Zinc is a crucial micronutrient vital for various aspects of neuronal function, including the direct facilitation of neurotransmission [1,2]. When zinc balance is disrupted, it can lead to pathological changes that result in irreversible neurodegeneration in the central nervous system and retina. Notably, the excessive activity of surplus zinc is particularly evident in the interneuron space [3,4]. Pigment epithelium-derived factor (PEDF) is a protein that possesses anti-angiogenic, anti-tumorigenic, and neurotrophic functions. Here, we report that increase in zinc content may be recognized by the key retinal neurotrophic factor PEDF. X-ray crystallographic analyses revealed that PEDF binds zinc ions at five distinct high-affinity intermolecular sites. This interaction reduces the protein's negative surface charge and facilitates reversible oligomerization, which in turn obscures the target recognition sites crucial for its neurotrophic and antiangiogenic functions, as well as its binding to extracellular matrix components like collagen.

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Optical control of intercellular calcium dynamics in induced pluripotent stem cell-derived cardiomyocytes with hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is one of the most common cardiovascular diseases that is mainly caused by mutation encoding sarcomere-associated proteins [1]. Since there is still no efficient treatment for HCM, more understanding of the pathogenicity of this disease will help to improve early diagnostic and therapeutic methods. Using Induced Pluripotent Stem Cells-derived cardiomyocytes model is of great importance that enable us to study HCM features [2]. Two iPSCs lines including a control cell line m34Sk3 and patient-derived cell line HCM-11f3 were differentiated into cardiomyocytes. Optical mapping was conducted at different timepoints of the differentiation process. Results showed differences in HCM features like calcium concentration in different stages of differentiation. To study the mechanisms of disturbances in calcium dynamics, we developed a joint *in vitro - in silico* approach to describe the cause-and-effect relationship between changes in cell membrane potential and intracellular calcium release. The combination of two optical mapping labels (voltage-dependent dye FluoVolt, calcium-dependent fluorescent dye Fluo 4, AM) and computer modeling made it possible to identify characteristic differences in calcium dynamics between control cardiomyocytes (m34Sk3) and hypertrophic cardiomyocytes (HCM11f3). This study will enable us to develop a deeper understanding of the relationship between abnormal calcium and HCM as well as the occurrence of arrhythmias, and further develop effective therapeutic methods to treat these diseases.

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Optical insights into stroke: assessing cortical activity and hemodynamic changes after photothrombosis in mice

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Despite extensive research, stroke continues to rank among the leading causes of mortality. The challenge of translating findings from animal studies to clinical settings remains significant. There is an urgent need to develop novel and effective strategies for investigating stroke outcomes in experiments. One such approach could be the wide-field optical imaging (WFOI).

We used adult C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J mice (Jackson Laboratory) (n = 19) that had undergone a "cortex-wide cranial window" operation. Photothrombotic stroke was induced in the left somatosensory cortex. The WFOI was used to record spontaneous hemodynamic and calcium activities in the cortex as well as those induced by sensory stimulation of the limbs (before, 1 and 7 days after stroke).

It was discovered that the most responsive parameters to the stroke were the correlation coefficient between hemodynamic and calcium signals in the cortical somatosensory area, which exhibited a statistically significant change on the 1st day following the stroke ($r = 0.46 \pm 0.24$ (mean±sd) prior to the stroke, $r = 0.09 \pm 0.06$ 1 day after the stroke, $p = 0.03$). Additionally, the interhemispheric correlation coefficient of brain activity during a resting state also showed a significant alteration ($r = 0.24 \pm 0.03$ prior to the stroke, $r = 0.02 \pm 0.24$ 1 day after the stroke, $p = 0.0001$). It was shown that the size of the stroke lesion obtained by the WFOI method in mice was highly correlated with the size of the lesion determined by classical histology methods ($r^2 = 0.49$, $p = 0.035$).

Therefore, WFOI enables the detection of both morphological and functional consequences of stroke over time in chronic experiments.

Optimization of Enzyme and Mediator Concentrations in Chitosan-Based Membranes for Enhanced Performance of Second-Generation CGM Devices

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According to the Russian Diabetes Association, citing the International Diabetes Federation, as of January 1, 2016, approximately 415 million people aged 20 to 79 suffer from diabetes worldwide. This growing number underscores the urgent need for effective glucose monitoring and control. Continuous glucose monitoring (CGM) is crucial in diabetes management, providing continuous glucose data and enabling timely therapy adjustments. Despite advances in CGM technology, current devices still face challenges, particularly in improving the accuracy and stability of readings over time. A key component of CGM systems is the membrane, which must exhibit high selectivity and sensitivity to glucose while remaining stable long-term. This study aims to investigate the impact of glucose oxidase concentration and type, as well as mediator concentration (neutral red), on the performance of membranes in second-generation CGM devices. Chitosan was used as the matrix, and glutaraldehyde was employed as the cross-linking agent. Membranes were fabricated using chitosan and applied to screen-printed electrodes. Various concentrations of glucose oxidase (types II and VII) and neutral red mediator were tested. Bovine serum albumin was used to maintain protein concentration stability within the membrane. The performance of the electordes was evaluated through static response analysis (current change relative to glucose concentration change), dynamic response analysis (current change rate on the linear segment of the amperometric curve), detection range, and sensitivity. Variations in glucose oxidase concentration and type significantly affected membrane performance. Glucose oxidase type VII showed a superior response to glucose concentration changes, while the optimal concentration of neutral red improved signal stability. Optimization of glucose oxidase and mediator concentrations, using chitosan and glutaraldehyde, enhances the performance of second-generation CGM devices. These findings contribute to the advancement of CGM technology.

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Optosensoric analysis of the effect of carbonic anhydrase inhibitor on epileptiform activity in a model of epilepsy on hippocampal slices of transgenic mice

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Epilepsy is a common neurological disease characterized by excessive activation and synchronization of neuronal networks and seizures. One third of patients with epilepsy suffer from drug-resistant forms [1] and it is important to search for new therapeutic strategies. For example, nowadays the importance of ionic homeostasis in epilepsy is widely discussed, especially H^+ and Cl^- ions contribution. One of indirect ways to treat epilepsy is carbonic anhydrases inhibitors (CAIs) [2]. Carbonic anhydrases are enzymes that catalyze the reversible hydration of carbon dioxide. However, the mechanism of CAIs action needs clarification. In this study we used transgenic mice specifically expressing in neurons sensor of Cl^- and H^+ (ClopHensor) [3,4]. Effects of CAI 6-Ethoxy-2-benzothiazolesulfonamide (EZA) was analyzed at recording local field potential (LFP), intracellular H^+ and Cl^- in the CA1 zone neurons of hippocampal slices. Epileptiform activity was induced by 4-aminopyridine (4-AP). The obtained results demonstrate that: (i) application of EZA leaded to intracellular acidification in neurons; (ii) EZA reduced the amplitude of synaptically induced intracellular H^+- and Cl^--transients; (iii) there was a decrease in the LFP induced by Shaffer collaterals stimulation and a simultaneous increase in paired potentiation under the EZA application; (iv) epileptiform activity in the 4-AP model of epilepsy in brain slices resulted in strong multiphasic acidification and a significant increase of $[Cl^-]_i$; (v) EZA reversibly prevented the generation of epileptiform activity and reduced the rise time of LFP changes during synaptic stimulation in 4-AP model of epilepsy in hippocampal slices.

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Oxidised cholesterol decreased P2X receptor mediated activity in rat trigeminal nerve

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Migraine is a chronic neurovascular disease affecting about 30% of population. Activation of trigeminal neurons provides generation and conduction of nociceptive signalling in the central structures of nervous system and underlies headache pain. Cholesterol is an essential part of the cytoplasmic membrane of all cells including neurons. Oxidised forms of cholesterol like 24S-hydroxycholesterol (24-HC) are able to cross blood brain barrier and affect various systems of the body.

The aim of this work was to study the effects of 24-HC on the activity of trigeminal afferents and mast cell degranulation evoked by ATP.

A rat hemiskull preparation was used to record the electrical activity of peripheral trigeminal nerve. To evaluate effect of 24-HC on mast cells in meninges hemiskull preparation were incubated in ATP (100 μM) and were stained by Toluidine blue.

Application of ATP increased action potential frequency by 480 % compared to baseline activity ($n=6$, $p<0.05$). Preliminary incubation of preparation in 24-HC (1 μM) decreased ATP induced nociceptive activity and frequency of AP increased by 200 % compared to control ($n=7$, $p<0.05$). Moreover, 24-HC application in rat hemiscull preparation did not affect the background activity of trigeminal nerve during 40 min of incubation. Incubation of brain meninges in a solution containing 100 μM ATP increased the rate of degranulated mast cells to 11±3.3% ($n=10$) compared to control values: 1.9±0.87 ($n=5$). Pre-incubation in 24-HC suppressed the effect of ATP, reducing the rate of degranulated mast cells to 5±1.26 ($n=4$).

The obtained data indicate that 24-HC reduces ATP-induced trigeminal nerve activity, and increases mast cell stability.

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PCMMs nanomaterial study on *Caenorhabditis elegans* elucidated them as multufuctional bioprobes

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Multifunctional nanomaterials which are active in the near infrared (NIR) region and can be physically guided (laser light, magnetic field, ultrasound etc.) hold a great promise in several biomedical applications, such as drug delivery, cell biology, biosensing and bioimaging [1]. In this study, we developed a multifunctional photoluminescence coding magnetic microspheres (PCMMs), studying their physical and chemical properties, such as photoluminescence characteristics, size, and surface charge. We were interested to exploe the possibility of using PCMMs to study the biological systems [2]. To reach this goal we investigated the possibility of the PCMMs imaging in the C. elegance animal model [3]. We performed estensive toxicity screening of 10 types of luminescent magnetic microspheres (LMM) and 5 types of carbon dots (CD), which were embedded into LMM. We also explored the delivery and localization of tested nonotarticles inside of the nematodes body.We found out that tested particles are not cytotoxic for living C. elegance tissues at the optimized range of the concentrations having trend of nematodes lifespan extention. Our fluorescent microscopy study revealed the CDs and LMM localization in the intestinal part of nematodes body. Our results demonstrated the possibility of detecting photoluminescence coding magnetic microspheres PCMMs in the body of living organisms, which means the potential for application of this nanoscale drug delivery system in the future research in humans.

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Physical model of the nuclear membrane permeability mechanism

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Nuclear cytoplasmic transport is mediated by many receptors that recognize specific nuclear localization signals on proteins and RNA and transport these substrates through nuclear pore complexes. Facilitated diffusion through nuclear pore complexes requires the attachment of transport receptors. Despite the relatively large tunnel diameter, some even small proteins (less than 20–30 kDa), such as histones, pass through the nuclear pore complex only with transport receptors. Over several decades, considerable material has been accumulated on the structure, architecture, and amino acid composition of the proteins included in this complex and the sequence of many receptors. We consider the data available in the literature on the structure of the nuclear pore complex and possible mechanisms of nuclear-cytoplasmic transport, applying the theory of electrostatic interactions in the context of our data on changes in the electrokinetic potential of nuclei [1] and our previously proposed physical model of the mechanism of facilitated diffusion through the nuclear pore complex (NPC) [2]. According to our data, the main contribution to the charge of the nuclear membrane is made by anionic phospholipids, which are part of both the nuclear membrane and the nuclear matrix, which creates a potential difference between them. The nuclear membrane is a four-layer phospholipid dielectric, so the potential vector can only pass through the NPC, creating an electrostatic funnel that “pulls in” the positively charged load-NLS-NTR trigger complexes. Considering the newly obtained data, an improved model of the previously proposed physical model of the mechanism of nuclear-cytoplasmic transport is proposed. This model considers the contribution of electrostatic fields to the transportation speed when changing the membrane’s thickness in the NPC basket at a higher load.

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Pichia pastoris as an industrial production platform for regenerative growth factors

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Growth factors are a large group of protein molecules that have been proven to play crucial role in wound healing. For regenerative technologies development, especially the ones that require human stem cell propagation and differentiation, having access to high quality virus- and prion-free recombinant growth factors is an essential part nowadays [1]. Establishing a cGMP compliant production of recombinant growth factors may increase accessibility of those to the biopharmaceutical industry, but the low production amount needed, lower production site efficiency and therefore the higher total cost is a limiting factor if we consider manufacturing just one growth factor type. A possible solution may be establishing an industrial production platform which can be used to manufacture similar types of growth factors using similar bioprocesses. Out of the three key expression hosts (the others being *E. coli* and mammalian cell cultures such as CHO) routinely used in modern biotechnology, methylotrophic yeast *Pichia pastoris* offers correct target protein folding and high productivity while keeping upstream and downstream operation costs comparatively low. We demonstrated that *P. pastoris* can be a promising industrial platform for recombinant growth factor production by designing two similar processes to produce rhPDGF-BB and rhFGF-2, two major cytokines with similar properties [2]. rhPDGF-BB and rhFGF-2 produced within the suggested platform showed purity and activity matching those offered commercially. Similar molecules, such as human nerve growth factor (hNGF-β), may also be produced this way, thus offering a potential boost to regenerative technology research.

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Pipeline for bacterial genomes assembly and annotation

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In agriculture, bacteria are used to create biological preparations that stimulate the growth of agricultural plants, provide their protection from phytopathogens and pests, as well as probiotics for farm animals. This use is possible due to the unique set of proteins and low-molecular compounds produced by bacteria. At the same time, each bacterial strain is capable of producing only a certain set of compounds encoded in its genome. Thus, deciphering the spectrum of produced compounds based on genomic sequencing data is an important task. We have developed a software pipeline that assembles the bacterial genome from raw reads and annotates the resulting genome, identifying its key features, and combines different bioinformatic tools. The annotated properties of bacterial strains include the presence of genes encoding virulence factors, a number of bactericidal and fungicidal compounds, as well as the prediction of insecticidal properties of strains, which allows for a relatively rapid comprehensive assessment of the potential of the analyzed bacterial strains for agriculture to protect agricultural plants from pests and pathogens.

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Planned infrastructure of the SRF “SKIF” for crystallization of biopolymers and their structural studies

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Determination of three-dimensional structures of macromolecular compounds is a key element in the solution of a wide range of problems in science and in applied research. Among these, the most important are: understanding the mechanisms of biochemical processes, developing new drugs, and creating proteins and enzymes with desired functions. Currently, approximately 184,600 (or 84%) of experimentally determined structures of macromolecular compounds have been obtained using X-ray crystallography, with more than 80% of the cases for raw data collection synchrotron radiation was used.

X-ray crystallography requires the presence of a single crystal. Many synchrotron radiation centres in the world have their own facilities for working with biopolymers and crystallising them in the immediate vicinity of the synchrotron. The plan of the "SKIF" project also includes this facility in the laboratory building within walking distance of the synchrotron. This laboratory building is currently under construction. The report will present the capabilities of the planned facilities (laboratory space dimensions, temperature regime, humidity) and the necessary equipment for working with biopolymer solutions, crystallization, crystal storage and its transport to the synchrotron station.

