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POSTERS

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About these abstracts

Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are **not copyedited** prior to publication.

We are unable to make **corrections of any kind** to the abstracts once they are published.

Indexing

Abstracts published in *The FEBS Journal* Supplement for the 42nd FEBS Congress will be included individually in the Conference Proceedings Citation Index published by Web of Science.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented.

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POSTERS

Monday 11 September
13:00–15:00

Synthetic Biology

P.1.1-001
Optogenestat –mini photo bioreactors for *in vivo,* real time characterization and and evolutionary tuning of bacterial optogenetic circuits

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Gene circuits have been dynamically characterized by using chemical effectors molecules, which has been limited by diffusion of effector across the cellular membrane. Optical method for creating on-demand protein signals in live cells would bypass limitations and, in principle, enable the dynamical characterization of virtually any gene circuit that responds to changes in protein concentration. Current standard protocol to characterize such optogenetic circuit using flow cytometer is tedious, labor intensive and cumbersome. So far very limited data is available for different growth conditions. In this work, we engineer a bioreactor of working volume ~10 mL specifically designed for optogenetic characterization of light sensing *E. coli*. We develop an integrated bioreactor that uses optogenetic control for quantitative, up/down control of gene expression and monitors the measurement of the relevant parameters during the microbial growth. The optical density, fluorescence detection for green fluorescence protein reporter as well as the input stimuli is provided by light emitting diode (LED) at multiplexed wavelength. The light sensing *E. Coli* harbors a synthetic two component system (TCS) circuit from CcaS-CcaR system used in the chromatic adaption of cyanobacteria *synechocystis* PCC 6803. The sensor histidine kinase CcaS is produced in a green-absorbing ground state. Absorption of green light flips CcaS to a kinase active red-absorbing state that phosphorylates the response regulator CcaR, which then binds to the *cpcG2* promoter and activates transcription. We aslo evolutionary tuning of such TCS by serial dilution transfer to reveal the interdependence of growth (fitness) and gene expression. With light illumination for photosynthesis, such bioreactors can also be used for characterization of synthetic genetic circuit of photosynthetic microorganisms such as *P. tricornutum.*

P.1.1-002
Light-induced pancreatic β-cell proliferation through endogenous opsin signaling

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Diabetes is a disease characterized by the loss of function and number of healthy pancreatic β-cells, with no permanent cure yet. A key therapeutic concept is based on regenerating β-cell mass by islet transplantation or inducing proliferation of β-cells by growth factors, respectively. These therapies, however, are either highly invasive or often non-specific and thus potentially carcinogenic. Here we propose an alternative approach based on light induced proliferation of β-cells that is less invasive and increases the spatio-temporal precision, potentially circumventing previous limitations.

Using RT-PCR, we found that several opsins, especially panopsin, and to lower levels melanopsin and rhodopsin, are present in rodent and human pancreatic islets. To test whether these opsins have a functional role, we analyzed the response of primary murine and human pancreatic islets to illumination with blue-green light. By using a nucleotide incorporation assay we determined the percentage of proliferating cells in primary human and murine islets, which showed increased proliferation after illumination compared to unilluminated control groups. Illumination resulted in elevated activation of the major proliferative MAPK/Erk and anti-apoptotic PI3K/Akt pathways, as determined by Erk1/2 and Akt phosphorylation levels using Western blot. Furthermore, we optimized the illumination protocol regarding wavelength- and intensity-dependence of proliferative pathway activation.

Taken together, our results show that pancreatic β-cell proliferation can be induced with high spatial and temporal precision using visible light, without addition of exogenous factors or gene transfer, indicating a potential mechanism for a novel therapeutic strategy.

P.1.1-003
Directed evolution of cellobiose dehydrogenase from *Phanerochaete chrysosporium* in yeast *Saccharomyces cerevisiae* for increased activity

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Cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in yeast *Saccharomyces cerevisiae* for extracellular expression. Gene library was constructed using random mutagenesis by error-prone PCR. Obtained library was screened with microtiter plate assay using modified DCIP assay. Several mutants were found that have higher kcat compared to wild-type enzyme. Both mutants and wild type expressed in yeast showed broad band in SDS electrophoresis due to high glycosylation level. Obtained mutants could be useful in lactobionic acid production and biosensor manufacturing.

P.1.1-004
Expanding the genetic code of a phototrophic organism

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The photoautotrophic fresh water cyanobacterium *S. elongatus* is widely used as a chassis for biotechnological applications as well as a photosynthetic bacterial model. In this study, a method has been established to expand the genetic code of this cyanobacterium thereby enabling the incorporation of unnatural amino acids into proteins. This was achieved through UAG stop codon suppression, using archaeal pyrrolysyl orthogonal translation system. We demonstrate incorporation of unnatural amino acids into green fluorescent protein with 20 ± 3.5% suppression efficiency. The introduced components were shown to be orthogonal to the host translational machinery. In addition, we observed

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that no significant growth impairment resulted from the integration of the system. To interpret the observations, we modelled and investigated the competition over the UAG codon between release factor 1 and pyl-tRNA$_{CUA}$. Based on the model results, and on the fact that 39.6% of the stop codons in *S. elongatus* genome are UAG stop codons, suppression efficiency in this organism is unexpectedly high suggesting a preference for a read-through event in a middle of a gene over translation termination.

P.1.1-005
Characterization of triple helix structures *in vivo* using a novel deep sequencing approach

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Recent transcriptome-wide studies demonstrated that regions throughout the human genome are actively transcribed. Within the group of non-coding transcripts, the diverse class of long non-coding RNAs (lncRNAs) has gained widespread attention due to its involvement in transcriptional regulation. Despite promising studies of RNA-dependent functional effects, it remains unclear how these RNAs target genomic loci. One important, yet controversial mode of genomic targeting is the formation of RNA-DNA triple helix (triplex) structures. In triplex formation, a single-stranded nucleic acid molecule interacts with a duplex strand via Watson&Crick-independent basepairs termed Hoogsteen bonds. Since their discovery, triplexes have been studied intensively *in vitro* and gained renewed interest after the explosion of lncRNA findings. Here, we provide a novel tool termed Triplex-Seq to detect triplex formation in living cells using a deep-sequencing approach. Briefly, the triplex-forming oligonucleotides (TFOs) consist of two parts: (i) a triplex-forming motif unique for each third strand molecule and (ii) a short index sequence shared among all TFOs. A library of these TFOs was constructed using degenerated bases. Furthermore, we integrated a set of purine-rich putative triplex target sites (TTS) into a human artificial chromosome maintained in Chinese hamster ovary cells. Following transfection of the library, a subset of TFOs is expected to bind the TTS via Hoogsteen basepairing. Next, triplexes will be crosslinked, co-precipitated third strands will be selectively enriched and analyzed using next-generation sequencing. Based on our preliminary data, we anticipate that the combination of the synthetic biology-based approach and the use of deep sequencing technologies for studying nucleic acid triplex interactions will provide a platform for deciphering the triplex code *in vivo* and holds promise to elucidate the targeting mechanism for lncRNA transcriptional regulators.

P.1.1-006
Production of glycosylated yeast alcohol dehydrogenase in *Escherichia coli* dual plasmid expression system

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Biotransformation tools, enzymes, nowadays represent a prospective trend to substitute ineffective and often environmentally harmful organic synthesis with biocatalysis. Yeast alcohol dehydrogenase (ADH) is an industrially valuable enzyme used in the synthesis of green leaf volatiles, compounds utilized in the food, fragrance or pharmaceutical industry. We focused on ADH production in the host organism *Escherichia coli* that serves as a relatively simple and accessible means of recombinant proteins production. Current possibilities of protein overexpression in prokaryotic organisms have recently been extended with recombinant protein glycosylation. We decided to apply this novel approach in *E. coli* expression system producing ADH. The glycan structure conjugated to the enzyme should provide a reliable way to achieve oriented covalent immobilization of ADH that would bring benefits such as reusing and potentially higher enzyme stability. We have successfully constructed an expression system based on *E. coli* encompassing the high-copy number vector pJexpress401-ADH-CGH coding ADH conjugated with C-terminal D-Q-N-A-T repetitions, and the low-copy number plasmid pACYC-pgl2 containing genes coding enzymes participating in the mechanism of glycan assembly. Here we present the first promising results from immunoblotting and lectin-blotting detection of ADH-CGH production in bioreactor and purification of the cell lysate indicating successful *in vivo* glycosylation of this enzyme.

P.1.1-007
New strategies in production of thermophilic catalase-peroxidase AfKatG in *Escherichia coli*

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The increasing need of recombinant proteins forces us to think of new production strategies or improving the old ones. The limitation to the highest possible amount of a protein obtained from any fermentation system is cell density. Therefore production strategies affect it on various levels. Originally, different media, growth conditions or vectors were used but with new genetic tools we are able to use genetic/ genomic engineering to optimize protein production. The protein of our interest is thermophilic catalase-peroxidase AfKatG from thermophile *Archaeoglobus fulgidus*. *Escherichia coli* as a widely used production system is able to grow rapidly to high densities in inexpensive media. That's why the first strategy for production was using different media. We proved complex media with relatively lower cell density to be better for production of fully active (with catalase activity 6000 – 9000 U/mg and peroxidase activity 25 – 37 U/mg) and thermostable enzyme (1st melting point 97 °C, 2nd 104 °C). To improve peroxidase activity single mutation were done. Using same conditions only inclusion bodies were obtained, thus we tried other strategies. The best one appeared to be combination of lower expression temperature (20 °C) and coexpression with chaperones. The produced enzymes can be directly used in numerous industry requesting both catalase and peroxidase activity at higher temperatures.

P.1.1-008
Polyhydroxyalkanoate production from moderately halophilic *Bacillus* strains

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Polyhydroxyalkanoates (PHAs) are biodegradable polyesters that can be synthesized by various species of bacteria as an intracellular carbon and energy storage in an environment that is carbon-rich but poor in certain nutrients such as phosphorus and nitrogen. Development of eco-friendly, bio-degradable plastics from cheap and renewable resources is becoming increasingly important but PHAs hold only a relatively small fraction of the biopolymer market share because of their relatively high production cost and concurrent availability of low-cost petrochemical plastics. Among the PHA producing bacterial strains, *Bacillus* which are taxonomically very diverse and capable of growing in large scale culture are the most popular source of PHAs. Many *Bacillus* species including moderately halophilics from different environments have been screened for PHA production and more screening studies are needed.

In this study, five moderately halophilic *Bacillus* strains including *Bacillus siamensis-ATY1, Bacillus tequilensis-ATY2, Bacillus licheniformis-DBA2, Bacillus safensis-D8* and *Bacillus pumilus-DB5* which were isolated from salted sheep skins were screened for PHA production. Besides conventional substrates such as; starch, D-fructose and D-glucose, agro-industrial wastes; anaerobic fermentation liquids derived from cheese whey, olive oil plant wastes and the leather industry were used for PHA production. High PHA productivities were achieved by all the strains and among the strains *Bacillus Pumilus* had the highest capacity. This strain accumulated PHA to 80.66% of its cell dry weight when olive oil mill wastewater was used as the carbon source. The biopolymer which was produced was identified and characterized. Based on our findings, the strains produce polyhydroxybutyrate (PHB). To reduce the production cost, sterile and non-sterile tap water and sea water were used in the production medium and promising results were obtained for PHB production.

P.1.1-009
Synthetic genetic circuits enabled cellular nano-toxicity sensors

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Nanoparticle (NP) adaptation in nanotechnology has emerging usage in many fields; meanwhile unique properties of NP (i.e. high surface-to-volume ratio) arises safety issues on human as well as environment. Since NPs are highly catalytic, they create potential toxicity to cells. Thus, early detection of toxicity might be helpful to take precautions in advance. In this work, we aim to build a whole cell nanotoxicity sensor by using synthetic biology approaches which is capable of sensing toxicity caused by NPs. We use cell's natural stress response, heat shock protein response (HSPR), in our nanotoxicity sensor. HSPR is responsible for keeping cells alive under any stress conditions (temperature shift, chemical exposure, starvation etc.). In our design, we use promoters of HSPs to control over-expression of a reporter gene (i.e. *gfp*) in the presence of toxicity caused by NPs or heavy metals. At the beginning, we compare toxicity responses of different HSP promoters to determine the most sensitive and efficient nanotoxicity sensor. Next, we design different approaches to tune sensitivity of the sensor. For this manner we use riboregulators to provide tight control over the signal, we use some mutagenesis strategies on promoter regions of HSPs, we re-engineer quorum sensing, which is the bacterial communication systems, accordingly our nanotoxicity sensor, or we make co-expression studies to shot down the system at steady state with a special heat shock repressor protein (HspR) found in *Mycobacterium tuberculosis*. Recently, we integrate toxicity sensor system in eukaryotic cells (i.e. HEK293) to compare toxicity role on different cell lines representing different tissue and organs. As a conclusion, this study is composed of sets of bacterial and eukaryotic nanotoxicity sensors facilitating genetic circuits based sensing in the presence of toxicity. In further steps, the circuit will be extended to be able to detect the source of the toxicity along with the toxicity way.

P.1.1-010
Hispidin-3-hydroxylase: a luciferin biosynthesis enzyme of glowing fungi

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Bioluminescence is on the front edge of biological sciences both as a fundamental research topic and as a tool for different applications. Despite the fact that this topic is being extensively researched none genetically encodable eukaryotic bioluminescent systems are known. Our research group has recently established the structure of fungal luciferin: 3-hydroxyhispidin, a derivative of a known secondary metabolite hispidin ((E)-6-(3,4-dihydroxystyril)-4-hydroxy-2H-pyran-2-on). During the past year we have also found two novel enzymes: luciferase and hispidin-3-hydroxylase (H3H) from the bioluminescent fungus *Neonothopanus nambi*. The second enzyme catalyzes hydroxylation of hispidin and is a key enzyme of fungal luciferin biosynthesis. Using a bioinformatics approach we identified close H3H homologues in other bioluminescent fungi, e.g. *Panellus stipticus*, *Mycena cytricolor, Armillaria mellea*. We also proved experimentally the function of H3H in heterologous expression systems, such as yeast *Pichia pastoris*, bacteria and mammalian cell cultures. Moreover, we have constructed functional chimeric fusion proteins, containing both luciferase and H3H. We believe that our work is a first and important step towards the development of the first fully genetically encodable eukaryotic bioluminescence system. This work was supported by the Russian Science Foundation grant 17-14-01169.

P.1.1-011
Synthetic-evolution approach reveals context-specific regulation of a mitotic kinesin-5 nano-motor

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Kinesin-5 mitotic nano-motors play essential roles in mitotic spindle dynamics, by cross-linking and sliding apart antiparallel microtubules of the spindle. Recently, we found that the *S. cerevisiae* kinsesin-5 Cin8 is phospho-regulated by Cdk1 at three sites in its catalytic motor domain, which governs its localization to the mitotic spindle during mitosis. We also determined that each of these Cdk1 sites plays a different role in regulation of Cin8. Here we tested the rigidity of phospho-regulation of Cin8, and

examined whether novel Cdk1 sites created by a single amino acid replacement can mimic the known phospho-regulation or create new phenotypes. For this purpose, we systematically generated Cin8 mutants carrying a novel Cdk1 site as a sole source for Cdk1 phospho-regulation. We found that out of 29 novel sites that we have generated, only one site in the motor domain, in high proximity to a native Cdk1 site, recapitulated the phospho-regulation of the adjacent native site, although several sites were created nearby. This result suggests that phospho-regulation of Cin8 by Cdk1 at this site is rigid and highly dependent on the structural context. Two other sites resulted in novel phospho-regulation of Cin8; however they resulted in a less optimized phospho-regulation. Interestingly multiple-sequence-alignment revealed that one of these sites is present in other organisms, suggesting that phospho-regulation at this site is flexible throughout evolution and may occur according to the needs of the organism.

P.1.1-012
Expression and purification of capsid proteins of Aichi virus and *in vitro* reassembly of empty virion

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Aichi virus is a positive-sense single-stranded RNA virus (+RNA virus) from the *Kobuvirus* genus of the *Picornaviridae* family and a human pathogen. Symptoms of its infection are nausea, gastroenteritis and fever and approximately 80–95% of adults worldwide have antibodies against the virus. Relatively low but still significant virulence deems *Aichi virus* as a suitable model for investigation of the live cycle of +RNA viruses. Our model aims to encompass the biology of many dangerous human pathogens such as HCV or West Nile virus. *Aichi virus* reassembly requires viral capsid proteins (Vp0, Vp1 and Vp3) in high purity and sufficient quantity. In order to obtain structural proteins we cloned them in form of polyprotein as they are present in *Aichi virus* genome and also separately into different expression systems – (bacterial, insect and human cells). GST-His tagged polyprotein was produced in insect cells, but with insufficient yield. In *Escherichia coli* strains no detectable expression of viral polyprotein was observed. In contrast, Vp0 cloned separately was expressed in *E. coli* BL21 and was obtained in high purity and amount. Antibodies against Vp0 for further virion detection are being prepared. Due to apparent toxicity of Vp1 and Vp3 they were expressed in *E. coli* BL-21 pLys strain. However, in this system both Vp1 and Vp3 are extensively degraded during the protein purification. To overcome the protein degradation, polycistronic expression vector was designed.

The project was supported by the Czech Science Foundation grant number 15-21030Y and by Ministry of Education of the Czech Republic – LO1302. The Academy of Sciences of the Czech Republic (RVO: 61388963) is also acknowledged.

P.1.1-013
Tuning of acyl-ACP thioesterase activities directed for tailored fatty acid composition

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Medium chain fatty acids have driven significant attention as sources of biofuels in recent years. Acyl-ACP thioesterase catalyzes the termination of de novo fatty acid synthesis, which is considered as the key enzyme to determine the carbon chain length. Though recombinant medium chain acyl-ACP thioesterase (TE) affects the fatty acid profile in heterogeneous cells, it is still intractable in tailoring of the fatty acid composition merely by engineering a specific thioesterase. In this study, it was quantified the activity of a C8-C10 specific thioesterase FatB2 from Cuphea hookeriana on C10-ACP was twice as high as that on C8-ACP based on a synthetic C8-C16 acyl-ACP pool *in vitro*. Whereas *in vivo*, it demonstrated that ChFatB2 preferred to act on C8-ACP instead of C10-ACP since 84.9% C8 fatty acids composition was obtained in the ChFatB2 engineered *E. coli* strain. To achieve C10 fatty acid as the major product, ChFatB2 was tuned by rational design based on structural understanding and enzymatic analysis. A surface I198E mutant was identified to redistribute the C8-ACP flow and C10 fatty acid was accomplished as principal composition with 57.6% of total fatty acids *in vivo*. The study systematically elucidated the inconsistency of TE activities *in vitro* and in genetically engineered cells, demonstrated that the relative activity of TE competing to KAS enzymes directly determined the fatty acid composition. Whereas structural and enzymatic analysis revealed that TE relative activity can be regulated by interaction with ACP moiety of the substrates. Tuning of thioesterases activities based on TE-ACP interaction provides a prospective strategy for tailored fatty acid synthesis.

P.1.1-014
Theranostic microrobots: programmable biofilm secretion for *in vivo* drug delivery and cancer immunotherapy

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Smart responsive cancer therapies can be envisioned by bacteria as programmable nanorobots that specifically target tumors and exhibit toxicity to cancer cells. However, bacterial cancer therapies have several limitations such as potential symbioses concerns, intrinsic bacterial toxicity and septic shock due to immune response. Escherichia Coli Nissle 1917 (EcN) is a non-pathogenic probiotic bacteria that has been used to treat gastrointestinal diseases and specifically colonizes solid tumors. Therapeutic approaches such as releasing toxic cargo of bacteria inside the cancer cells can be effectuated by invasion of cancer cells via bacterial internalization. Bacterial amyloid fibers can mediate internalization of bacteria into eukaryotic cells. Furthermore, bacterial amyloids can trigger innate immune response and activation of host immunity can suppress tumor growth. In this study, we aim to engineer EcN using synthetic gene circuits for diagnosis as well as the selective eradication of solid tumors. In general, we intend to program the bacteria to secrete biofilm proteins from different microorganisms in a tumor-micro environment responsive manner. We hypothesize that biofilm secretion will trigger the internalization of engineered bacteria into cancer cells. Upon internalization, therapeutics will be secreted into cancer cells. In addition, the innate immunity triggered by secretion of biofilm proteins will cause further tumor suppression. In conclusion, genetically engineered bacteria as a tool to eradicate tumor cells holds great potentials to overcome limitations of current therapies, as well as acting as a diagnosis tool. Enhanced internalization of therapeutic bacteria by tumor cells via different biofilm proteins as well as biofilm mediated host immunity can increase therapeutic effects. Increasing the internalization capacity and host immunity via biofilm proteins will enable efficient usage of EcN as tiny smart robots to selectively destroy tumor cells.

P.1.1-015
Synthetic quorum sensing wired cell consortia for controlled release of proteins from cell surface

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Synthetic biology paves the way for developing living machines which are able to perform certain tasks. These microrobots can be programmed to sense, and produce molecules upon certain signals. Besides, controlling their movements is possible with different signals such as light or chemicals. All information required for engineering such cellular system is found in nature, and owing to biodiversity among the organisms, infinitely diverse cellular machines can be constructed for various applications. One of the excellent examples of those is quorum sensing phenomena which cells utilize to communicate each other. In this work, we aimed to develop and characterize a cell consortia that is able to release protein in a controlled manner. To establish the controlled release, a reporter protein, sfGFP, with TEV recognition site is displayed on the cell surface via Ag43 autotransporter shuttle protein. Ag43 protein cleaves itself into two subunits namely α and β. β subunit forms β barrel into outer membrane then translocates its α domain. After translocation, α domain remains intact with β barrel via noncovalent interaction (1). The release of cargo protein from cell surface can be accomplished by production of extracellular TEV protease via another cell. 'AND' logic gate is aimed to constructed between the production of extracellular TEV protease and protein display cassette to control the release of protein. The released capacity and kinetic will be assayed in different induction times. Later on, the cells will be wired with LuxI/LuxR quorum sensing module and release of protein from cell surface will be assigned in different cell densities. Herein, we propose a novel cell consortia for controlled release of proteins from the cell surface. The proposed cell consortia have many different applications such as cellular catalyst and theoretics.

Reference

1) Van der Woude MW & Henderson IR (2008). Regulation and function of Ag43 (flu). *Rev. Microbiol. 62*, 153–169.

P.1.1-016
Developing multi input whole cell biosensors

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Biosensors are widely used to sense many biomedical markers related with disease conditions. Commonly, recognition element senses the target compound, the response is converted to a detectable signal that can be measured in different ways such as electrochemical, optic, acoustic, mechanic, calorimetric. Yet, specificity of molecules used as recognition layer, renewal of recognition layer, could be listed as some drawbacks of conventional systems. Whole cell biosensor can be a cheaper alternative for conventional sensors. For environmental, food and biomedical applications, a large number of microbial biosensors have been developed in recent years. Using teach of Synthetic biology multi-input cellular sensor was developed. Multi input logic gates are one of the novel approach in synthetic biology. Reprogramming cells that can recognize specific analytes and developing signals according to these signals with the help of the synthetic biology enabled synthetic circuits is main goal of the project. This study aims to create a multi input logic gates in a single cell in order to use as a biosensor for kidney failure and presence of heavy metal ions. In order to obtain this biosensor, plasmids are designed, cloned and sequence verified in order to sense urea, uric acid, cadmium, arsenic, lead and copper. Ongoing studies mainly based on the characterization of the circuits via fluorescence measurements and protein expression analysis. After finishing characterization of all circuits, logic gates are constructed via using these circuits. As a conclusion, development of multi input logic gates of these circuits could be used as a biomedical sensor for kidney failure and heavy metal detection at the end of the project.

P.1.1-017
Local anesthetics affect pore-forming activity of cyclic lipopeptide syringomycin E

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Syringomycin E is a well-studied CLP which forms anion-selective voltage-dependent ion channels in model lipid membranes. Here the effects of local anesthetics (LAs) on the conductance and dwell time of SRE-channels have been studied. Planar lipid bilayers composed of DOPC and bathed in 0.1 or 2 M KCl have been formed by the Montall and Müller technique. It has been shown that at 0.1 M KCl aminoamides, lidocaine (LDC), mepivacaine (MPV), and prilocaine (PLC), have increased the conductance of the SRE-channels (g) on 15% via alteration in the membrane surface potential. The effects of aminoesters, tetracaine (TTC), procaine (PC), and benzocaine (BZC), have been in accordance to their effects on the dipole potential of lipid bilayers. TTC has enhanced g on 30%, PC has not changed it, while BZC has reduced g on 50%. Aminoamides and PC have not affected the dwell time of SRE-channels (τ), while TTC and BZC have significantly reduced it by about 4 times. This effect might be related to uncoupling action of TTC and BZC on the lipid bilayer. Effects of aminoesters were practically independent on the concentration of electrolyte in the bathing solution, while aminoamides have significantly influenced the shape of current-voltage curve of SRE-channels and have drastically reduced τ at 2 M KCl contrary to 0.1 M KCl. We have hypothesized that in the conditions of LA's and SRE's charges screening by high concentration of counter ions in bathing solution, aminoamides are able to interact with channel-forming SRE-molecules. Measuring the cooperativity of binding it has been shown that two LA molecules interact with single SRE-channel. The possible structural determinates of the interaction are discussed.

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DNA Damage and Repair

P.1.2-001
Identification of proteins interacting with abasic sites in DNA: an avenue toward a discovery of moonlighting functions

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Abasic (AP) sites are among the most common DNA lesions. Certain proteins can interact with AP sites to form Schiff bases, which can be stabilized by borohydride treatment. Several types of AP DNA were used to trap proteins in human cell extracts by

this method. In the case of duplex AP DNA with protruding ends (DDE-AP DNA), the major crosslinking products had molecular masses of about 100 and 45 kDa. Using peptide mass mapping based on MS data, we identified the proteins forming these adducts as the Ku80 subunit of Ku protein and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The main function of Ku is participation in double-strand break repair by nonhomologous end-joining, and GAPDH is widely known as a glycolytic enzyme. However, both proteins have many moonlighting functions. Then we studied interaction of purified Ku and GAPDH with AP DNA. We demonstrated that efficiency of Ku crosslinking to AP sites depends on DNA structure. Ku was shown to cleave AP sites in DDE-AP DNA by β-elimination; it prefers apurinic sites over apyrimidinic ones. Moreover, Ku can initiate an APE1-independent pathway of AP site repair. In cancer therapy, temozolomide and APE1 inhibitors are often used. As a result, apurinic sites appear and their repair should be blocked. But Ku is often expressed efficiently in tumor cells. Thus, Ku may initiate a backup repair pathway leading to drug resistance. We also found that GAPDH forms both borohydride-dependent and independent adducts with AP sites. Its NAD^+-binding site participates in interaction with AP DNA. GAPDH does not cleave AP sites, but crosslinks to AP sites cleaved by AP lyases. The level of GAPDH-AP DNA crosslinking depends on oxidation of GAPDH SH-groups. We assume that under oxidative stress irreversible binding of GAPDH to AP sites upon its translocation to the nucleus may be a suicidal event that could be one of the factors leading to cell death.

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P.1.2-002
Clustered DNA lesions repair by NER system

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Clustered damages are multiple closely spaced lesions inside one or two turns of the duplex DNA. In this research we investigated the interactions of nucleotide excision repair (NER) proteins with clusters comprising model DNAs of various structures. Clustered DNA damages can be result of highly contaminated environment or intensive combined therapy of certain diseases.

The set of the model DNA bearing clustered damaged was created and their interactions with NER proteins were analyzed. DNA-duplexes contained efficiently processed by NER system as the isolated lesions nAnt or dC^{FAB} in one strand and DEG residue (diethylene glycol insertion, AP site analog) in the complementary strand. The substrate properties of model DNAs, the efficacy of its recognition by XPC-HR23B and the properties as affinity probes for crosslinking were analyzed.

It was revealed that the bulky damage (nAnt or dC^{FAB}) removal efficiency depends on the position of DEG. The specific excision level for DNA with DEG, located at a distance 3-4 nucleotide from bulky damage, was significantly lowered compared to the excision level detected for DNA, containing single bulky damage. DEG, shifted by 10–20 nucleotides, does not result in dramatic excision lowering.

Using the equilibrium fluorescent titration we have evaluated the affinity of sensor protein XPC to model DNAs. It was shown that DNAs, bearing clustered damages, form with XPC more stable complexes as compared to DNA, containing single bulky lesion. It should be noted that the affinity parameters not always are in direct correlation with the damage removal efficiency. When used as a probes for cell extract proteins crosslinking DNAs demonstrated high selectivity and efficacy.

The data obtained are important for understanding the probable fate of bulky adducts-containing clusters of different chemistry and topology in mammalian cell.

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P.1.2-003
Protective role of curcumine against genotoxicity induced by cisplatin

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Curcumin (diferuloylmethane) is a polyphenolic, highly pleiotropic molecule which posses great therapeutical potential and enormous range of pharmacological activities, such as anti-inflammatory, antimicrobial, neuroprotective, antioxidant and anticancer activity. In the last decades, many studies reported the protective effects of curcumin on human lymphocytes against different genotoxic agents. Cisplatin is a anti-cancer chemotherapy drug which is well known as a genotoxic agent simply because of it's ability to generate oxygen/nitrogen free radicals during chemotherapy and causes DNA damage. It is used for therapeutical treatment of numerous human cancers and animal tumors. The main mechanism of action is linked to its ability of interfering with DNA repair mechanisms and inducing apoptosis in cancer cells. The majority of antineoplastic drugs, such as cisplatin, posses strong genotoxic effects. The aim of this study is to investigate the protective effects of curcumin against genotoxicity induced by cisplatin in human peripheral blood lymphocyte cells *in vitro*. The study was conducated with usage of micronuclei assay on three healthy, nonsmoking male volunteers in age of 25–30. Whole blood samples were incubated with cispatin, applied as 50 mg/mL concentrate for solution for infusion and curcumin, as pure analitical standard, at concentrations of 25, 50 and 75 μg/mL. The lymphocyte cultures were then mitogenically stimulated to allow the evaluation of micronuclei in cytokinesis-blocked cytome assay. The results obtained from this study indicate a possible protective role of curcumin against genotoxicity inuduced by cisplatin and have important implications for patients undergoing cisplatin therapy. Future therapeutical guideliness can be applied for patients undergoing chemotherapy, that are consisted of increased therapeutical use of curcumine with aim of protecting DNA and reducing side effects of different genotoxic agents.

P.1.2-004
Conformational dynamics of human dioxygenase ABH2 in the course of action on methylated DNA

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Aberrant DNA methylation is a common threat to a living cells. Depending on their type, these modifications are processed in some different enzymatic pathways. One of them is the direct

oxidative demethylation catalyzed by AlkB family of Fe(II)- and α-ketoglutarate-dependent dioxygenases. Among these dioxygenases human enzyme ABH2 accomplishes the removal of 1-methyladenine (1mA) and 3-methylcytosine (3mC) residues from DNA.

Having two cofactors ABH2, obviously, performs complex kinetic mechanism of action. Resolving this mechanism and describing conformational changes of ABH2 is a key to understanding the structural basis of direct DNA demethylation process and DNA repair in general.

In our study we investigated steady-state and pre-steady state kinetics of human ABH2 using PAGE and stopped-flow methods. The detailed kinetics of conformational transitions in the course of ABH2 action on DNA containing 1mA and binding of ABH2 apoenzyme and its cofactors has been studied providing more deep insight on the mechanism of catalysis.

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P.1.2-005
The O-GlcNAcylation/Phosphorylation balance of DNA-PKcs in response to DNA double-strand breaks

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O-GlcNAcylation is a reversible post-translational modification (PTM) of Serine and Threonine residues, implicated in the regulation of numerous intracellular proteins. Since its discovery in the early 1980s, O-GlcNAcylation has been extensively studied in the context of cellular stress. This PTM is closely linked to phosphorylation since O-GlcNAcylation sites can also be phosphorylation sites and these modifications may be competitive or synergistic for two adjacent residues. DNA-PKcs is a key factor in the Non Homologous End Joining (NHEJ) pathway of DNA double-strand break (DSB) repair. In response to DSB, DNA-PKcs is known to go through an important number of phosphorylations on Ser-/Thr- residues gathered in clusters. Despite a unique report showing that the activity of ataxia telangiectasia mutated protein ATM kinase is affected by O-GlcNAcylation, the O-GlcNAc/Phosphorylation balance in DNA Damage Response (DDR) is poorly studied. It has been shown that DNA-PKcs is an O-GlcNAcylated protein, but the role of this PTM in the DDR is still unclear. To assess the O-GlcNAc/Phosphorylation balance of DNA-PKcs, we immunoprecipitated DNA-PKcs from HeLa cells. Immunoblot analyses using anti-DNA-PKcs or anti-O-GlcNAc antibodies were then performed. We then analysed the activity of DNA-PKcs in the presence or absence of bleomycin- or camptothecin-induced DNA damages after O-GlcNAc modulation. Our results demonstrate for the first time that DNA-PKcs was *O*-GlcNAcylated in Hela cells, and sensitive to O-GlcNAc modulation induced by PUGNAc, DON or siRNA OGT. Also, O-GlcNAc level of DNA-PKcs seems to affect its phosphorylation and its kinase activity. Taken together, these results suggest the potential implication of DNA-PKcs O-GlcNAcylation in the DDR. Others aspects of DNA-PKcs O-GlcNAcylation need further studies. A better characterisation of the DNA-PKcs O-GlcNAc/Phosphorylation balance in DDR will help us to evaluate its impact on NHEJ efficiency during DSB repair.