The report will also present the planned experimental capabilities of the station 1-2 "Structural Diagnostics" for structural studies, including the collection of raw data for the single crystal X-ray crystallography method.

Platelet phospholipid membrane mediates activation of coagulation factor X by factor IXa

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Blood coagulation is a cascade of proteolytic reactions, the key processes of which appear to be mediated by platelets phospholipid membrane. Given reactions of coagulation occur in unstable condition like thrombus, we have developed a mathematical model with surface- and membrane-dependent substrate delivery with rejection of stationarity in the model.

We develop and analyze a computer-based model of the activation of coagulation protein factor X, formulated as a system of ordinary differential equations (ODES) to distinguish pathways of Factor X activation and to evaluate the effect of platelet membranes on the system at their high concentrations.

In order to accurately describe the latest experimental data, we have validated the model and determined the values of binding constants, complex formation, and catalysis. This mathematical model has also allowed us to investigate the process of complex formation in details. We have described and confirmed the existence of two activation pathways: membrane-dependent and solution-dependent enzyme delivery. We found their contributions to be comparable, and the ability to switch between these two complexes (from solution-dependent to membrane-dependent) enables factor IXa to not bind directly to the membrane, but to achieve binding by "falling onto" the membrane as part of activating the complex. The main advantage of our model is its consideration of the variability of the process, allowing us to simulate increased platelet concentrations, simulating a blood clot. As a result, we believe that the relationship between the formation of Factor Xa and the concentration of platelet membranes has a bell-shaped form, with a peak at a concentration between 1.5 and 2×10^6 platelets per milliliter. This concentration corresponds to the expected number of platelets that can form a thrombus, so we can use it as a basis for modeling under thrombus conditions.

Post-Stress neuroinflammation and MAP2 expression in adult and aged rats with contrasting excitability

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Nervous system excitability is recognized as a risk factor for post-stressor disorders. This study aimed to assess post-stress neuroinflammation, MAP2 expression and behavior disturbances in adult and aged rats with contrasting excitability levels.

Methods: The study used five-month-old male rats selected strains: high-threshold (HT, low excitable) and low-threshold (LT, high excitable). Each experiment involved 24 rats, divided into four groups. The stress model involved 15 days of daily exposure to emotional and pain stress (Gecht protocol). Rats were assessed at 7, 24 days (5-6 months old), and 6 months post-stress (1.2 years old). Before sacrifice, rats underwent "Open field" (OF) and "Elevated plus maze" (EPM) tests. RNA was extracted from the amygdala, hippocampus, and prefrontal cortex, and mRNA levels were measured using RT-PCR. 50 μm slices were stained for MAP-2 using primary antibody (Abcam, ab32454) and secondary Alexa Fluor 594 (Thermo Fisher Scientific), and examined with a Leica DM4000 fluorescence microscope at 20x magnification.

Results: After 24 days, both strains showed reduced motor and exploratory activity in the EPM, with LT rats experiencing broader changes. In older LT rats, motor activity in the OF decreased significantly. Differences between the strains also emerged in older age: LT rats spent less time in the center of the OF and had lower overall motor activity compared to HT rats. TNF mRNA levels were elevated in the amygdala of LT rats and the hippocampus of HT rats 24 days after stress. In LT rats, MAP-2 fluorescence decreased in the Cg1 PFC following stress, while it increased in the IL zone of the PFC in aged rats. Additionally, aged HT rats had higher MAP-2 fluorescence in the Prl PFC, and aged LT rats showed higher fluorescence in the amygdala and CA1 hippocampal region compared to younger groups.

These results suggest that age and nervous system excitability may play a role in how organisms respond to stress, offering insights that could contribute to a better understanding and potential treatment of age-related stress disorders.

Potassium ion channels gating process study with wavelet based bispectrum analysis

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Potassium channels are found in biological membranes of all natural organisms, where they provide stabilization of the potassium gradient, signaling and other functions. Due to the almost complete absence of an inactivation process, voltage gated potassium channels from snail *Lymnaea st.* neurons and *Vero* cells are ideal objects for measuring long-term data series of transchannel current continuous recordings lasting up to 15 minutes. We have previously [1, 2] investigated the effects of long-term memory in the lifetime sequences of these channels using the R/S, BFA and multifractal methods, but in this case we analyzed the open and closed lifetime sequences separately. The cross-relationships of these sequences with the possibility of identifying the master/slave interactions have not been studied. To solve these problems, an adequate tool is bispectral analysis based on the wavelet transform [3]. We have studied the sequences of lifetimes of natural potential-dependent potassium channels and a model channel based on the modified stochastic model of A.A. Grinevich. An increase in the relationship between the processes of opening and closing the gate mechanism with an increase in the transmembrane potential in natural potassium channels was established, as well as similar behavior in the model ion channel, which additionally confirms the correctness of the provisions underlying the model.

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Prediction of the interaction between NK cell receptor KIR2DS4 and HLA-C*05-peptide complex

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The ability of NK cells to establish antigen-specific responses has been demonstrated in various infections. NK cell receptors of the diverse family of Killer-cell Immunoglobulin-like Receptors (KIR) interact with HLA class I molecules, and this interaction is peptide-dependent [1]. The activating receptor KIR2DS4 enables NK cell degranulation following interaction with specific peptides presented within HLA-C*05 [2]. However, the mechanism underlying the differential NK cell response depending on a peptide remains poorly understood and lacks explanation based on the structure of ligand-receptor interaction. Using AlphaFold 3 [3], we generated models of KIR2DS4-peptide-HLA-C*05 complexes to analyze the contact interfaces. We confirmed the substantial role of the aromatic ring in 8th amino acid of peptide sequences in mediating interactions with KIR2DS4. Even with the same amino acid at the 8th position, different peptides exhibited variability in polar contacts with KIR2DS4. Our results may contribute to the prediction of KIR-HLA interactions and facilitate the identification of specific peptides capable of activating NK cells.

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Properties of luxR proteins from psychrophilic bacteria in application to expression vector construction

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Quorum sensing (QS) systems allow bacteria to communicate and coordinate their behavior by exchanging specific low-molecular compounds called autoinducers (AI). For example, the marine psychrophilic bacteria *Aliivibrio logei* regulate luminescence by synthesizing N-acylated derivatives of L-homoserine lactone (acyl-HSL) and detecting these AIs by transcription regulators LuxR1 and LuxR2. The properties of these receptor proteins became the subject of our study. We demonstrated that LuxR1 and LuxR2 have differences in specificity to acyl-HSLs with different acyl group lengths, and also have different optimal DNA binding sites. This results have found application in the construction of temperature-switchable acyl homoserine lactone-regulated expression vectors [1]. In systems based on these vectors, expression of target proteins depends on temperature and on the concentration of the AI in the medium simultaneously: at temperatures from 20 °C to 30 °C, expression is induced by the appearance of 3-oxo-hexanoyl homoserine lactone, and when the temperature increases to 37 °C, expression stops completely. Expression vectors were tested on a number of target genes in various recipient strains of *Escherichia coli*, the content of the target protein reached 50% of the total cellular protein.

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Protein-protein interaction within Landau-Ginzburg-Wilson approach

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Some proteins can form aggregates under various environmental conditions, leading to decreased biological activity or complete loss of function [1]. Studying the mechanism of protein coaggregation can reveal the etiology of neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's, and help develop prevention methods. While widely used molecular dynamics methods with full-atom models require significant computing power, simplified protein models are gaining interest. The Landau-Ginzburg-Wilson approach allows modeling of single-chain protein temperature dynamics, representing protein structure in terms of a kink solution of a generalized discrete nonlinear Schrödinger equation [2]. To account for multiple protein chains, pairwise Van der Waals interactions based on amino acid sequences are considered, introducing an 8-6 Lennard-Jones potential [3] into the full energy calculation. This proposed model combines field theory methods and classical molecular dynamics approaches. The stability of the final aggregate structure is investigated by simulating heating/cooling trajectories using the Glauber algorithm. This approach is applied to study human amylin aggregation—a polypeptide that forms fibrils at high concentrations, such as in type 2 diabetes [4]. Additionally, it examines the aggregation potential of α-synuclein, a biomarker for Parkinson's disease [5].

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Putative zinc binding site of proteorhodopsins with the DTG/DTS motif

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Proteorhodopsins are the largest known family among rhodopsins [1]. The phototrophy they mediate is of great importance in marine ecosystems. Representatives of this group play a significant role in the proton gradient of various organisms. Of all their diversity, there is a subfamily of mirror proteorhodopsins (MPRs) with a DTG/DTS motif. A characteristic feature of MPRs is an atypical pH dependence. It was shown that MPR *Spa*R (*Sphingomonas paucimobilis* rhodopsin), in addition to the peculiarity of its work depending on pH, is inhibited by zinc ions [1].

In this work, we show that *Mp*R (*Methylobacterium populi* rhodopsin), the protein from the same MPRs family, has a similar pH sensitivity and not inhibited by zinc ions. The data were obtained using time-resolved spectroscopy. Taking into account the differences in the sequence between *Spa*R and *Mp*R, it is possible to assume in more details about the amino acids responsible for the formation of the binding site with zinc ions, and also to isolate *Mp*R and similar proteins into a separate subfamily. Further work will be devoted to detailing the specific amino acids responsible for interaction with zinc using site-directed mutagenesis.

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Recombinant Fusion Vaccine Candidate Based on the Genetically Inactivated S1 Subunit of Pertussis Toxin and RTX Domain of Adenylate Cyclase Toxin for Enhanced Cellular Immune Response

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The development of vaccines capable of inducing a robust cellular immune response is essential for combating infections such as pertussis. In this study, we designed a recombinant genetically inactivated antigen based on the S1 subunit of pertussis toxin (PT). To enhance the immunogenicity, this antigen was fused with the RTX domain of adenylate cyclase toxin (ACT), which was shown to act as an adjuvant able to deliver antigens into antigen presenting cells and eliciting T-cell activation [1]. The genetic inactivation of the S1 subunit was achieved through 9K/129G mutations, which eliminated the enzymatic activity of the toxin while preserving its immunogenic epitopes, as previously demonstrated in clinical trials [2]. The recombinant S1 subunit of PT was purified from inclusion bodies of E. coli BL21(DE3) cells. Our *in vitro* and *in vivo* studies demonstrated that the S1 subunit alone effectively activates CD4+ and CD8+ T cells. Furthermore, the fusion antigen, also expressed in BL21(DE3) cells, has emerged as a promising candidate for further vaccine development. We propose that this novel fusion antigen has the potential to improve the efficacy of pertussis vaccines by enhancing the cellular immune response.

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Reconstitution of the *Staphylococcus aureus* 50S ribosomal subunit and GTP-binding protein YsxC complex in vitro for cryo-EM structural analysis

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Staphylococcus aureus, a bacterium colonizing approximately 30-50% of the global population, with around 20% being persistent carriers, is a leading cause of hospital-acquired infections [1]. Its capacity to develop resistance to multiple antibiotics, including methicillin, poses a significant public health challenge. Understanding the interactions between proteins and ribosomes is vital for identifying key proteins involved in bacterial protein synthesis. These proteins could serve as targets for new drugs designed to disrupt ribosomal function and inhibit the growth and reproduction of pathogenic bacteria. One such promising target is the GTP-binding protein YsxC, though its interaction with the ribosome remains poorly understood.

To explore this, we utilized biochemical purification techniques to isolate protein and ribosomes, enabling the in vitro reconstruction of the 50S-YsxC complex. Before conducting cryo-EM experiments, negative staining was employed to assess the quality of the ribosomal subunits.

Our work established optimal conditions for purifying the YsxC protein and the 50S ribosomal subunit from *Staphylococcus aureus*. As a result, we obtained the 50S-YsxC complex ready further structural analysis using cryo-EM.

The protocols developed in this study set the stage for future cryo-EM experiments, with the aim of revealing the high-resolution structure of the protein-ribosome complex. These findings have the potential to greatly enhance our understanding of protein-ribosome interactions and facilitate the development of novel antibacterial therapies.

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Registration and mathematical modeling of the NADH photobleaching dynamics in cardiac tissue

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In this study, we propose a novel approach to noninvasive optical detection of cardiac tissue ischemia. The nonlinear dynamics of NADH molecules fluorescence (fNADH) changes under the influence of constant radiation (365 nm) was used as an initial source of information [1]. This allowed us to obtain a map of ischemic regions in real time, as well as to separate reversible and irreversible stages of ischemic damage. The aim of our work was to build an accurate quantitative assessment of ischemic damage; for this purpose, we developed a mathematical model describing the balance of NADH and NAD+ concentrations during photobleaching and the resulting glutamate dehydrogenase (GDH) activity. The theoretical part of the work allowed us to draw two conclusions: (i) the change in fNADH over time nonlinearly depends on both the initial NADH and NAD+ concentrations and the GDH activity, (ii) the inverse problem has a unique solution for each case: the fNADH signal shape allows us to determine the NADH and NAD+ balance at an arbitrary power of absorbed excitation radiation. The possibility of using an arbitrary power value allowed us to move from a qualitative to a quantitative assessment. Both conclusions were tested in a series of experiments on isolated perfused rat hearts and monolayers of human cardiomyocytes obtained by directed differentiation from induced pluripotent stem cells. The obtained results could be useful both for fundamental study of ischemic injury processes and for practical application with construction of ischemic maps of hearts stored in cardioplegic solution during transportation or long-term open-heart surgery. This study was supported by Russian Science Foundation grant #24-21-00162.

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Regulation mechanisms of the microalgal metabolome for biotechnological uses

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Among the organisms widely used in obtaining bioproducts, microalgae are very popular prokaryotic and eukaryotic photosynthetic organisms. The spectrum of biologically active compounds obtained from algae is quite broad: proteins, carbohydrates, lipids, vitamins, pigments, enzymes, etc. The biomass of algae is rapidly growing, which increases its value as a biotechnological raw material. In this regard, microalgae are considered raw materials for producing agricultural feed, biofuels (biodiesel, bioethanol, biogas, biohydrogen), cellulose, and products with pharmacological, food and cosmetic value. In the course of the work, the ability of microalgae from different phylogenetic (Chlorophyta, Heterokontophyta, Cyanobacteria) and ecological groups (marine, freshwater, soil) to accumulate lipids, valuable fatty acids, chlorophyll a was analysed in a laboratory experiment assuming several gradations of nutrient starvation conditions (nitrogen and phosphorus limitation). Also, the unification of the used nutrient media by the content of nitrogen and phosphorus as a necessary condition for the implementation of the subsequent comparative analysis was carried out; the ranges of the required content of nitrogen and phosphorus in the cultivation medium were established [1], a standardised approach for lighting modes was proposed [2].