P.1.2-006
1,N6-α-hydroxypropanoadenine is effectively repaired by *E. coli* AlkB dioxygenase

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1,N^6-α-hydroxypropanoadenine (HPA) is a six-membered adduct formed in reaction of adenine with acrolein (ACR). ACR is a mutagenic agent originated from different sources including cigarette smoke, exhaust fumes and overcooking. It is also generated endogenously during oxidative stress as a by-product of lipid peroxidation. *E. coli* AlkB dioxygenase (EcAlkB) is a DNA repair enzyme that remove alkyl lesions from bases *via* an oxidative mechanism restoring native DNA structure. It belongs to the superfamily of 2-oxoglutarate and Fe(II) dependent dioxygenases. AlkB is induced within *E. coli* system of adaptive response to alkylating agents (Ada response). Our *in vivo* data show that HPA has mutagenic properties and, generated in plasmids, causes mainly A→C and A→T transversions and, less frequently, A→G transitions. We established the optimal pH, Fe(II) and αKG concentrations for enzymatic reaction. Our data proved that the protonated form of HPA is preferentially repaired by AlkB, though the reaction is stereoselective. Moreover, the number of reaction cycles carried out by an AlkB molecule remains limited and reached 38 ± 4 enzymatic cycles before its total inactivation. Molecular modeling of the AlkB/HPA complex demonstrated that the R stereoisomer in the equatorial conformation of the HPA hydroxyl group is strongly preferred, while the S one seems to be susceptible to AlkB-directed oxidative hydroxylation only when HPA adopts the *syn* conformation around the glycosidic bond. In addition to the biochemical activity assays, substrate binding to the protein was monitored by differential scanning fluorimetry allowing the identification of the active protein form with cofactor and cosubstrate bound and monitoring substrate binding. Our results reveal that AlkB – type dioxygenases repair hydroxypropanoadduct to adenine *in vivo* and *in vitro* indicating that they substrate specificity is broader than previously reported.

P.1.2-007
Characteristics of spontaneous γH2AX foci accumulation in long-term cultured human mesenchymal stem cells

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Expansion of mesenchymal stromal/stem cells (MSCs) used in clinical practices may be associated with accumulation of genetic instability. Understanding temporal and mechanistic aspects of this process is important for improving stem cell therapy protocols. We used γH2AX foci as a marker of a genetic instability event and quantified it in MSCs that undergone various numbers of passage (3-22). We found that γH2AX foci numbers increased in cells of late passages, with a sharp increase at passage 16-18. By measuring in parallel foci of ATM phosphorylated at Ser-1981 and their co-localization with γH2AX foci, along with differentiating cells into proliferating and resting by using a Ki67 marker, we conclude that the sharp increase in γH2AX foci numbers was ATM-independent and happened predominantly in proliferating cells. At the same time, gradual and moderate increase in γH2AX foci with passage number seen in both resting and proliferating cells may represent a slow, DNA double-strand break related component of the accumulation of genetic

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instability in MSCs. Our results provide important information on selecting appropriate passage numbers exceeding which would be associated with substantial risks to a patient-recipient, both with respect to therapeutic efficiency and side-effects related to potential neoplastic transformations due to genetic instability acquired by MSCs during expansion.

P.1.2-008
Structural basis for the recognition and processing of DNA containing bulky lesions by the mammalian nucleotide excision repair system

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Mammalian NER eliminates the broadest diversity of bulky lesions from DNA with wide specificity. At that the double incision efficiency for structurally different adducts can vary over several orders of magnitude. Therefore, great attention is drawn to the question of the relationship among structural properties of bulky DNA lesions and the rate of damage elimination. The synthetic DNA structures (model DNA) which imitate NER intermediates and substrates, e.g. double-stranded DNA bearing an appropriate modification are widely used instruments of NER investigations *in vitro*.

Our present work concerns the properties of several structurally diverse model DNAs containing bulky modifications. We evaluated the impact of these lesions on spatial organization and stability of the model DNA. Their affinity for the damage sensor XPC was also studied.

According existing concepts, it was expected, that the values of melting temperature decrease, bending angles and KD values clearly define the model DNAs substrate properties, but the experimentally estimated levels of the substrate properties were far away from these expectations.

Molecular dynamics simulations have revealed structural and energetic basement of the discrepancies observed.

A several lesion-specific regions of DNA secondary structure stabilization and destabilization were found, and their possible impacts on efficiencies of DNA damage recognition and subsequent excision was suggested.

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P.1.2-009
Cancer cell senescence: the role of DNA damage response and NF-kB pathways revisited

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Senescence of cancer cells is an important outcome of treatment of many types of cancers. Cell senescence is a permanent cell cycle arrest induced by stress conditions, including DNA damage. DNA damage activates DNA damage response (DDR), which includes members of the phosphatidylinositol 3-kinase-related kinase (PIKKs) superfamily, protein kinases ATM, ATR and DNA-PK. The so far collected data indicates that ATM, with its downstream targets: CHK2, p53 and p21, is the key protein involved in DDR-dependent senescence. The so called senescence-associated secretory phenotype-SASP relies on ATM/CHK2, but not on p53 signaling. Moreover, genotoxic agents used in cancer treatment can activate NF-κB, which also induces the transcription of SASP genes. In this study we asked the question whether there is a connection between DNA damage-induced ATM and NF-κB signaling pathways in HCT 116 cells undergoing senescence upon doxorubicin treatment. First, we showed that doxorubicin induced DDR-dependent cell senescence, which was successfully abrogated by a PIKK inhibitor, caffeine or simultaneous silencing of all three kinases using three specific RNAs. Furthermore, we have documented, that an interaction between ATM and the IKK (IκB kinase) regulator NEMO does not exist. Moreover, we have shown that the silencing of the NF-κB component, p65, reversed SASP, but not cell senescence, thus proving that DNA damage induced independently the ATM and NF-κB signaling pathways. Next, we asked the question whether all PIKKs are equally involved in DDR signaling of cells undergoing senescence. It appeared that silencing of a single kinase ATM or ATR or simultaneous of two of them did not protect from cell senescence thus proving that DNA-PK can replace them and that all three kinases can be activated during DNA damage-dependent senescence. Moreover, they can compensate each other.

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P.1.2-010
Nuclear poly(A)-binding protein 1 is an ATM target and essential for DNA double-strand break repair

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The DNA damage response (DDR) is an extensive signaling network that is robustly mobilized by DNA double-strand breaks (DSBs). This network is based on a core of DDR-committed players and transitory recruitment of additional proteins from other physiological arenas. The primary transducer of the DSB response is the serine-threonine protein kinase, ataxia-telangiectasia, mutated (ATM), which is activated following DSB induction and in turn phosphorylates a plethora of effectors in various DDR pathways. In this study we uncover a new branch of the ATM-mediated DNA damage response, in which the ATM target is the protein nuclear poly(A)-binding protein 1 (PABPN1) – an RNA binding protein (RBP) that usually regulates different aspects of RNA processing and stability. We speculated that PABPN1 phosphorylation by ATM in response to DNA damage might exert a global effect on alternative cleavage and polyadenylation (APA) of mRNA, a widespread post-transcriptional phenomenon regulated by PABPN1, but 3′-RNA-seq technology ruled out this possibility. Rather, we found that PABPN1 is physically and functionally recruited by the DDR to assist in DSB repair. We map the phosphorylation site of PABPN1 to Ser95 and show that this phosphorylation is critical for timely DSB repair. We find that PABPN1 is important for nonhomologous end-joining (NHEJ) and homologous recombination repair (HRR). The suboptimal repair through HRR in the absence of PABPN1 appears to stem from a defect in deep 5′ to 3′ end-resection, a prerequisite for HRR. Another phenomenon observed upon PABPN1 depletion is the prolonged DSB-induced cell-cycle arrest at the G2/M boundary. Importantly, mass spectrometry analysis captured DNA damage-induced interactions of

phospho-PABPN1 with well-established DDR players as well as other RNA metabolizing proteins; the temporary teaming up of these various proteins with PABPN1 may form a functional module that is recruited by the DDR from the RNA metabolism arena.

P.1.2-011
The molecular basis for yeast persisters

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Mutation rate balances the need to protect genome integrity with the advantage of evolutionary innovations. It has been proposed that microorganisms increase their mutation rate when stressed, perhaps addressing the growing need for evolutionary innovation. Such a strategy, however, is only beneficial under moderate stresses that allow cells to divide and realize their mutagenic potential. By contrast, severe stresses rapidly kill the majority of the population with the exception of a small minority of cells that are in a phenotypically distinct state termed persistence. Although persister cells were described many decades ago, the stochastic event/s triggering persistence remain unknown.

We report that spontaneous DNA damage triggers persistence in budding yeast by activating the general stress response, providing protection against a range of harsh stress and drug environments. We further show that the persister subpopulation carries an increased load of genetic variants, unrelated to their stress survival. We propose that this coupling of DNA damage to phenotypic persistence could increase genetic diversity, specifically in severe stress conditions where diversity is beneficial but the ability to generate de-novo mutations is limited.

P.1.2-012
Effect of downstream enzymes of base excision DNA repair pathway on the action of human DNA glycosylase MBD4

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The processes of DNA methylation and demethylation are the basis for the epigenetic regulation of gene expression, which plays a major role in cellular differentiation, genome imprinting, carcinogenesis, and manyage-related changes in organisms. It is known that different enzyme systems are involved in DNA demethylation: DNA methyltransferases, dioxygenases, and DNA glycosylases. DNA glycosylases initiate the process of demethylation through the excision of the methylated DNA base, and hence, other enzymes, such as AP-endonucleases, DNA polymerases, and DNA ligases, are necessary to restore the original nucleotide. Human methyl-CpG-binding domain 4 (MBD4) removes the 5-hydroxymethyluracil (5-hmU) residues formed as an intermediates in a multistep pathway of DNA active demethylation. The aim of this work was to elucidate the effect of downstream DNA repair proteins of base excision pathway (AP-endonuclease APE1, DNA polymerase β, PCNA and XRCC1) on the stages of specific site recognition and catalysis by MBD4. For this purpose the conformational dynamics of DNA substrates was recorded by following the efficiency of the fluorescence resonance energy transfer (FRET) between FAM/BHQ1 dye pair. Our data revealed that APE1 accelerates MBD4 reaction rate, whereas polymerase β was proved to slow it down. The proteins PCNA and XRCC1 did not effected significantly the MBD4 action.

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P.1.2-013
Systematic computational mapping of known and novel factors in the HRR pathway

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In 70% of hereditary breast and ovarian cancers (HBOC) the initial driver genes are unknown. The vast majority of the known HBOC driver genes such as BRCA1, BRCA2 and PALB2 are involved in DNA damage response, and specifically in homologous recombination repair (HRR). In recent years HRR impairment was discovered to be associated with many other types of cancer including pancreatic and prostate cancers, and gliomas, often as in breast cancer without mutations in the known HRR genes. We therefore hypothesize that at least part of the missing heredity in HBOC and other cancers is caused by unidentified HRR genes. In this work, we systematically mapped genes connected to the HRR pathway, as possible causes and targets for therapy in HBOC and other cancers. We developed a new Clade optimized Phylogenetic Profiling (cladeNPP) method that uses the pattern of conservation and loss of proteins across 600 eukaryotes, while identifying co-evolution even if it happened only in specific clade across the tree of life. Using cladeNPP, we were able to identify many new possible HRR interacting genes that would be missed by existing phylogenetic profiling methods. We are currently experimentally characterizing the function of two genes that we found to co-evolve with BRCA1, and preliminary results show these genes play a previously unknown role in response to DSBs. We further integrated the cladeNPP method with multiple other data sources, including screens detecting genes involved in HRR, co-expression and protein-protein interactions to form a list of 255 genes with possible involvement in HRR. The pipeline we constructed is flexible and modular, and can be easily applied for additional pathways.

P.1.2-014
Genotoxic effects of environmental pollutant zinc and copper pyrithione on zebra fish (*Danio rerio*)

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Zebrafish (*Danio rerio*) is becoming a more widely used and increasingly powerful model organism for many fields of modern biomedical research including vertebrate development, disease modelling, and functional neuroscience. Growing of living organisms such as bacteria, protozoans, algae, and crustaceans can accumulate in large numbers onsurfaces like pipes, tanks, and ships' hulls, resulting in corrosion, clogging and contamination, known as biocides. Zinc and copper pyrithione (Zn-Cu pyrithione) are well known antifouling materials and biocides. The aim of the present study is to determine the effects of these

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environmental pollutants on aquatic toxicology and the possible negative effects on humans by evaluating DNA/RNA oxidative damage and further epigenetic modifications.

The zebrafish (length 3–4.5 cm) were exposed to different concentrations of Zn/Cu Pyrithione starting from 2 μg/L. Stocking density was 20 fish/15 L including 4 different groups as Zn Pyrithione, Cu Pyrithione, Zn+Cu Pyrithione and control groups. Fish samples were taken under ice anaesthesia for histologic and DNA analysis after 24 and 96 h exposure periods. To evaluate the DNA/RNA oxidative damage a total zebra fish was homogenised for DNA isolation, hydrolysed and damage was measured by commercial kit as EIA. DNA/RNA oxidative damage as 8-hydroxy-2′deoxyguanosine (ng/mL) was statistically significantly different compared to control group. Hyperemia, epithelial lifting on the secondary lamella of the gill tissues and hydropic degeneration on the liver tissues were observed.

In conclusion, as marine pollution and deterioration of ecosystems are directly affect the humans. the results presents awareness among environmental pollution, marine pollution and health problems. The toxic effects of environmental pollutants on DNA damage and antioxidant systems on different stages of organisms on the food web provide basic data to understand and estimate the effects on the human beings.

P.1.2-015
Multiple roles of ribosomal protein S3 with respect to DNA repair, cancer and protein quality control

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Our lab has identified that eukaryotic ribosomal protein S3 (rpS3) has a DNA repair endonuclease activity for damaged chromosomal DNA. We also discovered that this protein is involved in the damage processing of mitochondrial DNA damage through the translocation from the cytoplasm to the mitochondria upon excessive oxidative stress conditions. The function of rpS3 in the DNA repair processing is connected with cell viability and protein quality control. It also has multiple extra-ribosomal functions through different post-translational modifications in humans and yeasts which determine the fate of rpS3 between a ribosomal component and a non-ribosomal soluble protein.

Recently, we have discovered that this protein is secreted after glycosylation in the ER. It is secreted only from various cancer cell lines and cells from cancer patients but not in normal cells. We also confirmed that rpS3 is secreted more into media from the more invasive cancer cell lines. The secretion pathway turned out to be a ER-Golgi dependent pathway. We propose that secreted rpS3 could be used as a useful marker for cancer or cell invasiveness.

P.1.2-016
Role of AID/APOBECs in micronuclei formation: AID-ing chromosomal instability

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Genetic abnormalities are the main cause of variability within organisms and are the basis of occurrence of genetic diseases and cancers. Many human diseases not induced by external causes are caused by changes in the genetic information due to chromosome mis-segregation resulting in Chromosomal instability (CIN). Micronuclei (MN) are an effective cellular indicator of CIN, and elevated frequencies of MN are observed in most solid tumors and pre-neoplastic lesions. Micronuclei are the result of abnormalities in the nuclear envelope formation associated with aberrant DNA replication and repair. In order to investigate the role of the AID/APOBECs as inducers of CIN we are using a quantitative assay for chromosome mis-segregation developed by exploiting the human artificial chromosome (HAC) present in human fibro-sarcoma HT1080 cells. In these cells the HAC kinetochore can be conditionally inactivated, thus leading to formation of micronuclei. Interestingly, we observed elevated levels of formation of micronuclei after transient expression of AID, APOBEC1, APOBEC3A and APOBEC3G in presence of CytochalasinB (CytoB) an actin polymerization inhibitor. Remarkably, even in absence of co-treatment with CytoB, elevated levels of MN were observed especially in the case of AID and APOBEC1. To further verify whether the induction of C to U changes and the downstream activation of the DNA repair pathways, we inhibited the Uracil-DNA glycosylase (UDG) using a bacterial UDG inhibitor (UGI). Indeed, a diminution of the levels of MN formation in cells expressing AID and APOBEC1 in the presence of UGI was readily observed, indicating that MN formation can indeed be triggered downstream to the activation of the DNA repair pathways. Considering that the AID/APOBECs have been associated to the onset of cancer through their ability to mutate DNA, our finding show another possible cancer-inducing effect of these deaminases: their ability to induce chromosomal instability.

P.1.2-017
DNA-dependent protein kinase activity assessed by quantum dot–based microarray

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Therapeutic efficacy against cancer is often based on a variety of DNA lesions, including DNA double-strand breaks (DSB). DSB could be repaired by the both major pathways homologous recombination (HR) and non-homologous end-joining (NHEJ), in which Rad51 and DNA-dependent protein kinase (DNA-PKcs) represent central proteins, respectively. In last decade, the function of these DNA repair proteins has been described as a potential mechanism of resistance in tumor cells. Therefore, the proteins of reparatome have become targets for treatments aiming to improve the efficacy of anticancer therapy. Given the importance of DNA-PKcs activity in NHEJ, the therapeutic efficacy of targeting DNA-PKcs is frequently described as a strategy to prevent repair of treatment mediated-DNA damage in cancer cells. The screening of new inhibitors, acting as sensitizers, require the development of high-through-put tools to identify and assess the most effective molecules. Here, we describe the elaboration of antibody microarray dedicated to NHEJ pathways focusing on the DNA-PKcs activity in response to DNA damage. By combining protein microarray with the semiconductor fluorescent quantum dots (QDs) detection, we show that it is possible to follow the modification of proteomic and phosphoproteomic cellular profiles induced by inhibitor during the response to DNA damage. Finally, we discuss applications of developed promising tool to screening kinase inhibitors and then targeting DSB repair to improve chemo- or radiotherapy in cancer treatment.

P.1.2-018
Dynamics of multistep damage recognition by 8-oxoguanine–DNA glycosylases

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8-Oxoguanine DNA glycosylases of different structural families excise the oxidative product 8-oxoguanine (oxoG) from DNA. Despite a wealth of available structural and kinetic information, not much is known how these enzymes efficiently distinguish oxoG from a large excess of undamaged DNA bases. Also, due to their ability to find specific non-canonical bases in DNA and introduce targeted breaks at these points, DNA glycosylases look promising as DNA-modifying tools. Here we combine molecular dynamics simulations and evolution analysis to explore the mechanism by which human 8-oxoguanine DNA glycosylase (OGG1) and its bacterial counterpart (Fpg) discriminate between oxoG and guanine (G). The key predictions of the simulations have been validated by site-directed mutagenesis and kinetics. Based on the free energy profiles, we found that hOGG1 and Fpg are remarkably similar in their damage recognition mechanisms despite lack of structural homology. They distinguish 8-oxoG and G using the bases' specific features not only at the active site, but also at earlier stages during base eversion. Glycosylase mutations or DNA modifications abolishing the key identified transient protein–DNA interactions along the eversion pathway significantly decreased the activity of both enzymes. The similarity suggests that lesion recognition through multiple gating steps may be a common theme in DNA repair. In Fpg, we have identified an evolutionarily conserved cluster of amino acids far from the active site that likely participates in the enzyme specificity at a very early stage of DNA binding. Furthermore, we have analyzed 104 Fpg structures with a randomly mutagenized active site loop searching for conformational predictors of the enzyme activity. Based on these results, we propose a scheme for in silico selection of DNA glycosylases with altered specificity based on the Fpg scaffold.

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P.1.2-019
DNA repair in transcription intermediates by helix–two-turn–helix family DNA glycosylases

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Oxidative DNA lesions are constantly generated by both endogenous and environmentally induced reactive oxygen species. Repair of oxidative base lesions proceeds via base excision repair pathway. In bacteria, Fpg and Nei DNA glycosylases, belonging to the helix–two-turn–helix (H2TH) structural family, remove oxidized purines and pyrimidines, respectively. Interestingly, human H2TH family glycosylases, NEIL1, NEIL2, and NEIL3, have been reported to prefer oxidized lesions in DNA bubbles or single-stranded DNA. It had been hypothesized that NEIL proteins might be involved in the repair of lesions in transcription bubbles associated with RNA polymerase molecules. However, direct action of H2TH family enzymes in transcription bubbles has not been shown, and structural features of bubbles required for efficient excision, except for their length, have not been studied. We have investigated the excision of 8-oxoguanine (oxoG) and 5,6-dihydrouracil (DHU) by *E. coli* Fpg and Nei and human NEIL1 and NEIL2 from single-strand oligonucleotides, perfect duplexes, bubbles with different number of unpaired bases (6–30) and containing the lesion in different positions, D-loops with the third strand made of DNA or RNA, and from complexes with *E. coli* RNA polymerase. Fpg, NEIL1 and NEIL2 efficiently excised the lesion located inside a bubble. Fpg and NEIL1 was generally more active than NEIL2 in excision of 8-oxoG from ssDNA and bubbles. Nei, on the other hand, was active only on DHU located in dsDNA. Fpg and NEIL1 also have shown activity in D-loops with RNA. The presence of an additional unpaired 5′-tail of DNA or RNA didn't affect the activity. The activity of Fpg was observed in pre-assembled transcriptional complexes with *E. coli* RNA polymerase and depended on the position of the lesion in the bubble, possibly reflecting local accessibility of the lesion within the elongation complex.

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P.1.2-020
Fumarase is involved in DNA double strand break resection through a functional interaction with Sae2

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Fumarase is a mitochondrial enzyme of the tricarboxylic acid cycle and can be readily detected in the cytosolic compartment of all organisms. We have previously discovered that the cytosolic population of fumarase is important for the DNA damage response (DDR) against double strand breaks (DSBs). Nevertheless, the particular role of the enzyme in DDR pathways is not fully understood. Recently a comprehensive study suggested that in human cells the homolog of fumarase is important for the non homologous end joining (NHEJ) repair pathway. Here we show that cytosolic depletion of fumarase in yeast prolonged the interaction of Mre11 with the DSB and subsequently delayed the onset of repair by the homologous recombination (HR) pathway. The knockout of the NHEJ component yKu70 decreased the DSB susceptibility of cytosolic fumarase depleted cells. These results suggest the involvement of cytosolic fumarase in the DSB resection process, which is essential for the repair of DSBs by HR. Further investigation showed that overexpression of fumarase in Mre11 nuclease dead strains, or exposure of such cells to fumarate, reduced their DNA damage sensitivity. The elevated susceptibility to DSBs, following cytosolic fumarase depletion, was suppressed by the overexpression of Sae2 nuclease. Fumarase overexpression, however, had no effect on the DNA damage susceptibility of Sae2 depleted yeast. Measurement of DSB resection kinetics, 0.29 kbp from the break site, showed significant inhibition of resection following the depletion of cytosolic fumarase. Notably, overexpression of Sae2 reconstituted the DSB resection ability of the cytosolic fumarase depleted cells. A split-ubiquitin assay suggested that cytosolic fumarase and Sae2 physically interact *in-vivo*. These results suggest that fumarase is involved in the DSB resection process, through a functional interaction with Sae2.

P.1.2-021
Promoter G-quadruplexes refolds to double stranded DNA under photoinduced damage by cationic porphyrins

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For a number of oncogenes, it is known that guanine rich promoter sequences form the G-quadruplex spatial structures, which are involved in the regulation of their expression. Stabilization of such structures in most cases leads to inhibition of expression. On the other hand, it is known that oxidative stress leads to DNA damage primarily via guanine modifications. In our work, we investigated the interaction of cationic porphyrin derivatives with a number of promoter sequences of oncogenes (BCL2, KIT, MIC, KRAS, NRAS) and photo-induced action of these compounds on the promoter DNA sequences. The double helix of DNA appeared to be more resistible to porphyrin induced photodamage comparing to the G-quadruplex structure of promoters sequences. The location of oxidized guanines in the potential quadruplex sequences of the oncogenes was determined. Oxidation of guanines leads to destruction of the G-quadruplexes structure and formation of the double helix of DNA. The mechanism of spontaneous or porphyrin induced mutations in potential quadruplex sequences and their impact in oncogene expression are discussed.

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P.1.2-022
Oxidatively induced macromolecular damage in frontotemporal dementia and dementia with lewy bodies

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Dementia is a complex disease that mostly affects the elderly and represents a significant public health burden in the world. Dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD) are the most common forms of dementia, in which oxidative stress is significantly involved. Free radical-mediated oxidatively induced macromolecular damage plays a role in basic neurodegenerative mechanisms. In this study, oxidatively induced DNA, lipid, protein damage markers and N^{ε}-carboxymethyllysine (CML) levels were studied in peripheral blood of DLB (n = 20), FTD (n = 21) patients, and controls (n = 45). In addition, the mRNA expressions of DNA repair enzymes, human 8-oxoguanine DNA glycosylase (hOGG1) and human endonuclease VIII-like 1 (hNEIL1), were measured with RT-PCR in order to evaluate DNA damage/repair balance together. DNA base damage products were determined by isotope dilution GC-MS/MS. The levels of 3-nitrotyrosine was measured by ELISA; malondialdehyde (MDA) and CML levels were investigated with HPLC. Regarding the base damage products studied, the levels of 4,6-diamino-5-formamidopyrimidine were significantly higher in DLB and FTD patients as compared to control subjects ($P < 0.05$). There were no significant changes in 8-hydroxyguanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 5-hydroxy-5-methyl-hydantoin and 5-hydroxycytosine levels. The thymineglycol, 5-hydroxymethyl-uracil, 5-hydroxyuracil, 5,6-dihydroxyuracil damages were not detected neither in patients nor in the control groups. MDA levels in DLB, and FTD patients were significantly higher than those in controls ($P < 0.05$), whereas CML, 3-nitrotyrosine, hOGG1 and hNEIL1 levels did not differ significantly between the groups. This study is noteworthy, since markers of oxidative macromolecular damage and expressions of DNA repair enzymes have been studied for the first time in this extent of dementia patients.

P.1.2-023
A new regulatory pathway responding to hyper-acetylation of histone H3K56

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Acetylation of histone H3 on lysine 56 is a modification that marks newly synthesized histones and sites of DNA damage. Deacetylating this residue is equally as important as its acetylation. In the model organism *Saccharomyces cerevisiae* two redundant proteins of the sirtuin family, Hst3p and Hst4p, keep the acetylation levels low outside the S-phase of the cell cycle. In the absence of those deacetylases (in cells that are *Δhst3 Δhst4*) there is hyper acetylation of H3K56 throughout the entire cell cycle and on both new and old histones, conferring genomic instability that leads to phenotypes of defective growth and thermosensitivity. Here we report that cells experiencing hyper acetylation of H3K56 share a lethal interaction with the lack of the protein kinase Dun1p, in a manner that is independent of *DUN1*'s known role in upregulation of dNTP levels in the cells. We also report that Dun1p is a negative regulator of the alternative clamp loader Ctf18p, but not of the other alternative clamp loaders, Elg1p and Rad24p. We find that other proteins involved in the S-phase checkpoint, such as Mrc1, as well as some of the downstream targets of Dun1p, such as Npl3p, are also part of this regulatory pathway. In contrast, the E3 ubiquitin ligase Rtt101p, similarly to Ctf18p, is under the regulation of Mrc1-Dun1-Npl3 pathway. Neither Rtt101p nor Ctf18p interfere with the acetylation of H3K56, but seem to act downstream of it. Together, our findings argue that in the presence of hyper acetylation of H3K56, Dun1p, Mrc1p and Npl3p play a critical role in maintaining viability against harmful effects caused by the actions of Ctf18p and Rtt101p.

P.1.2-024
Inherited mutations in the DNA helicase RTEL1 compromise telomere elongation by telomerase in Hoyeraal-Hreidarsson syndrome

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Telomeres protect the chromosome ends from being recognized as double strand breaks, activating the cellular DNA damage response, and causing cell cycle arrest and genomic instability. Telomeres are elongated by telomerase in a tightly regulated manner to compensate for sequence loss during replication and ensure a sufficient number of cell divisions throughout life, yet restrict lifespan and prevent cancer development.

Hoyeraal-Hreidarsson syndrome (HHS) is an inherited telomere biology disease characterized by accelerated telomere shortening and a broad range of symptoms, including bone-marrow failure, immunodeficiency, developmental defects and death at early age. Mutations in the DNA helicase RTEL1 were found to cause HHS, among other mutations in genes implicated in

telomerase biogenesis, recruitment or activation. RTEL1 functions in genome-wide replication and inhibition of tri-repeat expansion and fragility. It was also suggested to functions at telomeres in the resolution of G-quadruplexes and t-loops during DNA replication. However, a direct role of RTEL1 in telomerase action has not been.

To further understand the role of RTEL1 in telomere length maintenance, we examined the telomere phenotypes resulting from RTEL1 mutations in HHS patient cells, and the suppression of these phenotypes by inducible ectopic expression of various variants of RTEL1.

In addition, we expressed a recombinant RTEL1 protein in insect cells to study its biochemical activities *in vitro*, and examine the precise substrate specificity, catalytic activity and protein-protein interactions of the protein. Our results support the role of RTEL1 in G-quadruplex resultion during telomere replication. In addition, they indicate a novel essential role of RTEL1 in telomerase action. Thus RTEL1 is essential for extending the proliferation capacity of telomerase positive cells and supporting the renewal of tissues with rapid cellular turn over, as well as cancers.

P.1.2-025
Dynamic changes in the chromatin landscape of the small intestine of DNA mismatch-repair deficient and irradiated mice: insights from a multi-scale model

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Cancer is a consequence of accumulating genomic aberrations accompanied by changes in the epigenome that can modulate gene expression patterns and their stability. Sources of genomic variations are e.g. an impaired DNA mismatch-repair (MMR) system, which is known to be recruited by the histone mark H3K36me3. Recently, it has been demonstrated that DNA damage represses the H3K36me3 demethylase KDM2b, which potentially improves the recruitment of MMR. Whether and how defects in the MMR machinery impact on these processes is largely unknown. We have measured histone modification profiles of the small intestine of irradiated, MMR-deficient and control mice to reveal long-term changes in the epigenetic landscape of the tissue induced by (1) temporary DNA damage and (2) constantly accumulating genetic instabilities. Thereby, we found significant alterations already in the intestine of minimal irradiated mice, among them a global increase in H3K36me3 and a local enrichment of the H3K4me3 histone mark at the promoter of polycomb target genes, which are essential for stem cell fate decisions. Similar changes are detected in untreated MMR-deficient tissues prior to tumour formation. We hypothesise that the H3K4me3 gain following DNA damage results in a bivalent cell state and protects the respective gene promoter from DNA methylation and, thus, the cell from severe changes in the expression of tissue-(un)specific genes. In order to evaluate this hypothesis, we integrate our molecular findings into a well-established, 3D model of the intestinal crypt. This extension allows us to quantitatively study the impact of changes in the epigenetic landscape of individual cells on the composition and the development of the crypt following genetic perturbations and during ageing.

P.1.2-026
APOBEC1, an RNA/DNA editing path towards cancer

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The AID/APOBECs are cytosine deaminases acting on nucleic acids and exerting their activity in diverse physiological contexts. Yet, their common ability to mutate nucleic acids represents a double edged sword, and their mutational signature can be found in 60% of cancer types. Among them, APOBEC1 is the only family member to physiologically target RNA. APOBEC1 has been linked to cancer development in mice but its oncogenic mechanisms are not yet well understood. APOBEC1 is overexpressed in oesophageal adenocarcinomas and in Barrett's oesophagus, a closely associated dysplasia. Here we show that expression of APOBEC1 induces both a mutator phenotype and chromosomal instability in human cells, likely through direct targeting of genomic DNA. These are features that can be found both in the tumours and in the dysplasia. Moreover, analysis of the RNA-seq data from The Cancer Genome Atlas reveals a number of transcripts targeted by APOBEC1, whose editing could affect the cellular homeostasis. These findings suggest that APOBEC1 could support the oncogenic process from its very early stages through a dual path: the induction of genetic alterations through its ability to target DNA, and alteration of the cellular state through RNA editing.

P.1.2-027
Effects of RTK family-mediated Rad51 phosphorylation on homologous recombination DNA repair pathway

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Human cells are subjected to environmental and endogenous stresses that can damage DNA and affect the genomic integrity. Among these damages, the double strand breaks (DSB) of DNA are the most severe alteration and are repaired by homologous recombination (HR) pathway. Rad51 protein plays a central role in this mechanism and its regulation is essential to achieve the faithful repair of DNA. The Rad51 activity is regulated through some post-translational modifications such as phosphorylations but its dysregulation could promote the cancer cell resistance to therapeutic treatments.

In the last decade, many studies have provided an evidence for the relation between receptor tyrosine kinase (RTK) signaling and the response to DNA damage pathways. RTK family can indeed affect the DNA repair pathways. Many studies demonstrate the involvement of RTK on HR process in cancer cells, but the direct link with Rad51 has not been fully shown.

In this context, we have tested and identified several RTK proteins which are directly able to phosphorylate Rad51 protein *in vitro*. The phosphorylation sites were identified using different mutants of RAD51 in which each tyrosine was replaced with a phenylalanine residue. We confirmed these preliminary results by using mass spectrometry approach. We then studied the impact of these Rad51 phosphorylations on its recombinase activity and on the HR DNA repair pathway.

The elucidation of physiological role of Rad51 phosphorylations induced by the receptor tyrosine protein kinases can allow

identifying the interaction networks involved in the resistance to anticancer therapy. Therefore targeting HR process through Rad51 and RTK signaling might open up to a new strategy for sensitization of resistant cancer cells.

P.1.2-028
Modulation of DNA-dependent protein kinase by a reactive nitrobenzoxadiazole compound

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The expression and activity of DNA-dependent protein kinase (DNA-PK) is related to a DNA repair status in the response of cells to exogenous and endogenous factors. Recent studies indicate that EGFR is involvedin modulating DNA-PK. It has been shown that a compound NSC 228155 (NSC), bearing a nitrobenzoxadiazole (NBD) scaffold, enhances tyrosine phosphorylation of EGFR and triggers downstream signaling pathways. Here, we studied the behavior of DNA-PK and other DNA repair proteins in prostate cancer cells exposed to compound NSC. We showed that both the expression and activity of DNA-PKcs (catalytic subunit of DNA-PK) rapidly decreased upon exposure of cells to the compound. The decline in DNA-PKcs was associated with enhanced protein ubiquitination, indicating the activation of cellular proteasome. However, pretreatment of cells with thioglycerol abolished the action of compound NSC and restored the level of DNA-PKcs. Moreover, the decreased level of DNA-PKcs was associated with the production of intracellular hydrogen peroxide by stable dimeric forms of Cu/Zn SOD1 induced by compound NSC. Our findings indicate that reactive species of oxygen and electrophilic intermediates, generated and accumulated during the redox transformation of NBD compounds, are primarily responsible for the rapid modulation of DNA-PKcs functions in cancer cells.

P.1.2-029
Expanding the mitochondrial links to the DNA damage response

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Genome integrity is constantly battered by genotoxic agents. These can induce DNA damage that leads to cell death if not properly repaired. Most studies on the DNA repair process have focused on yeast and mammals, in which histone chaperones have been revealed as key regulators for DNA to be accessible to repair machinery. However, knowledge of their exact role in DNA damage response is far from complete, in particular in plants. Our recent studies reveal that the closely related histone chaperones human SET/TAF-Iβ and plant NRP1 are similarly involved in nucleosome assembly following DNA break in humans and plants, respectively [1,2], and that both histone chaperones interact with cytochrome *c* in the cell nucleus upon DNA damage. We have used Nuclear Magnetic Resonance (NMR), Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR) and Molecular Docking (MD) to provide a structural insight into the complex formed by cytochrome *c* with each histone chaperone. Cytochrome *c* competitively hinders the binding of SET/TAF-Iβ and NRP1 to core histones, thus locking their histone binding domains and inhibiting their nucleosome assembly activities [1,2]. These findings also indicate that the underlying molecular mechanism of nucleosome disassembly/reassembly needed for DNA repair is highly conserved throughout evolution.

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P.1.2-030
About how bacterial photolyases may assist in repairing UV-damaged human DNA

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Ultraviolet (UV) irradiation produces inflammation, degenerative aging and skin cancer. Among others, UVA/B causes direct and indirect DNA damage (oxidative stress and protein denaturation), meanwhile UVC mainly causes direct DNA damage by formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine (6,4) pyrimidone photoproducts (6,4PP). The Nucleotide Excision Repair (NER) system repairs these lesions, but in addition to NER, the Base Excision Repair pathway produce glycosylases and other enzymes that restore the DNA. However, the simplest way to repair a CPD or 6,4PP lesion is carried out by the enzyme photolyase that directly reverses the damage. Photolyases are found in all living forms, except placental mammals and some marsupials. Our hypothesis of work was that photolyases produced by UVC-resistant bacteria may efficiently fix the damage of human UV-irradiated DNA. A collection of Antarctic UVC-resistant bacteria was assessed and their UV-resistant behavior was characterized, then CPD- and 6,4-photolyases were searched into the draft genomes (results already published). Based on bacterial UVC-resistance properties, two coding sequences for CPD- and 6,4-photolyases were chosen and produced by DNA recombinant technology and purified to homogeneity. Their activities were analyzed using comet assay (CHO and HaCaT cell lines) and highly sensitive and specific monoclonal antiCPD or anti-6,4PP antibodies (on calf thymus DNA; ELISA experiments). All experiments were subjected to statistical analysis. Results showed that the bacterial CPDphotolyase repairs 90% and 80% of induced DNA-damage in CHO and HaCaT cell lines (comet assay), respectively; and 50% of calf thymus damaged DNA as observed using ELISA. Currently, we are analyzing the repair potential of the recombinant 6,4- photolyase. Finally, we are prone to produce low costly photolyases with a high repairing ability on eukaryotic DNA, mainly for cosmetics and pharmaceutical uses.

Proteomic Approaches in Cell Biology

P.1.4-001
Alterations in the proteome of leaves of cold-tolerant and cold-sensitive grapevine cultivars during long-term cold stress

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Grapevine is one of the oldest crops in the world. However, grapevine is also very sensitive to low temperatures, which limits its cultivation in many parts of the world. In order to gain a better understanding of cold tolerance mechanisms in grapevine, we investigated changes in the proteome of leaves of cold-sensitive and cold-tolerant cultivars in response to prolonged cold-stress. The isolated proteins were separated by two-dimensional electrophoresis (2-DE) and identified with tandem mass spectrometry type MALDI TOF/TOF. Many of the identified proteins demonstrated a similar tendency in the level of their accumulation in leaves of both the cold-sensitive and cold-tolerant cultivar under cold stress. However, a few proteins presented a specific profile of accumulation in leaves from the varieties with different tolerance to cold, or else a change in their accumulation was noted during stress in leaves of one of the two analysed cultivars. The most significant differences concerned the proteins involved in carbohydrate and energy metabolism (malate dehydrogenase, ferredoxin NADP reductase, chlorophyll a-b binding protein), genetic information expression and protein modification (ribosome recycling factor) and defence (chaperonin 20 kDa). The proteins distinguished in this research can play the role of potential biomarkers of grapevine sensitivity to cold.

P.1.4-002
Exosomes of human placenta: analysis of morphology and proteins

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Syncytiotrophoblast cells constitutively secrete exosomes of endosomal origin during the pregnancy. The placenta-derived exosomes are important in intercellular communication and immune function. Placenta exosomes carry proteins and RNA molecules. Exosomes from different sources were mostly obtained by differential centrifugation and ultracentrifugation. It was shown these exosomal preparation contained from several dozen to hundreds protein. In our work the exosomes were isolated from placenta of 10 healthy women by differential centrifugation and ultrafiltration. Exosome preparations were additionally purified by gel filtration from impurity proteins and their complexes. Morphology of vesicle preparations was characterized with transmission electron microscopy (TEM) and size distribution was determined by nanoparticle tracking analysis (NTA). Electron microscopy revealed the presence of spherical vesicles, with a typical cup-shape and diameters ranging from 40 to 100 nm, microparticles without membranes (20-60 nm) were also observed within the preparations. NTA showed a particle size distribution ranging from 45 to 295 nm in diameter with an average size of 91 ± 25 nm. TEM with anti-CD63 and anti-CD81 immune labeling demonstrated the presence of exosomes in 40–100 nm membrane particles. Exosome proteins were identified before and after gel filtration by MALDI MS and MS/MS spectrometry of protein tryptic hydrolysates derived by SDS PAGE and 2D electrophoresis. Purified exosomes contained only from ten to twelve different major proteins: secreted proteins, iron transport proteins, cytoskeleton-related proteins, tetraspanins. Our results demonstrating the decrease of number of major proteins identified in preparations of exosome after gel filtration may be important in further studies of exosomes' biological functions.

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P.1.4-003
Quantitative proteomics analysis of proteins involved in leaf senescence of rice

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Enhancing grain production of rice (*Oryza sativa* L.) is a top priority in ensuring food security for human being. One approach to increase yield is to delay leaf senescence and extend the available time for photosynthesis. Liang-You-Pei 9(LYP9), one of the super hybrid rice, possesses big advantages of high yield and high disease resistance, but is age-sensitive, which often decreases grain yield. In the present study, to have a better understanding of the complex mechanism of leaf senescence of LYP9, and identify proteins associated with leaf senescence and establish links between protein expression changes and leaf senescence, iTRAQ-based quantitative proteomics approach was employed to investigate the protein expression profiles in flag leaves at early, middle and late stages of grain-filling of rice LYP9. Totally 5067 proteins were identified. Compared with the proteins in the flag leaves at early stage of grain-filling of LYP9, 240 proteins up-regulated and 188 proteins down-regulated in the leaves of middle stage, and 387 proteins up-regulated and 202 proteins down-regulated in the leaves of late stage, respectively. In addition, 39 identified proteins were constantly up-regulated and 18 identified proteins were constantly down-regulated in the leaves from early to middle and late stages of grain-filling, respectively. Among them, chloroplast chaperonin 10 (Cpn10), geranylgeranyl diphosphate reductase, Mg chelatase subunit ChLD, porphobilinogen deaminase (PBGD), protochlorophyllide (Pchlide) reductase B and thioredoxin-like protein CITRX might have involved in the leaf senescence of rice. These findings provided valuable information for understanding the age-sensitive of LYP9, and offered a foundation for future improve LYP9.

P.1.4-004
Decoding the bacterial responses to anti-infective agents through proteomic approaches

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Almost all bacterial pathogens depend on cell density mediated cell to cell communication called quorum sensing (QS) to coordinate their physiological and pathogenic determinants (virulence factors and biofilm) required for antibiotic resistance and to establish infection under *in vivo* conditions. Hence, inhibition of QS/biofilm formation in clinically important pathogens has become one of the promising targets to quench the virulence and emergence of multiple drug resistance. QS and biofilm inhibitors (collectively known as anti-infective agents) work by targeting the expression of genes involved in virulence and biofilm production and hence the possibility of the emergence of resistance is meagre. Numerous anti-infective agents have been identified against a wide range of pathogens but their mode of action remains unclear. Proteins are the real players involved in all

biological processes. Hence in the present study, two dimensional gel based proteomic profiling followed by mass-spectrometric identification of differentially expressed proteins of bacterial pathogens grown in the absence and presence of anti-infectives were done to shed more light on molecular mechanisms and identification of promising druggable targets. Vanillic acid was found to attenuate the QS and biofilm formation in *Serratia marcescens* by down-regulating the proteins involved in surface layer formation, fatty acid biosynthesis and amino acid metabolism. Furthermore, proteomic analysis revealed the ability of L-Ascorbyl 2, 6-dipalmitate to down-regulate the expression of intracellular peptidases in methicillin-resistant *Staphylococcus aureus* to attenuate the pathogenicity and biofilm formation and not by directly affecting the QS signalling process. Hence, it is advocated that studying the responses of bacterial pathogens to anti-infective agents would pave the way for identification of novel molecular mechanism(s) and hidden protein target(s). Further, this target protein(s) and pathways could be useful in designing/screening of novel anti-QS and antibiofilm agents to combat the QS and biofilm mediated infectious diseases.

P.1.4-005
The combination of Surface Acoustic Wave technique with mass spectrometry provides a novel tool to study metabolic pathways

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Surface Acoustic Wave (SAW) technique has emerged as a powerful tool to study a wide range of affinity biomolecular interactions at near physiological conditions, ranging from protein-protein to protein-peptide or protein-DNA interactions. Its combination with proteomic approaches allows the direct characterization of proteins in biological samples, providing new tools to analyse interacting proteins related to the same metabolic pathways.

In the current work, this combination was selected to study the pathway of epithelial growth factor (EGF) protein and its receptor (EGFR). It has been reported that overexpression of EGFR is related with inflammation diseases and cancer. Briefly, the growth factor EGF was immobilized on a chip surface of a SAW biosensor. Samples of prostate homogenates of 12-week old rats with aged-related hyperplasia, were injected and passed through the chip surface. Affinity binding phenomena of prostatic homogenate proteins with the immobilized EGF protein and also with its receptor were first measured via the SAW biosensor. Interacting proteins were then eluted from the chip surface and submitted to a classical bottom-up proteomic analysis (enzymatic digestion and nanoliquid chromatography coupled to tandem mass spectrometry of the peptide mixture). Not only EGFR, which is a member of the Erbb family, but also Erbb2, Erbb3 and Erbb4 were found. Moreover, other proteins described as interactors of EGFR such as hornerin, clatherin, myosin or filaggrin were detected.

The eluted proteins were related with EGF and EGFR metabolic pathway. All of them have been previously reported to play crucial roles in inflammation diseases, cancer proliferation and metastasis when they are overexpressed in cells.

Our results point out that the combination of the SAW technique with mass spectrometry is a valuable novel tool to deeply study metabolic pathways.

P.1.4-006
SDS-PAGE analysis of heat shock proteins of *Enterococcus faecalis* and *Enterococcus faecium* isolates from urine and feces samples

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In this study, the synthesis of heat shock proteins (HSP) was investigated in enterococci isolated from urine and stool samples of humans. For this purpose, a total of 30 *E. faecalis* isolates, 20 (62.5%) from 32 urine and 10 (16.6%) from 60 stool samples were identified by multiplex PCR technique. A total of 30 *E. faecium* isolates were identified as 8 (4.5%) from 177 urine and 22 (47.8%) from 46 stool samples. In protein band analysis performed on SDS-PAGE; *E. faecalis* and *E. faecium* reference strains were subjected to different heat treatments at various application times for preliminary testing to determine optimal temperatures and application times for HSP synthesis. According to the treatment of *E. faecalis* reference strain with 42°C and 45°C temperatures for 30 min. separately, new and different protein molecules as 38, 68 and 120 kDa molecular weight, were determined respectively. After treatment of *E. faecium* reference strain at 42°C and 52°C temperatures for 30 min. respectively, it synthesized new and different protein molecules at 67, 73, 80 and 120 kDa molecular weight at both heat treatments. Protein molecules of 38 (3.3%), 43 (50%) and 120 (80%) kDa were detected at 42°C in 30 *E. faecalis* isolates. Same protein molecules were also observed at 45°C. While only 3 *E. faecium* isolates synthesized 120 kDa protein at 30 min with 42°C treatment, 38, 43, 56 and 120 kDa protein molecules were observed at 52°C.

P.1.4-007
Separation and fractionation of peptide mixtures in proteomic analysis using simple capillary gradient chromatographic setup

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Separation and (pre-)fractionation of a complex peptide mixtures, glycopeptides and glycans using a comprehensive, fast and straightforward chromatographic system in a microliters volumes is demonstrated. The introduced chromatographic system consists of a capillary column, a microsyringe, a steel weight, a holder, a laboratory stand and either a MALDI target or tubes for collecting eluate fractions and the handling does not require special

skills of operator. The processing of one sample which includes pre-wetting, equilibrating, sample loading, washing and microgradient elution either onto a MALDI target (simple peptide mixture) or into microtubes for second dimension of the 2D LC proteomic experiment (complex peptide mixture).

The procedure starts with the preparation of a capillary column (15 min) followed by its pre-wetting, equilibrating, sample loading and washing (15-20 min). Next, the microgradient is easily created in the syringe by a consecutive aspirating of solutions with decreasing concentrations of acetonitrile. In the final step, the sample is either directly eluted on MALDI target or collected to tubes for a further analysis (5–10 min). The key features are: (i) the application of an easy, very fast and low-cost analysis of samples by an alternative chromatographic method highly compatible with mass spectrometry or other downstream proteomic procedures of the sample preparation; (ii) the unique variability of the system setup due to a smart split-free microgradient elution allowing multi-dimensional chromatographic separations. This system nicely contributes to the repertoire of common liquid chromatographic systems and provides an alternative way to achieve excellent chromatographic separations.

P.1.4-008
Derivatization of peptides captured on reversed stationary phase for their improved de novo sequencing using LC MALDI-TOF MS/MS analysis

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De novo sequencing of enzymatically derived peptides may be important in cases when the protein sequence is not present in protein databases or when the protein is posttranslationaly modified. Tandem mass spectrometry analysis sometimes produces complex fragmentation spectra and modification of peptides for simpler spectra interpretation may be important.

Most peptide derivatization protocols are usually carried out in a reaction tube in a solution containing necessary ingredients for completing the reaction. This is usually followed by removal of reagents and purification of peptides prior to mass spectrometry analysis. The work here presents an alternative way for derivatization of peptides captured on C18 reversed stationary phase in a homemade microcolumn. First, peptides are loaded on the stationary phase, then they are modified with O-methylisourea and afterwards with 4- sulfophenylisothiocyanate. The reagents are removed after completing the reactions by washing the column and the modified peptides are separated on a MALDI target plate for tandem mass spectrometry analysis, simplified data interpretation and their *de novo* sequencing.

Presented protocol simplifies the peptide derivatization for obtaining simpler tandem mass spectra, which leads to easier *de novo* sequencing of peptides. Moreover, the necessary amount of derivatization reagents is decreased and the purification step may be combined with direct reversed phase chromatography separation of modified peptides.

Molecular Basis of Diseases

P.1.5-001
Assesment of the prevalence of factor II and factor V Leiden gene polymorphisms related to cardiovascular diseases in healthy blood donors

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Global life expectancy has been increased significantly in recent years and this situation has lead to an increased interest in age-related diseases. Cardiovascular diseases are serious causes of mortality and morbidity in the worldwide and in Turkey. Previous studies have been shown that factor V Leiden (FVL, G1691A) and factor II (FII, G20210A) mutations are most common genetic risk factors for thromboembolism. Therefore, we investigated the frequency of FVL and FII polymorphisms among healthy blood donors depending on age and weight in Blood Bank Centre of Balcali Hospital.

The study was conducted by age and weight on 96 donors. The patients subjected to the study were separated into two age groups between the ages of 20–39 and 40–59. In addition, the donors were divided into different groups respectively to their body mass index normal weight, and obese. Peripheral blood was collected into EDTA-containing tubes for genetic analyses. DNA was extracted from buffy coat using DNA blood kit (Qiagen, Germany) and stored −80°C for later use. The FVL and FII polymorphisms were determined by PCR using TIB, MOLBIOL, LightMix kits (CN: 40-0593-64, 40-0594-64).

The results revealed that when donors were evaluated for Factor II gene polymorphism, the 9.09% of donors with a normal weight of 20–39 age group were heterozygous (G/A) and others were homozygous (G/G). All obese donors were found homozygous (G/G) genotype genes. All donors of 40–59 age group with normal weight were homozygous (G/G) and 4.37% of donors in obese group was heterozygous (G/A) genotypes for Factor II genes. When donors were evaluated for Factor V Leiden gene polymorphism, 9.09% of donors with the normal weight of 20–39 age, 9.09% of donors with the normal weight of 40–59 age and 9.09% of donors of the obese group with 40–59 age were heterozygous (G/A) genes. Factor II and Factor V Leiden gene polymorphism related to cardiovascular diseases were not related to the age and weight.

P.1.5-002
The change of products of lipoperoxide cascade in sperm or rats on exposure of dust-salt aerosols of Aral Sea

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Influence of environmental toxicants disturbs morphological parameters of sperm and leads to inhibition of the antioxidant capacity. This leads to inhibition of expression of proteins in plasma and sperm. The aim of this study was to investigate the laws of formation of disturbances of spermatogenesis under the influence of dust-salt aerosols of Aral Sea in Experiment.

The object of the study were white mongrel male rats weighing 180–200 g in number of 27 animals, of which 7 ones were in the

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control group. The animals were kept on a standard diet of a vivarium at ordinary temperature and humidity conditions in the natural change of light and darkness. Biochemical studies of animal sperm conducted not later than 1 h after slaughter and examined by the classical method of catabolites lipoperoxide stage and activity of enzymes of antioxidant protection.

We studied the quantitative content of catabolites of lipoperoxide cascade and parameters of antioxidant protection in sperm of rats inhaled a seed at dose 2 mg/m^3.

We have found that within 60 days of the experiment there is a complex oscillatory behavior of the products lipoperoxide cascade of physiological parameters in rat's sperm.

The content of the primary products lipoperoxide cascade (conjugated dienes) in spermatozoa after gavage in rats by inhalation at a dose of 2 mg/m^3 is increased as compared with the control group (356.3 ± 18.2 mmol/l and 96.0 ± 11.0 mmol/l respectively).

The content of secondary products lipoperoxide cascade (malondialdehyde) in spermatozoa rats increased compared to control (0.65 ± 0.028 mmol/l and 3.7 ± 0.15 mmol/l respectively).

Based on the results obtained in acute inhalation seed dust-salt aerosols of Aral Sea in a dose of 2 mg/m^3 in the body of rats observed the development of oxidative stress. This is evidenced by the activation of free radical processes and the depletion of the antioxidant defense system in the spermatozoa of rats, which is the main link of reproductive disorders in rats.

P.1.5-003
CYTOKINE profile of action coal-radiation factors at the remote period

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Cytokine belongs to signal molecules that forms and regulates defense mechanism against various factors that trigger toxic effect on the body especially when it is exposed to inflammations and immune responses. This paper studies toxicological properties of coal dust (CD) and sublethal doses of radiation its effect at the remote period on cytokine status of lab rats. For this experiment 4 groups of lab rats (control, CD, γ–Rad, CD+γ-Rad) were exposed to gamma-radiation of 6 Gy on the radiotherapeutic equipment "Teragam" and to inhalation exposure of 50 mg/m^3 of CD in exposure chamber every 4 days during 3 months period.

As a result we evaluated by using immune-ferment method the activity in peripheral blood of Tx1 an 2 types (IL-2, IL-6, TNFα) of pro-inflammatory cytokines and interferon of II type (IFNγ).

Results evaluation of exposed group showed contradictory behavior comparing with the intact group. In the first group (Cd) the increase of cytokines had occurred, where IL2-15%, IL6-25%, TNFα – 9%. In the second group (γ–Rad) the decrease in IL and TNFα by 20–52%. The IFNγ indicators displayed increase in both groups (CD–65%, radiation–48%).

The combined dust-radiation exposed group has showcased progressive decrease in IL2 and TNFα indicators, which were rather smaller comparing with other exposed groups (~50%). The consistency of IFNγ with respect to control groups was rather higher, IL6 was significantly higher.

Combined effect of CD and γ-Rad had showcased significant changes, which could be characterized by the defection of regulatory components of immune system, where the deregulation of cytokine emission can cause various autoimmune, inflammatory and tumor diseases including pneumoconiosis.

P.1.5-004
Plasma N-glycome composition can identify individuals at increased risk of type 2 diabetes development

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Better understanding of type 2 diabetes and its prevention is a pressing need. Changes in human plasma N-glycome are associated with many diseases and represent promising diagnostic and prognostic biomarkers. Variations in glucose metabolism directly affect glycosylation through the hexosamine pathway, but studies of plasma glycome in type 2 diabetes are limited. We hypothesize that glycans can predict the development of type 2 diabetes. Using chromatographic approach, we analyzed N-linked glycans from plasma proteins of 59 patients who developed hyperglycemia during ICU hospitalization due to an acute illness (a known predictor of type 2 diabetes) and compared them with glycans from 49 similar ICU patients who remained normoglycemic. Samples were taken after the cessation of inflammatory process was confirmed (based on blood count and CRP). Individuals at higher risk of diabetes presented increased branching of plasma N-glycans. Using total plasma N-glycome data, we created a mathematical algorithm with good diagnostic power (sensitivity and specificity 96.6% and 85.4%, respectively) to identify people at increased risk for development of type 2 diabetes. Plasma protein N-glycosylation has a good ability to identify people at increased risk of type 2 diabetes. This might offer a potentially new approach for improvement in diabetes prevention, and consequent reduction in the incidence of this widespread disease.

P.1.5-005
Adjuvant potential of plant viruses with different structure and their virus-like particles

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Previously we have demonstrated that spherical particles (SPs), generated by thermal remodelling of tobacco mosaic virus (TMV), have high immunopotentiating activity. Evidence is being accumulated showing that plant viruses and their virus-like particles (VLP) can serve as effective adjuvants. Nevertheless there is no information about the influence of the virions and VLP structure to their adjuvant properties. Here we compared ability of two helical (TMV, rod-shaped rigid virion; potato virus X (PVX), flexible virion) and two spherical agents (brome mosaic virus (BMV), icosahedral virion; TMV SPs, RNA-free spherical particles) to improve immune response to the model antigen. Mice were immunized with mixture of model antigen and one of four adjuvant agents. Total IgG titer and IgG isotypes (IgG1, IgG2a and IgG2b) titers were measured. In comparison with individual antigen TMV, PVX and SPs induced approximately ten times higher IgG titres to the model antigen. Icosahedral agent (BMV) induced only two times higher IgG titers. Although TMV, PVX and SPs were comparable in stimulation total IgG, there were differences in IgG isotypes titres. SPs stimulate mostly

IgG1 (many infectious diseases are characterized by prevalence of IgG1 in immune response) and IgG2b. IgG1 titer after immunization with SPs was the highest among four adjuvant agents. The adjuvant properties of TMV and PVX were similar, all three isotypes are represented approximately the same. BMV generated the poorest immune response, which were mostly due to IgG2b. Apart from the analysis, being described above, which were carried out according to standard immunization protocol, the second blood collection was done in 12 weeks after last immunization in order to investigate duration of immune response. The level of antibodies decreased in all cases; however SPs demonstrated the best ability to induce prolonged immune response. Funding: Russian Science Foundation (grant No. 14-24-00007).

P.1.5-006
Mixed infections of TMV-based recombinant viruses allow creating multivalent influenza vaccines and studying assembly of chimeric particles in plants

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Recombinant viruses based on tobacco mosaic virus (TMV) genome and carrying three versions of conserved Influenza A M2e epitope on the surface of chimeric particles were created by our group previously. They conferred protection against homo- and heterologous influenza strains. Additional virus containing hydrophobic fusion peptide (fp) from hemagglutinin was constructed as well (Petukhova et al., 2013, 2014, 2015). Symptoms and development of mixed Agrobacterium-mediated infections of *Nicotiana benthamiana* (TMV-M2e and TMV-fp) were significantly distinguishable from separate inoculations. For example, we observed white mild chlorosis of non-inoculated leaves instead of systemic necrosis caused by TMV-fp. We tried either separate injections of agrobacteria coding for TMV-M2e and TMV-fp into different leaves or mixed cultures into the same leaf. First variant led to softer symptoms and lower accumulation of modified coat proteins (CP). Simultaneous expression of different antigens in systemic leaves was confirmed by Coomassie staining and Western blotting. Preparations of chimeric viral particles from upper leaves were purified employing the routine method useful for wild type (wt) TMV and analyzed by SDS-PAGE. Three structural proteins that reacted with anti-TMV serum were found: CP-M2e-ala, CP-fp and CP without epitope (ratio 40:20:40); CP-M2e-ser, CP-fp, CP without epitope (ratio 60:0:40); CP-M2e-cys, CP-fp, CP without epitope (ratio 0:0:100). Electron microscopy proved that all multivalent particles resemble TMV-wt in morphology except for increased amount of shorter virions and curved geometry described for TMV-Cg strain in *Arabidopsis thaliana* stems and apical leaf petioles (Serrano et al., 2008).Thus, in addition to the possibility of creating multivalent vaccines, mixed TMV-based infections allow to study fundamental aspects of viral long-distance movement and particle assembly.

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P.1.5-007
Cord endothelial dysfunction can be sensed by circulating red blood cells?

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Cigarette smoke can exert marked effects on the outcome of *in utero* development and even mediate long-lasting health consequences. Our aim was to find adequate procedures to detect a long term impact of maternal smoking on intrauterine life. A better understanding of the pathophysiological complications emerging during fetal development necessitates studies of the umbilical cord (UC). Therefore we studied the UC, which is primarily responsible for the proper fetal development. The UC vessels are direct elongation of the developing embryo's vascular system, and particularly exposed to harmful agents not retained by the placenta. In parallel we examined the red blood cells (RBCs) from molecular aspects. Our experiments were built around the endothelial nitric oxide synthase (eNOS), because the umbilical vessels lack innervation, so the endothelial cells (ECs) and RBCs have crucial role in controlling blood flow and maintaining physiologic conditions. Blood from neonates born to smoking (Sm) and non-smoking (nSm) mothers was taken immediately after birth from the UC arteries. Small pieces of UCs were fixed for morphological and immunohistochemical analysis. We found elevated levels of prooxidants such as $ONOO^-$, H_2O_2 with corresponding enzyme activities, refer to an increased oxidative stress in Sm samples. Structurally we found abnormal morphology and unequivocal apoptotic condition of the ECs in the Sm samples. We found that the eNOS levels and its activity were significantly dropped in Sm samples, in a vessel specific manner. These results are clearly indicates that the structural changes induce functional changes within the ECs layer, with impaired stress responsiveness. Blood samples showed very good correlation with these findings. Additionally the changes in eNOS levels and activity are associated with greater number of unusual shape of RBCs, and decreased ability for deformation. Our research was carried out in the framework of the GINOP 2.3.2-15-2016-00040 grant.

P.1.5-008
Activation of G protein-coupled estrogen receptor mediates anti-inflammatory effect in Crohn's disease

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It is suggested that estrogens exert an anti-inflammatory effect in gastrointestinal tract. However, impact of G protein-coupled estrogen receptor (GPER), which is responsible for rapid non-genomic action of estrogens, on pathogenesis of Crohn's disease (CD) is still unknown. The aim of our study was to determine contribution of estrogen signaling through G protein-coupled estrogen receptor in Crohn's disease. Real-time PCR and Western blot analysis showed overexpression of GPER both at the mRNA and protein level in colon tissues obtained from Crohn's disease patients compared to normal colon epithelium. Moreover, analysis demonstrated that

increased expression of GPER is associated with the reduced methylation of one of two CpG island identified in the promoter region of *GPER* gene. GPER-mediated immunomodulatory effect was shown in the studies using semi-chronic trinitrobenezenesulfonic acid (TNBS)-induced mice model of colitis. In mice model GPER activation by GPER-specific agonist, G-1 led to improvement of macroscopic score and reduction of inflammation area, ulcer score and myeloperoxidase (MPO) activity, while GPER inhibition by GPER-specific antagonist, G-15 had no effect on colon inflammatory parameters. Reduction of the microscopic damage and decrease of immune cell infiltration after administration of GPER-specific agonist, G-1 but not G-15, GPER-specific antagonist was proved by histological analysis. Results indicate that abnormalities in the G protein-coupled estrogen receptor signaling network may contribute to the development of Crohn's disease. Activation of G protein-coupled estrogen receptor attenuate inflammation in Crohn's disease, which suggests that GPER may play significant role in CD maintaining remission.