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Revealing the Changes in Regulatory Elements Landscape Driven by SIRT6 Deficiency and Aging in the Brain

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SIRT6 is a deacetylase and mono-ADP-ribosylase playing a crucial role in aging as well as in the progression of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [1]. While diverse characteristics of SIRT6 functioning have been already investigated, some aspects of its mechanism of action, particularly its regulatory elements and how they change with aging, remain poorly understood. Our work aimed to investigate alterations in the regulatory landscape, specifically enhancers, caused by brain-specific SIRT6 deficiency and brain aging in mouse. To address this problem, we employed a multi-omics approach, integrating ATAC-seq and Hi-C profiles of young (4 months), mid-aged (10 months), old (20 months) and SIRT6-KO (10 months) cortical neurons with publicly available enhancer marks (ChIP-seq) and enhancer-promoter annotation to predict brain-specific enhancer sequences, including those within *SIRT6* locus. Predicted enhancers generally demonstrated higher GC content and CpG island density compared to non-enhancer regions, confirming the quality of the obtained annotation. Interestingly, both GC and CpG site contents were significantly lower in aged cortical neuron enhancers, compared to those in other experimental groups, suggesting age-specific mechanisms of enhancer methylation. Differential analysis of chromatin accessibility within enhancer regions revealed 30 significant regions in SIRT6-KO and 482 regions in old samples relative to the mid-aged controls. Thus, although both aging and SIRT6 deficiency lead to regulatory changes in cortical neurons, aging appears to have a greater impact on chromatin accessibility and enhancer landscape compared to SIRT6 knockout alone.

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Ribosomal antibiotic Madumycin II disturbs protein synthesis machinery in a pleiotropic manner

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Elucidation of molecular mechanisms of inhibition by antibiotics aims several goals. Firstly, it provides an opportunity to modify existing therapeutic drugs to increase their potency and to overcome bacterial resistance. Secondly, it allows to deepen our knowledge about structure and functions of the drug's cell target. The bacterial ribosome inhibitor Madumycin II (Madu) of the streptogramin A group was discovered in the 20th century [1], but it is still a poorly studied compound. Our investigation of the Madu action applying functional biochemical tests and structural biology methods demonstrates a complex of changes in a large multicomponent protein biosynthesis machinery in the early stages of elongation caused by the antibiotic. On the one hand, Madu binds in the peptidyl transferase center and sterically interferes with the acceptor end of P-site tRNA causing it to rotate by 180 degrees from its normal location [2]. This effect is observed not only in the case of deacylated P-site tRNA, but also in the aminoacylated tRNA form with one, two or even three amino acid residues. On the other hand, Madu allosterically targets A-tRNA disrupting its contacts with the ribosome, destabilizing the entire tRNA body and forcing dissociation during proofreading step. Flipped-out conformation of P-site tRNA and A-tRNA flexibility make peptidyl transferase reaction impossible in the presence of Madu.

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Role of mechanosensitive Piezo1 channels in the migration of malignant cell lines

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Piezo1 are mechanosensitive calcium-permeable ion channels that play crucial role in cellular mechanotransduction and associated signaling processes. Several lines of evidence point out pathophysiological role of Piezo1 in the reactions of malignant and tumor cells. High expression of hPIEZO1 mRNA (according to Human Protein Atlas database) is correlated with poorer prognosis of patient survival in lung, ovarian and cervical cancers. We investigated the functional role of Piezo1 channels in the migrative ability of these cancers using cultured cell lines: A549 as a model of lung, PA-1 as ovarian and m-HeLa c11 as a cervical cancer, respectively. To elucidate the role of Piezo1 in cell migration we utilized gap wound healing assay with silicone 2-well inserts. To stimulate Piezo1 activity we used synthetic compound Yoda1, a selective chemical activator of Piezo1 channels. We showed that Yoda1 reduced the degree of wound healing by PA-1 and HeLa cells to a different extent but had no effect on the wound closure by A549 cell line. Fluorescent staining showed that a number of cells were Piezo1-negative in all cell lines (the highest number of those detected in A549 cell population). Calcium imaging showed the different degrees of cell responses to Yoda1 between the cells in population as well as between cell lines used in study. Yoda1 decreased of PA-1 and m-HeLa cell proliferation and viability, whereas A549 cells were insensitive to the reagent. Importantly, m-HeLa cervical carcinoma was significantly more sensitive to Yoda1 comparing to PA-1 ovarian carcinoma cells, and this correlated with the degree of Yoda1 effect on cell motility. Thus, we demonstrated the significant differences between the number of functional Piezo1 channels in the plasma membrane of lung, ovarian and cervical cancer cells. Our data imply that selective activation of Piezo1 by Yoda1 could be considered as potential approach to decrease the migration of ovarian and cervical cancer cell lines.
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Role of the antioxidant skq1 in the pathogenesis of mptp-induced neurodegeneration using adult fish danio rerio

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Oxidative stress and mitochondrial dysfunction play a key role in neurodegenerative diseases. When mitochondrial function is impaired, oxidative stress increases, and the cellular energy potential decreases, which is critical for neurons, particularly in the substantia nigra, the region most vulnerable in Parkinson's disease. In our study, the neurotoxin MPTP was used to model this condition in Danio rerio (zebrafish). This neurotoxin inhibits the activity of mitochondrial complex I, leading to the generation of reactive oxygen species (ROS) and the destruction of dopaminergic neurons, inducing symptoms resembling Parkinson's disease. To counteract this pathological process, the renewable antioxidant SkQ1 was employed. Due to its positive charge, SkQ1 integrates into the inner mitochondrial membrane, where it accepts free electrons escaping from the electron transport chain, thereby preventing damage to the membranes of the matrix.

Two studies were conducted: behavioral and biochemical. The results of the behavioral experiments demonstrated that incubation in an SkQ1 antioxidant solution prevents Parkinson-like movements in adult fish exposed to MPTP. The biochemical study revealed the key role of superoxide dismutase 2 (SOD2) and the glutathione system in protecting the mitochondrial membrane from ROS. It also showed that the SkQ1 antioxidant aids in neutralizing ROS, thereby supporting the mitochondrial antioxidant system during oxidative stress.

The research results demonstrate the effectiveness of the SkQ1 antioxidant in mitigating oxidative stress occurring at the mitochondrial membrane during the degeneration of dopaminergic neurons in Danio rerio, and they also enable the exploration of other potential therapeutic properties of this antioxidant.

ROS generation in mitochondria and cytosol during optogenetic cytosol alkalization in human cells

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Optogenetics provides possibilities for a precise control of a vast variety of cellular processes [1]. For example, microbial rhodopsin-based tools provide possibilities for cytosol pH control [2]. Also, recently, cytosol alkalization in human cells by outward proton pump Arch3 was shown to induce intrinsic, mitochondria-mediated apoptotic pathway of cell death [3]. It is known that cytosol and mitochondrial matrix alkalization, ROS production in mitochondria and Ca^{2+}-signaling are tightly interconnected in mammalian cells, and, under certain conditions they favors mPTP opening and cell death [4].

In the present work we studied whether ROS generation occurs under the optogenetic cytosol alkalization with a genetically-encoded fluorescent sensor Hyper7 [5]. Arch3 was expressed in plasma membrane of HeLa cells, and cytosol alkalization by Arch3 leads to mitochondrial ROS generation as well as cytosol ROS level elevation. We propose that ROS production may be one of the key steps in optogenetic cytosol alkalization-induced cell death. Elevation of cytosol pH and ROS imbalance are common for cancer cells, understanding of interactions of these factors is important for cancer biology and our work might help to address the problem.

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SAXS-based structural modeling of the complex of histone-like HU protein with N-terminal fragment of Lon protease

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Small-angle X-ray scattering (SAXS) allows a reconstruction of the structure using a one-dimensional small-angle X-ray scattering profile. In combination with molecular modeling and dynamics (MD), it is widely used to study proteins and their complexes. This combination allows a selection of the model whose structural characteristics are the closest to the parameters determined by SAXS. In this work, SAXS was used for an assessment of complexes formed by the 328 N-terminal amino acid (aa) residues of Lon protease from *E. coli* (Lon) and histone-like protein HU from *M. gallisepticum* (HUMgal). Full-length Lon protease (a multidomain hexameric protein with a molecular weight (MW) of more than 600 kDa) is an important component of the protein quality control system in all kingdoms of life, while small (MW 20 kDa) dimeric HU proteins belong to the nucleoid-associated proteins specific to the kingdom of bacteria. Like many DNA-binding proteins, HU proteins are native substrates for bacterial Lon proteases. The Lon-HUMgal complex was obtained by gel filtration chromatography. The SAXS experiment was carried out in the BioMUR station of the Kurchatov synchrotron radiation source. The HADDOCK program, AI-based AlphaFold2 via ColabFold, and MD were used to design structural models of the Lon-HUMgal complex. The CRYSOL program was applied to calculate the theoretical SAXS curves and compare them with the experimental data allowing the selection of the most reliable models. We showed that HUMgal binds to the N-terminal barrel-shaped fold formed by the 100 N-terminal aa residues of Lon protease, while the binding site on the HUMgal molecule could not be precisely determined, since the two types of models had similar reliability. The results obtained will help to clarify the mechanism of recognition and processing of substrates by complex molecular machines, an example of which is Lon protease. The work was supported by the Russian Science Foundation (grant #21-74-20154); SAXS experiment was partially supported within the framework of the thematic plan of the NRC Kurchatov Institute.

Search for antigen-specific T-cells using the effect of trogocytosis

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It is important to know antigen-specific T-cells clones for selective immunotherapy and full control of its efficacy. For example, in the case of autoimmune diseases, antigen-specific CD4+ T-cells serve as a target, and their number and phenotype are an indicator of the effectiveness of therapy in the case of infectious diseases and cancer. However, the search for and identification of antigen-specific T-cells is a complex task that currently has no universal and generally accepted approaches. In our work we use *in vitro* stimulation with antigens followed by sequencing of T-cell receptors of cells proliferating in response to the stimulus. In this case, 10 mL of blood allows us to test the response to 6-8 antigens. We have found that the B-cell marker CD20 is present on activated T-cells. By labeling membrane proteins, we have proven that this phenomenon is related to trogocytosis, the phenomenon of transfer of membrane proteins between cells as a result of their interaction. We have proposed to use this effect for multiplex detection of specific T-cells to several antigens in one experiment simultaneously by labeling the membrane of antigen-presenting cells with fluorescent probes according to the antigens loaded in them.

Sequence-based prediction of enzyme optimal pH

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Enzymes are crucial catalysts that accelerate chemical reactions in the body, but they function optimally only within a specific pH range. This property makes it essential to design enzymes that work at a desired pH, particularly for biotechnological applications such as laundry detergents and brewing. Predicting the optimal pH for enzyme activity can be achieved in silico, significantly reducing the need for labor-intensive and time-consuming laboratory experiments. In our research, we developed a sequence-based machine learning model to predict the optimal pH value for enzymes. [2] To ensure the robustness of our approach, we rigorously validated the model using various splitting strategies, including those based on sequence similarity, enzyme classification, and protein family annotation. [3,4] Our model demonstrated high accuracy even for sequences with low similarity to the training set. We also explored the impact of different protein language models as a source of sequence embeddings on our machine learning approach, finding that Transformer-based Large Language Models (LLMs) outperformed traditional RNN, LSTM, and CNN models. Our method not only holds the potential to predict whether a specific point mutation will increase or decrease an enzyme's optimal pH but is also efficient enough to screen large protein databases to identify sequences with the desired pH value.

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Serine proteases alpha-chymotrypsin and plasmin inhibit the motility processes of leukemia cell lines that express functionally active ENaC-like channels

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Ion channels are involved in the regulation of many physiological and pathophysiological functions in the cells and tissues. Epithelial sodium channels (ENaC) could affect the development of cancer processes, including proliferation, migration, regulation of cell volume and membrane potential. In previous work, we showed that extracellular serine protease trypsin activates amiloride-insensitive ENaC-like channels in human myeloid leukemia K562 cells; the expression of four (alpha-, beta-, gamma- and delta-) ENaC subunits was determined. The aim of this work was to study the role of serine proteases of various specificities in the regulation of ENaC-like channels activity and related processes in leukemia cell lines.

In single-channel whole-cell patch-clamp experiments, direct activation of ENaC-like channels was found after extracellular application of serine proteases alpha-chymotrypsin (5 μg/ml) or plasmin (10 μg/ml) in leukemia K562 and U937 cell lines. The involvement of proteolytic cleavage of these serine proteases in the stimulation of ENaC-like channels in K562 cells was confirmed using specific protease inhibitors (SBTI or alpha2-antiplasmin, respectively). In vitro migration experiments showed for the first time that proteolytic activity of alpha-chymotrypsin and plasmin significantly inhibited migration and invasion ability of K562 cells. Then, we determined the similar effect of proteases on motility processes of different human leukemia cell lines (U937, HL-60, KG-1), expressing functionally active ENaC-like channels. However, no effect of alpha-chymotrypsin on the migration of MOLT-4 leukemia cells, which do not express ENaC-like channels, was detected. It could be concluded that extracellular serine proteases can be considered as universal regulators of sodium handling and migration ability of human leukemia cell lines through ENaC-like channels.

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Sex differences in age-related changes in functional activity and expression of ETAR and ETBR in the aorta and myocardium of rats

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Age-related cardiovascular diseases, particularly hypertension, are linked to the development of endothelial dysfunction in the blood vessels. A significant amount of attention has been paid to the investigation of age-associated changes in endothelial-mediated vasodilation, which is regulated by nitric oxide. Nevertheless, endothelial cells also generate powerful vasoconstrictor peptides, the most significant of which is endothelin-1 (ET-1). The aim of this study was to evaluate the sex differences in age-related changes in the functional activity and expression levels of endothelin receptor subtypes - ETA and ETB - in the aorta and myocardium of rats. Materials and methods: The strength of aortic contractions was measured isometrically, and gene expression levels were assessed using PCR analysis. Results: It was observed that the sensitivity of young male aortas to ET-1 stimulation was significantly greater than that of young females. In the process of aging, in contrast, the contractile response of the aorta to the effects of ET-1 significantly increases in female rats, while the vascular response in males remains unchanged. At the same time, in both sexes, there is a 1.5-fold decrease in the expression of vasoconstrictor ETAR genes with age in the aorta. In older female rats, there is a significant reduction in the levels of mRNAs that mediate the vasodilatory effects of ET-1. Conversely, in male rats, ETBR expression increases by 1.5 times with age. Sex differences were also revealed, suggest a shift in the balance between ETA and ETB receptor expression in different parts of the aged rat heart. Conclusion: The study has identified features of age-related changes in the activity and expression of endothelin receptors in the vascular and cardiac tissues of rats, which may influence the risk of developing hypertension and myocardial dysfunction, depending on the gender of the animals.