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P.1.5-009
Production of a novel HBV chimeric antigen in mammalian and plant cells and characterization of the immune response elicited in mice

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Hepatitis B Virus (HBV) causes approximately 600,000 deaths/year due to associated liver complications. The present therapies are not efficient enough to cure the disease and prevention through vaccination remains the most effective approach to control the infection. The current yeast-produced vaccine against HBV is based on the small (S) envelope protein which is able to self-assemble into highly immunogenic subviral particles (SVPs). However, the vaccination programs are limited in developing countries because of the high costs. Moreover, despite the efficacy and safety of the commercial vaccine, around 10% of the vaccinated individuals develop poor immune response. In this context, our study aims to develop a more immunogenic HBV antigen by combining epitopes derived from both large (L) and S surface proteins. An immunogenic fragment from the preS1 domain of L was inserted within the antigenic loop of S to form a new chimeric antigen. The resulting chimera and the wild-type S protein were produced in HEK293T and *N. benthamiana* cells, as a low-cost alternative system. The properties of the newly-obtained protein (N-glycosylation pattern, SVPs formation) are similar to S in either expression system, as shown by biochemical and electron microscopy analysis. Chimera secretion from mammalian cells and functional exposure of both S and preS1 epitopes were evaluated. Antigens purified from HEK293T medium and tobacco leaves were used for immunization studies in mice. Analysis of the immune response indicated significantly higher IgG titers and selection of HBV-specific T cells in mice receiving the chimera, as compared to those receiving S. Further characterization of the mice sera was performed and specific anti-S and anti-preS1 antibodies were detected. To conclude, the novel S/preS1 antigen with improved immunogenicity may represent a promising alternative to the conventional HBV vaccine. This work was supported by the EEA Norway-Romania Program, Grant 5SEE/2014.

P.1.5-010
Fibrosis modulation in scleroderma by epigallocatechin-3-gallate

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Objective: The aim of the present study was to evaluate the potential protective effects, against fibrosis, of gallocatechin-3-gallate (EGCG) in bleomycin induced scleroderma model.
Materials and Methods: Thirty-two healthy female Balb-c mice (22 ± 5 g body weight) were used in this study. The mice were randomly divided into four groups, control groups (n = 8), Bleomycin group (n = 8), Bleomycin + EGCG (n = 8) and EGCG (n = 8). At the end of experimental period all mice were sacrificed; blood samples and liver and skin tissue specimen were collected. Western blotting was used to quantify matrix metalloproteinases (MMP) - MMP-1, MMP-8, MMP-13, p-SMAD 2/3 and SMAD 2/3 from protein homogenates of the skin tissues. TGF- β expression was determined by real-time PCR. Immunohistopathological (alpha-SMA) and histopathological examinations of skin tissues were also done.
Results: When compared to sham and control, experimental groups (EGCG-treated group) were observed to have reduced connective tissue fibrosis in the dermis area ($P = 0.000$), according to Masson Trichrome results. EGCG groups were observed to show a significant reduction in fibrosis at the dermal surface area ($P = 0.022$) using hematoxylin measurements. MMP-1, MMP-8, p-SMAD 2/3 protein levels and TGF- β mRNA expression were slightly decreased in the EGCG Groups compared with the control groups ($P < 0.05$).
Conclusion: These results suggest an antifibrotic role for EGCG.

P.1.5-011
Monitoring of tear fluid of selected diseases

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Tear fluid is a biological material which is highly investigated in recent years as the source of biomarkers of ocular and systemic diseases. Among the advantages of tear collection are mainly noninvasiveness and simple access. This work is focused on the utilization of synchronous fluorescence fingerprint and atomic force microscopy as the novel diagnostic and monitoring tools in patients with glaucoma and diabetes mellitus. Tears were collected from healthy subjects, patients with diabetes mellitus and glaucoma by the cellulose minisponges and by flushing the ocular surface with *saline* and stored at −80°C. The synchronous fluorescence spectra were measured on Perkin-Elmer Luminescence Spectrophotometer LS 55. The surface of tear was analyzed by atomic force microscopy on Dimension FastScan Atomic Force Microscope in tapping mode. The modified fluorescence spectra, the different position of fluorescent peak and changed morphology of tear microscopic structure were observed in glaucoma and diabetic patients in comparison with healthy subjects. The results showed increased fluorescence intensity of tears in patients with glaucoma ($F = 983$) in comparison with tears of patients with diabetes mellitus ($F = 525$). The fluorescence spectra and atomic microscopic structure of tears of

patients with different stages and length of diabetes mellitus exhibited slightly different pattern. The fluorescence intensity increased from (F = 525) in patients with diabetes mellitus lasting 10 years to (F = 771) in patients with diabetes mellitus lasting 20 years. Monitoring of tear autofluorescence and microscopic structure of tears could be useful rapid untraditional method for diagnostics of glaucoma and diabetes mellitus and the sensitive monitoring tool in patients with different stage of diseases.

P.1.5-012
Single nucleotide polymorphism rs3753381 associated with myasthenia gravis affects SLAMF1 enhancer activity

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The cell-surface protein SLAMF1 (CD150) encoded by *SLAMF1* gene is a member of a family of costimulatory receptors expressed on a variety of hematopoietic cells. SLAMF1 is abundantly expressed on the surface of the mature activated lymphocytes and is involved in formation of the germinal center together with other costimulatory molecules.

Previously we determined that the activity of *SLAMF1* promoter critically depends on binding of transcription factor EBF1. Disruption of EBF1 binding site resulted in a significant drop of the level of *SLAMF1* gene expression in the Raji and MP-1 B-lymphoblastoid cell lines, and in primary B cells. Additionally, three transcriptional enhancers in human *SLAMF1* locus that increase the activity of the promoter and contain EBF1 binding sites were identified.

In the current study, the two enhancer elements in *SLAMF1* gene locus, in which the autoimmune-associated single nucleotide polymorphisms rs3753381 and rs11265455 emerge, were additionally characterized. By means of bioinformatics and mutational analysis of regulatory elements that overlap with polymorphic positions both of the named polymorphisms were studied to influence on *SLAMF1* gene expression in B cells. The locus, in which rs3753381 resided, was shown to possess the enhancer activity, as in the Raji and MP-1 B-cell lines its minor variant (G → A) associated with myasthenia gravis demonstrates a 2-fold increase of the reporter gene expression. This result is consistent with the fact that such polymorphism apparently improves the binding properties of the sites for the transcription factors from RXR, FOX and NFATC families. Alongside, the mutational disruption of any of these binding sites resulted in the decreasing of the enhancer's activity in both MP-1 and in Raji cells. Hence, the minor variant of the studied polymorphism possibly contributes to the progression of the myasthenia gravis disease via the modulation of the *SLAMF1* expression, most likely in pathogenic B-lymphocytes.

P.1.5-013
Inhibition effect of Alzheimer's disease Aβ peptide on the proteolitic activity of the N-end rule antiapoptotic pathway

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According to the amyloid hypothesis, oligomerization of beta-amyloid peptide (Aβ) plays a key role in the development of Alzheimer's disease (AD). Mutations of individual amino acid residues in this peptide lead to occurring of familiar, early-onset cases of AD. Such Aβ mutants have higher neurotoxicity and aggregation ability in comparison to unmodified peptide. Using flow cytometry, we have shown that Aβ carrying the "Taiwanese" mutation (D7H-Aβ) and "English" mutation (H6R-Aβ) induce apoptosis more efficiently than Aβ. Apoptotic effect of both peptides is associated with reduced mitochondrial potential of cells, which indicates the start of the mitochondrial apoptosis pathway. The N-end rule pathway is one of the major antiapoptotic systems of the mammalian cells, allowing to selectively destroy specific proapoptotic fragments of proteins. This pathway targets proteins containing N-terminal degradation signals called N-degrons, polyubiquitylates these proteins and thereby causes their degradation by the proteasome. The ubiquitin reference technique was employed in degradation assays to investigate the ability of Aβ and its mutants to inhibit the N-end rule pathway. We have defined D7H-Aβ and H6R-Aβ have greater inhibition effect on the N-end rule pathway activity than Aβ. Utilizing proteins with different destabilizing N-terminal residues, we have identified that R-transferase is a component of this pathway inhibited by amyloid peptides. Thus, Aβ-induced suppression of the N-end rule pathway activity may lead to neurons' death in AD.

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P.1.5-014
Prophylactic effect of recombinant banana lectin on TNBS-induced colitis in BALB/c mice

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Recombinant banana lectin (rBanLec), which structurally and functionally highly resemble to its naturally occurring counterpart, is recognized as modulator of local immune response in the murine colon. The aim of this study was to investigate prophylactic effect of rBanLec on murine model of colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS).

Experimental colitis was induced in BALB/c mice by a single intrarectal (i.r.) administration of 2.5 mg TNBS / 50% EtOH. Mice were treated with rBanLec (i.r. 100 μl rBanLec/PBS; rBanLec concentration: 0.1 μg/ml for rBL0.1 group, 1 μg/ml for rBL1 group, and 10 μg/ml for rBL10 group) 24 h prior to induction of colitis. Colitic non-treated BALB/c mice were used as a referent. The effect of rBanLec pretreatment was assessed at the peak of pathology. Mice were monitored for weight loss. Collected

colonic samples were evaluated for neutrophil infiltration (H&E staining), myeloperoxidase (MPO) activity, NO production, cytokine profile (IL-12, TNF-α, IL-10 and TGF-β) and expression of Th-marker key transcription factors (T-bet for Th1, GATA-3 for Th2, RORγt for Th17).

The significant reduction in disease severity was marked for rBL1 ($P < 0.005$) and rBL10 ($P < 0.0005$) groups. That was in line with reduced neutrophil infiltrations in colon of rBL1 and rBL10 mice. Besides, all inflammation-related parameters (MPO activity, NO production, IL-12 and TNF-α levels, and T-bet and ROR-γt expression) were lower in colonic samples collected from rBL1 and rBL10 mice in comparison to referent ones. Unexpectedly, the levels of IL-10 and TGF-β were also reduced. However, the ratio TNF-α / IL-10 calculated for individual samples were in positive correlation with the observed intensity of disease.

Obtained results show that rBanLec stimulation, in a positive dose-dependant manner, modulates local immune milieu in colon in a way to diminish deleterious potential of subsequently induced inflammatory response.

P.1.5-015
Nothofagin inhibits allergic reaction through the reduction of mast cell activation

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Mast cell functions as a major component in immunoglobulin (Ig) E-mediated allergic diseases such as asthma, atopic dermatitis and rhinitis. Nothofagin, a major component in green rooibos, has been reported with its biological benefits such as septic responses and vascular inflammation. However, the anti-allergic effect of nothofagin has not been investigated. In this study, we assess the inhibitory effects of nothofagin in allergic reactions using mast cells and animal model. To verify mast cell activation *in vitro*, RBL-2H3 and rat peritoneal mast cells were sensitized with anti-dinitrophenyl (DNP) IgE and stimulated with DNP-human serum albumin. Nothofagin prevented histamine and β-hexosaminidase releases by reducing intracellular calcium influx in a dose-dependent manner. Nothofagin inhibited IgE-stimulated gene expression and secretion of tumor necrosis factor-α and interleukin-4. Furthermore, those results came from down-regulating the activation of Lyn, Syk, Akt and nuclear factor–κB. In passive cutaneous anaphylaxis model, topical administration of nothofagin suppressed local pigmentation and ear thickness. Taken together, we suggest that nothofagin might be a potential candidate for the treatment of mast cell-involved allergic inflammatory diseases.

P.1.5-016
Inhibitory effect of esculetin on atopic dermatitis-like skin lesions

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Atopic dermatitis (AD) is a common chronic inflammatory skin disorder accompanying with scratching and pruritus. We investigated the effects of esculetin, isolated from *Cichorium intybus* L, on AD-like skin lesions and underlying mechanisms of action. For induction of AD, we exposed the ears of BALB/c mice with house dust mite (*Dermatophagoides farinae* extract, DFE) and 2,4-dinitrochlorobenzene (DNCB). For 4 weeks, repeated alternative treatment of DFE/DNCB caused AD-like skin lesions. Oral administration of esculetin reduced AD symptoms based on ears thickness and scratch numbers. The immunoglobulin (Ig) E and IgG2a levels in serum, mast cell and eosinophil cell infiltration in skin tissue, and serum histamine levels were also decreased by the esculetin. In addition, esculetin suppressed Th2, Th17 as well as Th1 cytokine such as interleukin (IL)-4, IL-13, IL-31, IL-17, and interferon-γ in the ear tissue. Further, we investigated the effect of esculetin in keratinocyte activation, a hallmark of the pathogenesis of acute and chronic stage of AD. As results, esculetin suppressed gene expression of Th1, Th2, and Th17 cytokines and activation of nuclear factor-κB and STAT1. Taken together, the results suggest that esculetin attenuated AD-like symptoms, suggesting that esculetin might be a potential therapeutic candidate for the treatment of AD.

P.1.5-017
Large scale transcriptomics and proteomics study reveals neoplastic potential in endometrium of patients with stage IV ovarian endometriosis

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Endometriosis is defined as the presence of endometrial-like glands and stroma in extrauterine site. Two mechanisms have been put forward to explain the observed association between endometriosis and gynecological cancer: (1) endometriosis cells undergoing transformation to malignancy by aberrant factors in extrauterine niche, and (2) the coexistence of endometriosis and ovarian cancer due to shared risk factors and antecedent mechanisms. However, earlier studies revealed that eutopic endometrium from patients with ovarian endometriosis was different from normal endometrium. In the present study, the potential role of eutopic endometrium in predisposing endometriosis patients to gynecological cancer was examined by investigating the differences in the (i) large scale transcriptomics between endometrium of patients with stage IV ovarian endometriosis ($n = 12$) and without endometriosis ($n = 12$) using whole genome human expression microarray followed by qRT-PCR validation, and (ii) steady state protein expression in endometrium between patients with stage IV ovarian endometriosis ($n = 13$) and without endometriosis ($n = 14$) by employing large-scale gel-free 2D proteomic experiments, followed by QTOF LC-MS and immunochemistry for validation of the results. 22 gene products and 8 unique proteins which are involved in the process of anti-apoptosis, cell cycle, angiogenesis, epithelial-mesenchymal transformation and telomere maintenance and known to form the basis of malignant transformation were identified in eutopic endometrium from patients with stage IV ovarian endometrioma. Thus, the endometrium in patients with stage IV ovarian endometriosis possesses many features of neoplastic process with the potential for malignant transformation. Further studies are necessary to derive new insights and novel classification of molecular pathogenesis of endometriosis. Approved by the Institutional Ethics Committee, AIIMS-Delhi. Supported by DST and UGC, Government of India.

P.1.5-018
Effects of MGF E peptide pretreatment on the cell morphology, mobility, proliferation and osteogenic differentiation of BMSCs under severe hypoxia

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Bone marrow-derived mesenchymal stem cells (BMSCs) can be applied in bone tissue repair. Nevertheless, severe hypoxia surrounding the injured sites always inhibits cell proliferation and osteogenic differentiation of BMSCs, which hinders the bone defect healing. Previous studies showed that mechano-growth factor (MGF) E peptide played a neuroprotective role against hypoxia-ischemia, but whether MGF E peptide can also prevent BMSCs from severe hypoxia is still unknown. In this study, the effects of MGF E peptide on the biological behavior of BMSCs under $CoCl_2$-stimulated severe hypoxia were detected. The results demonstrate that hypoxia promotes hypoxia-inducible factor-1α (HIF-1α)) stabilization and transferring into the nucleus. 500 μM $CoCl_2$ significantly causes cell contraction and reduces cell adhesion, migration, proliferation and osteogenic differentiation. The expression levels of osteoblast-specific genes (alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx2), and bone gamma-carboxyglutamic-acid-containing proteins (BGP)) in BMSCs under severe hypoxia are also markedly decreased after differentiation culture for 0, 7, and 14 days. Fortunately, MGF E peptide inhibits HIF-1α stabilization and nuclear translocation. After pretreated with MGF E peptide, cell area and roundness can be partly restored, and cell adhesion, migration and proliferation of BMSCs can also be recovered. Furthermore, MGF E peptide pretreatment improves the ALP activity and accelerates the calcium deposition of BMSCs under severe hypoxia. MEK-ERK1/2 and PI3K-Akt signaling pathway are involved in MGF E peptide regulating the morphology, proliferation and osteogenic differentiation of BMSCs. In conclusion, this study provides new evidence for the role of MGF E peptide in regulating biological behavior of BMSCs under severe hypoxia, which may have potential application for bone defect healing (Supported by the National Natural Science Foundation of China (11672051)).

P.1.5-019
Novel developmental roles of class IA phosphatidylinositol 3-kinase (PI3K) revealed by graded genetic activation of the catalytic p110α subunit in human induced pluripotent stem cells

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Heterozygous activating mutations in the human *PIK3CA* gene, encoding the catalytic p110α subunit of class IA phosphatidylinositol 3-kinase (PI3K), are frequently found in human cancer and have recently also been found in rare individuals with patchy or mosaic overgrowth, grouped under the clinical entity PIK3CA-related overgrowth spectrum (PROS). Harnessing the power of CRISPR/Cas9 gene editing and human induced pluripotent stem cells (hIPSCs), this work presents the first isogenic human developmental cell models of activating p110α mutations in an otherwise normal diploid genetic background. The strongly-activating cancer "hotspot" H1047R variant ($p110\alpha^{H1047R}$) was knocked into hIPSCs either heterozygously or homozygously to explore the effects of graded p110α activation on self-renewal, multi-lineage differentiation and growth factor signalling. Embryoid body differentiation assays revealed that hIPSCs homozygous for $p110\alpha^{H1047R}$ fail to differentiate despite removal of the self-renewing factors TGFβ and FGF2. This differentiation-resistant phenotype was characterised by sustained expression of a panel of key self-renewing genes, including *NANOG, FGF4* and *GDF3*, and failed induction of markers of all three germ layers. In contrast, embryoid bodies hetorozygous for $p110\alpha^{H1047R}$ showed robust downregulation of self-renewing genes comparable to wildtype counterparts, but exhibited dysregulated induction of several mesoderm and endoderm markers, mirroring clinical observations of lineage-specific distribution of this variant in PROS patients. Signalling studies revealed that p110α rather than its homologue p110β is the class IA PI3K isoform transducing growth factor stimulation in pluripotent stem cells, with substantial defects in this regulation upon constitutive genetic p110α activation. In conclusion, this work has elucidated important aspects of class IA PI3K signalling in developmental biology of relevance to PROS patients and cancer.

P.1.5-020
Structure and function studies of RNA-binding proteins with FAST motifs and a RAP domain

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The FASTK family (Fas-activated Serine/Threonine Kinase) contains six human proteins (FASTK, FASTKD1-5) which localize to the mitochondria and have been functionally linked to cellular respiration and a rare mitochondrial disease. While human FASTK was initially annotated as an atypical Ser/Thr kinase later studies dispute this annotation. Structurally, FASTK proteins contain an N-terminal mitochondrial targeting signal, FAST motifs (FAST1 and FAST2) and a C-terminal RAP domain. The N-terminal part is predicted to be highly globular with small disordered regions. The FAST motifs are putative RNA binding domains with a novel α-helical repeat fold that does not display sequence similarity to any other known helical repeat motifs. Interestingly, the RAP domain is found in many members of the recently identified class of octotricopeptide repeat (OPR) proteins, which are believed to play a role in chloroplast RNA biology. The OPR proteins have been shown to bind RNA, but their structure or RNA binding specificity is unknown. The RAP domain is overrepresented in *Plasmodium*, and hence structural information of this domain is relevant to the field of malaria. FASTK family members are connected with mitochondrial RNA metabolism. For instance, FASTK binds multiple sites along ND6 mRNA and modulates degradosome activity. FASTKD2 is required for mitochondrial ribosome biogenesis, while FASTKD5 for maturing mRNAs precursor. Recently it was shown that FASTKD1 depletion increase ND3 mtRNA level. In contrast FASTK, FASTKD2, FASTKD4, and FASTKD5 are all positive regulators of specific mitochondrial mRNAs. Our project aims to provide for the first time structural and novel biochemical information about the relatively understudied FASTK family. This will have relevance to drug design therapeutic strategies, particularly of cancer and inflammation and will likely reveal new folds of RNA binding domains thus contributing to the general knowledge of the rules that govern RNA recognition.

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P.1.5-021
Characterization of the LPS-induced secretome of vascular stem cell: effects on endothelium

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The vessel wall has been reported as a reservoir for different types of vascular stem cells, particularly the mural layers of postnatal vessels contain the multipotent mesenchymogenic population. Increasing evidences suggest that the secretome of stem cells is able to modulate response of resident cells, including endothelial cells, in the pathogenesis of different diseases. Considering the pig an excellent model for translational medicine, we recently isolated and characterized a population of MSC-like cells from porcine aorta, named porcine Aortic Vascular Precursor Cells (pAVPCs) with pericyte-like properties. The aim of the present research was to investigate the ability of pro-inflammatory lipopolysaccharide (LPS) to influence pAVPCs secretome, furthermore the effects of secretome on LPS-induced endothelial cell death was tested. pAVPCs were seeded in a 24 well plate and treated with LPS (10 or 0.1 μg/ml) for 4 h. A preliminary screening on inflammatory pathway was performed on cells by an RT2 Profiler PCR Arrays (84 genes) and on culture media by Luminex Multiplex Elisa Assays (13 cytokines and chemokines). A significant increase of cytokines (TNF-α, IL-1α, IL1-β, IL-6 and IL-8) and chemokines (CXCL2, CXCL10, CCL1, CCL20, CSF-2) mRNA was detected even with a different degree of intensity. At protein level multiparametric Elisa confirmed a significant increase of IL-6 and IL-8, while IL-1α, IL-2, IL-4, IL-10, IL-18, CSF-2, IFN-γ decreased; TNF-α and IL1-β has never been detectable. Then, conditioned media of pAVPCs were added to LPS-treated endothelial cells and showed a clear protective action against LPS cytotoxicity evaluated by MTT test. Overall, our results indicated that pAVPCs strongly responded to LPS and their conditioned medium is able to protect endothelial cells against pro-inflammatory stimulus.

P.1.5-022
Quantification of oxidative stress, different bio-markers can tell different stories

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The commonly used term "oxidative stress" (OS) is a concept, intuitively defined as an excess of pro-oxidative compounds (mostly reactive oxygen species, ROS) over antioxidants. However, ROS are essential for normal body function and OS is therefore tightly controlled by a number of mechanisms. Many methods have been developed to quantitate "the level of OS". Most of these methods are based on the steady state concentration of different biomarkers.

Unfortunately, OS depends on the method of determination and cannot be quantitated in terms of a universal criterion. Yet, the approach of "Bad free radicals, good antioxidants" is still quite common and the paradigm that people under OS belong to high-risk groups that would benefit most from antioxidant supplementation is still accepted by many researchers.

This approach led to the production of many commercially available kits, by many producers, many of whom produce more than one kit, to assay the cellular OS and the concentrations of ROS in body fluids, aiming at identifying individuals under OS "that will gain from supplementation of antioxidants". According to a new market research report, published by Markets and Markets, the annual global Oxidative Stress Assays Market was about half a Billion $ million in 2015, and is expected to reach $736 million by 2020.

This approach is problematic (i) OS cannot be quantitated by a universal criterion, (ii) many assays of antioxidants are conducted in solutions, unlike in biological systems, peroxidation, where it occurs at lipid-water interfaces, and (iii) the reasonable assumption that antioxidants are particularly effective under oxidative stress does not accord with the results of epidemiologic studies.

Our interpretation of the available results is that different biomarkers used in different assays reflect different types of OS, namely that the different biomarkers tell us different stories. Much work is still required to characterize the different types of OS.

P.1.5-023
Suppression of calcium store-dependent activity of L-type channels affected in Alzheimer's disease cell model

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Presenilin-1 (PS1) gene is responsible for 40% of Familiar Alzheimer's disease (FAD) cases. PS1 forms catalytic subunit of gamma-secretase complex. Despite most of FAD-related mutations in PS1 are loss of function mutations, there is a range of mutations with retained catalytic function. M146V presenilin-1 is an example of this type of PS1 mutant. Cellular and animal FAD models expressing PS1 M146V have disturbed calcium homeostasis, including change in calcium concentration in endoplasmic reticulum (ER). STIM1 is a calcium sensor of ER. STIM1 activates store-operator calcium channels and inhibits L- and T-type voltage-gated calcium channels. Since voltage-gated calcium channels are important elements of calcium signaling in neuronal cells, we investigated STIM1-dependent L-type channel activity in the FAD cell models. Using TIRF microscopy technique we observed lower STIM1-YFP translocation to plasma membrane in response to depletion of calcium stores in PS1 M146V expressing cells. Hippocampal neurons (HN) are the most affected in AD patients. STIM1 is expressed in HN at a low level. Using Fura-2 calcium imaging we showed that shRNA knock-down of STIM1 increased voltage-operated calcium entry in mouse HN with depleted calcium stores. This increase was inhibited by verapamil. Further, we expressed PS1 wild-type and PS1 M146V in mouse HN. PS1 M146V neurons with depleted calcium stores have elevated voltage-gated entry similar to control neurons with knock-down STIM1 sensors. Excessive voltage-gated entry was canceled by nifedipin or verapamil. Our data suggest the importance of STIM1 sensor as an inhibitor of L-type channels in HN. Neurons expressing FAD mutant PS1 M146V have suppressed STIM1 dynamic, which leads to increase in activity of L-type channels in HN with depleted calcium stores.

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P.1.5-024
Autophagy and necroptosis may contribute to neuronal damage in rat retina during the development of AMD-like retinopathy

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Age-related macular degeneration (AMD) is a degenerative disease of the retina and the leading cause of blindness in the elderly. The pathogenesis of early events and the mechanisms of retinal cell death in AMD is not completely clear. In this study, we investigated the pathways of retinal cell death involved in the development of similar to AMD pathology in the senescence-accelerated OXYS rats at the early preclinical stages of retinopathy (age 20 days), at early (3 month) and progressive stages (18 month) of disease. Comparison of gene expression profiles of retina from OXYS and disease-free Wistar rats by RNA-Seq has shown that cell death in the retina of OXYS rats occurs due to apoptosis, as well as necrosis and autophagy. Increased level of apoptosis (TUNEL+ cells) was observed in OXYS rats at 20 days but not at advanced stages. Examination of autophagy proteins in retina by western blot analysis and immunohistochemistry (IHC) showed increased levels of Atg7 and Atg12-Atg5 conjugate proteins in OXYS retina compared to Wistar at the age of 3 mo. However, in the retina of 18-month-old OXYS rats with progressed stage of retinopathy the western blot and IHC revealed significantly decreased Atg7 and Atg12-Atg5 protein levels compared to age-matched Wistar rats. According to IHC Atg7, protein was expressed in the ganglion cell and outer plexiform layers, while the Atg5-12 complex was expressed in photoreceptors and retinal pigment epithelium. It is believed that the pro-survival function of autophagy could be achieved via suppression of necrotic cell death. It is interesting that the necrosome subunits Ripk1 and Ripk3 were detected in ganglion cell layer simultaneously with a decrease in autophagy in OXYS retina. Our data suggest that apoptosis is not the main form of the cell death during AMD and the dysfunction of autophagy may trigger necroptosis. Support by RFBR 15-04-02195 and budget project 0324-2016-0003.

P.1.5-025
Early calcium signalling remodelling in Alzheimer's disease – evidence from murine cultured hippocampal neurons

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A significant body of evidence suggests that calcium dysregulation plays a key role in the onset and progression of Alzheimer's disease (AD). We have studied how the endoplasmic reticulum (ER) functions in both maintaining calcium homeostasis and mediating intracellular signaling processes and how these functions might be disrupted in AD. Cultured hippocampal neurons were prepared from control and transgenic 3xTg-AD mice and TgF344-AD rats between 3–6 days old. Using calcium imaging, group 1 metabotropic glutamate receptor (I-mGluR)-mediated somatic responses were measured under basal conditions and also under conditions where the ER was "preloaded" with calcium (using a depolarising stimulus; 15 mM K^+), mimicking a 'learning event'. In non-transgenic neurons, from both murine models, I-mGluR activation combined with loading stimulus, evoked enhanced Ca^{2+} signals relative to I-mGluR activation alone. In contrast, we did not observe enhanced responses in transgenic neurons, suggesting a loss of this signaling function. Secondly, we observed significantly larger responses to I-mGluR activation under basal conditions, in transgenic neurons, suggesting a pathological increase in ER calcium levels. Pharmacological studies demonstrated that in non-transgenic neurons the coupling of I-mGluR stimulation to ER Ca^{2+} release is mediated via both PLC/IP_3R (primarily) and cADPR/RyR pathways. Furthermore, the relative contribution of these pathways in I-mGluR mediated signaling is altered in transgenic neurons. Finally, Hippocampal tissue from 3-week old 3xTg-AD mice exhibited altered expression of two key calcium signaling components, RYRs and BCl-2 (an ER Ca^{2+} release modulator). The fact that such stark alterations in calcium homeostasis and signaling have been observed in neurons from rodent models of AD at such a young age (≤6 days), suggests that calcium dysregulation may occur at a much earlier stage in the disease progression than previously thought.

P.1.5-026
Fenofibrate-induced inhibition of TGF-β/Smad signalling pathway attenuates fibroblast-to-myofibroblast transition in bronchial fibroblasts derived from asthmatic patients*

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Negligible effects of commonly used therapies on subepithelial fibrosis and bronchial wall remodelling of patients with asthma force us to search for the new potential compounds affecting this phenomenon. A particularly important process associated with the fibrosis of 'asthmatic' airway wall is the transition of local bronchial fibroblasts (HBFs) into α-SMA+ myofibroblasts (FMT) induced by profibrotic cytokines, primarily TGF-β_1. Our recent studies have shown that lipid metabolism also play a key role in this process. Cholesterol biosynthesis pathway inhibitors (such as statins) attenuate TGF-β_1-induced FMT in 'asthmatic' HBFs. Likewise, our current research proves that synthetic ligand of PPAR-α receptor – fenofibrate (FF) which is one of the most commonly used hypolipidemic agents – reduces the level of intracellular cholesterol in HBFs from asthmatics. Using immunofluorescence, western blot and qRT-PCR analyses we demonstrated that FF supressed the FMT efficiency (measured by α-SMA expression and incorporation into stress fibres) in HBFs through the inhibition of Smad2-dependent pathway in a dose-dependent manner [1–25 μM]. Those effects have corresponded with the decrease in expression of connexin (Cx)43 (a protein involved in the control of FMT in HBFs).

To sum up, we were the first to demonstrate, that FF could be applied as potential subepithelial fibrosis inhibitor in asthma. The effect of FF is broadly pleiotropic, because it works through pathways that are both lipid-lowering (such as activation of PPAR-α receptors) and non lipid-lowering (such as modulation of TGF-β/Smad2 signalling activity). We believe that our *in vitro* findings may be used in the future for effective therapy of bronchial asthma.

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P.1.5-027
ZnO nanoparticle – a potent anti-cholera agent

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Vibrio cholerae is the leading cause of diarrhea in the developing countries that calls for the development of a new strategy to combat the disease. There are attempts to use nanoparticles (NP) to fight infectious diseases. Earlier we demonstrated the antibacterial activity of ZnO NP on two biotypes (classical and El Tor) of O1 serogroup of *V. cholera*e where we showed that effect was more on El Tor – both in planktonic and in biofilm forms. Here we have used the proteomic approach to study the response of *V. cholerae* in presence of ZnO NP. We found overexpression of several chaperone proteins and outer membrane proteins on treatment with ZnO NP. Along with these, we also observed the change in expression of a protein that point towards the onset of Cpx pathway. Further, we observed that at a concentration which is not cytotoxic, ZnO NP can destabilize the cholera toxin structure, preventing it from the binding ganglioside GM1 receptor, and thereby abolishing its activity, irrespective of the two biotypes. The mechanism was validated in the animal model. Hence ZnO NP has antibacterial property as well as can inhibit the activity of cholera toxin already produced. The results throw up interesting possibility towards preventing cholera infection.

P.1.5-028
Structural basis for hijacking of human ACBD3 and PI4KB proteins by picornaviruses

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Picornaviruses are small positive-sense single-stranded RNA viruses that include many important human pathogens. Within the host cell, they replicate at specific replication sites called replication organelles. To create this membrane platform, they hijack several host factors including the acyl-CoA-binding domain-containing protein-3 (ACBD3) and phosphatidylinositol 4-kinase beta (PI4KB) [1]. We present a structural characterization of the molecular complexes formed by the non-structural 3A proteins from several picornavirus species and appropriate interacting domains of human ACBD3 and PI4KB [2, 3]. Specifically, we present a series of crystal structures of the 3A:ACBD3 complex as well as an NMR structure and SAXS analysis of the ACBD3:PI4KB complex. We show that the viral 3A proteins act as molecular harnesses to enslave the ACBD3 protein leading to its stabilization at target membranes, which leads in turn to the recruitment and activation of the PI4KB kinase. Our structural analysis explains how these viral-host protein complexes assemble at the membrane and identifies new potential targets for antiviral therapies.

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P.1.5-029
A new approach to the production of immunodominant epitopes of VP2 protein of infectious bursal disease virus

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Infectious bursal disease virus (IBDV) is an etiologic agent of a highly contagious immunosuppressive illness of young chickens – infectious bursal disease, also known as Gumboro disease. Immunosuppression caused by IBDV leads to an increased susceptibility of chickens to a wide range of secondary viral and bacterial infections, often resulting in the death of a bird. The only effective way to fighting this virus is vaccination. Although live and inactivated viral vaccines against IBDV have long been successfully used in poultry industry, the issue of creating safer recombinant vaccines based on the structural proteins of the virus is still extremely relevant. VP2 is the IBDV capsid protein folded into 3 domains: B (Base), S (Shell) and P (Projection). The tertiary structure of the P-domain is a β-barrel, in two loops of which there are epitopes recognized by neutralizing antibodies to the virus. We decided to generate separately recombinant P-domain of IBDV capsid protein. The fragment encoding amino acids 200–345 of VP2 was amplified from cDNA of DD1 IBDV strain and subsequently cloned into the plasmid pSAV-TEV. The resulting chimeric gene encoded mature streptavidin, the TEV proteinase site and the P domain of VP2 under the control of T7 promoter. The expression of the target gene was activated in *E. coli* Rosetta2(DE3) cells using IPTG. The chimeric protein SAV-sPD accumulated in inclusion bodies. Analysis of the purified protein by PAGE showed that SAV-sPD forms oligomeric complexes. Thus, we proposed a new approach to the generation of immunodominant epitopes of the VP2 protein of IBDV in a prokaryotic expression system. The use of streptavidin tag fused to the P-domain allows to increase the yield of the target protein, facilitates the oligomerization of the epitopes, and can be used for additional chromatographic purification.

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P.1.5-030
Modification of plant cell wall polysaccharides during the disease development caused by Pectobacterium atrosepticum SCRI1043

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Degradation of complex carbohydrates may result in generation of various products having different physiological properties. It is well-known that the breakdown of the plant cell wall polysaccharides by phytopathogens provides them with a growth substrate. In our study, we tested the possibility of the formation of "adventitious" cell wall degradation products that do not serve as a source of energy only for bacteria but also possess peculiar properties and thus influence the pathosystem formation. The composition of plant cell wall polysaccharides in tobacco plants infected by *Pectobacterium atrosepticum* SCRI1043 (*Pba*) was analyzed. The disease development led to the accumulation of pectic fragments (namely, of rhamnogalacturonan I), which were not tightly bound to the cell wall, had relatively high molecular weight (100 kDa), and contained covalently bound phenolic compounds. The phenolic compounds are known to provide cross-linking of carbohydrates after oxidation. The investigation of composition of phenolic compounds showed the presence of increased amount of these compounds in infected plant samples. On the model pathosystem it was shown that *Pba* forms specific 'multicellular' structures – bacterial emboli in the xylem vessels of infected plants. Using monoclonal antibodies, the fragments of rhamnogalacturonan I were marked in the lumen of xylem vessels. Besides, the reactive oxygen species (ROS) were detected in the vessels, that did not hamper the bacterial cells. Thus, the products of polysaccharide degradation play a crucial role in the formation of bacterial emboli by forming a gel that serves as a matrix for *Pba* cells.

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P.1.5-031
Polymorphism of thrombophilia genes of the blood coagulation system in women of the Kazakh ethnic group with a habitual miscarriage

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The aim of current work was to study the polymorphism of clotting thrombophilia genes, the F2 gene (rs1799963), the F5 gene (Leiden, rs6025), the F7 gene (rs561241), the F13 gene (rs5985), the FGB gene (rs4220), the ITGA2 gene (rs1126643), the ITGB3 gene (rs5918) and the PAI-1 gene (rs1799889). These genes were surveyed among women of the Kazakh ethnic group with Habitual miscarriage of pregnancy. DNA was isolated from the venous blood of the examined women. Gene polymorphism was studied by PCR using allele-specific primers ("SNPexpress" Lytech, Russia) on the RealTime CFX96 amplifier (BioRad, USA). The criterion for selecting women at risk was the presence of spontaneous miscarriages in the first two pregnancies without previous records of normal pregnancy. The control group consisted of women with two normal delivery and had no obstetric complications in the current pregnancy. 198 pregnant women participated this experiment, which were divided into a risk group (79) and control group (119). In calculating OR (95%CI) the frequencies of the polymorphic alleles and determining the frequency of distribution of genotypes of all the genes under study and their compliance to a Hardy-Weinberg equation, the SNPstats online program was used. Calculation of OR indicators was carried out according to five models of inheritance of characteristics – dominant, codominant, overdominant, recessive and log-additive. Statistically significant differences in the incidence of genotypes from investigated thrombophilia genes in women with habitual miscarriage and control group for all five models of inheritance were not revealed. There is a lack of homozygous genotypes according to the mutant alleles of F2 and F5 genes in both groups of the examined women. Also, the absence of a homozygous genotype for the mutant allele of the C/C gene of the ITGB3 gene was detected in both examined groups of women.

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P.1.5-032
Obtaining of VP3 protein of infectious bursal disease virus (IBDV) in two different expression systems

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Infectious bursal disease of chickens is one of the main problems in the poultry industry. Appearance in the late 1980's of very virulent strains of IBDV (the causative agent of this infection) led to an increase in mortality rates of up to 90%. A crucial point in control of this disease is the timing of vaccination of young chickens. The first 2 weeks after hatching they are protected by maternal antibodies, and premature administration of the vaccine strain can lead to neutralization of the virus and low vaccination effectiveness. Therefore, screening of chickens for immune status before and after the introduction of the virus is extremely important, and the development of effective test systems remains a topical issue. The aim of this work was to obtain a recombinant VP3 protein that could potentially be used in IBDV ab-screening tests. VP3 is a component of the IBDV virion, which acts as a scaffolding protein required for capsid assembly control. It is known that the first antibodies that appear in chickens after IBDV infection are directed to VP3. We decided to obtain recombinant VP3 in two expression systems: prokaryotic (*E. coli*) and eukaryotic (Expi293F). The fragment encoding VP3 was amplified from the cDNA of the very virulent strain DD1 IBDV circulating in Russia. This fragment was further cloned into the plasmid pETmin under the control of the T7 promoter and into the pIgL-His vector under the control of the CMV promoter. In both vectors, the VP3 gene was followed by the site encoding 6-His tag. After IPTG induction of *E. coli* BL21(DE3)Gold cells with pETminVP3 plasmid, recombinant VP3 accumulated in the cytoplasmic fraction in soluble form (the yield was 40 mg/l). The stable line Expi293F/VP3 obtained by us also ensured the accumulation of the target protein in the cytoplasm (20 mg/l). Both

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variants of VP3 were purified by metal-chelate chromatography on a Ni-Sepharose column.

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P.1.5-033
Development of producer strains of recombinant capsid protein VP1 of chicken anemia virus

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Chicken anemia virus (CAV) is a small icosahedral single-stranded DNA virus without a lipoprotein membrane. This virus causes serious anemia and immunodeficiency in young chickens. Outbreaks of chicken infectious anemia can lead to high chick mortality (up to 55% in the flock) and pose a serious problem for poultry industry. For this reason, the development of a recombinant subunit vaccine against CAV seems to be an extremely actual task. VP1 is the only capsid protein of CAV, represented in the virion in an amount of 60 copies. This protein carries epitopes responsible for the induction of neutralizing antibodies to the virus, and thus is thought to be a good candidate for use as an antigen for vaccine development. In this study, we obtained 2 recombinant VP1 producer strains, which can later be used to create a vaccine and diagnostic tests. The fragment encoding the full-length VP1 was cloned into the plasmids pET32a and pETmin to obtain 2 genes for subsequent expression of the capsid protein: one with a sequence encoding thioredoxin, the other without it. In both cases, the expression of the target gene was under the control of the inducible T7 promoter, and a site encoding 6 histidins was located before the stop codon. *E. coli* BL21(DE3)Gold cells were transformed with plasmid pTrxVP1$(H)_6$ (based on pET32a), expression was induced by IPTG at a final concentration of 1 mM. The chimeric protein Trx-VP1 accumulated both in the fraction of the inclusion bodies and in the soluble form in the cytoplasm in approximately equal amounts. *E. coli* Rosetta2(DE3) cells were transformed with plasmid pVP1$(H)_6$ (based on pETmin), expression was also induced by IPTG. Recombinant VP1 accumulated predominantly in the inclusion bodies, although a small part of the protein was also present in the soluble fraction.

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P.1.5-034
Increased vulnerability of primary NPC1-deficient neurons against Lovastatin is associated with signs of increased autophagy

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Niemann Pick C (NPC) is a fatal hereditary neurovisceral disorder associated with lysosomal dysfunction and subsequent cell loss. The disease is caused by mutations in either of two genes, npc1 and npc2, coding for the lysosomal proteins NPC1 and NPC2. Npc1-mutations account for about 95% of patients. Published data suggest a cell-autonomous cause of death for neurons. Using a NPC1-mouse model, we obtained survival curves of primary neuron cultures virtually identical to those from wildtype (wt) littermates. After challenging the neurons by a variety of different stressors (glutamate, hydrogen superoxide, osmotic shock, cholesterol-loading and cholesterol-depletion, and inhibition of HMG-CoA reductase (HMGCoA-r) by Lovastatin (Lova), only one difference emerged: NPC neurons are more vulnerable against Lova, i.e. less Lova is needed to evoke an identical magnitude of cell death. Addition of geranylgeranylpyrophosphate (GGPP), an intermediate of the mevalonate pathway (which is blocked by Lova), rescued both NPC1-deficient and wt neurons from Lova. HMGCoA-r levels in NPC1 and wt neurons, however, are equal. Lova, as a statin considered to induce autophagy, does this in fact GGPP-dependently in both wt and NPC1 neurons. Remarkably, concentrations of Lova already able to induce an increase in autophagy markers LC3B and p62 in NPC1-deficient neurons are insufficient to induce this in wt neurons. Notably, the cultured NPC1-deficient neurons are found associated with already increased levels of LC3B and p62 under basal (untreated) conditions. Thus, we reasoned that less Lova is needed to reach a threshold in autophagic impairment required to induce cell death. In line, pre-treatment with cyclodextrin (CD), known to restore the impaired lysosomal degradation in NPC, normalized the vulnerability against Lova. This suggests that treating NPC patients by inhibition of endogenous cholesterol biosynthesis should be combined with a CD treatment in order to avoid toxic effects.

P.1.5-035
Study of formation and progress of endometriosis on the molecular level

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Background: One of the promising markers for detection and monitoring of endometriosis is hypoxia inducible factor-1 (HIF-1), that has been found to be upregulated by several signal pathways, including NF-*kappa*B. Transcription of HIF-1 gene is significantly higher in eutopic endometrium in comparing to healthy endometrium. HIF-1 usually induces pro-survival (CAIX, Glut-1, VEGF) genes. Another suitable marker of endometriosis progression is beta-catenin, which affects the regulation of transcription of genes involved in the Wnt signaling pathway and cell adhesion. So far, there is no reliable marker of the development and progression of endometriosis from patient serum, and therefore the use of molecular methods appears to be one of the possible and prospective pathways. Aims: Detection of expression changes of β-catenin and HIF1alpha at mRNA levels in the serum of patients with different stages of endometriosis compared to the control group. Correlation of obtained results with demographic and clinical characteristics. Methods: The experimental group (n = 60), consisting two groups of patients: patients with suspected endometriosis and patients with histologically confirmed endometriosis. Expression of individual genes was detected by qRT-PCR and the results were compared with the control group

(n = 50). Results and conclusion: The mRNA level for HIF1 was 9.4% higher and for beta-catenin 21% higher than in controls. In the histologically confirmed endometriosis group, a significant increase in HIF1 mRNA was detected, 50% higher than in controls, as well as β-catenin with mRNA levels increased by 56%. Increased expression of HIF1alpha and beta-catenin genes is associated with the progression of endometriosis and is characterized by its early stages. Detection of specific markers is therefore a highly current topic and can assist in the development of both new therapeutic applications in the treatment of endometriosis.

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P.1.5-036
Normal cell derived microparticles used as delivery system for microRNAs protect against atherosclerotic vascular disease

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Cell-derived microparticles (MPs), endothelial progenitor cells (EPCs) and circulating microRNAs (miRNAs) have attracted major interest as biomarkers and potential regulators for atherosclerotic vascular disease. We evaluated the possible protective role of MPs and EPC-derived MPs (MPEs) of healthy origins in atherosclerosis development as well as the mechanisms responsible for their repair capacity. The experiments were performed on hamsters divided into: (1) simultaneously hypertensive – hyperlipidemic (HH) by combining two feeding conditions for 4 months, to induce atherosclerosis; (2,3) HH with retro-orbital sinus injection containing MPs or MPEs, from control hamster, one doze per month for 4 months of HH diet, to prevent atherosclerosis; (4) controls (C), age-matched normal healthy animals. The results showed that: (1) MP/MPE transplantation suppresses the development of atherosclerosis processes via: (i) alleviation of dyslipidemia, hypertension, circulating EPC levels, cytokine/chemokine profiles; (ii) structural and functional remodeling within the vessel wall and heart; (2) MPs operate as protective and delivery system for miRNAs in circulation; (3) MPs and MPEs protect against atherosclerotic vascular disease via transfer of miR-10a, miR-21, miR-126, miR-146a to circulating late EPCs. It mentioned that, the favorable effects of MPEs are similar to those of MPs. The data indicate that MP and MPE transplantation can counteract HH diet-induced detrimental effects by their miRNA transfer to circulating EPCs mediating their function. These promising findings using MPs and MPEs as therapeutic tools for transferring miRNAs in an atherosclerotic animal model give hope to patients with cardiovascular disease.

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P.1.5-037
Platelets stimulate the functional properties of late endothelial progenitor cells in atherosclerosis

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Endothelial progenitor cells (EPCs) and platelets have an essential role in vascular regeneration and endothelial repair, but the mechanisms underlying the interaction between these cells are poorly understood. The purpose was to evaluate the effect of platelets on functional properties of late EPCs, *in vitro*, in the direct co-culture conditions, and to investigate the involved mediators, in experimental induced atherosclerosis. The late EPCs were obtained in culture from peripheral blood isolated from two animal groups: hypertensive-hyperlipidemic hamsters (HH) and control hamsters (C). In parallel experiments, late EPCs from C group (late EPCs-C) and late EPCs from HH group (late EPCs-HH) were co-incubated with or without platelets obtained from C and HH groups. Results: Platelets from control animals promoted the capacity of late EPCs-C to form colonies, proliferate and migrate and improved the late EPCs-HH functional properties, while the platelets from HH group diminished the functional abilities for both late EPCs-C and late EPC-HH. The increased SDF-1α, MCP-1, VEGF, PDGF and reduced CD40L and IL-1β,-6,-8 levels were found in media from late EPCs-C or late EPCs-HH co-incubated with platelets from C group; the platelets from HH groups had an opposite effect on these molecules. In addition, the control platelets enhanced the miR-223 and IGF-1R expressions in late EPCs-HH. Conclusions: *In vitro* exposure to control platelets had a positive effect on functional properties of late EPCs from atherosclerotic animals. The current study contributes to understanding the EPC-platelet relationship and lead to new perspective of EPC used for both repair and maintenance of existing vasculature, and for the treatment of cardiovascular diseases.

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P.1.5-038
The crystal structure of Lyme disease agent Borrelia burgdorferi surface protein BBE31 that is vital for successful colonization of a mammalian host

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Lyme disease is a tick-borne infection caused by a spirochete *Borrelia burgdorferi* which is transmitted to a mammalian host after a bite of infected *Ixodes* tick. Initially *B. burgdorferi* is located in the ticks gut but to infect the new host the spirochete during the ticks blood meal should travel to the ticks salivary glands through the hemolymph from where it can spread to the new host organism. It has been described that dozens of *B. burgdorferi* outer surface proteins are up-regulated during the ticks feeding process likely for the purpose to assist in the takeover of the new host organism.

We have determined the crystal structure of a key outer surface protein BBE31 from *B. burgdorferi* and *B. spielmanii*, known to be essential for the transfer of *B. burgdorferi* from the ticks gut to the hemolymph after a ticks bite. The protein exerts the function by interacting with the *Ixodes scapularis* tick gut protein TRE31. Analysis of the primary and tertiary structure of BBE31 reveals that the protein belongs to the *B. burgdorferi* pFam54 paralogous gene family and has a structural similarity with another *B. burgdorferi* outer surface protein BBA64 – a protein known to be essential for the transfer of *B. burgdorferi* from the ticks salivary glands to the mammalian host. The 3-d structure of a protein playing an important role in the initial phase, before the spirochete is physically transferred to the new host, can help to provide new insights into the molecular details of the transmission process to potentially use the knowledge for development of new strategies to fight against the Lyme disease.

P.1.5-039
Kinetic stabilizers of the cystathionine beta-synthase regulatory domain as potential pharmacological chaperones for homocystinuria

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Pathogenic missense mutations in human cystathionine beta-synthase (CBS) rarely targets key catalytic residues. Instead, these mutations cause structural perturbations, which result in misfolding of the mutant enzymes with subsequent aggregation or rapid degradation of the proteins. Loss of CBS function leads to classical homocystinuria (HCU). CBS contains two sets of binding sites for S-adenosylmethionine (SAM) that independently regulate the enzyme activity and kinetically stabilize its regulatory domain. We examined the hypothesis that CBS activation may be decoupled from kinetic stabilization and thus CBS regulatory domain can serve as a novel drug target for HCU. We determined the effect of SAM and its close structural analogs on CBS activity, their binding to and stabilization of the regulatory domain in the absence and presence of competing SAM. Binding of S-adenosylhomocysteine and sinefungin lead to stabilization of the regulatory domains without activation of the enzyme. Direct titrations and competition experiments support specific binding of these two SAM analogs to the stabilizing sites. Binding of these two ligands also affects the enzyme proteolysis rate supporting the role of the stabilizing sites in CBS dynamics. Our results indicate that binding of SAM to regulatory and stabilizing sites in CBS may have evolved to display an exquisite thermodynamic and structural specificity towards SAM as well as the ability to transduce the allosteric signal responsible for CBS activation. Thus, ligands may be developed to function as kinetic stabilizers or pharmacological chaperones without interfering with the physiological activation of CBS by SAM.

P.1.5-040
Adapter activity of inositol phosphatase SHIP2 enables sustained MAP kinase activation by fibroblast growth factor receptor (FGFR) via recruitment of SRC-family kinases to the FGFR signaling complex

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FGF growth factors govern cell behavior via sustained activation of ERK MAP kinase, but factors regulating continued ERK activity in FGF signaling remain unclear. Here, a mechanism is described by which FGFs regulate sustained ERK activation. When using proteomics to uncover novel mediators of FGFR signal transduction, we identified SHIP2 as a phosphorylation target and interactor of FGFR1, FGFR3 and FGFR4. SHIP2 knock-out effectively converted sustained FGFR-ERK activation into a transient signal, and rescued the cell phenotypes triggered by pathological FGFR-ERK signaling. Chemical inhibition of SHIP2 activity or expression of inositol phosphatase-inactive SHIP2 mutant had no inhibitory effect on FGF-mediated ERK activation, suggesting that adapter but not catalytic activity of SHIP2 is important for maintenance of the ERK signal. Indeed, SHIP2 recruited SRC-family kinases to the active FGFRs, which assisted the FGFR-mediated phosphorylation and assembly of FRS2 and GAB1 adapter complexes that relay the signal from FGFR to RAS/ERK signaling module. Our data uncover a molecular mechanism underlying sustained ERK activation by FGFRs, identify SHIP2 as an essential mediator of canonical FGFR signaling, and demonstrate SHIP2 role in pathological FGFR3 signaling in skeletal-dysplasia.

P.1.5-041
Phosphorylation of beta-amyloid peptide prevents inhibition of Na,K-ATPase and alters its amyloidogenic properties

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According to amyloid hypothesis of Alzheimer disease (AD), accumulation of beta-amyloid peptide (Aβ) in brain tissue is a trigger of downstream pathological cascades, which initiates development of the disease. For the majority of AD cases the initiating factor is still unknown, and such form of the disease is termed sporadic AD (sAD). Possible factor that triggers sAD is aberrant post-translational modification of Aβ. It is known that various Aβ modifications such as isomerization, pyroglutamylation and truncation can increase its aggregation, neurotoxicity and ability to inhibit long-term potentiation of neurons. We have studied the influence of phosphorylation of Ser8 residue on Aβ 1-42 properties.

Earlier we have found that Aβ 1-42 binds to Na,K-ATPase with high affinity, which in turn leads to the inhibition of its activity; this effect may be one of the Aβ physiological functions, however accumulation of Aβ and long-term inhibition of Na,K-ATPase can lead to disruption of neuronal function. Using purified Na,K-ATPase and lysates of SH-SY5Y neuroblastoma cells, treated with amyloid peptides, we have shown that phosphorylation of Aβ 1-42 abrogates its inhibitory effect on Na,K-ATPase.

Moreover, injection of phosphorylated Aβ 1-42 into transgenic mice that suffer from cerebral amyloidosis leads to significant decrease in amyloid burden, whereas injection of non-modified peptide does not affect the number of amyloid plaques in mice of the same line. According to our data, differences in biological effect of amyloid peptides can be concerned with weaker Zn-dependent aggregation of phosphorylated Abeta compared to intact Aβ 1-42. Thus, phosphorylation of Aβ 1-42 Ser8 residue dramatically changes its properties and the nature of this change suggests protective role for such modification of the Aβ peptide.

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P.1.5-042
ZIKA virus protease NS3 interaction with human fibroblasts proteome

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ZIKA virus, belonging to *flaviviridae* systematic group, is a human pathogen, spreading with mosquito *Aedes africanus* from eastern Africa to Asia, Europe and both Americas. During infections of adults, progression of symptoms is usually mild and does not cause serious health problems (elevated temperature, rash, other influenza like symptoms). In rare cases infection might be responsible for development of *Guillain-Barre* syndrome. Unfortunately, during infection of pregnant women, this virus promotes development of microcephaly in fetuses and then in newborns.

Mechanisms of viral activation in the eukaryotic cells are not clear. Especially there is no agreement in the literature what are the major protein goals of the viral protease NS3, which activity is necessary during the early stages of infection. As the genome of ZIKA virus is known since 2006, we decided to check, with the aid of proteomic approach, what are the main targets for NS3 protease in the human cells. Identification of proteins involved in infection progress may lead to design of effective antiviral therapy, which can be easily translated into other viral infections from *flaviviridae* family. In our investigations we used NS3 protein cloned in the *E. coli* model. After enzyme purification and activity check, NS3 protease was introduced into the proteome of human fibroblasts received from the primary cell cultures. To find the differences between naïve and NS3 protease treated proteomes, labelling and additional enzymatic truncation along with nanoLC-MS/MS system was used.

Results allowed for identification of potential protein goals involved in viral infection inside the fibroblasts. Our results can be easily translated into the whole f*laviviridae* family with their typical representatives like Denga or West Nile viruses.

P.1.5-043
Transcriptomic profile of early and mid-secretory endometrium revealed by RNA-seq: a paired sample cohort study

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Inner uterine lining or endometrium is unique constantly self-renewed adult tissue that is vital for embryo implantation. As the footrace for universal endometrial receptivity markers continues, RNA-seq remains the most powerful tool for transcriptomic marker discovery. In this study, we aimed to describe endometrial maturation mechanisms at transcriptome level with respect to cell types' contribution and identify novel robust biomarker candidates for endometrial receptivity. The gene expression profiles of early secretory and mid-secretory endometrium samples obtained from 35 healthy fertile women (aged 23–36) across one menstrual cycle were analysed in three cohorts using paired sample study design. Additionally, mid-secretory endometrium transcriptome profiles of healthy women were compared to those from women with recurrent IVF failure (RIF; n = 38; aged 26–49). EdgeR software was used for cohort-level differential expression analyses, and meta-analysis was performed using METAL software. DeconRNAseq package was used for tissue deconvolution analysis. As a result, 3591 significantly differentially expressed genes (DEGs) were identified in the receptive endometrium of healthy women. Among these, 2284 DEGs (1174 up-regulated and 1110 down-regulated) were significantly differentially expressed in all cohorts (fdr < 0.05). The genes with the highest expression rate change were common endometrial markers and genes associated with endometrial functioning. Novel potential biomarkers for endometrial receptivity are suggested, based on their significance level, expression change and detection rate. The comparison of receptive phase samples from healthy women and RIF patients identified 36 DEGs, that were significantly differentially expressed (fdr < 0.05) and had similar effects in all cohorts. Tissue deconvolution analysis allowed to adjust for differences in stromal and epithelial cell proportions, enabling to detect transcripts with true expression rate changes.

P.1.5-044
Investigation of mitochondrial stress inducers on human bone marrow derived mesenchymal stem cells for mitochondrial disease model

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Mitochondrial dysfunction is the cause of many critical diseases. Modelling mitochondrial dysfunction in a dish can be accepted as the first step of therapeutic studies. We aimed to establish an

in vitro damage model to represent mitochondrial dysfunction in healthy human bone marrow derived Mesenchymal Stem Cells (hBM MSCs). The present study was designed to test the effects of mitochondrial stress inducers including ethidium bromide (EtBr), rotenone (Rot) and hydrogen peroxide (H_2O_2) on different mechanisms of mitochondrial function of hBM MSCs. In this study, MSCs were exposed to specific concentrations of stress inducers (400 μM H_2O_2, 500 μM Rot, 1 μM EtBr). To assess the state of cells via mitochondria-related indicators, ROS and Mito-Tracker were evaluated by flow cytometer, ATP levels were measured by luminometer and CytC activity were monitorized by spectrophotometer at specific time intervals during 3 days. ROS production increased in MSCs at 72nd hour of induction with all stress inducers compared to control values. Although increased ROS production is associated with increased cell damage, it may indicate increased mitochondrial function as well. When the cells are exposed to stress condition, it could protect itself and mitochondrial activity may be expected not to be low at that point. The idea was supported with the highest ROS and ATP levels at the 72nd hour of H_2O_2 induction among all stress inducers. Interestingly, at the 48th hour after induction with Rot,ROS production (0.95 ± 0.13), ATP (0.73 ± 0.36) and mitochondrial (0.98 ± 0.05) activity were decreased compared to control. These findings suggest that the 500 μM of Rot induction for 48 h could be an optimal damage model for being used in further studies. Although MSCs can be advantageous to study damage models with their proliferative capacity, heterogeneity of them is the biggest obstacle and due to this reason our results were not consistent and the differences did not show statistical significance.

P.1.5-045
Protein profiling in chronic kidney disease assessed by high-throughput technologies

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Background: Chronic kidney disease (CKD), despite being a silent epidemic disease, represents one of the main causes of mortality in general population. Recent advances in proteomic technology have provided an excellent opportunity to achieve high-throughput screening as well as testing that could help early diagnosis, evaluation and prognosis in CKD. The present study aims to assess the relationship between bone/vascular alterations and the circulating level of 6 biomarkers in CKD patients with different stages.
Methods: Two proteomic technologies – xMAP array and SELDI-ToF MS (surface-enhanced laser desorption/ionization time-of-flight mass spectrometry) were assessed to quantify a panel of 6 biomarkers (IL-6, TNF-α, OPG-osteoprotegerin, OPN-osteopontin, OCN-osteocalcin, FGF-23 and Fetuin-A). A total of 106 serum samples (86 with CKD – stages 4, 3, 2 and 20 normal controls) were analyzed using CM10 ProteinChip Arrays. Serum protein profiles from CKD and normal patients were analyzed with the ProteinChip Data Manager Software 3.0.7.
Results: The proteomic spectra obtained were compiled, normalized, and mass peaks with mass-to-charge ratios between 2 and 100 kDa were identified. Peaks information was analyzed using univariate statistics and 10 significantly different protein peaks were selected, with AUC values ranging 0.750–0.930 and $P \leq 0.05$. The results obtained by SELDI-ToF-MS analysis confirm those obtained by xMAP array.
Conclusions: The biomarkers panel shows great potential for early detection, clinical evaluation and prognosis in CKD patients. The present study reflects the clinical utility of a multiplexed biomarker panel in CKD and was found to be more relevant than one single biomarker to detect patients in early CKD stages. Proteomic techniques shed light on clinical evaluation for CKD staging and prognosis.
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P.1.5-046
The presence of miR-29 family in skin hypertrophic scars and normal skin

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Wound healing is a complex process. Wounds sometimes can heal excessively or inadequately. In this case, patients have to live with huge scars. Hypertrophic scars are fibrous growths and remain within the boundaries of the original wound. Despite many studies that have examined the pathophysiology of hypertrophic scars, the underlying causes and the best treatment modalities are still unknown.

The miR29 family in humans includes hsa-miR-29a, hsa-miR-29b-1, hsa-miR-29-2, and hsa-miR-29c. Firstly, miR29a was found in Hela cells by Lagos-Quintana, followed by the subsequent discovery of miR-29b and miR29c. The particular interest of miR29 family members is due to their ability to inhibit the synthesis of ECM proteins. miR29 family has been shown to possess anti-fibrotic function. miR29 family members are taking an active role in fibrotic processes of various tissue such as liver, skin, heart, kidney. . .etc.

In this study, we aimed to investigate the presence of miR29 family members: miR29-a, miR29-b-1, miR29-b-2, miR29-c in skin hypertrophic scar tissue and normal skin specimens. miRNA gene expression was evaluated with semi-quantitative real time PCR. To confirm the results of PCR and to show the localization of miR29, we used the *in situ* hybridization method.

Our data showed that the significant decrease in miR-29b-1 gene expression in skin hypertrophic scar tissue in comparison to healthy control skin. According to in situ hybridization results, miR-29b is primarily localized in nucleus in both skin specimens.

These data suggest that the downregulation of miR-29b may play a role in the progression of abnormal scar formation.

P.1.5-047
Cell cycle and oxidative stress in Huntington disease dermal fibroblasts

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Mitochondria are the powerhouse of the cells and gained a central role in the modulation of metabolism, regulation of cell proliferation and apoptosis. There are many reports of mitochondrial dysfunctions present in Huntington disease (HD) patients cells including increased oxidative stress. Recently, also cell cycle deregulation in neurodegenerative diseases has become an interesting research field. It is still under debate whether observed changes in nervous cells may contribute to the

peripheral tissue and be relevant in search of possible disease biomarkers, therefore, it required further investigation. Recent studies suggest that oxidative stress and cell cycle may be intertwined at the molecular level. The aim of the present study was to characterize cell proliferation, cell cycle and reactive oxygen species (ROS) level in human skin fibroblasts obtained from HD patients at different stages of the disease compared to healthy controls. To outline the profile of potential changes, evaluation of the results with the development of the disease has been conducted. Our results indicate possible connections between oxidative stress and the cell cycle progression in HD dermal fibroblasts. Our studies have shown that the changes in analyzed factors may be an important factor in the disease development and contribute to the understanding of the molecular basis of HD development. The common role of mitochondria dysfunction appears to be very promising, and could lead to new strategies in the development of therapeutic agents for Huntington disease.

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P.1.5-048
Roles of MAPK pathways in human embryo lung fibroblasts induced by silica and carbon black

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Silica is one of the most serious occupational hazard factors which can lead to lung fibrosis after long-term inhalation. Carbon black (Carbon Black, CB) is a kind of amorphous carbon, light and very fine black powder. The surface area is very large, and strong polymerization. CB is a common environmental pollutant, mainly from incomplete combustion of fossil fuels and biofuels. CB can be discharged into the atmosphere in the form of PM2.5, which has an important impact on human health and the environment. CB produces heat or cooling effects on the environment by directly absorbing light, increasing the reflection of snow and ice, or directly acting on the clouds. Human embryo lung fibroblasts (HELF) cells was used to analyzed the effects of Connective Tissue Growth Factor (CTGF) and MAPK exposed to carbon black and silica by Werstern blots, luciferase assay and immunofluorescence technique. MTT experimental results showed that cell survival rate did not decrease significantly between the concentration of CB between 60–240 g/ml, and the corresponding cell survival rate is between 65%-80%. So 100 g/ml CB was used in the following experiments. The results of electron microscope showed that the carbon black particles were not observed in HELF cells, but the silica particles could be observed. The cell model of CTGF siRNA (T-CTGF) was successfully constructed. Exposures of 100 g/ml silica for 12 h or 100 g/ml carbon black for 24 h can cause elevated levels of CTGF significantly in HELF. Carbon black could induce significant changes of MAPK, AP-1 and cytokines IL-6 and IL-8 in HELF. And these changes of cytokines IL-6 and IL-8 could be introduced by the MAPK signal transduction pathway, but not dependent on AP-1. Combined with the results of the previous studies, silica can cause abnormal signal pathway in MAPK/AP-1 cells. So it means that MAPK plays an important role in the particle exposure.