Sigma-1 receptor antagonists suppress Ca^{2+} responses induced by immunomodulators Glutoxim and Molixan in macrophages

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Pharmacological analogues of oxidized glutathione (GSSG), disulfide-containing drugs Glutoxim® (disodium salt of GSSG with d-metal at nanoconcentration, «PHARMA-VAM», St. Petersburg) and Molixan® (complex of Glutoxim with inosine nucleoside), have found clinical application as broad-spectrum immunomodulators and cytoprotectors in the complex therapy of bacterial, viral and oncological diseases. Clinical studies have shown that Molixan is effective: in the prevention and treatment of COVID-19 infection; leads to a more rapid regression of the disease severity to a milder form; in preventing the development of new diseases and in slowing down the aging processes. Earlier, we have discovered for the first time that Glutoxim and Molixan induce intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-operated Ca^{2+} entry in rat peritoneal macrophages [1]. Sigma-1 receptors are ubiquitous multifunctional ligand-operated molecular chaperones in the endoplasmic reticulum membrane with a unique history, structure, and pharmacological profile. They bind ligands of different chemical structure and pharmacological action and modulate a wide range of cellular processes in health and disease, including Ca^{2+} signaling [2]. Using Fura-2AM microfluorimetry we have shown for the first time that two structurally distinct sigma-1 receptor selective antagonists – typical neuroleptic haloperidol (butyrophenone derivative) and compound BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine) - significantly suppress both Ca^{2+} mobilization from Ca^{2+} stores and subsequent store-operated Ca^{2+} entry, induced by Glutoxim or Molixan. The data obtained invite us to suggest the involvement of sigma-1 receptors in the complex signaling cascade triggered by Glutoxim or Molixan and leading to $[Ca^{2+}]_i$ increase in macrophages as well as in the modulation of store-operated Ca^{2+} entry in macrophages.

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Single-point mutations prediction using ProteinMPNN

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Protein engineering is a powerful approach providing notable potential benefits to various fields such as biotechnology and medicine. Recently, machine learning models have been more widely adopted in various scientific challenges, including protein engineering. One such model is ProteinMPNN, a transformer trained to solve the reverse folding problem: predicting protein sequence based on structure [1]. While no claims about increased stability of redesigned molecules were originally made by the developers, over time the model was used to successfully redesign numerous proteins, some showing increased stability and function [2], [3]. We have developed an algorithm to predict potentially beneficial single-point mutations using ProteinMPNN. A version of this algorithm allows for comparison between ProteinMPNN output and publicly available single-point mutations datasets for certain proteins. For 4 different proteins, relation between the thermodynamic effect of a mutation and ProteinMPNN prediction log probability was evaluated, possibly indicating correlation. Overall, the presented method is versatile and useful in evaluating the quality of the underlying model. With more and more similar models coming out for public use, it becomes increasingly important to compare them with simple common benchmarks. Single-point mutation prediction might be one such benchmark with easily accessible experimental data and clear interpretation.

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Singlet oxygen is protective against β-amyloid-induced neurotoxicity

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The main component of the senile plaques in Alzheimer's Disease is shown to be neurotoxic in oligomeric form. The mechanism of β-amyloid toxicity includes abnormal calcium signaling, induction of oxidative stress and mitochondrial dysfunction induced by oxidation of DNA and activation of the DNA repair enzyme poly(ADP-ribose)-polymerase (PARP), which consumes nicotinamide adenine dinucleotide that reduces substrate availability [1,2]. A 1267 nm laser shown to be able to generate singlet oxygen in cells and tissues and activate ATP production. We studied the effect of laser-induced singlet oxygen on β-amyloid toxicity in primary co-culture neurons and astrocytes.

We have found that laser-induced singlet oxygen reduced the effect of β-amyloid on NADH depletion, decreasedmitochondrial membrane potential and protected cell agains cell death induced by full peptide βA-1-42 or short βA25-25. This protective effect could be due to the reduction of the oligomerization of βA by singlet oxygen which we found on the full peptide 1-42.

Thus, laser-induced singlet oxygen is protective against βA-induced toxicity.

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SIRT6: a master-regulator of mitochondrial transcription and metabolism in the mouse brain

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NAD^+-consuming deacetylase SIRT6 is widely expressed in most mammalian tissues and plays a critical role in maintaining genome and epigenome stability by regulating key cellular signaling and metabolic pathways, protecting from DNA damage accumulation, telomere dysfunction and inflammation [1]. Due to these abilities, SIRT6 plays a significant role in brain function, particularly in the context of normal and pathological aging. Brain SIRT6 levels decline with both aging and Alzheimer's disease, and its complete inactivation has been previously associated with premature aging syndromes [2]. However, the extent and precise details of the regulatory mechanisms by which it impacts transcriptomics and metabolomics programs in the aging and diseased brain require further investigation.

To build a bridge from reduced SIRT6 activity to age-related brain pathology, we generated murine brain-specific SIRT6-deficient and control models and performed integrative multi-omics analysis of the obtained RNA-seq and LC-MS profiles. Our results demonstrate that the absence of SIRT6 in the mouse brain primarily disrupts mitochondrial function and manifests as a global reduction in mitochondria-related gene expression, metabolic alterations in tricarboxylic acid (TCA) cycle and impaired mitochondrial biogenesis. Interestingly, down-regulated differentially expressed mitochondrial genes are predominantly localized to the mitochondrial inner membrane (MIM) and involved in ATP synthesis. In line with these findings, we observe expression level reduction of key genes associated with mitochondrial protein import machinery, belonging to TIM (e.g. *Timm23*, *Timm21*, *Timm10*, *Timm9*) and TOM (e.g *Tomm22*, *Tomm7*, *Tomm5*) translocation complexes. Finally, Gene Set Enrichment Analysis reveals a number of neurodegenerative disease pathways, including Alzheimer's and Parkinson's diseases, significantly affected by SIRT6 loss, with more than 60% of core enrichment genes related to mitochondrial functions. Thus, our findings emphasize a vital role of SIRT6 in maintaining brain health by protecting it from oxidative stress and age-related diseases.

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Site-specific spin labeling of GPCR in native membrane

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Electron paramagnetic resonance (EPR) spectroscopy is a method that allows researchers to investigate the structure and conformational dynamics of either soluble or membrane proteins. A perspective object that can be studied using EPR are G-protein-coupled receptors (GPCRs), which serve as targets for a variety of pharmacological drugs [1]. One of the stages of sample preparation for the EPR experiment is site-specific labeling of the protein with a spin label. Such labeling occurs because of the formation of disulfide bonds between the thiol groups of the label and the protein's cysteines. Often researchers perform this treatment on purified protein, while it is in micelles [2], but this can lead to labeling of transmembrane cysteine residues, what in most of the cases is undesirable. We treated GPCR with spin label MTSL both on purified protein in micelles and while it was in native membranes. We showed that treatment of the purified receptor while it is in detergent micelles with the MTSL results in labeling of transmembrane cysteines. At the same, while labeling GPCR in native membrane, such effect is not observed.

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Structually modified DARPins as a basis to developing diagnostics radiopharmaceuticals

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Search and development of new molecules as structural components of radiopharmaceuticals selectively labeling tumor sites with increased concentration of molecular targets is one of the most urgent challenges of all modern scientific research in radionuclide diagnostics and therapy of oncological diseases. Here we present the results of work on the creation of series structurally modified targeted proteins based on DARPins (Designed Ankyrin Repeat Protein) G3, EC1, E01 adapted for selective labelling by short-lived metal radionuclides. For this purpose, different amino acid tags were added to the N'- and C'- termini of DARPins molecules to enable the last ones binding either directly with short-lived metal radionuclides (HHHHHH, HEHEHE), or indirectly, via various chelators attaching to proteins through single reactive sulfhydryl groups in tags (EEEC, GGGC). An unified and flexible method of protein expression and purification had been developed, that allowed to obtain the high purity proteins suitable for clinical application. Most of the modified DARPins had been comprehensively characterized by complex of modern physicochemical methods, including mass spectrometry, plasmon resonance, Ligand Tracer and etc. The innovative radiopharmaceutical "[99mTc]-(He)3G3" based on the modification of DARPin $(He)_3$-G3 had successfully passed phase I clinical trials. It was shown, that "[99mTc]-$(HE)_3$-G3" is safe providing a low radiation load dose on the human body and well tolerated by patients [1].

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Structural and dynamical study of interaction between REGN10987 Fab and S-proteins of Delta and Omicron SARS-CoV-2

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The use of neutralizing antibodies is one of the approaches to COVID-19 treatment. Understanding how neutralizing antibodies recognize different SARS-CoV-2 strains is the key to development of drugs and vaccines against coronavirus infection. In this work we present structure of the full-length trimer of the S-protein of the delta variant of SARS-CoV-2 in complex with a recombinant analogue of the REGN10987 antibody's Fab. The structure was obtained by cryo-electron microscopy at a resolution of 2.3 Å. The binding regions of the receptor-binding domains of the S protein (RBD) with the Fab fragments of the REGN10987 antibody (RBD/Fab) were obtained at a resolution of 3.2–3.4 Å. Two RBDs of the S protein were in the 'down' state, while the third RBD was in the 'up' state. In both RBD conformations, binding of the Fab fragment was observed in the receptor binding motif (RBM) region, which blocks recognition and subsequent binding of the S protein to the ACE2 receptor. The use of molecular dynamics allowed us to explain the observed differences in the interaction of Fab with Delta and Omicron RBD variants. Thus, this study provides insight into the contribution of known mutations in the RBD of both the omicron SARS-CoV-2 variant and other strains to the ability of the virus to evade binding to the REGN10987 antibody.

Structural and functional characteristics of proton half-channels in bacterial F_oF_1-ATP synthase revealed by molecular dynamics simulations

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Adenosine triphosphate (ATP) is essential for various biochemical processes. In the cell, ATP is produced by a universal molecular motor known as F_oF_1-ATP synthase, utilizing the energy from a proton electrochemical gradient. Although recent structural studies have improved our understanding of the proton half-channels locations, many questions remain unanswered. A major unresolved issue is how proton transfer is coupled with ATP synthesis.

The study focuses on examining the structure of half-channels and analyzing potential proton movement areas. This is achieved through molecular dynamics simulations of the membrane F_o factor of ATP synthase from *E. coli* embedded in three types of lipid bilayer that represent different biological cell states.

The structural and functional characteristics of the inlet and outlet half-channels were obtained. A comprehensive set of spatial positions of polar amino acid residues and water molecules that significantly influence proton transport was revealed, along with the localization of three conserved structural clusters of water molecules (W1-W3). Additionally, stable spatial positions of significant amino acid side chains in the *a*-subunit were detected [1]. The impact of cardiolipin content in membrane on the hydration of half-channels was examined [2]. A mutation analysis was performed to investigate the role of functional elements of the protein structure in the proton transport [3]. The findings from molecular dynamics simulations of the mutant protein indicated that substitutions of some conserved polar amino acids resulted in dramatic alterations in the occupancy and capacity of the structural water clusters (W1-W3), up to their complete disappearance and consequently to disruption of the proton transport chain.

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Structural characterization of the *Gallus gallus* 80S ribosome

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Recent decades have seen major advancements in understanding ribosome structure and translation. However, there remain some groups of organisms whose ribosomes have not been studied in detail, but investigation of the structural organization of these ribosomes can provide insight into the evolution of the translation apparatus and have practical implications.

The first studies on the avian ribosome date back to the 1970-1980s, but due to methodological limitations they were never completed [1,2]. In our work, we present the high-resolution structure of the *Gallus gallus* 80S ribosome derived from cold-treated chicken embryos. The 80S ribosomes containing elongation factor eEF2 with GDP (eEF2), SERPINE1 mRNA binding protein 1 (SERBP1), and tRNA in the P/E position is commonly referred to as translationally inactive. This class of the ribosomes shows common features with complexes already studied in mammals [3,4].

Modeling of the expansion segments of *G. gallus* 28S ribosomal RNA showed specific features in their structural organization and allowed us to describe areas where differences between mammalian and avian ribosomes occur. The resulting information could provide insights into the evolution of these expansion segments. This study of the avian ribosome forms a solid molecular background for future structural and functional studies of translation and its regulation in birds, as well as filling the evolutionary gap in the ribosome structure between lower eukaryotes and mammals.

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Structural features of the copper-binding protein CopC from a haloalkaliphilic bacterium

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The family of periplasmic copper-binding proteins CopC plays an important role in copper homeostasis in cells of gram-negative bacteria. In the genome of the haloalkaliphilic bacterium *Thioalkalivibrio paradoxus*, the CopC gene is located near the gene encoding the copper-containing enzyme - thiocyanate dehydrogenase (TcDH) [1,2]. We suggested that CopC could participate in the incorporation of copper ions into the active center of TcDH.

We obtained the structure of CopC from *Tv. paradoxus* (tpCopC), which had a number of differences from homologous proteins of the family. tpCopC is a dimer, while homologous proteins are monomers. The sequence of tpCopC contains an insert of 28 amino acids that form an α-helix and loops. We suggested that this insert could involved in the formation of intermolecular contacts during the interaction of tpCopC with other proteins.

We showed that copper ions can be carried from the copper-binding motif (His-brace) of CopC to the active site of TcDH *in vitro*. Thus, this study reveals new structural and functional features of the CopC family.

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Structural investigation of histone-like HU proteins and their complexes with DNA oligonucleotides by SAXS and complementary methods

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The structure and functions of the bacterial nucleoid are controlled by nucleoid-associated proteins (NAPs). The most abundant NAPs in dividing bacterial cells are histone-like HU proteins. Previously, structural ensembles of HU protein conformations from pathogenic mycoplasmas *Spiroplasma melliferum* and *Mycoplasma gallisepticum* were obtained by NMR spectroscopy. A structural study of these mycoplasma proteins was carried out using small-angle X-ray scattering (SAXS). The occurrences of individual conformations from the ensemble obtained by the NMR method were estimated based on the scattering data from HU protein solutions [1]. In particular, an approach based on the characterization of equilibrium mixtures in terms of volume fractions of their components was utilized. In addition, a structural study of complexes of these mycoplasma proteins with DNA oligonucleotides was carried out using such complementary methods as molecular dynamics (MD) and SAXS [2]. Structural MD models were screened to determine the best fit to the solution scattering data of the corresponding complexes. The overall shapes of the complexes were also independently reconstructed using multiphase *ab initio* bead modelling. The results shed light on the mechanisms of interaction of HU proteins with DNA, thereby improving the understanding of their functioning.