P.1.5-049
The protective effect of paricalcitol on renal ischemia/reperfusion injury in rats through the inhibition of p38 MAPK signaling pathway

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Renal ischemia/reperfusion injury (IRI) is a serious medical condition that might lead to acute kidney failure. Also increased reactive oxygen species during renal IRI results in activation of p38 mitogen-activated protein kinase (MAPK) which causes induction of inflammatory responses. Paricalcitol is an active vitamin D analog which has a therapeutic potential in various renal diseases. However, the effect of paricalcitol on renal IRI has not been studied yet. Hence, we aimed to investigate the effects of paricalcitol on oxidative stress, inflammation and the possible role of p38 MAPK induced by renal IRI in rats. 20 wistar albino rats were randomly divided into 3 groups; Sham, IR, IR+paricalcitol. IRI was performed through bilateral clamping of the pedicles 45 min ischemia followed by 24 h of reperfusion. Paricalcitol (0.3 μg/kg, i.p) was administered 24 h before ischemia. High performance liquid chromatography (HPLC) and a colorimetric kit were used to analyze malondialdehyde (MDA) and superoxide dismutase (SOD), respectively. Also real time-PCR was used to analyze mRNA expressions of TNF-α and Interleukin-1. Total p38 and phospho-p38 (p-p38) protein expressions were analyzed with western blot. MDA levels were increased significantly in the IR group compared to the sham group. Paricalcitol pretreatment decreased the MDA levels significantly. SOD levels were decreased in the IR group compared to the sham group. Paricalcitol administration increased significantly the SOD levels. mRNA expressions of TNF-α and Interleukin-1 were found to be significantly higher in the IR group compared to the sham group. Paricalcitol pretreatment caused a significant decrease in both mRNAs' expressions. Besides, p-p38 MAPK protein expression was significantly higher in the IR group. Paricalcitol pretreatment decreased significantly the p-p38 MAPK. In conclusion, our study suggests that paricalcitol may represent a potential strategy to attenuate renal IRI.

P.1.5-050
Age-related alterations in hippocampal neurotrophin signaling pathway are involved in development and progression of Alzheimer's disease-like pathology in OXYS rats

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Alzheimer's disease (AD) is the most common type of age-related dementia worldwide, and the precise mechanisms of its progression are not fully understood till now. Age-related alteration of neurotrophic signaling may contribute to AD-associated neurodegeneration since neurotrophins manage neuronal survival, death and synaptic plasticity. To investigate a link between age-related alterations of neurotrophic signaling pathway (NSP) and progression of AD we used OXYS rats as a suitable model of sporadic form of AD. The RNA-seq data obtained for the hippocampus were used to analyze changes in NSP. ELISA was used to quantify level of Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus. Western-blot analysis was used to quantify levels of TrkB and phTrkB receptors in the hippocampus.

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Immunohistochemistry was used to localize proBDNF, mature BDNF, TrkB and p75NTR receptors. According to KEGG pathway, there were no differentially expressed genes related to NSP in 20-days-old OXYS and Wistar rats (control). At the age of 5 months *Shc4* gene was up-regulated in OXYS rats. However, at the age of 18 months 6 genes related to NSP were down-regulated in OXYS rats compared to Wistar rats. Analysis of protein content showed that BDNF level was increased in OXYS rats at 3 months of age and decreased with age. Proapoptotic proBDNF became prevailing form of BDNF and its co-localization with p75NTR was increased in OXYS rats at 18 months of age. Activation of TrkB (phTrkB/TrkB ratio) was decreased in OXYS rats at the age of 18 months. Obtained results showed no differences in NSP when AD-like pathology in OXYS rats is absent (20 days of age). Activation of NSP during manifestation of AD-like pathology in OXYS rats (3–5 months of age) may be considered as compensatory process. Considerable alterations of NSP coincided in time with active progression of sings of disease in OXYS rats (18 months of age). The work was supported by Russian Scientific Foundation grant (16-15-10005).

P.1.5-051
Detection of molecular changes during progressive aneurysm of thoracic aorta

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Aortic functions as well as regulation of aortic wall homeostasis depend on changes in the structural components of the extracellular matrix (ECM) which are affected by multiple molecular signalling pathways. The proteolytic theory of ascendant thoracic aneurysm (TAA) development envisions increased concentrations of MMP and reduced concentrations of TIMP in the aorta acting in concert to increase tissue degradation, leading to expansion of thoracic aorta. Several cytokines and chemokines that are produced (TNF-α, interferon-γ, IL-1, 2, 6, and IL-8) are upregulated and promote the recruitment of other inflammatory cells to the aortic wall. Aims: Correlation of the diameter of TAA with gene expression of inflammation markers (IL-6, CRP) and components of ECM (Emilin-1, MMP9, TIMP) on mRNA level for detection of the degree of pathological process of TAA formation. Methods: The experimental group (n = 58) consisted of patients suffering from thoracic aortic aneurysm, regurgitation and aortic valve stenosis, who were divided into three groups according the diameter of the aortic aneurysm (stage 1: 43 $\pm$ 2.3 mm, stage 2: 51 $\pm$ 2.8 mm and stage 3: 59.5 $\pm$ 3.7 mm). Expression of individual genes was detected from tissue samples using qRT-PCR and the results were compared with the control group (n = 10). Results conclusions: We found increased expression of mRNA for IL-6 rising from stage 1 to the maximum in stage 3 (650% higher) versus aortic controls. The rising concentrations of CRP mRNA were associated with aneurismal size, proved by Spearman correlation. We found increase in mRNA levels of MMP9 with maximum in stage 3 about 490% higher than controls, also affected the expression of TIMP, which mRNA levels were elevated too. Obtained results could help surgeons decide if the progression of aortic aneurysm is too fast and give them a chance to improve the lifetime and healthcare of patients suffering with progressive TAA.

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P.1.5-052
Mitochondrial impairment in Nogo-B receptor deficiency

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Recently we identified a glycosylation disorder due to mutation R290H in C-terminal domain of Nogo-B receptor (NgBR) in two brothers (P1, P2) with scoliosis, retinitis pigmentosa and neuronal defects. NgBR is evolutionary conserved protein localized in the membrane of endoplasmic reticulum. Together with prenyltransferase, NgBR creates complex for dolichol synthesis, which is essential for N-glycosylation. Dolichol intermediates are localized also in membranes of Golgi apparatus, lysosomes or mitochondria. Furthermore, dolichol biosynthetic pathway is interconnected with biosynthesis of coenzyme Q10 – part of oxidative phosphorylation system (OXPHOS).

Aim of our study was to analyze the impact of NgBR deficiency on mitochondrial ultrastructure and functions.

Skin fibroblasts (P1, P2), muscle (P1), heart and brain (P2) were analyzed. Activities of OXPHOS complexes were measured by spectrophotometry, protein amount of selected subunits of OXPHOS was detected by immunoelectrophoretic methods, mitochondrial network was visualized using fluorescent microscopy, mitochondrial ultrastructure and tissue organization were detected by transmission electron microscopy.

Decreased cristae number, swollen mitochondria and unequally distributed network were revealed in fibroblasts. Reduced levels of OXPHOS subunits CoxII and NDUFA9 as well as lowered total amount of complex IV were found in fibroblasts. Increased number of mitochondria and tissue fibrosis was detected in heart. Upregulation of complex II and decreased Q10 content was found in frontal cortex.

Our results indicate secondary mitochondrial dysfunction due to a breakdown of the glycosylation pathway. The study of mitochondrial metabolism in congenital disorders of glycosylation may contribute to the elucidation of pathomechanisms in unclear metabolic diseases.

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P.1.5-053
Effects of chronic exposures of selected heavy metals on antioxidant enzyme activity of freshwater snails Lymnaea natalensis

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Anthropogenic activities lead to the release and accumulation of heavy metals in aquatic bodies. A report on the Bulawayo Lower Mguza dam which receives domestic and industrial effluent from Bulawayo city, in Zimbabwe, revealed high levels of the heavy metals cadmium, copper, lead and mercury in water. The current study therefore was aimed at investigating the effect of chronic exposure of these metals on one of the antioxidant enzymes, glutathione S-transferase (GST), of *Lymnaea natalensis* snails. Groups of snails were exposed to heavy metals at concentrations

reportedly found in the Wright dam, for 28 days. Water and feed were changed daily, and samples collected at 1, 7, 14, 21 and 28 day intervals before analysing for inhibition of GST activity. All heavy metals caused inhibition. Exposure to cadmium caused 58 – 60% inhibition from day 1- day 21, with a significant decrease ($P < 0.005$) on day 28 (30%). Inhibition increased for both copper and mercury exposures between days 1 and 21 with high inhibitions on day 21 of 70% and 80% respectively. Day 28 showed a decrease in inhibition, albeit, insignificantly ($P > 0.005$) with copper as compared to the mercury exposures which showed 25% inhibition. Variation in inhibition was observed with the lead exposures. The results suggest that chronic exposures may inhibit GST activity for a certain period, after which, inhibition subsides, possibly due to adaptation.

P.1.5-054
Evaluation of the effect of clusterin rs2279590 single nucleotide polymorphism in pseudoexfoliation syndrome risk and on clusterin protein level in aqueous humor

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Pseudoexfoliation syndrome (PES) is an age-related systemic disorder of extracellular matrix characterized by the presence of fibrillar deposits in the anterior segment of eye. Clusterin (CLU) is a multifunctional glycoprotein that is accumulated in PES material. CLU expression level is unexpectedly low in aqueous humor of PES patients which could be due to single nucleotide polymorphisms (SNP) on *CLU* gene. This study aimed to investigate the role of rs2279590 T/C SNP, the association between rs2279590 SNP and CLU protein level in aqueous humor in PES risk. The study population for SNP study consisted of 130 PES patients and 169 controls, while for aqueous humor analyses consisted of 9 PES patients and 17 controls. All samples were obtained from Gülhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey. Genomic DNAs were isolated from whole blood of subjects by a commercial kit. Genotypes were assigned by real-time PCR. Total protein concentrations were determined by Bradford protein assay and CLU concentrations were determined by ELISA in aqueous humor samples. T allele frequency was 0.408 in PES patients and 0.388 in controls for CLU rs2279590 ($P = 0.618$). According to preliminary results of aqueous humor, CLU concentration was found as 0.169 ± 0.140 μg/ml in PES, 0.319 ± 0.400 μg/ml in controls ($P = 0.280$). Aqueous humor CLU proportion in total protein concentration was found as $0.034 \pm 0.099\%$ in PES, $0.174 \pm 0.194\%$ in controls ($P = 0.433$). Aqueous humor CLU concentration and the proportion of CLU in total protein was found as highest in TT genotyped PES patients and controls while lowest in CC genotyped ones. These are the preliminary findings of a larger research project and CLU rs2279590 SNP, aqueous humor total protein and CLU concentrations are analyzed for the first time in Turkish population in PES. This work does not point out a relation for polymorphic allele in the risk for PES.

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P.1.5-055
The association between connective tissue growth factor (CTGF) rs6918698 polymorphism and pseudoexfoliation syndrome, and the effect of rs6918698 on aqueous humor CTGF concentration

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The production and accumulation of abnormal fibrillary material in many ocular tissues causes pseudoexfoliation syndrome (PES). Since excessive synthesis of microfibrillar components occurs in PES, growth factors may have roles in pathophysiology. Connective tissue growth factor (CTGF) is a protein expressed in several tissues, including the anterior chamber of eye. CTGF gene has genetic polymorphisms including rs6918698 G/C single nucleotide polymorphism (SNP) at position −945, in promoter. The presence of C allele for rs6918698 is critical for transcriptional suppression of the *CTGF* gene which would reduce CTGF production. Aim of this study was to investigate the effect of rs6918698 polymorphism on CTGF level in aqueous humor and for the risk of PES. Study population consisted of 130 PES patients and 169 controls for SNP study. 8 PES patients and 16 controls were studied for aqueous humor CTGF concentration. Blood and aqueous humor samples were collected by Gülhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey. Genotypes were assigned by PCR followed by RFLP analysis. CTGF levels in aqueous humor were assigned by ELISA. The frequency of CTGF rs6918698 polymorphic allele C was 0.477 in PES, and 0.432 in controls ($OR = 1.199$, $P = 0.273$). Aqueous humor CTGF concentration was found as 3.70 ± 1.66 ng/ml in PES ($n = 8$), 4.21 ± 2.44 ng/ml in controls ($n = 16$) ($P = 0.903$). In GG genotype, it was found as 3.48 ng/ml in PES ($n = 1$), 2.83 ± 0.69 ng/ml in controls ($n = 5$) ($P = 0.435$). In GC genotype, it was found as 4.41 ± 2.0 ng/ml in PES ($n = 4$), 4.65 ± 2.61 ng/ml in controls ($n = 9$) ($P = 0.873$). In CC genotype, it was found as 2.81 ± 1.20 ng/ml in PES ($n = 3$), 5.66 ± 4.19 ng/ml in controls ($n = 2$) ($P = 0.319$). These are the preliminary findings of the first study analyzing the relation between CTGF SNPs and aqueous humor CTGF levels. This work did not point out a role of rs6918698 for the risk of PES or CTGF level in aqueous humor.

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P.1.5-056
Pro-inflammatory S100a9 protein involved in the amyloid-neuroinflammatory cascade in Alzheimer's disease serves as a robust biomarker differentiating early stages of dementia

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We have demonstrated that pro-inflammatory protein S100A9 plays a critical role in the amyloid-neuroinflammatory cascade leading to amyloid depositions and amyloid neurotoxicity in Alzheimer's disease (AD). S100A9 proved to be as a robust biomarker differentiating early stages of cognitive impairment in AD in conjunction with others such as Aβ(1−42) and tau-proteins. CSF samples from 104 stringently diagnosed individuals divided into

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five subgroups were analyzed, including nondemented controls, stable mild cognitive impairment (SMCI), mild cognitive impairment due to AD (MCI-AD), AD, and vascular dementia (VaD) patients. ELISA, dot-blotting, and electrochemical impedance spectroscopy were used as research methods. The S100A9 and Aβ(1−42) levels correlated with each other: their CSF content decreased already at the SMCI stage and declined further under MCIAD, AD, and VaD conditions. Immunohistochemical analysis also revealed involvement of both Aβ1−42 and S100A9 in the amyloid-neuroinflammatory cascade already during SMCI. Tau proteins were not yet altered in SMCI; however their contents increased during MCI-AD and AD, diagnosing later dementia stages. Thus, S100A9 and three other biomarkers taken together and reflecting different underlying pathological causes can accurately differentiate dementia progression and also distinguish AD from VaD.

P.1.5-057
Association of ApoB and CETP genetic variants with Type 2 Diabetes-related traits in population from Bosnia and Herzegovina

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Genome Wide Association (GWA) studies have shown the association of SNPs rs673548, rs693 in ApoB gene and rs1800775 in CETP gene with parameters of type 2 diabetes (T2D) and diabetic dyslipidemia. The aim of this study was to investigate the association of these single nucleotide polymorphisms (SNPs) and Type 2 diabetes and dyslipidemia in the population of Bosnia and Herzegovina (BH).

Our study involved 352 patients with T2D and 156 healthy subjects. Biochemical and anthropometric parameters were measured in all participants. DNA was extracted from the peripheral blood for the purpose of genetic testing. Polymorphisms in ApoB (rs673548, rs693) and CETP (rs1800775) genes were analyzed by using Sequenom IPLEX platform. Our results demonstrated significant associations for rs180075 polymorphism in CETP gene with levels of fasting insulin (P = 0.020; P = 0.027; P = 0.044), triglycerides (P = 0.046) and ALT (P = 0.031) activity in control group. In group of diabetic patients, results showed a significant association of rs673548 in ApoB gene with levels of fasting insulin (P = 0.008), HOMA-IR (P = 0.013), VLDL-C (P = 0.037) and CRP (P = 0.029) and rs693 in ApoB gene with BMI (P = 0.025), systolic blood pressure (P = 0.027), fasting insulin (P = 0.037) and HOMA-IR (P = 0.023) levels. Significant associations were also observed for rs1800775 in CETP gene with triglyceride (P = 0.023) levels.

This is the first study that examined the impact of variations of candidate genes on a wide range of metabolic parameters in BH population. Obtained results suggest an association of variations of ApoB and CETP genes with specific markers of T2D and dyslipidemia. Further studies would be needed in order to confirm these genetic effects in other ethnic groups as well.

P.1.5-058
IL10 gene -1082G/A polymorphism analysis in patients with keratoconus from Ukraine

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Keratoconus (KC) is a bilateral non-inflammatory corneal ectasia characterized by progressive thinning of the cornea. It is estimated that the prevalence of KC varies from 50 to 230 individuals per 100,000 in the general population, dependent on ethnicity. KC is the most common indication for corneal transplantation in Ukraine. Keratoconus is a multifactorial disease, likely caused by the interaction of multiple disease susceptibility genes and environmental factors. Anti-inflammatory cytokine gene IL10 is expressed in injured corneal epithelium. Mice model experiments demonstrated that IL10 gene polymorphism -1082G/A (rs1800896) significantly affects the level and functional activity of encoded protein. Taking into the account that anti-inflammatory cytokines provide elimination of inflammatory cells preventing ulceration, melting and neovascularization in cornea. We have chosen IL10 rs1800896 as possible candidate for KC genetic susceptibility. In our study rs1800896 was analyzed in group of 106 KC-patients and 100 healthy controls by PCR with following EcoNI RFLP analysis. Comparative analysis revealed that frequency of rs1800896 homozygous (AA) was higher in patients with KC (25%) comparing to control group (19%). This data did not reach the threshold for significance but show a trend toward association between rs1800896 homozygous genotype (AA) and KC development. The involvement of new patients with KC and family members analysis now in progress to provide additional evidence that IL10 gene polymorphism -1082G/A is likely to contribute to KC risk.

P.1.5-059
The differential role of Ku in the HIV-1 replication: influence on integration and transcription

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The human Ku heterodimer is composed of two subunits: Ku70 and Ku80. Its main function is the binding of double-strand DNA breaks during the first steps of the NHEJ repair process. The involvement of the Ku heterodimer in the HIV-1 replication has been shown in various reports. Ku affects different stages of the viral replication cycle particularly the integration and transcription. The integration can be influenced by the functioning of Ku in the DNA repair machinery as well as by a direct stabilization of the viral integrase (IN) by the Ku70 subunit. Earlier we have shown that a stable complex is formed by recombinant HIV-1 IN and Ku70 when both proteins are expressed in bacteria. We performed a mutational analysis and showed that the main binding site within IN and Ku70 is located in the N-terminal domain of Ku70 (a.a. 1–250) and the helix α6 of IN (a.a. 200–220) that links its catalytic and C-terminal domains. Moreover, alanine substitutions of amino acid residues E212 and L213 of IN significantly impede the complex stability not only when it

is tested on recombinant proteins but also when the complex is immunoprecipitated from cellular lysates. Additionally we have shown that the cellular stability of HIV-1 IN is indeed positively affected by Ku70 while the influence of Ku80 subunit is rather weak. Furthermore, the integration of a single-round HIV-based luciferase reporter vector in cell lines with a stably reduced Ku70 expression is significantly weakened. Using luciferase reporter assay in human cells that are either depleted of or superexpressing Ku70/Ku80/both we have shown that it is the Ku80 subunit that is essential for positive transcription regulation. Altogether, our results suggest a differential role for the two subunits of the Ku heterodimer in the HIV-1 replication cycle. The work was supported by RFBR grant 17-04-01178 and RSCF grant 17-14-01107.

P.1.5-060
Modulation of hERG channel activity by scFv antibody fragments

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Human ether-a-go-go related gene (hERG) potassium channels play a critical role in the repolarization of the cardiac action potential. Changes in hERG channel function underlie LQT2 syndrome and are associated with cardiac arrhythmias and sudden death. The amino-terminal cytoplasmic region of the hERG channel contains a Per-Arnt-Sim (PAS) domain which has been shown to be determinant of the characteristic slow deactivation of the channel, whereas the carboxy-terminal region contains a cyclic nucleotide binding (CNBh) domain. LQT2-associated mutations map both to the PAS domain and the CNBh domain. In order to specifically modulate hERG channel function we have generated single chain variable fragments (scFv′s) using phage display technology that bind to the PAS domain. Stable production of two scFv proteins from *E. coli* has allowed the biochemical characterization of their interaction with isolated PAS domain: scFv2.10 binds to the flexible PAS-Cap region with a Kd ~250 nM whereas scFv2.12 binds to the globular region of the PAS domain with a lower affinity of Kd ~4 μM. Using *in vitro* binding assays we are currently mapping the region on the PAS domain where scFv2.12 binds and looking at the effect of both molecules on the interaction between the PAS and CNBh domain. Addition of purified scFv protein to HEK293 cells expressing hERG channel resulted in both giving an increase in channel deactivation, however, there was a clear difference in their effect on channel inactivation. Addition of scFv2.10 slowed the time of entry into the inactivated state whereas scFv2.12 accelerated the time of recovery from inactivation: interestingly for both molecules this resulted in an increase in total current through the hERG channel. We are currently exploring these molecules as unique biological tools for modification of hERG channel function.

P.1.5-061
Analysis of miRNA expression during the progression of bladder cancer

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Background: Bladder cancer is one of the most common urological cancer and remains a source of significant morbidity and mortality. Standard surveillance strategies of repetitive cystoscopy can be finessed through risk stratification, but are still invasive and associated with both expense and patient discomfort. Molecules of miRNAs are involved in the most important cellular processes, including apoptosis and proliferation. The main function of miRNAs is post-transcriptional regulation of gene expression. Ability of miRNAs to inhibit translation of oncogenes and tumor suppressor genes implies their involvement in the carcinogenesis. More than half of the genes encoding the miRNA are associated with the development and progression of various forms of cancer. Aims: Based on a study of the current literature, we decided to identify miRNA expression profiles in urine of patients suffering from different types and stages of bladder cancer. Methods: For isolation of total miRNA from collected urine samples (n = 20) was used urine purification kit. Isolated miRNAs were transcribed into cDNA using microRNA RT kit, in combination with the set of primers for amplification of specific miRNAs. Final evaluation was done using Taqman Advanced miRNA assay. Statistical analysis was done and results were correlated with control group (n = 10). Results and conclusion: Our results showed that specific miRNAs were in urine significantly overexpressed in comparing to control group. Expression levels for most of analyzed miRNAs reached the 2 or 3 times higher levels in samples of patients with non-infiltrating urothelial carcinoma in comparing to infiltrating form of bladder carcinoma. This comprehensive experimental approach will allow to collect new findings on the progression or eventual recurrence of the urothelial carcinoma, thus contributing to a more accurate prognosis of the disease and improving the quality of life of patients.

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P.1.5-062
The study of molecular causes of disorders of sexual development in patients from Ukraine

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Disorders of sexual development (DSD) constitute an important group of rare human diseases, with more than one affected baby in every 4500 births. DSD are often associated with complications such as ambiguous genitalia, infertility and increased susceptibility to testicular or ovarian cancer. Unfortunately, our knowledge of the genetic networks controlling sex determination is limited. The goal of our study is to identify mutations underlying unresolved DSD phenotypes – in novel DSD genes, or regulatory regions that lead to atypical gene expression. Clinical data and blood samples are being collected by the Regional Centre's of medical genetics of Vinnytsya, Zaporizhzhya, Kherson,

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Chernigiv, Poltava, Zhytomyr, Simferopol and the State Institution V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine. The cohort of 43 patients with different DSD phenotypes was collected. Using GTG-banded chromosome analysis we identified 8 46,XX males and 35 46,XY females (28 46,XY DSD (15 among 28 with androgenic insensivity), 2 46,XX DSD, and 2 45,X/46,XY DSD, 1 46,XX(26)/46XY (3) DSD, 1 46,XY9phgh, 1 46X, del(x)(q21)). After PCR and FISH analysis with CEP X, and WCP1-22,X,Y probes there of five 46,XX males were determined as SRY-positive with Xp; Yp translocation. In one of the rest three 46,XX males we analyzed of CYP21A2 deletion/conversion in homozygous status. Sanger sequencing analysis revealed intact SRY gene in 18 46XY females from our cohort. As well frequent rs279895, rs376062302, rs531364677, rs200423545 were determined in DMRT1 gene and rs915034 in NR5A1 gene which were not associated with DSD. Exome sequencing analysis of two 46,XX SRY-negative male and seven 46,XY SRY-positive females are in progress. WES were performed on an Illumina HiSeq 4000 system. Only the unique and novel DNA variants will be validated by conventional sequencing and analyzed in the parents.

P.1.5-063
IPSC – based model for the study of molecular mechanisms of spinocerebellar ataxia type 1

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The genetic reprogramming technology allows generation of pluripotent stem cells from somatic cells. These cells, called induced pluripotent stem cells (iPSCs) have the ability to self-renew, and to differentiate into any type of somatic cells. Neurodegenerative disorders are of particular interest for the disease modeling with IPSCs and their derivatives due to the limited access to the cells of human brain for research applications or the limitations of of existing animal models. Spinocerebellar ataxia type 1 (SCA-1) belongs to the class of polyglutamine neurodegenerative diseases. This is an autosomal dominant disease caused by the expansion of CAG repeats in the ATXN1 gene encoding the ataxin-1 protein, but the exact functions of both normal and mutant ATXN1 are largely unknown. SCA-1 is caused by Purkinje cell death and degradation of the olive-cerebellar tracts, which leads to impaired coordination of movements, and cognitive disorders. The severity of the disease depends on the length of the CAG tract. In order to establish the first iPSC-based model of SCA-1 we obtained and characterized iPSCs from fibroblasts of 2 patients diagnosed with SCA-1 by lentiviral transduction of OCT4, SOX2, c-Myc, KLF4. All iPSCs express pluripotent state markers (Oct4, SSEA-4, Tra-1-60), have normal karyotype, and form embryoid bodies. The directed differentiation of SCA-1 iPSCs into neurons confirmed their ability to give rise to Aldolase C-positive Purkinje neurons. We also generated a set of modified antisense oligonucleotides allowing allele-specific ATXN1 knockdown. Established system is suitable for SCA-1 mechanisms study, drug validation and screening. This work is supported by the Russian Science Foundation grant #14-15-00930.

P.1.5-064
B cell depletion by anti-CD20 antibodies entrains trabecular bone loss in mice

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B cell depletion by anti-CD20 antibodies (Abs) has revolutionized the treatment of B cell malignancies and autoimmune disorders. As B cell homeostasis is linked to bone metabolism we addressed the effect of anti-CD20 treatment on bone mass.

Ten-week-old female C57BL/6J mice were administered two doses of 75 μg anti-mouse CD20 Abs two weeks apart. After 4 weeks, B cell content and immune-profile of bone marrow (BM) cells were examined by flow cytometry. Bone morphometric analysis was performed in the distal femur using μCT. For trans-differentiation experiments, we tested the osteoclastogenic potential of flow-sorted B cell subsets *in-vitro*.

In mice with >90% splenic B cell depletion (5 out of 7), anti-CD20 treatment induced a significant bone loss, i.e. 25% decrease in trabecular bone mineral density (30.1 ± 2.5 *vs* 40 ± 3.7 mg HA/cm^3), 44% decrease in connectivity density (10.3 ± 1.5 *vs* 18.9 ± 2.8/mm^3), 13% reduction in trabecular number (2.8 ± 0.13 *vs* 3.24 ± 0.13/mm), and 14% increase in trabecular separation (p+, $P < 0.05$). *In-vitro* stimulation by M-CSF and receptor activator of nuclear factor κ-B ligand (RANKL), induced the transdifferentiation of pro-B cells ($CD19^+CD43^{high}IgM^-$), but not pre-B cells ($CD19^+CD43^{low}IgM^-$), nor immature B cells ($CD19^+IgM^+$), isolated from non-treated mice, into TRAP+ multinucleated osteoclasts (OC) ($16\% \pm 3.7$ *vs* $0.8\% \pm 0.28$ and $0.5\% \pm 0.1$ OC area, respectively). Moreover, only $CD115^+$ pro-B cells, but not $CD115^-$ pro-B cells, gave rise to bone-resorbing OC ($18 \pm 6.5\%$ OC area *vs* none).

This is the first report demonstrating adverse skeletal outcomes of pharmacological depletion of mature B cells. Our data also suggest that transdifferentiation of pro-B cells into osteoclasts plays a role in this clinical condition.

P.1.5-065
Structural insight on the trigger mechanism of abnormal cleavage of the amyloid precursor protein transmembrane domain

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The initial steps of the Alzheimer's disease (AD) pathogenesis remain puzzling still. More than half of mutations associated with familial forms of AD were found in the amyloid precursor protein (APP) transmembrane (TM) domain and juxtamembrane (JM) regions. The pathogenic mutations presumably affect structural-dynamic properties of the APP TM domain, changing its conformational stability, lateral dimerization and intermolecular interactions, which can result in enhanced and alternative cleavage by γ-secretase in membrane. We designed highly productive system of cell-free expression and easy purification procedure for APP JM-TM fragments of different length, as well as the

fragments with familial AD mutations. The system allows us to produce milligram quantities of the $^{13}C/^{15}N$-labelled APP fragments for detailed NMR characterization of structure, dynamics, and oligomerization. The fragments were solubilized in detergent micelles and lipid bicelles for acquiring of proper high-resolution NMR spectra despite low sample stability and aggregation. MD-relaxation of obtained NMR structures in hydrated explicit lipid bilayers provided a detailed atomistic picture of intra- and intermolecular interactions. "Australian" (APP L723P) mutation is identified to be associated with autosomal-dominant AD. We detected enhanced flexibility and partial unfolding of the C-terminal region of the TM-helix of L723P mutant compared to wild-type peptide, which can facilitate the APP proteolysis at the ε-site. The L723P mutant gradually converts from α-helical to β-conformation accompanied with high molecular weight aggregates formation. The mutant APP TM fragments are shown to be promising objects for elaboration the molecular aspects of γ-secretase proteolysis. Understanding of the principle of different length amyloidogenic peptides generation is necessary for adequate tactics for AD treatment. The work is supported by the Russian Foundation for Basic Research (project #17-04-02045-a).

P.1.5-066
Invasive properties of uropathogens and catheter-associated biofilms formation

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The ability to long-term persistence of uropathogens in the organism can be linked both to biofilm formation and to the capacity to penetrate the eukaryotic cells. We investigated the ability of *Klebsiella oxytoca* strain to form biofilm *in vivo* (urological catheter) and *in vitro* (plastic microplates) conditions. Using the method of scanning electron microscopy (SEM) we examined the surface of the urological Foley catheter from a patient with prostate hyperplasia. On the inner surface of the catheter we observed biofilm in which bacterial cells, matrix and blood cells were visualized. On the surface of the outer silicone layer of the catheter the biofilm with another architecture was detected. There were identified a large number of fibrin fibers, blood cells and the matrix inside it. The *K. oxytoca* strain was isolated from the biofilm of the catheter and produced extended-spectrum beta-lactamases (ESBL). Adhesive and invasive properties of bacteria were investigated. Using the method of yeast agglutination assay we showed that bacteria express type 1 fimbriae. When cultured on natural urine bacteria formed long filamentous cells, which apparently increased the adhesive properties of the pathogen. By the method of SEM we showed that the bacteria adhere on the surface of carcinoma cells of the bladder T-24 thus causing changes in the morphology of cancer cells. Gentamicin method has demonstrated the ability of *K. oxytoca* strain to invade the T-24 cells. It showed that the cultivation on natural urine increased the invasive properties of bacteria twice in comparison to the cultivation on the LB medium. Treatment of biofilm by the drug preparation of extracellular serine proteases of *Bacillus pumilus* 3-19 (10 U/ml activity) reduced the density of the biofilm by 50%.

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P.1.5-067
The essentiality of CSNAP for CSN function

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The COP9 signalosome (CSN) is an evolutionarily conserved, eukaryotic protein complex, which regulates the ubiquitin-26S proteasome pathway by controlling the activity of cullin-RING-ubiquitin ligases. As such it is critical for key cellular and developmental processes, including signal transduction, cell cycle progression, DNA repair and cancer. Recently, we discovered that CSNAP (CSN Acidic Protein) is the ninth, stoichiometric subunit of the CSN complex. Despite the fact that this subunit displays less than 2% of the CSN total mass, our recent results show that it has high functional significance. Depletion of CSNAP from cells results in morphological changes, alterations in cell cycle progression and alterations in the DNA damage response. Moreover, based on quantitative proteomic results we demonstrate that CSNAP impacts the CSN-CRL interaction network and hence the cellular levels of 26S proteasome substrates. Overall, overall our results indicate that CSNAP is essential for the proper function of the CSN complex.

P.1.5-068
Decoding HCMV latent gene expression program using single cell transcriptomics

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The ability of the Human cytomegalovirus (HCMV) to maintain lifelong infection is attributed to the virus' ability to establish a latent state in specific types of cells, best characterized of which are cells of the hematopoietic lineage. Reactivation from latency in immunocompromised individuals leads to life threatening illness, emphasizing the need to better understand HCMV latent state. Although there are a few well-characterized latent transcripts, the full transcriptional program during latency remains unclear. We harnessed the power of single cell RNA-seq to map HCMV transcriptome during latent infection in monocytes and hematopoietic progenitors. Importantly, this approach allows the exclusion of transcriptional reads originating from lytic cells and enables the identification of diverse latent gene programs within a cell population. Surprisingly, our analysis reveals the absence of a unique latency associated viral gene expression program. Instead we find that the low level gene expression in latently infected cells resembles late lytic gene expression profile. Since cells that carry latent virus *in-vivo* are very rare, obtaining high-throughput data of naturally latent cells requires immense sequencing output. We therefore took advantage of the massive RNA-seq atlas generated by GTEx consortium to examine the viral transcriptional program during natural latency. Importantly, viral gene expression as was unveiled from these samples resembled the single cell RNA-seq data; we identified low expression of late lytic viral genes but no evidence for latency specific program. Finally, this systematic survey in diverse human tissues revealed that HCMV persistence *in-vivo* is not limited to the hematopoietic system and is more prevalent than previously estimated. Overall, our analysis transforms the current view on HCMV latency and illustrates how advancement in genomics technologies can help shed light on complex host-pathogen interactions.

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P.1.5-069
miR-132 overexpression is detrimental to embryonic development, whereas its conditional peripheral excess leads to lipid accumulation

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MicroRNA-132 (miR-132) is a short non-coding RNA which has been shown to regulate diverse pathways, including neuronal development and acute stress reactions, epigenetics and inflammation. However, the biological impacts of miR-132 excess during embryonic development and in murine non-neuronal tissues have not yet been compared. Here, we report that global overexpression of miR-132 under the ubiquitous H1 promoter impairs normal embryonic development. Thus, fertilized eggs microinjected with an miR-132 gene under the control of an H1 promoter yielded predicted numbers of transgenic embryos at embryonic day E10.5. However, by day E14.5, some transgenic embryos underwent a resorption process; and no newborn pups were positive for the transgene, after multiple rounds of injections. In comparison, wild-type embryos showed 100-fold increases in endogenous miR-132 expression from E9.5 to P0. Together, this suggested that unregulated overexpression of miR-132 interferes with normal embryonic development between E10.5-E14.5. A bioinformatics survey followed by pathway enrichment analysis predicted multileveled involvement of miR-132 targets in insulin signaling, fatty acid biosynthesis, glycolysis, axon guidance, dorsal-ventral axis formation and TGFβ signaling, possibly attributing embryonic demise to impairments in part of these pathways. Supporting this notion, we recently showed that transgenic mice with inducible peripheral miR-132 overexpression presented phenotypes of hepatic steatosis and hyperlipidemia, increased body weight, serum LDL/VLDL and liver-triglyceride levels, accompanied by increased hepatic pro-steatotic transcripts and decreased hepatic miRNA-132 target transcripts. Furthermore, antisense miR-132 suppression limited liver hyperlipidemia in fattened mice. Our findings highlight metabolic regulation as a key function of miR-132, and predict that its impact may both interrupt embryonic development and exacerbate lipogenesis in the adult liver.

P.1.5-070
The role of dual phosphatase MKP1 in progressive hearing loss

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Age-related hearing loss (ARHL) is is the most prevalent sensorial impairment of the elderly according to WHO. It is mainly caused by the death of irreplaceable cochlear cellular populations. ARHL is commonly associated with cognitive deficit, social isolation and depression. The onset and progression of the pathology rely on genetic factors that are not well characterized, and are often aggravated by environmental factors such as noise exposure or ototoxic agents. The stress kinases, p38 and JNK, are activated in response to insults that compromise cell integrity. Their activation precedes cellular loss in different scenarios of cochlear insult and its pharmacological inhibition has proved to be otoprotective in animal models.

The MAPK phosphatases are natural regulators of the activity of stress kinases and central elements in the cellular response triggered by these enzymes but their role in hearing loss has not been studied. ABR and DPOAE hearing thresholds were measured, data analysis showed that *Mkp1*$^{-/-}$ mice suffered premature and progressive hearing loss. Higher ABR latencies of wave I indicated delayed transmission. Functional decline along life correlated with morphological and cellular cochlear alterations. Histological analysis and immunohistochemistry revealed loss of sensory cells in the organ of Corti, degeneration of afferent spiral neurons and loss of the spiral ligament fibrocytes and increased macrophages infiltration. Gene expression data (RNA-Seq and RTqPCR) confirmed that null mice showed pathological differences when compared with wild type mice, concretely deregulation GSH synthesis and cycle enzymes, altered balance of pro- and anti-inflammatory cytokines, apoptosis mediators. In summary, we show here that MKP1 deficiency causes an exacerbated inflammatory response and accelerates progressive hearing loss. This Work was supported by grant FEDER/SAF2014-AGEAR. AC and JMB are supported by pre-doctoral contracts from CSIC.

P.1.5-071
The antimicrobial effect of the 5-((-)-bornyloxy)-2(5H)-furanone derivative on gram-positive bacteria

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Infection diseases caused by the multidrug resistant Gram-positive bacteria forming rigid biofilms that are non-permeable to antibiotics appears a great challenge in healthcare. Therefore the therapies based on combinations of antibiotics or use of agents blocking bacterial biofilm formation seems to be a promising strategy. In this context, some derivatives of 2(5*H*)-furanone are known to exhibit antimicrobial effects and interfere with the AI-2 signaling pathways of Gram-negative bacteria. On the other hand some other 2(5*H*)-furanone derivatives repress the growth of Gram-positive bacteria. Here we show that chiral 5-((-)-bornyloxy)-2(5*H*)-furanone derivative inhibits the growth of Gram-positive (*Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus* spp.) with no effect on Gram-negative (*Escherichia coli*, *Pseudomona aeruginosa*, *Klebsiela pneumonie*, *Salmonella typhimurium*, *Enterobacter aerogenes*) bacteria. The minimal inhibitory concentrations determined by the broth microdilution method in 96-well microtiter plates according to the EUCAST rules for all Gram-positive bacteria were found in the range of 8 – 16 mg/l. The concentrations suppressing the biofilm formation of Gram-positive bacteria were equal to the respective MICs suggesting that this compound demonstrates rather antimicrobial than antibiofilm activity. Interestingly, the bactericidal effect was observed only for *Bacillus* spp. purportedly its selective action, while bacteriostatic effect was detected for *S. aureus*, *S. epidermidis* and *Micrococcus luteus*. In summary, the 5-((-)-bornyloxy)-2(5*H*)-furanone derivative represents a promising chemotype for anti-bacillary drug design.

P.1.5-072
GLUT10 – lacking in arterial tortuosity syndrome – facilitates dehydroascorbic acid transport in the endoplasmic reticulum and nuclear envelope

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GLUT10 belongs to a family of transporters that catalyze the uptake of sugars/polyols by facilitated diffusion. However, neither the subcellular localization of GLUT10, nor its transported ligand(s) have been clearly identified. Loss-of-function mutations in the gene encoding GLUT10 are responsible for arterial tortuosity syndrome, a rare connective tissue disorder mainly characterized by tortuosity, stenosis and aneurysm formation of the main arteries. The results presented here showed a perinuclear distribution of GLUT10 as demonstrated by immunocytochemistry in fibroblasts from healthy controls. Immunoblotting revealed that GLUT10 protein was present in the microsomal fraction of the cells. Dehydroascorbic acid transport and accumulation was markedly reduced in fibroblasts from arterial tortuosity syndrome patients and in GLUT10 shRNAi-silenced immortalized human fibroblasts whose plasma membrane was selectively permeabilized. Re-expression of GLUT10 in patients' fibroblasts restored dehydroascorbic acid transport activity. Measurement of dehydroascorbic acid uptake in subcellular fractions of fibroblasts showed that endoplasmic reticulum transport was reduced in patients. GLUT10 protein produced by *in vitro* translation and incorporated into liposomes efficiently transported dehydroascorbic acid. Lower intracellular ascorbate content was measured in patients' fibroblasts incubated in the presence of physiological concentration of ascorbate. The present results demonstrate that GLUT10 is a dehydroascorbic acid transporter in the endoplasmic reticulum and nuclear envelope. Furthermore, they suggest that a reduced transport of dehydroascorbic acid into the endoplasmic reticulum and nucleoplasm – where ascorbate functions as a cofactor for Fe^{2+}/2-oxoglutarate dependent dioxygenases – can be a causal factor of the pathomechanism in arterial tortuosity syndrome.

P.1.5-073
Relationship between paraoxonase 1 (PON1) promoter (-107T/C) and coding region polymorphisms (192Q/R and 55L/M) and Pseudoexfoliation syndrome

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Pseudoexfoliation syndrome (PES) is an age–related systemic disease manifesting itself primarily in the eyes and is characterized by the accumulation of microscopic granular amyloid-like protein fibers. When these fibers occlude Schlemm's channels, intraocular pressure increases and this condition is called Pseudoexfoliation glaucoma (PEG). Glaucoma is one of the main causes of vision loss in the elderly. Hence, early recognition and appropriate management of PES and PEG are important in the prevention of blindness. However, the etiology of this disorder has not been clearly understood. Pathogenesis of PES was suggested to include oxidative stress. Paraoxonase 1 (PON1) is accepted as an important anti-oxidant enzyme. Expression level and activity and/or stability of this enzyme is affected by genetic polymorphisms in the promoter region (-107T/C) and coding region (192Q/R and 55L/M) of PON1 gene, respectively. The aim of this study was to evaluate the role of *PON1* -107T/C (rs705379), 192Q/R (rs662) and 55L/M (rs8545860) single nucleotide polymorphisms (SNP) in PES. Study population consisted of 70 PES patients and 70 control subjects. Blood samples were obtained from Gülhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey. Genomic DNAs were isolated from whole blood samples and all polymorphisms were determined by PCR-RFLP analysis. 192R allele frequency was found to be 0.257 in PES patients and 0.271 in controls (P = 0.786), while the frequency of 55M allele was 0.350 in PES patients and 0.364 in controls (P = 0.679). The frequency of -107C allele was 0.457 in PES patients and 0.429 in controls (P = 0.630). The results of this study did not show any association between PON1 -107T/C, 192Q/R and 55L/M SNPs and PES risk in a small Turkish population. These are the preliminary results of a research project analyzing the relationship between PON1 SNPs and the risk of PES and PEG.

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P.1.5-074
Fingerprinting analysis of metabolites in patients with rheumatoid arthritis and osteoporosis in Kazakh population

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Rheumatoid arthritis (RA) is the most common chronic autoimmune disease assosiated with severe destruction of joints and often accompanied by osteoporosis (OP). The main goal of this research is identifying metabolically interpretable genetic facrors predispoding to manifestation and progression of RA, along with its associated complication - OP as well as to positive/negative response to RA treatment, in Kazakh ethnic sample. Collection of clinical material and assesment of all participating individuals in this project was carried out in the Almaty's Rheumatology Center, the University Clinic of the Kazakh National Medical University (KazNMU), and the RA Center of the Semey's State Medical University. All participants in this project were included in the study only after the signing of a written agreement, approved by the Ethics Committee of the KazNMU. We conducted metabolic studies on the groups of patients with RA and patients with RA complicated with OP. There was conducted an isolation of polar and non-polar metabolites from serum, which were further separated into the HILIC bond column by HPLC-MS with the TOF (time-of-flight) detector. HPLC-MS was conducted three times for each sample. Fingerprinting analysis of metabolites by free acces software revealed differences between the case and control cohorts and also demonstrated notable discrimination between the RA and RA-OP cohorts. We intend to perform further MS/MS of all those samples in order to discover and approve every single metabolite. As a result of our research we want to determine specific metabolically interpretable and reliable genetic factors along with metabolic pathways, which might be shared or differed between RA and OP and might provide insight into the extent to which the process of degenerative changes occurs and progresses.

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P.1.5-075
UROD disorders: characterization of different mutations in the Czech population and one novel mutation in the UROD gene in the Egyptian population

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Uroporphyrinogen decarboxylase (UROD) is a cytosolic enzyme that decarboxylases uroporphyrinogen III producing coproporphyrinogen III in the fifth step of the heme biosynthetic pathway. Three metabolic disorders results from the decrease in the catalytic activity of the UROD enzyme: sporadic porphyria cutanea tarda (s-PCT), familial porphyria cutanea tarda (f-PCT), and hepatoerythropoietic porphyria (HEP). s-PCT results from the slight decrease in the UROD activity and is limited to the liver. No mutations are found in the UROD gene. f-PCT, autosomal dominant trait, due to a heterozygous mutation in the UROD gene. In HEP, the UROD catalytic activity markedly diminish between 5% to 30% of that of normal in all tissues due to homozygous mutation or compound heterozygous mutation in the UROD gene. We investigated the molecular defect in six Czech patients with fPCT and two Egyptian patients with HEP that were refered to our laboratory. Three different previously reported UROD defects were identified in six patients with fPCT from Czech origin. Non-sense mutation p.[Gln206];[=] in exon 6 in the UROD gene lead to a stop codon suggesting the synthesis of a truncated protein. Three sequential point mutations (c.399-401 delins CCA) in exon 5 in the UROD gene leading to the substitution from a non-polar amino acid (Valine) to the polar amino acid (Glutamine) (V134Q). Finally, heterozygous splice site mutation in intron 9 c. 942+1 G>A of the UROD gene was identified leading to unproper splicing of the mRNA. In the Egyptian patients with HEP, a homozygous mutation c.163T>A in exon 3 of the UROD gene was identified leading to the substitution of phenylalanine to Isoleucine at position 55 (F55I). The relative activity of F55I mutant UROD was 19% of wild-type towards pentaporphyrinogen I. This work was supported by grants from Grant Agency of Czech Republic (14-36804G), Charles University in Prague (PROGRES Q26/1LF, UNCE 204011/2012).

P.1.5-076
Structural analysis of human ANGPTL8, a factor of important role in lipid homeostasis

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ANGPTL8 (angiopoietin-like protein 8) is a recently identified plasma protein that has the ability to modulate triglyceride (TG) metabolism. Disrupted TG metabolism (including dyslipidemia) is characteristic of the metabolic syndrome, type 2 diabetes and obesity. The mechanisms regulating those processes are extremely interesting and of potentially broad practical implications.

ANGPTL8 binds and allosterically inactivates lipoprotein lipase (LPL), a plasma triglyceride degrading enzyme. It was postulated that pharmacological counteracting of LPL inhibition by ANGPL8 may prove an effective therapy in dyslipidemia. However the mechanistic basis for regulation of LPL activity by ANGPTL8 remain unknown. The structure of ANGPTL8, LPL or complex of these proteins has not been determined, so it is not known how ANGPTL8 interacts with LPL and regulates the activity of the enzyme. To gain an insight into this mechanism we optimized the expression conditions for ANGPTL8 in the heterologous system in *E. coli*. We obtained an efficient expression of the protein in soluble form. Purification by affinity chromatography led to enriched ANGTPL8 preparation. We are currently optimizing further purification by ion exchange chromatography and SEC. Based on bioinformatic analysis, possible unstructured regions, which could potentially have a negative impact on crystallization were removed. Crystallization trials have been made for all protein variants, but up to date were unsuccesfull. In the future we are going to evaluate the expression and crystallization of orthologues from different species.

P.1.5-077
Immunoprofile of coronary heart disease

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Objectives: Coronary heart disease (CHD) is a complex human disease associated with inflammation and stress. The underlying mechanisms and diagnostic biomarkers for the different types of CHD remain poorly defined. Mimotope Variation Analysis (MVA), a high-throughput next generation phage display platform allows to delineate individual's humoral immune response associated with CHD.

Results: We performed MVA from sera of human subjects with acute coronary syndrome (ACS) myocardial infarction (MI), and healthy control (HC). Quantitative serologic profiles (immunoprofiles) of millions of peptide antigens from 2 µl of sera sample were obtained. Clustering analysis of the top 5000 peptide antigens of individual samples revealed a highly prevalent serological response to common pathogens (i.e., *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, and herpes simplex virus 1 and 2) in diseased. Interestingly, in MI patients the profile of pathogenic response was targeted to different antigens (epitopes) as compared with other cohorts. For example, the *Helicobacter pylori* response was targeted to a specific epitope that was neither CagA nor VacA indicating to a novel virulence pathway associated with an acute form of disease

Conclusion: Our studies using the state-of-the-art immunoprofiling approach help to understand the underlying biological mechanisms involved in the pathogenesis of CHD; common infections may serve as potential biomarkers to differentiation MI from ASC and HC.

P.1.5-078
Identification of autoantibodies against fructose-1,6-bisphosphatase isolated from serum of autistic children

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Autism is a complex disease characterized by behavioral deficits, systemic metabolic abnormalities and the presence of serum auto-antibodies targeting key proteins in the brain. Aberrations of brain energy metabolism in autistic patients may involve mitochondrial metabolic dysfunctions within the CNS, a hypothesis supported by frequent manifestations of lactic acidosis in autistic children, apparently caused by a decrease in the rate of lactate utilization in gluconeogenesis. Because the levels of fructose 1,6-bisphosphatase (FBPase) and its substrate are at the core of the gluconeogenic pathway and both might be involved in regulation of cell survival, we hypothesized their link with neurometabolic conditions and symptoms often observed in autistic children. Using immuno-analysis techniques, we found anti-FBPase IgG in autistic patient sera that cross-reacts with liver and muscle FBPase (FBP1 and FBP2). This autoantibody does not affect FBPase enzymatic activity or its susceptibility to AMP, suggesting that the antigenic region is not in the FBP1 functional domains. We also evaluated the cytotoxic effect of these autoantibodies in cell culture and *in vitro* assays using N2a cell line. The results obtained by MTT assay and immuno-active caspase 3 suggest that the presence of anti-FBPase IgG is not harmful to the cell. We conclude that autistic patients have a high level of autoantibodies, targeting both FBPase isoforms with undetermined metabolic and cellular effects. Nevertheless, the possibility remains that these antibodies may have a differential effect on the FBPase isoforms that disrupts their protein-protein interaction with other structural or metabolic targets. Sponsored by FONDECYT 1141033.

P.1.5-079
Metabolomic characterization of Wolfram syndrome 1 deficient mouse

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Wolfram syndrome 1 is a rare autosomal recessive neurodegenerative disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Mutations in the WFS1 gene encoding wolframin glycoprotein can lead to endoplasmic reticulum stress and unfolded protein response in cells, but the pathophysiology at organism level is poorly understood. In this study several organs (heart, liver, kidneys and pancreas) and bodily fluids (trunk blood and urine) of 2- and 6-month old *Wfs1* knock-out, heterozygote and wild-type mice were studied by untargeted and targeted metabolomics using liquid chromatography-mass-spectrometry. The key findings include significant perturbations in pancreas and heart metabolism before the onset of related clinical signs, glycosuria that precedes hyperglycemia and implies a kidney dysfunction prior to the onset of classical diabetic nephropathy. Glucose use and gluconeogenesis is intensified in early syndrome stages, but later the energetic needs are mainly covered by lipolysis. Furthermore, in young mice we detected liver and trunk blood hypouricemia, which in time turns to hyperuricemia.

P.1.5-080
Serum vitamin D levels in patients with chronic obstructive pulmonary disease

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Background: Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disorder characterized by irreversible and progressive limitation of expiratory airflow. COPD is now considered to be a systemic disorder with multisystem involvement. As an immunomodulatory effector, vitamin D can not only boost innate immune responses upon infection but also regulate adaptive immune responses. Our aim was to investigate serum 25-hydroxy vitamin D levels in patients with chronic obstructive pulmonar disease (COPD).
Methods: 83 control, 139 COPD patients were enrolled to this study. Participants with known systemic diseases, including cardiovascular disease, renal disease, gastrointestinal disease, pulmonary disease, acute infection, chronic inflammation and cancer were excluded. Serum 25-hydroxy vitamin D levels were analyzed with API 3200 ABSCIEX LC-MS/MS system.
Results: Serum serum 25-hydroxy vitamin D levels were significantly higher in controls [18.2 (5–130) ng/mL] compared to patients [12.8 (2–128) ng/mL] ($P = 0.006$).
Conclusions: Vitamin D deficiency might be used to find out the mechanisms underlining COPD's clinical progression. Treatment with vitamin D analogs may contribute to decrease the symptoms. The size of this contribution must be confirmed in prospective observational and intervention studies.

P.1.5-081
Secretoneurin attenuates Ca2+-dependent arrhythmogenesis

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Circulating secretoneurin (SN) levels are reported to predict mortality in patients with myocardial dysfunction. SN has also been observed to inhibit Ca^{2+}/calmodulin-dependent protein kinase II δ (CaMKIIδ) activity, but the binding site of SN to CaMKIIδ has not been investigated. Additionally, whether elevation of SN protects against Ca^{2+}-dependent arrhythmia is not established. Using

pull down experiments and structural homology modeling, SN binding was mapped to the substrate binding site in the catalytic region of CaMKIIδ. SN attenuated isoproterenol (ISO)-induced autophosphorylation of Thr287-CaMKIIδ in Langendorff hearts, and inhibited CaMKIIδ-dependent ryanodine receptor 2 (Ser2814-RyR2) phosphorylation. SN was also observed to decrease RyR2 open probability in lipid bilayer experiments. In line with CaMKIIδ and RyR2 inhibition, SN treatment decreased Ca^{2+} spark frequency and dimensions in cardiomyocytes during ISO challenge. Ca^{2+} wave frequency was reduced, which corresponded with lower incidence of delayed after-depolarizations and fewer spontaneous action potentials. SN treatment also reduced the incidence of early after-depolarizations during ISO; an effect paralleled by reduced magnitude of L-type Ca^{2+} current. Based on these protective actions of SN, we investigated SN levels in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) who are prone to Ca^{2+}-dependent arrhythmia. Circulating SN levels were increased in CPVT patients, while levels of established biomarkers were unchanged. In conclusion, SN interacts with the substrate binding site of CaMKIIδ, thereby inhibiting its activity. A consequent reduction in RyR2 and L-type Ca^{2+} channel opening reduces incidence of early and late after-depolarizations. Production of SN may be an endogenous protective mechanism in patients with pathological cardiomyocyte Ca^{2+} handling, supporting its role as an emerging biomarker.

P.1.5-082
The mutational profiles of PIK3CA and TP53 genes and some peculiar ultrastructural aspects in breast cancer

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The *PIK3CA* gene is involved in the phosphatidylinositol 3-kinase/AKT cellular signaling pathway mediating proliferation and cell survival processes, thereby regulating tumor cell growth. The *TP53* is one of the most investigated prognostic or predictive markers in many human cancers. The purpose of the current study was to investigate the mutational status of the *TP53* and *PIK3CA* genes in breast cancer; the molecular results were completed by some ultrastructural details of the tumors – stroma interface during invasive growth of mammary carcinoma. In order to perform molecular analysis and transmission electron microscopic investigations, 22 samples of fresh breast tumor tissue, have been analyzed by Sanger method, respectively the routine TEM protocol. In *PIK3CA* gene, mutational frequency was 36.36% (8/22). Three mutations (37.5%) were identified in exon 9, helical domain and five mutations (62.5%) in exon 20, kinase domain. Regarding the frequency of somatic mutations in *TP53* gene, the mutation rate was 27.3% (6/22) with a predominance of deletion mutations (66.67%). Concerning the fine analysis of the tumor – stroma interface, we refer only to the ability of tumor cells themselves and telocytes (a recently described cell phenotype) present also inside of the peritumoral stroma to produce small lipoprotein sacks termed *extracellular vesicles*. Our results regarding the mutational status of *PIK3CA* and *TP53* genes are in line with those in international database. As regards the ultrastructural details, we have to consider the existence of extracellular vesicles containing an appreciable diversity of (macro)molecules (proteins, segments of genomic DNA, multiple forms of RNA, including miRNA, lipids, metabolites) that act as mediators involved in cell-cell and cell-extracellular matrix interactions, therefore having a high potential to be involved in mammary tumor progression and control.

P.1.5-083
Investigation of serum sclerostin levels in children and adolescents with type-1 diabetes mellitus

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Introduction: Interaction between diabetes mellitus (DM) and osteoporosis is a complex issue and needs careful evaluation. This interaction is especially important in bone metabolism of children with type-1 DM. Sclerostin is one of the factors modulating the Wnt/b-catenin pathway which is essential for normal osteogenesis. Sclerostin supresses mineralization of osteoblastic cells, inhibits osteoblast proliferation and promotes osteoblast apoptosis. The aim of the present study was to measure serum sclerostin levels in children and adolescents with type-1 DM and compare with age- and gender- matched control subjects.
Materials and Methods: The study included 40 children and adolescents with type-1 DM (19 males and 21 females) aged from 7 to 17 years and 40 healthy controls (18 males and 22 females) aged from 6 to 17 years. Serum sclerostin levels were measured by ELISA method using commercially available kit.
Results: Glucose and hemoglobin A1c (HbA1c) levels of children and adolescents with type-1 DM were significantly higher than that of the controls (P = 0.000). However, there was no significant difference between sclerostin levels of the groups.
Discussion and Conclusion: Our result showed that serum sclerotin levels were not changed in children and adolescents with type-1 DM.

P.1.5-084
Immune responses in patients with chronic renal failure before and after transplantation

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Chronic renal failure (CRF) is the end result of chronic renal diseases that characterized progressive loss in the number and function of nephrons. Worldwide, there are up to 400-500 million patients with chronic renal failure, and number of patients is increasing. Thus, the search markers at a very early stage of the kidney disease, as well as rejection, being a challenge for the medical community. The goal of this investigation was to analyze the serum level of 27 cytokines in Chronic kidney insufficiency (CKI) patients.

We examined serum from 240 patients and conditionally healthy people using cytokine protein array kit Bio-Plex Pro™ Human Cytokine 27-plex Assay. Patients were divided into next groups: 1) conditionally healthy donors, 2) patients at pre-dialysis stage, 3) patients received hemodialysis, 4) patients before transplantation, 5) patients after 1 year from transplantation, 6) patients more than 1 year after transplantation.

The cytokine levels of IL-1b and IL-2, and receptor inhibitor IL-1Ra were below level of detection. The level of IFN-g was not significantly different in all groups. The levels of the remaining

measured cytokines IL-4, IL-5, IL-6, IL7, IL-8, IL-9, IL-10, IL-12 b, IL-13, IL-15, IL-17, chemokines IP-10, RANTES, MIP1a, MIP1b, MCP1, eotaxin, growth factors VEGF, FGFb, PDGFbb, G-CSF and GM-CSF, as well as TNF-a were significantly higher (p value < 0.05) in the dialysis patient group and before treatment by compared with other groups. The data suggested that both cellular and humoral immunity in all patients with CRF was activated.

To identify early rejection markers among the cytokines, a data from patients after rejection (8 patients) and other patients after transplantation was separately analyzed in R environment (www.r-project.org). However, no significant differences in the level of cytokines from these groups of patients were observed.

P.1.5-085
The interaction of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) and alpha-actinin 2 is altered in GNE myopathy M743T mutant

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GNE Myopathy is a rare neuromuscular recessive disorder caused by missense mutations in GNE, the key enzyme of sialic acid biosynthesis. In an attempt to elucidate GNE functions that could account for the muscle pathophysiology of this disorder, the interaction of GNE with α-actinins has been investigated. Surface plasmon resonance and microscale thermophoresis analysis revealed, that *in vitro*, GNE interacts with α-actinin2, and that this interaction has a 10-fold higher affinity compared to the GNE-α-actinin1 interaction we previously showed. Further, GNE carrying the M743T mutation, the most frequent mutation in GNE myopathy, has a 10-fold lower binding affinity to α-actinin2 than intact GNE. This decrease could eventually affect the interaction, thus causing functional imbalance of this complex in skeletal muscle, that could contribute to the myopathy phenotype.

In vivo, using bi-molecular fluorescent complementation, we show the specific binding of the two proteins inside the intact cell, in a unique interaction pattern between the two partners. This interaction is disrupted in the absence of the C-terminal calmodulin-like domain of α-actinin2, which is altered in α-actinin1. Moreover, the binding of GNE to α-actinin2 prevents additional binding of α-actinin1 but not vice versa. These results suggest that the interaction between GNE and α-actinin1 and α-actinin2 occur at different sites in the α-actinin molecules and that for α-actinin2 the interaction site is located at the C-terminus of the protein.

P.1.5-086
Tau toxicity on plasma membrane Ca2+-ATPase (PMCA) is prevented and reversed by calmodulin

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The maintenance of intracellular free Ca^{2+} at the properly low level in eukaryotic cells involves a system of high-affinity Ca^{2+} membrane pumps. These transporters use the energy of ATP hydrolysis to pump cytosolic Ca^{2+} out of the cell (Plasma Membrane Ca^{2+}-ATPase, PMCA) or into internal stores (Sarco Endoplasmic Reticulum Ca^{2+}-ATPase, SERCA, and Secretory Pathway Ca^{2+}-ATPase, SPCA). Increasing evidence point out a link between Ca^{2+} homeostasis dysregulation, aging and aging-related diseases such as Alzheimer's disease (AD). AD is characterized by the accumulation of β-amyloid peptide in senile plaques and neurofibrillary tangles of tau protein. In this work, we show that calmodulin, the major endogenous activator of PMCA prevents the inhibition of the Ca^{2+}-ATPase activity of PMCA by tau and also reverses the inhibition of PMCA by tau. Furthermore, functional studies with native and truncated variants of human PMCA4b pointed out that tau binds to the C-terminal tail of PMCA, in a site located between the transmembrane domain 10 and the calmodulin binding domain. These results point toward a relationship between PMCA and neurodegeneration by tau protein, and the role of calmodulin as a neuroprotective agent against one of the major molecular hallmarks of Alzheimer's disease.

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P.1.5-087
Activity of the reproductive axis in the adult female is modified by pre-pubertal stress

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Environmental conditions in early life can have a substantial influence on female reproductive function. Earlier studies suggested that the challenging environment encountered by young pre-pubertal women may affect their subsequent fertility: menarche was delayed, they had lower salivary progesterone levels and early menopause. Variation in reproductive function might be determined by epigenetic regulation and, we hypothesized, this might be due to changes in expression of key genes in the reproductive axis. We established a mouse model for early life immunological stress in order to investigate its effects on reproductive function and elucidate the mechanisms involved. After weaning, female pups were divided into two groups: one treated for 7 d with 1.5–2.5% DSS in the drinking water to induce mild colitis, while the other served as untreated controls. Vaginal opening was assessed as a measure of puberty, and was significantly delayed by 3.4 days compared to untreated littermates. Furthermore, ovarian *Pgr* mRNA levels were significantly lower in DSS-treated mice compared to controls, at 2–3 and 8–12 month. Similarly, ovarian *Lhr* mRNA levels were lower in the treated mice at both time points, while mRNA levels of *Amh*, which is produced in pre-antral follicles, appeared higher in ovaries of the treated mice. These results suggest that ovarian gene expression is altered long-term by the pre-pubertal treatment, with possible implications for reproductive function and fertility. Our preliminary breeding experiments revealed that the DSS-treated mice are fertile, and the litter sizes significantly larger than those of untreated controls. Taken together, our results suggest that reproductive strategy in adult life is altered by pre-pubertal treatment, and indicate that this might involve an increase in numbers of follicles that start to develop in each cycle, perhaps to increase the likelihood of reproductive success which, in women, would also lead to an earlier menopause.

P.1.5-088
A conserved region of the HIV gp41 fusion protein exhibits dual functionality mediated by the plasma membrane that sheds light on its residue conservation

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The human immunodeficiency virus (HIV), utilizes its envelope glycoprotein gp160, specifically the gp41 subunit, to enter its host cell through membrane fusion. Recently, immunosuppression of T-cells has been reported as an additional function for gp41. The fusion promoting conserved pocket binding domain (PBD) of gp41 resides in an immune suppressive region of gp41. We hypothesized that the PBD plays a role in T-cell immunosuppression in addition to its role in membrane fusion. Using two independent cell culture model systems complemented by comprehensive biophysical techniques such as circular dichroism, fluorescence resonance energy transfer and nuclear magnetic resonance we revealed that the PBD is bifunctional, alternating between fusion facilitation and immunosuppression. We show that the latter activity is based on PBD-dependent stabilization a T-cell receptor binding conformation of gp41 in the membrane. Interestingly, the data suggests that successful membrane fusion does not seem to entirely rely on the PBD. Therefore, immunosuppression may be a possible driving force for the high degree of PBD conservation. This study reports for the first time a dual role for the gp41 PBD, providing insights into residue conservation and functionality. Taking into account its role in T-cell suppression described herein, this motif may serve as a promising future drug target as it mediates two key processes in the HIV infection cycle.