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Structural modeling of lipid bilayers using SAXS curves from liposomes

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Biological membranes (BMs), consisting of a lipid bilayer and peripheral or integral proteins, are intensively studied using numerous structural techniques, including small-angle X-ray scattering (SAXS). Various mimetics are often used to investigate exact features of this complex system. Among them, liposomes and micelles are the most popular due to their relative availability and ease of preparation. However, the use of SAXS for their study is a non-trivial task. The presence of regions of negative electron density in both the lipid bilayer and the micelles makes it impossible to reconstruct the structure from small-angle scattering curves in a homogeneous approximation. Therefore, special approaches are required. Recently, two related studies have described new algorithms for determining the structures of liposomes and micelles from SAXS data [1, 2]. In combination, they make it possible to describe in detail the structures of the mimetics, in particular their interactions with proteins, from the SAXS data [3]. In the present work, an additional multiphase approach [4] is described. This approach allowed an ab initio modeling of a lipid bilayer using simultaneously dummy atoms (beads) of different types (with different contrasts), which correspond to lipid or detergent hydrophobic tails and their polar heads. This modeling allows visualization of lipid bilayers and micelles, their heterogeneities and coacervation processes during self-assembly.

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Structural studies of the C-terminal peptide of the gram-negative targeting endolysin LysSi3 with broad bactericidal activity

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Antimicrobial resistance (AMR) is alarmingly increasing in medicine and is one of the major concerns of healthcare today. Due to this fact there is a growing interest in bacteriolytic enzymes that act to lyse bacterial cell wall especially against pathogens with AMR. These types of enzyme include bacteriophage-encoded endolysins which degrade the peptidoglycan (PG) cell wall polymer [1]. Endolysins are synthesized in the cytoplasm of infected bacteria at the final stage of their lytic development for specific degradation the PG polymers of the host bacteria. It leads to abrupt osmotic cell lysis and subsequent release of progeny phages. This phenomenon led to a growing number of studies aimed at using this class of enzymes as antibacterial agents. For endolysins targeting Gram-negative bacteria the specific activity against a broad host range is a known phenomenon, however, the molecular mechanisms specifying their broad spectrum of action are obscure. Moreover, phenomena of from-inside lytic action is widely documented for these enzymes, however, the mechanism of the outer membrane crossing is not clear.

The LysSi3 is a peptidoglycan hydrolyzing, lysozyme-like enzyme with predicted muramidase activity (GH24 family) and broad bactericidal activity against ESKAPE pathogens. In this work, the structures LysSi3 and its point mutant W120A in the C-terminal peptide with predicted antimicrobial activity were obtained using X-ray crystallography. The C-terminal peptide (110-154 residues) of the enzyme contains two α-helices and two β-sheets (a characteristic structure for antimicrobial peptides). The role of this peptide in the mechanism of LysSi3 action was assessed.

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Structural studies of translation regulation in prokaryotic and eukaryotic pathogenic microorganisms

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The control of pathogenic micro-organisms is the most important public health issue in the world. The development of new, highly selective antimicrobial agents requires an atomic-level understanding of the molecular mechanisms of interaction between inhibitors and their targets. One of the major targets of antibiotics is the ribosome of the bacterial cell. Eukaryotic ribosome inhibitors are no less interesting than antibiotics against bacteria because translation is the Achilles' heel of virus-infected or malignantly transformed cells: their protein biosynthesis needs far exceed those of healthy cells, making them particularly sensitive to translation inhibitors. Another public health concern is fungal infections.

By integrative structural biology approaches we investigated the general principles of inhibition of protein biosynthesis on ribosomes, mechanisms of antibiotic resistance, and showed the structural determinants that determine the difference in inhibition between bacterial and eukaryotic ribosomes [1-4]. These and subsequent structures of the functional ribosome provide the basis for a better understanding of how the structure of the ribosome determines its function and opens the door to the development of new antimicrobial drugs.

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Structural study of the carotenoid-xanthorhodopsin complex

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Proteorhodopsins (PRs) represent a diverse class of light-driven membrane proteins essential for the photosensory systems of over 50% of oceanic prokaryotes. These proteins use light energy through retinal-induced isomerization, triggering structural alterations essential for their function. Xanthorhodopsin (XR), a class of PR family, was discovered in 2005. The XRs exhibit a unique feature by using two chromophores for light absorption: a retinal moiety covalently bound within the protein and a carotenoid externally attached to transfer energy to the retinal molecule. The addition of a carotenoid significantly broadens the absorption spectrum of rhodopsin, improving light capture efficiency. However, the mechanism of carotenoid binding to XRs remains unclear.

In this study, we researched a rhodopsin belonging to the xanthorhodopsin class. Using X-ray crystallography and cryo-electron microscopy techniques, we obtained high-quality structural data in two distinct conformations: carotenoid-bound and non-carotenoid-bound states. Both obtained structures have a pentameric form. The progress in structure determination enabled a comprehensive analysis of carotenoid interactions with our rhodopsin, facilitating the unambiguous characterization of the binding pocket and elucidation of structural changes induced upon carotenoid binding. Notably, our study marks the first successful determination of a carotenoid-bound structure of this particular rhodopsin, shedding light on the complex mechanisms underpinning carotenoid binding in XRs.

Study of Complexation of AED peptide and Lysine Based Dendrimer with HisArg Spacers

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Previously, the behavior of second-generation lysine dendrimers with charged double lysine and arginine (LysLys, ArgArg) spacers between all branching points, as well as hydrophobic (LeuLeu, AlaAla, GlyGly) spacers and pH-dependent (HisHis) spacers was studied [1-7]. Their complexes with several drug peptide molecules (including AEDG, AED and GED) were also studied for the delivery of these peptides (see, for example [7]). Here we study interaction of AED peptide (AlaGluAsp) molecules with a dendrimer containing a histidine-arginine spacers (HisArg) where amino acid residues are different, and the charge of the His residue depends on pH. We performed molecular dynamics simulations [6,7] of complexation of 16 AED molecules with the dendrimer at two different pH values: a) pH > 7 with fully uncharged histidines and b) pH < 5 with fully protonated histidines. It was found that the dendrimer with protonated histidines can carry a larger number of AED tetrapeptide molecules.

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Study of the binding regions of human AMPA receptors peptide allosteric modulators by molecular modeling methods

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Positive allosteric modulators (PAM) are promising agents for fine regulation of membrane receptor activity. AMPA receptors are heterotetrameric ionotropic membrane proteins binding glutamic acid. They are an integral part of synaptic transmission and synaptic plasticity (e.g. long-term potentiation). AMPA receptors contain 4 domains in each subunit. The isosteric binding site is located in the GluR domain, which can be of four types (GluA1—GluA4), structural changes in which induce the opening of an ion channel. This domain is also ligand-binding (LBD) and presumably contains allosteric modulation sites [1].

In this work, the tetrapeptides Trp-Pro-Pro-Trp (WPPW) and Boc-Trp-Pro-Pro-Trp (BWPPW) were studied, which are peptidomimetics of the previously developed positive allosteric modulator PAM43 [2]. Peptides by their nature have a labile structure that allows them to take on various conformations. That is why peptide-based drugs are able to more gently engage the target and safely metabolize. The surface of the LBD AMPA receptor in the closed and open state with preservation of sterically important domains was studied by molecular docking using the AutoDock4 Lamarckian genetic algorithm (LGA) [3]. This approach made it possible to identify areas of potential allosteric modulation for peptides, taking into account structural changes associated with the presence of an orthosteric ligand in the system. Evaluation of the clustering of such results made it possible to specify the localization of the molecule despite the free rotation of the peptide molecule chain, in comparison with PAM43, for which simpler estimation algorithms (Vina) are used. This result shows that the genetic algorithm is most suitable for docking molecules of a peptide nature.

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Study of the domain architecture and three-dimensional structure of histidine kinases in Green Algae

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Histidine kinases (HKs) represent a vast family of proteins predominantly involved in signal transduction mechanisms, enabling cells to detect and respond to a wide array of intercellular and intracellular stimuli [1]. While their structural characteristics and distribution are well-documented in prokaryotes, data on eukaryotic histidine kinases remain incomplete, despite the existence of several phylogenetic studies [2]. Of particular interest are the histidine kinase rhodopsins (HKRs), identified in *Green Algae*, whose biological functions are not yet fully understood [3].

Green Algae, a group of autotrophic eukaryotes, play a crucial role in ecosystems by providing habitat and food for other organisms, contributing to nutrient cycling, and enhancing oxygen levels in aquatic environments through photosynthesis. The ability to perceive and respond to light is essential for the photosynthetic activity of Green Algae. While the mechanisms underlying these processes have been partially elucidated [4], further research is necessary.

The primary objective of this study was to conduct a bioinformatics analysis and to investigate the structural organization of HKs in *Green Algae*, focusing on the domains present, in order to infer their potential functions. Amino acid sequences were retrieved from the Uniprot database using the query "txid1148[Organism] AND (Histidine[All Fields] AND Kinase[All Fields])" as well as those classified under the EC nomenclature as HKs (2.7.13.-). Redundant sequences were removed, and the remaining sequences were analyzed using InterProScan-5.66-98.0 [5] to identify the presence of domains within the Pfam collection. Proteins were subsequently filtered to exclude sequences that lacked any domains characteristic of HKs, thereby minimizing the inclusion of erroneous entries. This process yielded 458 proteins that are likely HKs.

Among these proteins, two distinct subgroups were identified. The first subgroup, termed hybrid histidine kinases, includes proteins that, in addition to the histidine kinase domain (HK domain), also possess a receiver (REC) domain. A total of 269 such proteins were identified, constituting 58% of all HKs analyzed. The second subgroup, referred to as histidine kinase rhodopsins (HKRs), comprises hybrid histidine kinases that begin with a domain homologous to rhodopsin (Rhd). This group includes 97 proteins, representing more than a quarter of the HKs studied. We hypothesize that in this subgroup, the bacteriorhodopsin-like segment functions as a sensor for external signals, which are subsequently relayed through the conserved histidine residue to the response regulator. The most common domains and domain architectures were identified for both subgroups. Notably, more than a quarter of HKRs contain two REC domains rather than one.

For the HKRs, three-dimensional protein structures were predicted using AlphaFold3 [6], which in most cases corroborated the domain identifications.

However, in several instances, additional domains were detected that had not been identified by InterProScan. Further analysis using FoldSeek suggested that these could represent secondary REC domains, indicating that the Rhd-HK domain-REC-REC architecture may be even more prevalent than previously thought.

In conclusion, the identification of rhodopsin-like domains in a significant proportion of histidine kinases in photosynthetic Green Algae may imply an important functional role for proteins with this architecture in these microorganisms. Additionally, the presence of two REC domains may be a characteristic feature of these proteins, warranting further investigation.

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Study of the influence of 24-hydroxycholesterol level on short-term memory and the development of epilepsy in mice

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Oxysterols are products of cholesterol oxidation and play a significant role in a number of biological processes. It has been demonstrated that 24-hydroxycholesterol (24-OHC) has the ability to modulate neuronal functions, with potential relevance to hyperexcitability underlying seizures. The aim of the present study was to investigate the role of low concentration of 24-OHC in brain on pentylenetetrazole (PTZ)-kindling in mice. The occurrence and progressive development of seizures were induced by repeated systemic administration of PTZ at a subconvulsive dose. The mice treated with low-dose efavirenz, an allosteric activator of CYP46A1 which converts cholesterol into 24-hydroxycholesterol of with inhibitor of CYP46A1 enzyme - voriconazole. We estimated the development of PTZ-induced seizures by the Racine scale and short-term by T-maze test. Mice with high brain concentration of 24-OHC exhibited shorter latency to PTZ-induced seizure, increased seizure intensity and compared to control. In the voriconazole group there were an apparent improvement of the epileptic behavior and no differency in the epileptic latency and duration of PTZ-induced seizures. T-maze test did not reveal any significant differences between the two groups and the control. Our findings suggest that in the PTZ-kindling mouse model of epilepsy, depending on the level of 24-hydroxycholesterol in the brain, both anticonvulsant and proconvulsant effects can develop.

Study of the Influence of New Cardioprotective Compound Di(3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridinium) Salt 2-(nitrooxy) Butanedioic Acid (B6-NO) on Ion Channels of Cardiomyocytes

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Currently, the study of the occurrence of arrhythmias and ways to combat them remains a very relevant direction. One of the causes of arrhythmias is myocardial ischemia. Ischemia often leads to oxidative stress, during which free radicals oxidize the lipid membranes of cardiomyocytes. Thus, by damaging the membranes, oxidative stress leads to cell hypoxia and consequently to myocardial 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. Compounds with antioxidant activity can be used for the prevention of diseases associated with oxidative stress. Such compounds are synthesized at the Institute of Problems of Chemical Physics (IPCP) of the Russian Academy of Sciences. Di(3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridinium) salt 2-(nitrooxy) butanedioic acid (B6-NO) is such compound [1], which is a derivative of pyridoxine. B6-NO combines both antioxidant properties and NO-donor activity. It chelates iron ions by 94%, indicating the ability of B6-NO to block the Fenton reaction. Vitamin B6 (also known as pyridoxine) itself is a coenzyme involved in more than 100 metabolic reactions of amino acids, glucose, lipids, and DNA.

The excitation of the heart is regulated by ion channels. Therefore, this study aimed to investigate the influence of a new antioxidant compound, di(3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridinium) salt 2-(nitrooxy) butanedioic acid (B6-NO) on voltage-gated ion channels of cardiomyocytes, which play a crucial role in action potential formation. The study focused on voltage-gated fast sodium channels (Nav1.5).

The objective of the research was to examine the effect of the compound on the function of ion channels and determine the concentrations at which it exerts its influence. The study was conducted using the electrophysiological patch-clamp technique in the "perforated whole-cell" configuration on neonatal cardiomyocytes isolated from newborn rats.

It was found that di(3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridinium) salt of 2-(nitrooxy) butanedioic acid (B6-NO), has a suppression effect on the function of fast sodium channel Nav1.5, which started only at a concentration of 100 μM. The compound has a 30% suppression effect on the fast sodium channel Nav1.5 at a concentration of 100 μM, and 50 % suppression effect at a concentration of 200 μM.

However, an interesting fact is the bell-shaped dependence of the activity of the antioxidant B6-NO. Thus, the maximum effect of inhibiting lipid peroxidation is observed in the range of 5 μM to 50 μM [2], with no effect observed at lower or higher concentrations. Presumably, the antioxidant properties of B6-NO are manifested specifically in the initiation of lipid peroxidation by Fe^{2+} ions, which is likely associated with a specific mechanism of action of the compound on the Fenton reaction. The compound does not affect the function of fast sodium channels Nav1.5 at concentrations in which it exhibits the anti-oxidant effect.

Research on the effect of the compound on voltage-gated potassium ion channels and calcium channels is ongoing.

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Study of the Mechanism of Interaction Between the major alder allergen Aln g 1 and Respiratory Epithelium

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Allergy is a global health problem with a rapidly increasing prevalence but still lacking pathogenetic knowledge or optimal treatment. Homologs of Bet v 1 constitute one of the key groups of allergens causing spring pollinosis. This study focuses on the Aln g 1 allergen detected in European alder pollen, which causes allergic reactions of varying severity. We developed methods to produce recombinant Aln g 1 and its mutant D27A/L30A, which has reduced allergic properties, using the expression vector pET-His8 in *E. coli* BL21(DE3) cells. The ability of the proteins to bind hydrophobic ligands was confirmed. A real-time PCR method was applied to evaluate the effects of Aln g 1 on Calu-3 epithelial cells. The results of ΔΔCq analysis showed that Aln g 1 induced the expression of both pro- and anti-inflammatory cytokines (IL-33, TSLP, IL-1β, IL-13, IL-8), indicating its ability to induce immune system sensitization independently of Bet v 1. The mechanism of this interaction, it is probably comparable to the effects observed for protease allergens that activate receptors such as TLR-4 and PAR-2. In addition, the leakage of contents for liposome of different lipid composition was studied by assessing the increase in fluorescence due to the release of the fluorescent dye calcein. When Aln g 1 and D27A/L30A interacted with POPC and POPG liposomes, both proteins were found to be able to degrade them, but with different efficiencies. In the case of liposomes mimicking the composition of pulmonary surfactant (DPPC/DOPC/DOPG/DOPE), proteins were shown to degrade them with 50% efficiency. The data obtained provide a prerequisite for studying the molecular mechanism that facilitates pollen penetration into the human respiratory epithelium.

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Study of the mechanisms of antitumor immunity and tumor resistance during preventive vaccination with peptide antigens to melanoma B16

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Antitumor vaccines are one of the most promising cancer therapies today. RNA vaccines showed the highest effectiveness and became a prototype for the creation of an RNA vaccine against COVID-19 showed the highest effectiveness. The effectiveness of peptide vaccines is significantly lower, but in some cases they are indispensable, for example for non-encoded antigens. In this work, we investigated the effectiveness of antitumor immunity generated by a peptide vaccine against murine melanoma B16. We applied a prophylactic vaccination scheme 3 and 1 week before the inoculation of tumor cells and used our previously created database of tumor-specific T-cell clones (TSTC) to analyze lymphocyte repertoires. The prophylactic vaccine significantly slowed tumor growth and also enhanced the antitumor effect of anti-CTLA4 antibody therapy. At the same time, the effect of vaccination and therapy was manifested only in some mice, while in others, tumor growth was not affected or even accelerated. When comparing responding and non-responding mice to vaccination and therapy, we found that in the draining lymph nodes (dLNs) the former had a significantly higher clonality of CD8+ T-lymphocytes, and the latter had a significantly higher percentage of CD4+ TSTC. Tumor resistance may be due to TSTC dysfunction in these mice or other mechanisms of tumor immune escape. To get insight into these mechanisms, repertoires of tumor infiltrating T-cell are currently being analyzed, as well as flow cytometry data on their phenotype and the representation of individual subpopulations in the tumor and dLNs.

Studying Ferritin Self-Assembly Using the Smoluchowski Coagulation Model

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Ferritin is a protein complex responsible for storing iron in various organisms. One of the most important properties of ferritin is the spontaneous formation of a spherical hollow protein globule consisting of 24 subunits, this process is called self-assembly [1]. The self-assembly plays a crucial role in the functioning of such proteins; however, its molecular mechanism has not been fully studied yet [1]. Understanding the self-assembly mechanism might be useful for studying different oligomeric states of ferritin, which may help in the development of recombinant ferritin-based vaccines or drug delivery systems.

In this work, we simulated the self-assembly process using the Smoluchowski coagulation model [2], which is widely utilized in material science. The model considers how particles collide and stick together over time, forming larger clusters. It includes a set of differential equations to represent the change in the molar concentration of particles of a given size due to binary collisions. Using the Euler method, we numerically solved the Smoluchowski coagulation differential equations for various coagulation kernels, taking into account the limitations imposed by ferritin self-assembly – namely, the termination of the aggregation process upon the adhesion of 24 subunits. Additionally, we extended this model to describe the coagulation of two types of subunits. Our results explain the process of ferritin self-assembly over time, providing detailed information about the distribution of oligomers.

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Supramolecular clusters as a structural basis of the mitochondrial bioenergetic system

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The presence of stable clusters (supercomplexes) of respiratory chain enzymes called respirasomes in mitochondria is now well-established. At the same time, the structural connection of respirasomes with up- and downstream metabolic stages – NAD^+ reducing complexes and ATP synthases, respectively – remained unknown. This work using cryo-EM techniques demonstrates the presence of large supramolecular clusters uniting all of these bioenergetics enzymes in rat heart mitochondria.

Cryoelectron tomography definitively shows that in the inner membrane of mitochondria, respirasomes are ordered and clustered with ATP synthase dimers on the folds of the inner membrane. These results, when analyzed in light of modern data about the presence of short-distance lateral proton transport along biological membranes, clearly show that the detected clusters provide efficient coupling of the proton pumps with the main proton consumer, ATP synthase. Furthermore, the interaction of ketoacid dehydrogenase complexes with complex I (part of the respirasome) is demonstrated. This contact allows NADH produced by ketoacid dehydrogenase complexes to be transmitted locally to the NADH dehydrogenase (complex I). The presence of such a connection eliminates the need for free diffusion in the crowded environment of the mitochondrial matrix. In this environment, the charged NADH molecule has an extremely low free path length, and its delivery may limit the performance of the whole system. The formation of clusters completely solves this problem.

These results confirm the presence of microcompartments in cardiac mitochondria, where local and directed transfer of intermediate metabolites occurs between the active centers of molecular enzyme machines. Furthermore, the work shows that these compartments are much larger and couple more enzymes than previously thought. This allows mitochondria to achieve high densities of useful enzymes without loss in the rate of metabolite transfer between them. Moreover, these clusters are based on non-covalent interactions, so their organization is regulated by osmotic volume regulation and by changes in the phase state of lipids, allowing rapid and flexible rearrangement of enzyme clusters in response to different external and internal signals.

Surface plasmon resonance as a method of drug discovery

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Surface Plasmon Resonance (SPR) analysis has become a method of choice in drug discovery pathways. It is crucial for initial hit finding by screening of potential ligands and fragment libraries. During hit validation and lead structure optimization phases, it provides data of drug-target interaction kinetics, affinity and specificity as well as thermodynamics parameters. It also can be useful for evaluation of the pharmacokinetics and pharmacodynamics of lead compounds [1]. In our studies, SPR technology performed deciding role in establishment of the mechanisms of low-weight compounds regulation of protein-protein interaction [2, 3], discovery of new natural CYP51A1 inhibitor [4], and other cases of studying the ligand-protein interaction and protein-protein interaction regulation by the potential ligands. These works were performed within the framework of the Program for Basic Research in the Russian Federation for a long-term period (2021-2030) (№122030100168-2).

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Synthesis of photocontrolled combined PDT drug based on chlorine transmembrane chemotherapeutic carrier

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Doxorubicin (DOX) belongs to the anthracycline class of drugs and is widely used in anticancer therapy. It is a broad-spectrum, DNA-tropic antitumor antibiotic. However, it has a number of limitations, with low bioavailability being one of them. To utilize DOX in lower doses, it is essential to develop a targeted carrier system [1]. In this study, we used a Chlorin e6 derivative to create a combined drug for photodynamic therapy (PDT), which is conjugated via a photocleavable o-Nitrobenzyl linker. This approach has the potential to improve DOX bioavailability, as the chlorin e6 derivative used in the study can be considered a nucleus-targeting photosensitizer. The amphiphilic nature of our conjugate is also expected to enhance the bioavailability of DOX. [2, 3]. As a linker molecule, we choose a derivative of o-Nitrobenzyl, which is known for its suitable conjugation sites and good cleavage rate [4]. The use of a photocleavable linker offers a wide range of applications in terms of photocontrolled spatial separation of therapeutic agents within cells. As a result, we have proposed a novel approach to overcome low bioavailability of DOX. We obtained a photocleavable linker and its conjugate with natural chlorin derivative. All molecular structures were confirmed by a number of physicochemical methods and linker cleavage was studied.

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Testing of lux-biosensors in expeditionary conditions on the territory of the northern seas: Barents, Kara and Laptev

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Lux-biosensors are bacterial cells containing *luxCDABE* genes under the control of stress-induced promoters [1,2]. The objective of this work was to test lux-biosensors under expeditionary conditions to study the soil and benthic inhabitants of the northern seas: the Barents, Kara, and Laptev seas. We used *E. coli* MG1655 strain cells transformed with plasmids pColD-*lux*, pOxyR-*lux*, pSoxS-*lux*, pSVRAF, pAlkA-*lux* as lux biosensors [1,3], as well as cells of *B. subtilis* 168 with plasmids pNKAlkA and pNKDinC [2]. The Ocean-0.25 dredger obtained samples of bottom sediments, separated the bottom inhabitants from the substrate by washing. To assess the content of toxicants in the tissues of arthropods, the liquid fraction after centrifugation was used. Bottom sediments were a fraction of watered silt, sometimes with inclusions of sand.

It is shown that bottom sediments do not interfere with activation of lux-biosensors by standard toxicants. Soil samples were taken from four stations with coordinates 70.53959/58.22493, 73.35283/59.64791, 75.80513/69.9099 and 76.0552/73.97658. A study of the toxicity of the obtained sediment samples with all employed biosensors showed the presence of an effect only on DNA alkylation (promoter of DNA glycosylase P*alkA*).

Among the benthic inhabitants, following amphipods were tested: *Byblis longicornis; Haploos sibirica; Acerdies latipes latipes; Paroediceros lynceus*, as well as sea cockroach - *Mesidothea sabiniva* and sea spider - *Pantopoda sp.* Three of the four amphipods influenced PalkA with an induction coefficient of 2; 2.4 and 2; for *M. sabiniva*, the induction coefficient was 4.14; *Pantopoda sp.* - the strongest effect, showing an induction coefficient of 4.38.

The results obtained indicate that the bottom sediments apparently contain substances with a pronounced carcinogenic effect capable of alkylating the nitrogenous bases of DNA. Benthic arthropods, especially those with a life cycle of several years or more, are more likely to accumulate these alkylating substances in their bodies, as are amphipods, for which such studies have already been conducted.

Expeditionary work, collection of soil and bottom inhabitants were carried out at the expense of the floating university (№ 075-03-2024-117). The design of biosensors is supported by RSF 22-14-00124, the applicability of biosensors for the food industry was evaluated through the FSMF-2023-0010 project.

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Testing SAXS applicability for detection of illumination-driven structural changes in the purple membranes from *H. salinarum*

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Rhodopsins are a key tool in optogenetic research [1]. To study the structural changes that occur in their photocycle using X-ray diffraction, cryo-trapping under laser illumination is often used to stabilize the intermediates [2]. However, this can lead to a dramatical deterioration in resolution. One possible reason is the large amplitude of structural changes. To estimate their scale, small angle X-ray scattering (SAXS) can be used. As a model object to test the method, we used the well-studied photosensitive proton pump bacteriorhodopsin from haloarchaea *Halobacterium salinarum* (*Hs*BR). Within the native cell membrane, *Hs*BR forms two-dimensional hexagonal crystals called purple membranes (PM). High-performance methods for obtaining PM have been developed, which makes it a convenient object for the tests [3].

We investigated the effect of illumination for PM with wild-type and mutated (E204Q) *Hs*BR. SAXS data were collected at BL19U2 at the SSRF [4]. The most intense diffraction peak positions, corresponding to the (1, 1) Miller index, were compared for the datasets obtained with and without laser illumination. Wild-type *Hs*BR data were found to exhibit a peak position shift $\Delta q \sim -10^{-4}$ Å^{-1} (~3% of the integrated peak width) upon laser illumination. The relative error of the Δq ($\varepsilon_{\Delta q}$) is ~15%. Peak position shift was also observed for *Hs*BR$_{E204Q}$ data, but the $\varepsilon_{\Delta q}$ was ~50-80% because of the initially lower sample concentration, making it impossible to compare the wild-type and mutant cases. Hence, similar upcoming experiments should focus on obtaining a better signal-to-noise ratio, using synchrotron radiation sources and high concentration samples.

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The comparison of functional and proliferative activity between hTERT-NK cells versus iCasp9-NK cells

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Cancer progression is often accompanied with the acquisition of functional energy and aging by NK cells. As a result, persistent herpesviruses such as CMV and EBV reactivate. As it is known that the catalytic subunit of telomerase encoded by hTERT gene may enhance functional and the proliferative activity, we aimed to elucidate if NK cells modified for sustained hTERT expression acquire these beneficial characteristics. We examined the proliferative and functional activities of hTERT-NK cells and combined observations with RNA-Seq results. iCasp9-modified NK cells were chosen for control. Despite the similar growth rates of hTERT-NK cells and iCasp9-NK cells, hTERT-NK cells characterized with an increase in the expression levels of cell cycle genes and a better proliferative activity in the third month after isolation. Increased degranulation upon K562 target cell recognition simultaneously with a higher expression level of granzyme B was observed for hTERT-NK cells. An increased level of IFNg was also noted in hTERT-NK cells. These results reveal that hTERT-NK cells obtain additional advantages due to the stable hTERT expression. hTERT-NK cells are likely to perform high levels of functional activity over long time periods that is commonly needed during cancer treatment to perform immune surveillance and minimize relapse rates.

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The Effect of a Knot on the Thermal Stability of Protein 2EFV

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The 2EFV protein, isolated from *Methanocaldococcus jannaschii* archaea, has a unique structure with a knot in the form of a right trefoil that encompasses half of its amino acid residues, is the shortest of the proteins in the form of a knot [1]. In the work was performed the construction of a soliton protein model [2], which has an accuracy compared to the crystalline structure of 2EFV protein less than 0.44 Å, which is less than carbon fluctuations. Research using field theory and Monte Carlo techniques [3] shows that the 2EFV protein knot significantly improves its thermal stability compared to a similar unfolded variant. Simulations at different temperatures have shown that the knot creates a topological barrier, making it difficult to unfold the protein at high temperatures [4]. These results highlight the importance of the knots in ensuring protein stability and suggest that the knot in the structure of 2EFV protein may be an adaptation to extreme environmental conditions.

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The effect of new azobenzene derivatives on voltage-gated sodium channel in rat neonatal cardiomyocytes

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Voltage-gated sodium channels play pivotal role in cardiomyocite function: due to change in membrane permeability to sodium ions depolarization takes place and heart contraction occurs [1]. Therefore, one of promising treatment strategies for arrhythmia is photopharmacology, in particular use of AzoTAB (azobenzene trimethylammonium bromide) derivatives. AzoTAB blocks voltage-gated sodium channels in trans-form and transforms into cis-form and releases channels, by being exposed to mild-UV light [2]. However, due to AzoTAB toxicity, finding effective and less toxic derivatives is necessary. We performed whole-cell patch clamp techniques on neaonatal rat cardiomyocites affected by PLA-101 and PLA-001 (two azobenzyne derivatives synthesized by our collegues from MIREA). We found out that adding PLA-001 decreases current amplitude by 70%, PLA-101 decreases by 50%. Our results showed that PLA-101 and PLA-001 significantly reduce sodium current amplitudes, therefore are promising agents for photopharmacology.

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The effect of α-hairpinin EcAMP3 on the cell-free translation system of Gram-positive bacteria

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Plant antimicrobial peptides (AMPs) have been extensively studied in the last decades as potential antibiotics against pathogenic microorganisms with multiple resistances (for example, methicillin-resistant *Staphylococcus aureus*, MRSA). AMPs are characterized by a low molecular weight, positive charge, and amphiphilic structure. The main families of plant AMPs include defensins, thionins, lipid-transfer proteins, α-hairpinins, hevein-like peptides, snakins, knottins, and cyclotides. The precise mechanism of action is not known for each family. One promising family of AMPs with a poorly described mechanism of action is α-hairpinins. Their unusual gene structures, simple structural conformations, and potent antimicrobial activities make these peptides of great interest for both biotechnology and basic research. The α-hairpinins demonstrate antifungal and antibacterial activity, together with peptides displaying trypsin inhibitory and ribosome-inactivating activity [1]. In this study, we focused on EcAMP3 from barnyard grass (*Echinochloa crus-galli*) seeds. This is a small peptide from the α-hairpinins family consisting of 35 amino acid residues (~ 4.4 kDa). Unlike other peptides of the family, EcAMP3 has been shown to suppress the growth of some Gram-negative (*P. syringae* and *E. carotovora*) and Gram-positive (*C. michiganensis*) bacteria [1]. The molecular targets of EcAMP3 remain unclear. The spatial structure of this peptide is also unknown. We used the coupled transcription-translation cell-free system, based on *S. aureus* cell extract to analyze the influence of EcAMP3 on protein synthesis in Gram-positive bacteria. EcAMP3 in the concentration range of 5-50 μg/ml approximately equally inhibits the biosynthesis of the reporter protein (by ~30%). Increasing the concentration leads to greater inhibition of the cell-free system. At a concentration of 100 μg/ml (~ 23 μM), inhibition of the system by ~50% (IC_{50}) occurs. At a concentration of 500 μg/ml (~ 114 μM), inhibition of protein synthesis by ~ 67% is observed. The obtained results open the possibility for further experiments with other cell-free systems and structural studies.

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The influence of copper ions on eumelanin hydration examined by mid-infrared spectroscopy

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Melanins are promising natural materials for applications in bioelectronic devices such as organic electrochemical transistors [1], memristors [2], supercapacitors [3], and pH sensors [4]. Similar to bioorganics the properties of melanins are highly influenced by water and naturally occurring d-elements. Being a critical part of tyrosinase synthesis machinery, copper ions are widely presented in biologically derived melanins. In the current study we examine water and Cu2+ ions effects on eumelanin by infrared spectroscopy for the first time. Our findings reveal that copper ions significantly alter the properties of both melanin and hydration layers. Notably, with an increase of copper content, the fraction of 4-hydrogen bonded water molecules also increases, rendering general water behavior more ice-like. Copper ions shift the comproportionation reaction between quinone and hydroquinone moieties towards the formation of semiquinone radicals even in the dry system. Also, we demonstrate that these ions tend to decrease the contribution of some signatures of aqueous proton cations. The general picture explains the mechanisms of conductivity inhibition induced in melanin by copper ions via both trapping electron density of semiquinone radicals in corresponding complexes and by decreasing the proton diffusion efficiency via the transformation of water into a more ice-like structure.

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The investigation of interaction between plastocyanin and photosystem I in plants using molecular modeling

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Plastocyanin is an electron carrier protein in the electron transport chain of cyanobacteria and chloroplasts, carrying out electron transfer from cytochrome f of cytochrome-b6f-complex to photosystem I. In this work, we study the formation of the protein-protein complex between plastocyanin and photosystem I of *Pisum sativum* using molecular modeling. We presume that at first proteins diffuse under the influence of random forces and electrostatic interactions. The result of the diffusion is the formation of the encounter complex, which can then turn into the final complex in which electron transfer is possible [1]. We modeled the formation of the encounter complex using the Brownian dynamics method in which protein molecules are represented as solid bodies, moving through a continuous solvent medium [2]. Then we used the structure of the encounter complex as the initial structure for modeling the formation of the final complex using molecular dynamics. We ran three simulations lasting 1 ns and in one case out of three the final complex was formed. We propose that in the first step, the encounter complex is formed due to the electrostatic interaction between plastocyanin and the PsaF subunit of photosystem I. Then plastocyanin can rotate around the electrostatic contact and form the final complex due to the hydrophobic interactions. We propose that the crucial amino acid residues for this process are Q663, Q666, and S662 of the PsaA subunit, L626, Q608, and S629 of the PsaB subunit and P36, P86, F35, and L12 of plastocyanin.

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The IP3-dependent signaling pathway is involved in the regulation of atrophic processes in skeletal muscles during their unloading

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During muscle unloading accumulation of macroergic phosphates and Ca^{2+} ions occur in muscle fibers. Soleus unloading reduces the resting membrane potential, which is accompanied by the accumulation of [Ca^{2+}] in the sarcoplasm. ATP that ends up in the extracellular space can induce a delayed slow release of Ca^{2+} in the myoplasm via the P2Y2-PLC-IP3. We tested the hypothesis about a relationship between changes in cellular [Ca^{2+}] concentration, ATP accumulation and activation of the IP3-dependent signaling pathway during soleus unloading. 3 experiments were carried out: 1. with inhibition of L-type channels by nifedipine and 2. Inhibition of IP3-kinase, 3. Inhibition of IP3 receptors during soleus unloading of Wistar rats. It was found that during soleus unloading: 1. blocking DHPR prevents an increase in ATP and [Ca^{2+}] levels in the myoplasm ($p<0.05$) 2. inhibition of PI3K slows down m. soleus atrophy, prevents ATP accumulation in it, expression of ubiquitin ligase MuRF1 and Ub, an increase in IP3 levels and IP3R expression, an increase in the expression of Ca-dependent mRNA CaN, prevents a decrease in pCaMKII (Ca-calmodulin kinase II) ($p<0.05$); affects the regulation of anabolic signaling markers in unloaded muscles (IRS1 and 4E-BP); 3. inhibition of IP3 receptors prevents a decrease in the cross-sectional area of the m. soleus of fast and slow muscle fibers, which is associated with the prevention of a decrease in ribosomal biogenesis and an increase in the expression of autophagy markers ULK-1 and IL-6 ($p<0.05$). A decrease in Ca-dependent pCaMKII was prevented. Conclusion: There is a relationship between Ca influx into the fiber, ATP content and soleus atrophy. IP3K and IP3R are involved in the regulation of signaling during skeletal muscle unloading.

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The protective role of mangiferin and trans-cinnamic acid against oxidative stress in wild type and AAK-2 mutant *Caenorhabditis elegans* strains

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During cellular metabolism, reactive oxygen species and nitrogen species are produced which inherently results in the oxidative damage of DNA and proteins. As a survival mechanism, these stressors modulate gene expression and various signalling pathways in *C. elegans*. The resistance to various forms of stress peaks in early adulthood and thereafter decreases with age. In *C. elegans*, the AMP-activated protein kinase isoform AAK-2 is involved in stress responses, germ cell cycle arrest amid dauer formation, and lifespan maintenance.

In this study, we evaluated the effect of mangiferin and trans-cinnamic acid (at 10, 50, 100 and 200 µM) on percentage-survival, lifespan and motility of hydrogen peroxide-treated *C. elegans*. The nematodes (wildtype N2-Bristol and AAK-2 mutant strains) were exposed to 3% H_2O_2 corresponding to 1:10 v/v for 2 hours. Thereafter, the H_2O_2-stressed nematodes were observed using ZEISS-LSM-900 Confocal Microscope for the computation of %survival, motility and continued to be monitored until the last nematode alive (maximum lifespan-ML).

The treatment of oxidatively challenged wildtype *C. elegans* with trans-cinnamic acid at 50, 100 and 200 µM significantly ($p<0.05$) increased the %survival and ML of nematodes compared to the control. Furthermore, mangiferin at all doses investigated significantly ($p<0.05$) improved the survival rate and extended the lifespan of H_2O_2-stressed N2 nematodes by 4-folds. In the AAK-2 mutant strain, the %survival and ML were significantly increased in the nematodes treated with 100 and 200 µM of trans-cinnamic acid in a dose-related manner. There was also a dose dependent increase in %survival ratio and ML of nematodes treated with mangiferin. The 200 µM of mangiferin notably prolonged the lifespan of nematodes for 5 days and restored motility function in a-third of the total nematode population.

Our findings suggests that trans-cinnamic acid and more importantly, mangiferin markedly enhanced lifespan and stress tolerance in *in vivo* model of hydrogen peroxide-treated *C. elegans*.

The role of BK channels in the effects of sodium butyrate on colon contractile activity in a mouse model of irritable bowel syndrome

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Irritable bowel syndrome (IBS) is a multifactorial disorder, characterised by altered intestinal motility, visceral hypersensitivity and dysfunction of the gut-brain axis. Recent studies show that bacterial fermentation is affected in IBS, and changes in gut bacteria and their metabolites, such as short chain fatty acids (SCFAs), may play a role in causing and maintaining IBS symptoms. It is worth noting that there is evidence of a potential role for large conductance calcium-activated potassium channels (BK channels) in the effect of SCFAs on IBS, but their exact role is still unclear.

A series of experiments were conducted on mice, during which a model of IBS was induced by intracolonic instillation of 1% acetic acid. The strength of proximal colon segments contraction of the mice was evaluated under isometric conditions.

The administration of sodium butyrate resulted in a decrease in the spontaneous contractile activity in both the control and IBS groups. However, the inhibitory effects observed in the IBS group were less. Furthermore, it was determined that when BK channels were blocked with paxillin at a concentration of 1 μM, the effects of sodium butyrate on the amplitude of spontaneous contractions were not observed. In contrast, in the IBS group, its inhibitory effects were preserved.

Therefore, BK channels may be responsible for mediating the effects of sodium butyrate on the amplitude of spontaneous contractions in the control group. Moreover, our findings indicate that the inhibitory effects of sodium butyrate on BK channels inhibition are maintained in the IBS group, which suggests a potential role for these mechanisms in the pathogenesis of this syndrome.

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The role of cristae regulatory proteins in mitochondrial dysfunction in an experimentally induced hyperthyroidism model

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Mitochondria are complex bimembrane organelles that play a key role in cell signaling, metabolism and energy. A distinctive feature of their structure is the characteristic folds of the inner membrane called cristae. Proper architecture and arrangement of the inner mitochondrial membrane is essential for efficient respiration, realization of apoptosis mechanisms and control of organelle quality in the cell. Despite growing evidence that the mitochondrial inner membrane can be reconstructed, cristae membranes have been considered static for the last seventy years. Only recently has it been shown that cristae are constantly undergoing rapid dynamic remodeling. Studies in yeast and other organisms have led to the identification of four major pathways whose interactions result in the formation of cristae membranes. These include formation of mitochondrial ATP synthase dimers; assembly of the mitochondrial contact site and cristae organizing system (MICOS); remodeling of the inner membrane by dynamin-related GTPase (Mgm1/OPA1) and modulation of mitochondrial lipid composition. MICOS, F1FO-ATP synthase, OPA1, and inner membrane phospholipids such as cardiolipin interact to organize the ultrastructure of the inner membrane and rearrange cristae in response to cellular needs. Functional alterations in these proteins or in the cardiolipin and phosphatidylethanolamine biosynthesis pathway lead to abnormal inner membrane architecture and impair mitochondrial function. Since abnormal inner membrane architecture is associated with various pathologies such as cardiomyopathies, neurodegeneration and diabetes, understanding the role of different molecules in cristae biogenesis and dynamics would shed light on selected aspects of pathophysiology. An important task is to identify the constituents of abnormal cristae and their involvement in the pathogenetic process (using hyperthyroidism as an example). We investigated changes in cristae structure and possible correlation of these abnormalities with the content and localization of MICOS proteins.

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The role of local cytoskeletal rearrangement in clustering and activation of platelet GPVI receptors

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Tyrosine receptor clustering upon cell activation plays a pivotal role in signal transduction. The clustering parameters depend on the type and structure of the ligands and on the local cell membrane microenvironment. It has recently become clear that the local cytoskeleton rearrangement upon cell activation is an important player in signal transduction. It is believed that the impact of cytoskeletal depolymerization is explained by an increase in the local mobility of receptors and, consequently, the frequency of their collisions.

The aim of this study was to develop a computational model of clustering of platelet GPVI receptors. The model takes into account membrane lipid microenvironment and the ability of activated receptor to initiate cytoskeletal reorganization. A stochastic two-dimensional spatial computer model was developed in Python 3.9. Validation was performed based on experimental data from [1].

Interestingly, about one third of receptors were in the clustered state independently of cell activation. Upon activation this fraction statistically significant ($p<0.001$) increased by 5% and the degree of cytoskeleton depolymerisation increased by 15%. Without activation-induced cytoskeleton depolimerization the fraction of receptors in clusters was around 10%. Without the cytoskeleton limitation on receptors diffusion, the fraction of receptors in clusters did not depend on activation.

To conclude, the impact of local cytoskeleton depolymerization for platelet GPVI receptor could be assessed as 20%.

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The role of NLRP3 inflammasome in metabolic inflammation and its impact on behavior and brain aging

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Immunosenescence and chronic inflammation are hallmarks of aging and contribute significantly to brain aging and the deterioration of complex behaviors. Neuroinflammation within the hippocampus is particularly critical in the development of cognitive impairment and anxiety, although the precise mechanisms remain incompletely understood. This study aimed to investigate the dysregulation of insulin signaling and the mechanisms of metabolic inflammation ("metaflammation") in the brains of wild-type and NLRP3 knockout mice at different ages.

Our results revealed a pronounced upregulation of the NLRP3 inflammasome in the hippocampus during aging, leading to increased expression of phosphorylated metaflammation proteinases and inflammatory markers, as well as an increase in the number of senescent cells. This metaflammation was associated with increased anxiety and impaired social preference behavior in aged mice. Conversely, NLRP3 deletion ameliorated several age-associated behavioral and biochemical deficits, including improvements in signal memory, reductions in neuroinflammation, and attenuation of metabolic inflammation, although it did not alleviate anxious behavior.

These effects were associated with reduced IL-18 signaling and downregulation of the PKR/IKKβ/IRS1 pathway, as well as suppression of the senescence-associated secretory phenotype (SASP). The downregulation of PKR in the absence of NLRP3 suggests that inhibition of NLRP3-related pathways may slow the aging process and thereby attenuate cognitive decline. Therefore, genetic knockout of the NLRP3 inflammasome emerges as a promising therapeutic strategy to slow central nervous system aging. These findings highlight the potential of targeting NLRP3 to preserve cognitive function and delay the progression of age-related neurological disorders.

The role of the transmembrane domain in the activation of the insulin-like growth factor receptor (IGF-IR)

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The insulin-like growth factor receptor belongs to the family of receptor tyrosine kinases (RTKs), which play a key role in cell development and differentiation. RTKs consist of 3 parts: the extracellular part, which is responsible for ligand binding, the transmembrane domain (TM), and the intracellular part involved in cell signaling. The insulin-like growth factor receptor (IGF-IR) is a receptor tyrosine kinase that is activated by insulin—like growth factor 1 and 2, as well as insulin. IGF-IR plays a key role in cell growth, differentiation, and aging. Now, the exact mechanisms of activation and transmission of the intracellular signal of the insulin receptor family are still not completely clear. It is assumed that in the inactive state, the TM domains of IGF-IR are in a conformation that prevents the interaction of the cytoplasmic parts of the molecule.

To study the role of the transmembrane domain in the activation of the IGF-IR, we obtained mutant forms of the receptor containing double substitutions in the transmembrane domain. HEK293 cells were transfected with plasmid constructs combining mutant forms of IGF-IR. Then the cells were incubated in an F-12 medium, with the addition of insulin, and the cell lysates were analyzed by the Western blot method. The IGF-IR mutants V941E-A942R and V948E-G949R were not expressed in the HEK293 cell line. Surprisingly, the double substitution of G949E-G950R resulted in phosphorylation of the receptor in the absence of a ligand.

Then, we decided to make chimeric constructions in order to assess the impact of V941E-A942R and V948E-G949R mutations. To do this, we replaced the transmembrane domain of the insulin receptor-related receptor (IRR) with the transmembrane domain of IGF-IR. These receptors belong to the same family and have a high homology. IRR is activated by alkaline pH above 8. As a result, mutations V941E-A942R, V948E-G949R and G949E-G950R led to activation of the receptor at a pH of 7.4, unlike the wild-type receptor.

We have shown that the transmembrane domain plays an important role in the activation of the insulin-like growth factor receptor, and even point substitutions in its amino acid sequence can lead to the production of functionally active forms of the receptor, preferably due to the formation of salt bridges.

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The use of fluorouracil to create an acute irritable bowel syndrome model on mice

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Introduction Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract characterized by abdominal pain, inflammatory processes, imbalance of the intestinal microflora and peristalsis with frequent concomitant cognitive impairment. It is known that the use of the antitumor drug 5-Fluorouracil (5-FU) leads to gastrointestinal damage, epithelial hyperplasia and villous atrophy in the small intestine. The aim of this work was to form a model of IBS using injections of 5-FU.

Methods The experiment was conducted on 3 groups of mice: the control group (n=20) with injections of sodium chloride, the 5-FU group (n=30) with injections of 5-FU (66 mg/kg) and the AB group (n=25) with injections of a cocktail of antibiotics [1], which was used as a classical model of IBS. In this study, indicators such as weight, mortality were monitored, and colon hypersensitivity was also assessed by measuring the threshold intensity of the abdominal flexor reflex and the level of anxiety using the "Integral Anxiety Index" and "Open Field" tests.

Results There was no significant change in body weight in all groups, while significant mouse mortality was observed in the AB group of 16%. Visceral hypersensitivity was observed in animals in the AB and 5-FU groups. At the same time, in both experimental groups, anxiety significantly increased in all behavioral tests relative to the control group.

Conclusions In this study, it was found that in mice of the 5-FU group, the level of anxiety was increased, as well as visceral hypersensitivity was increased, which indicates that 5-fluorouracil can form a model of IBS.

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Transmembrane protein type1 SUSD2 is a part of pancreatic tumor microenvironment and indispensable for regulation of homeostasis in the mouse macrophages.

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Signaling mechanisms in the tumor microenvironment play a pivotal role in tumor genesis, and therefore it is important to search for novel stromal targets for drugs discovery [1]. We have previously identified, that Transmembrane protein type1 SUSD2 may present a significant interest [2]. Here we presented the results of immunohistological analysis of tumors tissue and immunofluorescence analysis of stromal culture, which revealed, that SUSD2 is expressed mainly in stroma and located on cell membranes. In experiments with RAW264/7 murine macrophages we showed, that SUSD2 gene expression is increased significantly after exposing cells to LPC for 24h, but did not lead to cell death, as previously was shown for other cultures [3], but instead, was accompanied by activation of anti-apoptotic signaling pathways (pAKT, pBAD) and decreased cell proliferation (PCNA, CyclinD), as well as suppression of TJ-protein Claudin. Surprisingly, that in the same time anti-apoptotic protein Survivin was also suppressed. Differences in the regulation of anti-apoptotic pathways may be associated with transitional state of macrophages from active inflammation to restoration of homeostasis after LPS treatment [4]. This may indicate possible mechanism of tumor drug resistance through the SUSD2 expression.

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Uncovering splicing-related functions of the NAD$^+$-dependent chromatin remodeler SIRT6 and its contribution during brain development, aging and degeneration.

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Alternative pre-mRNA splicing (AS) establishes one-to-many relationships between genes and isoforms. This capability is indispensable for the brain due to its complexity. Conversely, alterations in the process of AS and its regulation have detrimental outcomes, leading to aging and neurodegeneration. This is supported by studies that showed that the bulk of splicing changes occurring during aging are positively correlated to AS events seen in the brains of subjects with neurodegenerative diseases [1, 2]. SIRT6 is a NAD$^+$-dependent chromatin remodeler with neuroprotective and anti-aging properties [3]. Being a model for aging, it is increasingly targeted in a vast number of studies, however, no study has demonstrated its involvement with splicing functions in the brain. Yet, a significant number of brain-related isoform changes have been documented during aging and degenerative disorders. Here, we speculate on the involvement of SIRT6 with splicing functions in the brain. To this end, we performed RNA-seq based splicing analysis in mice devoid of SIRT6 functions across three developmental time points: mESC, NSC and adult cortex. We identified both common (32 genes) and temporal-specific (150 coordinate-based events) splicing signatures of SIRT6 silencing, and uncovered specific splicing temporal trends modulated by SIRT6 loss. Specifically in the cortex, a lack of SIRT6 results in differential splicing changes to genes like *Ahi1* and *Snrnp70*, which have been implicated in the pathophysiology of neurodevelopmental disorders including schizophrenia and neurodegenerative diseases like Alzheimer, respectively. Additionally, our analysis revealed that SIRT6 splicing functions contribute significantly to the aging-related and pro-longevity cellular pathways, including the ribosome, ubiquitin-mediated proteolysis, and ATP-dependent chromatin remodeling. The results of our analysis indeed uncover a novel role for SIRT6 in modulating splicing functions within the cell.

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Using liposomes loaded with fluorescent dyes to evaluate the membranolytic kinetics of modular nanotransporters

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Modular nanotransporters (MNTs) are a drug delivery system designed for targeted cancer treatment [1]. The endosomolytic module of MNTs facilitates pH-dependent pore formation in endosomes, which ensures the release of the MNT along with the delivered active component into the cytosol of the target cell following receptor-mediated endocytosis. The membranolytic activity of the module can be assessed by its ability to cause leakage of the contents of phosphatidylcholine liposomes loaded with a dye in a concentration that causes fluorescence self-quenching [2]. To study the kinetics of the process, we used a sulfo-cyanine dye, the fluorescence of which does not depend on pH. Using this approach, we measured and analyzed the kinetics of the early stages of membranolytic action of MNTs under different conditions for the first time. Our results helped us better understand the kinetics of the early stages of interaction of MNTs with the phosopholipid bilayer and demonstrated the conditions that are more suitable for further analysis.

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Utility of the R2C2 Method for Analysis of Sequencing Data of Highly Variable Immune cDNA Sequences Obtained from Illumina MiSeq and Oxford Nanopore Technologies (ONT)

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The production of single-domain antibodies, known as nanobodies, derived from IgNAR antibodies produced by sharks, presents a promising avenue for biomedical research. These nanobodies offer advantages over conventional antibodies due to their smaller size (15 kDa vs. 150 kDa), leading to reduced immunogenicity, increased tissue permeability, and simpler production processes. This study focuses on identifying and describing IgNAR isoforms in the brownbanded bamboo shark (Chiloscyllium punctatum).

In this study we identify Framework Region (FR) sequences, extracting Complementarity Determining Regions (CDRs), clustering CDR sequences, and performing statistical analysis of these regions, as it was described for other sharks [1]. The study involved obtaining shark blood samples, isolating lymphocytes, and extracting RNA. The Flair pipeline was used to analyze isoforms, combining long reads for structure and short reads for recombination region annotation. Among the identified isoforms, the most complete sequences were manually selected and annotated using domain search tools and relevant literature. CDR3 sequences, critical for antibody variability, were identified through specific FR regions using HHMER for domain identification and the seqkit toolkit for sequence extraction. Clustering of CDR3 sequences was performed using the CD-HIT-EST tool with an 80% identity threshold, followed by analysis of CDR3 length distribution and clonotype abundance with custom scripts in R and Python.

The study successfully annotated the FR and CDR regions within the Variable (V) region and extracted the highly variable CDR3 segment. Isoforms were classified based on the presence of amino acids like Cys and Trp within FR1, FR4, CDR1, and CDR3. While current results allow for the design of more specific primers for the region of interest, they do not provide comprehensive CDR3 characterization or reliable clonotype clustering.

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Viral rhodopsins of group 1 are cation channels

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Microbial rhodopsins are a diverse subfamily of light-sensitive membrane proteins extensively utilized across various fields of biology. Their distinctive feature is sensitivity to light, which induces conformational changes, thus enabling protein function. One of the new classes of microbial rhodopsins are viral rhodopsins identified in the genome of giant viruses [1]. Considering that the host of such viruses is phytoplankton, the function, physiological and biological role of viral rhodopsins can be of great interest for global ecology and climate.

OLPVR1 (Organic Lake Phycodnavirus Rhodopsin) and VirChR1 (Viral Channel Rhodopsin 1) are among the most studied viral rhodopsins [2]. Thus, recent studies have pointed out that OLPVR1 predominantly localizes to the endoplasmic reticulum while remaining functional, being a potential novel optogenetic tool wit unique properties [3]. Previous studies showed that VirChR1 is a cation channel [2]. In case of OLPVR1, its high amino acid homology with VirChR1, as well as other experimental data suggest its ion channel activity [2, 3]. However, direct experimental evidence of such channel properties, e.g., patch-clamp recordings, has been lacking. In this work, we investigated the functional properties of OLPVR1-based protein constructs using black lipid membrane (BLM) and patch-clamp methods. Our findings provide direct experimental evidence of ion channel properties in OLPVR1.

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Wide-angle X-Ray scattering analysis of haloarchaeal purple and claret membranes

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Purple membrane (PM) patches of *Halobacterium salinarum* cytoplasmic cell membrane resemble a two-dimensional hexagonal crystalline lattice, comprised of bacteriorhodopsin (*Hs*BR) trimers and polar lipids. Other archaeal rhodopsins, e.g., one from *Haloquadratum walsbyi* (*Hw*BR), can form similar clusters of claret membrane (CM) different from the PM due to bacterioruberin presence [1].

Here we report on *H. salinarum* and *H. walsbyi* cultivation, followed by PM and CM isolation. SDS-PAGE of the isolates shows the band for abnormally mobile *Hs*BR monomers (~17 kDa), but bands for both *Hw*BR mono- and trimers (~20 and ~64 kDa, resp.), indicating greater stability of the latter. Membrane structure was analyzed by wide-angle X-Ray scattering (WAXS) using home-lab instrument Rigaku MicroMax-007HF (Dolgoprudny, Russia) [2]. Diffraction pattern for PM was identical to previously reported. The peaks were resolved up to 6.6 Å, and described by a set of 2D Miller indices (h; k), where h = {1; 7}, and k = {-4; 0}. Meanwhile for CM, diffraction peaks distribution suggests coexistence of two distinct lattices.

WAXS data show significant difference of PM and CM latices. We hypothesize that qualitative lipid and pigment composition of the membranes could contribute to the differences. Thus, bacterioruberin, detected by UV-VIS spectroscopy, might be highly ordered across the CM, contributing to its unique WAXS 1D-profile. However, further research is required to verify this conclusion.

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α-Synuclein-related Mitochondrial Dysfunction in Aerobic Yeasts

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Parkinson's disease (PD) is a chronic progressive neurogenerative disease induced by several biomarkers [1]. One of them is the α-synuclein protein encoded by the SNCA gene, whose abnormal expression negatively affects cell homeostasis [1,2]. In recent years, a large amount of data has emerged on the role of oxidative stress in the neurodegenerative diseases development and the PD is not an exception [2]. The complex nature of PD pathogenesis leads to the need to simplify models for its study, since oxidative stress can occur in a cell for a variety of both external and internal reasons. Thus, yeast can be a prospective single-cell model that allows the study of conservative intracellular processes common in mammals [3,4]. In this study, PD models based on the aerobic yeasts Yarrowia lipolytica were created to investigate the α-synuclein effects on cellular bioenergetic disorders. Overexpression of α-synuclein in genetically modified yeasts was found to cause fragmentation of the mitochondrial reticulum, reduced tolerance to oxidative stress and increased levels of cell death in the presence of a prooxidant.

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β-barrel Deletion of Human 15-Lipoxygenase-2: A Promising Implication for Pancreatic Cancer Treatment

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Lipoxygenases play crucial roles in the oxygenation of membrane lipids and free fatty acids. Studies in their structure-function determination uncovered higher catalytic activity of rabbit 15-lipoxygenase following truncation of membrane binding-N-terminal β barrel domain [1,2]. Despite possible impairment in membrane binding ability, can deletion of N-terminal boost the catalytic efficiency of human 15-lipoxygenases whose overexpression and robust catalytic activity pose a significant threat to pancreatic tumors? We performed adeno-associated virus-mediated expression of 15-lipoxygense-2 (wild-type and N-terminal truncated mutant) and established a quantitative spectrophotometric assay for measurement of enzyme activities. Similar to rabbit 15-lipoxygenase, we found out that the rates of reaction of truncated mutant of human 15-lipoxygenase-2 was about 1.5 times higher relative to the wild type enzyme. Our results demonstrated that, at the expense of deletion of the N-terminal β-barrel domain, truncated human 15-lipoxygenase-2 can exhibit a greater lethal power deleterious to pancreatic tumors.

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