P.1.5-089
Adenosine signaling affects renin-angiotensin system and diabetic nephropathy pathogenesis

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Background: Progression of diabetic nephropathy (DN) is linked to intrarenal induction of the renin-angiotensin system (RAS) as well as increased levels of adenosine. The activity of RAS is dependent on the activity of peptidases that process the precursor angiotensin 1, such as aminopeptidase A (APA), generating products with poorly characterized effects on tissues and organs. Our aim was determine the role of adenosine and its receptors on RAS activity.
Methods: Rat glomeruli were treated ex vivo with 10 μM adenosine and pharmacological modulators of adenosine receptors for 24 h. APA induction by adenosine was determined by comparative shotgun proteomics using spectral count data and Quasi-Likelihood modeling. Changes in APA were confirmed by western blot and enzymatic assays. Physiological effects after *in vivo* administration of the adenosine A2B receptor antagonist MRS1754 (2.5 mg/kg/1 month) and APA derived peptides angiotensin 2-7 (Ang 2-7, 300 μg/Kg/day for 15 days), angiotensin 2-8 (Ang 2-8, 300 μg/Kg/day for 15 days) and angiotensin 2-10 (Ang 2-10, 300 μg/Kg/day for 15 days) were evaluated in healthy and diabetic rats.
Results: In glomeruli from diabetic rats ex vivo adenosine induced APA through A2B receptor. An antagonist of adenosine A2B receptor (MRS1754) blocked APA induction in DN. *In vivo* administration of RAS peptides generated from APA activity on angiotensin 1, had deleterious effects on rat physiology. The systemic hypertensive response were Ang 2-10 > Ang 2-8 and Ang 2-7 and proteinuria Ang 2-10 > Ang 2-8 > Ang 2-7. Peptides mediated renal injury inducing the fibrotic marker αSMA and decreased urinary creatinine.
Conclusion: Adenosine altered peptides repertory of RAS by inducing glomerular aminopeptidase A. Peptides derived from APA activity had deleterious effects on renal function.

P.1.5-090
Distinct ubiquitination signatures of serum proteins in Systemic Lupus Erythematosus

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Autoimmune diseases are in most cases multifactorial in their cause, with a mixture of genetic and environmental factors playing a role. Indeed, perturbations of the immune system and cellular proteostasis control, may elicit autoimmunity. Notably, alterations in post-translational modifications (PTMs), and particularly in ubiquitin, have been implicated in immune responses and autoimmunity. Here, we established a PTM Profiling approach to map the ubiquitination landscape of serum proteins from Systemic Lupus Erythematosus (SLE) patients compared to healthy individuals. Using this high-throughput system we monitored the ubiquitination status of thousands of proteins and identified distinct ubiquitination profiles that classified samples according to their physiological state. The set of differentially modified targets correlated with disease and should provide novel insight to the molecular basis of SLE.

P.1.5-091
Phenotypic and genotypic characterization of *Neisseria gonorrhoeae* resistant isolates in Bucharest, Romania

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The purpose was to characterize the *N. gonorrhoeae* recent isolates exhibiting antibiotic resistance to penicillin, tetracycline and fluoroquinolones using phenotypic methods, PCR and Antigen Sequence Typing (NG-MAST), a molecular typing method accepted at European level for this pathogen. Material and methods. 20 strains of *N. gonorrhoeae* were isolated *over a period of six months (January – June 2016)* from men urethral secretions sent to Synevo Central Reference Laboratory, Bucharest. All isolates were identified by mass spectrometry using MALDI Biotyper and the antibiotic susceptibility was determined by agar disk diffusion in accordance with CLSI, 2016. Simplex and multiplex PCR were performed on genomic DNA, in order to identify the penicillin resistance genes [TEM like β-lactamase; PenA and PonA; mutation in the promoter region of the *mtrR* gene; decrease in the permeation of the outer membrane porin *PorB1b* and also the mutations in the *pilQ* gene], tetracycline resistance genes (*tetM* gene and mutation in the *rpsJ* gene) and fluoroquinolone resistance (mutations in *gyrA* and *parC* genes).The resistant isolates were further characterized by NG-MAST. Results. A high level of resistance was noticed among isolates to ciprofloxacin and ofloxacin (80%) followed by tetracycline (65%) and β-lactam antibiotics (25% to penicillin and 10% to cefuroxime). The molecular study revealed the presence of the followed resistance genes: ciprofloxacin (*gyrA* – 45% and *parC* – 40%);

tetracycline (*rpsJ* - 100% and tetM - 10%); penicillins (*PilQ* - 95%; *mtr* - 90%; *PonA* - 35%; *PenA* - 15%; bla_{TEMlike} – 15% and *PorB* - 5%). Conclusions. The molecular typing of resistant isolates may provide the capacity to identify possible associations between ST and AMR (antimicrobial resistance) phenotype and patient characteristics, having as the final purpose to guide treatment algorithms and targeted prevention strategies.

P.1.5-092
MicroRNA expressions are altered in endometrial tissues of PCOS women

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Aim: To assess whether normally ovulating women and Polycystic ovary syndrome (PCOS) patients differ in tissue expression of several microRNAs (miRNA) in endometrium.
Background: Besides being the most common endocrinological disorder in reproductive age women, PCOS also affects fertility in a significant portion of couples seeking help for infertility. Patients with PCOS usually suffer from anovulation as a subfertility cause. However, PCOS carries the potential to disrupt endometrial intactness and hamper implantation, as well. We investigated the changes in the expression levels of a number of selected microRNAs in endometrial tissue samples of PCOS women and tried to detect an implied relationship. MicroRNAs are small RNA fragments that act as posttranscriptional regulators of various gene targets rather than encoding proteins themselves. miRNAs play a role in some biological processes. The abnormal expression of some miRNAs has been associated with various disorders. In humans, miRNAs are important in uterine functions, however, the endometrial aspects of PCOS related infertility and its implications in miRNA profiles have not been studied in detail. miRNA microarray shows that different miRNA profiles exist in mouse uterus between the implantation sites Endometrial disorders might be associated with the abnormal expression of miRNAs in the uterus. Methods:Total RNA will be isolated from endometrium samples taken from 15 PCOS patients and 15 normally ovulating controls, by using RNA purification kit. Then, the cDNA will be synthesized by using the reverse transcriptase kit from the isolated RNAs, and miRNA expressed from the cDNAs by RT-PCR using miR-146a, miR-155, miR-223, hsa-miR-30b, hsa-miR-30d, hsa-miR-145 and hsa-miR-494 primers.
Conclusion: Potential alterations in miRNA expression levels in PCOS women may shed light on candidate genes responsible for endometrial functions which are affected by hormonal irregularities.

P.1.5-093
Reduced levels of Tau protein in Familial Dysautonomia

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Familial Dysautonomia (FD), is a neurodegenerative disease, caused by a point mutation in the *IKBKAP*/*elp1* gene that leads to a severe reduction of ELP1 protein. ELP1 is a scaffold protein of the six subunits Elongator Complex,which regulates various cellular processes including transcription, translation and acetyl transferase activity through its catalytic subunit Elp3. Published work from our laboratory suggests that ELP1 plays a role in cytoskeleton regulation. Here we aim to decipher the role of Microtubule Associated Protein, a known key regulator of the neuronal microtubules (MTs) network, in FD using a human neuroblastoma and Drosophila Melanogaster models. We show that *IKBKAP* downregulation causes a severe reduction of tau protein levels *in-vitro and in-vivo,* reduced stability of Tau Protein, that is correlated with increase in Ubiqtinated TAU. Our findings suggests that ELP1 is involved, via post translation modification in regulation of tau protein levels. As tau dysregulation is associated with a vast range of neurodegenerative disorders understanding the role of ELP1 in regulation of tau levels could have wide implication.

Signaling Across Membranes: Receptors, Channels and Transporters

P.2.1-001
Aphid-evoked alternations in expression of glutathione transporter genes in maize (*Zea mays* L.)

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The objective of the performed survey was to assess influence of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) herbivory on transcriptional responses of two genes encoding glutathione transporters (i.e., *GT1* and *ABCC6*) in foliar tissues of maize (*Zea mays* L.) plants. The experiments were carried out on 14-day-old maize seedlings (Złota Karłowa and Waza cvs – susceptible and highly resistant to the aphids' infestation, respectively). The plants were colonized by 5, 10, 20, 40 or 80 apterous adult females of *R. padi* for several time intervals (1–96 h), whereas the control seedlings remained uninfested. The expression level of two studied genes was measured with the use of real-time qRT-PCR technique, and the obtained results were normalized to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene. It was found that *R. padi* infestation led to significantly higher upregulation of two examined genes (i.e., *GT1* and *ABCC6* – encoding glutathione transporter 1 and ATP-binding cassette transporter 6, respectively) in seedlings of more resistant maize genotype (Waza cv.) than in susceptible plants (Złota Karłowa cv.). In addition, transcriptional reprogramming of the target genes in maize tissues were dependent on duration of aphid exposure and insect abundance per plant. The findings indicated possible role of tested glutathione transporters in detoxification and complex defence reactions in maize plants subjected to aphid infestation.

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P.2.1-002
Regulation of VEGF-induced vascular hyperpermeability by the neuropilin 1 cytoplasmic domain

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The vascular endothelial growth factor (VEGF) is a secreted glycoprotein that can induce the growth of new blood vessels, known as angiogenesis, and stimulate vascular hyperpermeability. Upregulation of VEGF has been observed in ischemic disease, where angiogenesis is beneficial for recovery from hypoxia, whilst hyperpermeability causes damaging oedema. VEGF is expressed as three main isoforms, termed VEGF121, VEGF165 and VEGF189, of which VEGF165 is the most commonly studied. VEGF165 acts through two receptors to promote VEGF-signalling, the tyrosine kinase VEGF receptor 2 (VEGFR2) and the non-catalytic neuropilin 1 (NRP1). Although the short cytoplasmic domain (NCD) of NRP1 lacks kinase activity, *in vivo* studies from our group have recently demonstrated that this domain is required for VEGF165-induced hyperpermeability, even though it is dispensable for angiogenesis. Here, we investigate the role of the NCD in VEGF165-induced vascular hyperpermeability. Specifically, we have used *in vitro* models to study VEGF signalling pathways that depend on the NCD and to identify NCD binding partners that may be required for NRP1's role in promoting vascular hyperpermeability. We have further determined which of the VEGF isoforms require the NCD to induce permeability, and whether NCD use influences the potency of each isoform in evoking hyperpermeability. These findings will help develop our understanding of how VEGF-induced vascular hyperpermeability is promoted and how it can be mechanistically separated from VEGF-induced angiogenesis.

P.2.1-003
Cooperation of transport and sensing in C4-dicarboxylate signaling by DcuS sensor kinase of *E. coli*

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Bacteria depend on sensors for interaction and communication with their environment. Membrane bound sensor kinases (His kinases) of two-component systems represent the major device for signal perception and transmembrane signaling by bacteria [1]. Typically, the sensor kinases become activated by phosphorylation after binding of the stimuli to extra-cytoplasmic sensor domains. Rotational, scissors- and piston-type conformational changes are supposed to transfer the signal across the membrane.

The DcuS-DcuR two-component system of *E. coli* is a member of the CitA family of sensor kinases and controls expression of genes related to C4-dicarboxylate catabolism. The sensor kinase DcuS contains an extra-cytoplasmic PAS domain for fumarate binding. Transmembrane helices TM1 and TM2 constitute the membrane anchor of DcuS. Fumarate binding at the extra-cytoplasmic PAS domain triggers a long-range piston type movement of TM2 within the membrane whereas the position of TM1 is not affected [2]. The driving force for the shift of TM2 is provided by fumarate binding which causes contraction of the PAS domain [3, 4]. DcuS requires in addition the C4-dicarboxylate transporters DctA or DcuB for function [5]. The transporters form sensor complexes with DcuS and convert DcuS to the C4-dicarboxylate responsive state whereas free DcuS is in the permanent ON state. Details of TM1/TM2 interaction and dynamics during fumarate activation and signal transduction will be shown.

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P.2.1-004
Nicotine facilitates nicotinic acetylcholine receptor targeting to mitochondria but makes them less susceptible to specific ligands

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, which mediate fast synaptic transmission and regulate cell viability and proliferation. Previously we discovered the presence of nAChRs in mitochondria, where they regulate the early stages of mitochondria-driven apoptosis through activation of intramitochondrial kinases. However, the mechanism of nAChR functioning in and targeting to mitochondria is still unknown.

Nicotine has been shown to enhance maturation of nAChRs on the way of biosynthesis. We studied the content, state of glycosylation and functioning of nAChRs in the liver mitochondria of mice, which consumed nicotine with the drinking water during 7 days. The level of nAChR subunits in mitochondria *vs* non-mitochondria fractions and cytochrome c release from live mitochondria under the effect of Ca2+ were studied by Sandwich-ELISAs, and the nAChR-attached carbohydrate residues were identified by lectin-ELISA.

It was found that nicotine consumption stimulated targeting of nAChRs to mitochondria: the ratio of mitochondrial *vs* non-mitochondrial nAChRs enhanced from 0.78 ± 0.06 to 1.09 ± 0.08. Nicotine facilitated glycosylation of liver nAChRs: the non-mitochondrial a7 nAChR subunits contained more sialic acid, while mitochondrial a7 nAChRs were extra fucosylated compared to corresponding nAChRs of control mice. Finally, mitochondria of nicotine-consuming mice released more cytochrome c in response to 0.05–0.1 μM Ca2+ and were less sensitive to protective effects of a7 nAChR agonist PNU282987 and positive allosteric modulator PNU120956.

It is concluded that nicotine-induced extra-glycosylation facilitates the nAChR targeting to mitochondria but makes the nAChR molecules less susceptible to the binding or effects of specific ligands.

P.2.1-005
Glutamate transport systems in the spinal cord: new mechanistic targets for pharmacological modulation of excitatory signalling

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High-affinity glutamate transporters, GluTs (GLAST / EAAT1, GLT1 / EAAT2, EAAC1 / EAAT3, and EAAT4), as well as glutamate/cystine exchanger, xCT, are differentially expressed in sensory neurons, dorsal root ganglia (DRG) and surrounding glial cells in the spinal cord. Several pharmacological agents, believed to affect GluTs, including therapeutically promising new compounds, have been studied in co-cultures of DRG neurons and spinal glial cells. In such *in vitro* model system, that partially

recapitulates primary pain signaling path, both glial and neuronal GluTs and xCT undergo expressional changes, as well as post-translational modifications. Thus, for the first time, altered expression of a rare splice variant, GLT1c, has been demonstrated, both in rat and human spinal astroglia. Direct signaling through GluTs, a phenomenon recently reported by us, was also found to be involved in the modulation of pain signaling. Thus, physiological doses of some pro-nociceptive agents (e.g. capsaicin, cytokines) activate pro-apoptosis proteases, caspases, that precisely cleave spinal GluTs at their cytoplasmic C-terminal domains, but do not cause cell death. Both truncated C-terminal domains and bioactive peptides produced by the caspase-dependent cleavage functionally interact with other cytoplasmic or nuclear signaling complexes participating in aberrant pain signaling. For example, soluble C-terminal fragments of EAAT4 interfere with protein translation machinery via phosphorylation of PHAS1, and thus modulate the quantity of active GluT molecules in DRG neurons. In case of GLAST, C-terminus functionally interacts with the modulatory FXYD2/ gamma-subunit of Na+, K+ ATPase in spinal astrocytes and thus provides its targeting to the cell surface, while protelolytic cleavage reverses this process. The elucidated bioactive agents and regulatory pathways affecting glutamate signaling in the spinal cord can thus emerge as prospective drug prototypes/therapeutic targets.

P.2.1-006
Phloretin affects oligomerization membrane activity of fragment 25–35 of β-amyloid peptide through dissolving ordered lipid domains

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Amyloid beta oligomers are the predominant toxic species in the pathology of Alzheimer's disease. It is believed the prevailing mechanism for toxicity by amyloid oligomers includes ionic homeostasis destabilization in neuronal cells by forming ion channels (Arispe et al., PNAS, 1993). These selective, voltage-dependent, ion-permeable channel structures have been frequently studied using the model lipid membrane. We used planar lipid bilayer formed by monolayer-opposition technique and liposomes prepared by extrusing or the electroformation methods. Our results indicate that the channel forming activity of fragments 25–35 of beta-amyloid peptide signicant by increase at the addition of phloretin to membrane bathing solution. The results obtained by electron microscopy have demonstrated that the negatively charged dipole modifier interacts with positively charged fragments 25–35 of beta-amyloid peptide and influences on peptide oligomerization. We found that time course of beta-amyloid induced leakage of calcein from liposomes is characterized by two components: the fast one is related to sorption of peptide on the membrane and the slow one is related to the oligomerization of the peptides on the lipid bilayer surface. The introduction of the phloretin simultaneously with beta-amyloid peptide into the suspension of liposomes leads to significant reduction in times characterizing fast and slow components. We also demonstrated that the introduction of fragment 25–35 of beta-amyloid peptide to the suspension of liposomes caused amplification of phase segregation in the liposome membranes and 90% vesicles contained solid ordered domains. Addition of the phloretin to the liposomes modified by amyloid leads to disrupting the gel domains. We concluded that phloretin compensates the positive charge of the amyloid peptides and leads to the changes in their oligomerization status. The study was supported by RFS (14-14-00565) and SP-69.2015.4.

P.2.1-007
Production of functional Kir1.1b channels in protein-lipid nanodiscs

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Inwardly rectifying (Kir) potassium channels share similar topology with only two transmembrane helices per subunit and a large cytoplasmic C-terminus that tetramerizes into a cage that binds various ligands (e.g. phosphatidylinositol 4,5-bisphosphate, ATP or G-proteins) to regulate channel activity. Kir1.1b is a splice variant of KCNJ1 gene, which forms mitochondrial potassium channel inhibited by ATP (mitoK_{ATP}). Since mitoK_{ATP} resides in the mitochondrial inner membrane, it provides a potential way to regulate mitochondrial membrane potential and ROS production. Studies on mitoK_{ATP} are difficult due to very low amount of protein that could be obtained from mitochondria. We attempted to produce mitoK_{ATP} in lipid-protein membrane nanodiscs. These nanodiscs are build of truncated forms of apolipoprotein (apo) A-I which wrap around a patch of a lipid bilayer to form a disc-like particles, which allow for cotranslational insertion of membrane proteins into native environment. We were able to produce Kir1.1b channels *in vitro* as native tetramers in membrane nanodiscs indicating for proper channel assembly.

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P.2.1-008
Downmodulation of cholinergic neurotransmission by β3-adrenoceptor agonists depends on adenosine release and cyclic AMP activation of the exchange protein Epac in the human and rat urinary bladder

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The therapeutic success of β_3-adrenoceptor agonists, like mirabegron, for managing overactive bladder syndromes has generated a great interest in the discovery of their mechanism of action. Our hypothesis was that adenosine formed from the catabolism of cyclic AMP in the detrusor may act as a retrograde messenger via prejunctional A_1 receptors to explain inhibition of cholinergic activity by β_3-adrenoceptor agonists. Isoprenaline (1 μM) decreased [^{3}H]ACh release from stimulated (10 Hz, 200 pulses) human (-47 ± 5%) and rat (-39 ± 1%) detrusor. Mirabegron (0.1 μM, −57 ± 6%) and CL316,243 (1 μM, −37 ± 7%) mimicked isoprenaline inhibition; their effects were suppressed by blocking β_3-adrenoceptors with L748,337 (30 nM) and SR59230A (100 nM), respectively, in the human and rat detrusor. Mirabegron and isoprenaline increased adenosine release from the detrusor. Blockage of A_1 receptors with 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 100 nM) or of the equilibrative nucleoside transporters with dipyridamole (0.5 μM) prevented mirabegron and isoprenaline inhibition. Cystometry recordings in anaesthetized rats showed that SR59230A, DPCPX and dipyridamole reversed the decrease of the voiding frequency caused by isoprenaline (0.1–1000 nM). The inhibitory effects of mirabegron and isoprenaline on [^{3}H]ACh release were also attenuated ($P < 0.05$) by the selective inhibitor of the exchange protein directly activated by cAMP (Epac), ESI-09 (10 μM), both in human (-20 ± 7%) and rat (-5 ± 4%) detrusor strips,

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respectively, but not by the protein kinase A inhibitor, H-89 (10 μM). Data suggest that inhibition of cholinergic neurotransmission by β_3- adrenoceptors depends on downstream Epac activation both in human and rat urinary bladders. Whether this mechanism impacts on adenosine release via equilibrative nucleoside transporters and prejunctional A_1 receptors activation, remains to be elucidated.

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P.2.1-009
An attempt to isolate mitochondrial membrane protein complexes in membrane nanodiscs

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Interactions between proteins are fundamental for many biological processes. Mitochondrial respiratory complexes I, III and IV form functional supercomplexes in the mitochondrial inner membrane. Previously, we showed by means of blue native electrophoresis that mitochondrial large-conductance, calcium-activated potassium channel (mitoBK_{Ca}) functionally interacts with complex IV. However, little is known about this interaction. Here, we attempt to isolate mitoBK_{Ca} together with surrounding protein(s) into lipid-protein nanodiscs. These nanodiscs are build of engineered apolipoproteins, which wrap around a patch of a lipid bilayer to form a disc-like particles. Incorporation of membrane protein complexes into nanodiscs could stabilize interactions and allow for more detailed analysis of a protein complex.

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P.2.1-010
One receptor, two ligands, different responses: T-cadherin as a receptor for low density lipoprotein and adiponectin

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The glycosylphosphatidylinositol (GPI)-anchored protein T-cadherin (T-cad) is an unusual receptor. Despite lacking transmembrane and cytoplasmic domains, it has an ability to transduce in the same cell different (even opposite) signals upon binding two different ligands: low density lipoprotein (LDL) or high-molecular weight adiponectin. Recent studies have shown the role of T-cad in cardiovascular diseases, but the molecular mechanisms remain unclear. Both of these ligands are large molecular complexes sized about 25 nm. Similar to other GPI-anchored receptors the signal transduction upon ligand binding may be triggered by T-cad clustering. It was shown that clusters of GPI-anchored proteins are smaller than the resolution of light microscopy, thus we used Förster resonance energy transfer (FRET) to detect the proximity between the two molecules. For fluorescence labeling of T-cad, we inserted small peptide tag that was further conjugated with Alexa fluorescent labels on a surface of living cells. We have found that the FRET signal is acceptor density-independent, suggesting the non-random organization of T-cad molecules in the membrane. Our data revealed that LDL induces T-cad homoassociation and recruit Gai and Src-family kinases, which trigger the IP_3-Ca^{2+} signal. We have also demonstrated that the glycosylation at N382 in T-cad molecule is involved in T-cad clustering. N382I mutants lacking the N-linked sugar possessed an increased LDL-induced Ca^{2+}-response. Unlike LDL, the other T-cad ligand, adiponectin, does not induced Ca^{2+} response in HEK293 and smooth muscle cells. Moreover, adiponectin inhibited LDL-induced Ca^{2+} signaling. Thus we have established for the first time the ligand-induced clustering of T-cad and its raft-dependent signaling after LDL binding. We believe that the different effects of the two ligands can be explained by formation of distinct raft platform upon binding these ligands. The work was supported by the RSF project No. 14-24-00086.

P.2.1-011
Membrane potential regulation of C6 cells in culture under chronic electrical stimulation

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Nowadays the work of ion channels is of considerable interest. Voltage-gated ion channel permeability is directly connected with membrane potential. Inducing a change of membrane potential can be accompanied with a change of cell properties: functional and structural. One of these factors affected the value of resting transmembrane potential is external electric field. As a result, regulation of proliferative activity of cells in culture can be possible by application of external electric field. The aim of this study is to investigate changes of C6 cell membrane potential after external electrical field exposure. Stimulation of cells in culture was performed with uniform alternating electric field. The duration of the exposure was 12 h with different electric field strength (from 3 to 20 V/m) at frequency 10 Hz with different number of stimulation pulses in train (1, 3, 5, 7). Present work identifies different effect of electrical stimulation with various number of stimulation pulses in train. Depolarization of cells plasma membrane was observed after exposing them to 1 stimulation pulse in train. Cells stimulation with 3 pulses in train did not lead to any statistical significant change of membrane potential relative to control sample. But increasing of the resulting train duration to 10 ms (5 pulses) leads to hyperpolarization of plasma membrane. Also we had found different effect of electrical stimulation at several of field strength with 1 pulse in train. With higher electric field strength the plasma membrane is hyperpolarized. Under smallest field strength the plasma membrane is depolarized. Obtained results can be explained by changes in ion channels densities. Particularly, depolarizing effect may be caused by sodium channels, whereas hyperpolarizing effect – by potassium channel. Present study confirms the possibility of membrane potential regulation by external electric field. The observed effect depends on the parameters of electrical stimulation.

P.2.1-012
The role of ion channels and second messengers in the 1-34 parathyroid hormone fragment cardiac effects

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The ionized calcium, metabolism of which is regulated by parathyroid hormone (PTH), is a main trigger of cardiac pacemaker activity and electromechanical coupling of contraction. Previously we have shown the modulator-protective cardiac

effects of the 1-34 active fragment of PTH (1-34 PTH) in the patients with congestive heart failure. However, the molecular mechanisms of its action on the pacemaker and contractile heart functions remain unclear. The aim of the current study was the determination of the role of certain ion channels and second messengers in the 1-34 PTH cardiac effects using pharmacological analysis. The isolated frog heart functional activity was monitored by the photoelectrical method based on computer registration of the reflected laser beam from the contracting heart. The 1-34 PTH in a dose of 10^{-10} M was found to exert positive cardiac chronotropic and stabilizing inotropic effects, whereas in the control experiments the amplitude and rate of cardiac contractions were gradually decreasing. The specific inhibition of L-calcium channels by verapamil (10^{-5} M) resulted in significant decrease of the heart rate and abolition of hormone's effect. The 1-34 PTH stabilizing inotropic effect on heart was found dependent on cAMP since hormone's separate and theophylline (10^{-4} M) combined action was accompanied by equally high contraction amplitudes. In combination with the potassium channel blocker, aminopyridine (10^{-3} M) or the Na-K pump inhibitor, ouabain (10^{-3} M), 1-34 PTH induced the negative shift in the frequency but not in the amplitude of heart contraction. In conclusion, it can be suggested that the calcium channels can participate in the realization of 1-34 PTH effect on the cardiac pacemaker activity without excluding the possible involvement of sodium and potassium ions in the provision of the mentioned process. The inotropic action of 1-34 PTH is apparently accomplished by the cAMP mediated mechanism.

P.2.1-013
Trimers of dimers of SRII/HtrII full complex. Small angle scattering structural investigation

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Sensory rhodopsin II (SRII) from *Natronobacterium pharaonis* is a photoactive seven-helix membrane protein responsible for negative phototaxis. Exposion of light on SRII in complex with its transducer (HtrII) produces a cytoplasmic signal cascade by a transducer TM2 helix rotation. According to the structure of SRII in complex with a shortened transducer Htr_{114} (residues 1–114) (1H2S) the complex functions in dimeric form.

In this work, we investigate cluster formation of full complex SRII/HtrII. The protein complex was expressed in Escherichia coli strain Rosetta2 (DE3) and purified using standard methods. Solubilized in DDM complex SRII/HtrII was reconstituted to nanodiscs prepared from lipid DMPC and membrane scaffold protein MSP1E3D1.

We applied small angle X-ray (SAXS) and neutron (SANS) scattering experiments to obtain information about structure of SRII/HtrII complex reconstituted to nanodiscs. For the measurements, solutions of SRII/HtrII reconstituted to nanodiscs in series of sucrose concentrations and in series of H20/D20 ratios were prepared. The SAXS measurements were performed at the beamline BM29 (ESRF, Grenoble, France) and the SANS measurements were performed at the YuMO spectrometer (IBR-2, Dubna, Russia).

For SAXS and SANS curves, scattering invariants (radii of gyration, intensities at zero angle, Porod volumes) and pair distribution functions were obtained. In cases of SAXS and SANS, average scattering densities, partial volumes and total scattering lengths of complex in nanodiscs were calculated. Using DAMMIF program of ATSAS package *ab-initio* structures of SRII/HtrII reconstituted to nanodiscs were obtained.

It was shown that complex of sensory rhodopsin II with its transducer reconstituted to membrane mimicking system such as nanodiscs forms trimers of dimers. The possible role of forming trimers of dimers of the complex to negative phototaxis signaling is discussed.

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P.2.1-014
In vitro potency assays for the development of new Botulinum neurotoxin therapeutics

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Botulinum neurotoxin (BoNT) is a major therapeutic agent licensed in neurological indications such as dystonia and spasticity. Four clinical products are available in the Western hemisphere and of those, three are BoNT type A preparations and one is a BoNT type B preparation. Five other BoNT variants exist in nature, termed BoNT/C to BoNT/G, whose therapeutic potential has not been extensively exploited to date. In this study we assessed the *in vitro* potency of recombinant BoNT/A to BoNT/F using primary cultures of spinal cord (SCN), dorsal root ganglia (DRG) and cortical (ctx) neurons, all prepared from rat. All toxins were highly potent in all three models exhibiting potencies from the nM to the pM range. In all assays BoNT/A1 was the most potent toxin with pEC_{50} (log M) as follows: 11.80 ± 0.05 (n = 5), 11.01 ± 0.23 (n = 4) and 12.57 ± 0.08 (n = 7) (n numbers refer to experiments run in triplicate) for SCN, DRG and ctx systems, respectively. BoNT/B1 was by more than an order of magnitude less potent as compared to BoNT/A, with pEC_{50} (log M) as follows: 10.65 ± 0.23 (n = 3), 8.50 ± 0.11 (n = 4) and 10.82 ± 0.08 (n = 8) for SCN, DRG and ctx systems, respectively. In all three assays BoNT/C and BoNT/D were similar in potency to BoNT/A whereas BoNT/F, and to a lesser degree BoNT/E, were more similar to BoNT/B. The Pearson correlation values for toxin potency between the assays were r = 0.69 between SCN and DRG and r = 0.78 between SCN and ctx. Ctx was the most sensitive system to BoNTs and DRG was the least sensitive system. In summary, the three different cellular systems were broadly in agreement with each other. *In vitro* potency assays are a useful tool to benchmark the potency of BoNTs and can be used in the development of novel BoNT-based therapeutics.

P.2.1-015
Novel rhodopsin from octopus: from features to the development of production system in HEK293-G7 cells

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The retinal binding protein from octopus retina, octopus rhodopsin (OctR), is promising material for biomolecular photonic applications due to its unique properties. OctR is bistable and as a result – photoreversible. The extremely high ordering of octopus visual membranes, as well as its ability to capture single photons and sensitivity to light polarization suggests the possible use

of rhodopsins as a prototype for the photonic qubit detectors. At the same time heterologous expression of visual rhodopsins (Rho) remains a challenging task due to the difficulties of the producing in functional form in the majority of expression systems. The successful expression of recombinant invertebrate visual pigment (Gq coupled Rho) was developed in HEK293 cells for honeybee, white butterfly, jumping spider.

We cloned the novel full length *octR* gene from cDNA of *Octopus vulgaris* retina [Zhgun A.A., et.al., 2015]. After the alignment and topology imposition with structure of cephalopoda Rho from *T. pacificus* [PDB ID: 2Z73; Murakami M., et.al., 2008] we designed the model of OctR topology. According this model we proposed a number of OctR variants (full length opsin, C-end fusion with eGFP, delta 390-455 mutant less cluster with polyproline-rich repeats, with 6xHis- and c-Myc epitope tags from C-end and combinations thereof) and generated a set of constructs, based on pTRE-Tight vector (Clontech, United States) for expression in HEK293 cells under doxycycline-regulated promoter. We used HEK293-G7 cell line with constitutive expression of trans-activator protein tTA for promotor pTRE (Tet-Off system) [Shubin A.V., et.al., Applied Biochemistry and Microbiology, 2013. 49(9): p. 750–755]. The preliminary results of transient expression allow us to assume the proper folding of the OctR variants in HEK293-G7 cells. The data of the developing cell lines with OctR variants and regeneration with 11-cis retinal will be reported.

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P.2.1-016
Novel transmembrane protein c-Answer revealed by bioinformatic screening of genes present only in well regenerating animals

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The genetic basis of higher regenerative capacity of fishes, amphibians and reptiles, comparing to birds and mammals, is still poorly understood. Usually, it is thought that this is a result of restructuring of the corresponding regulatory network, which consists of approximately the same set of genes. We hypothesized that another cause might be a loss of some genes which are essential for regeneration. We propose a bioinformatic approach for systematic search of such genes. Our method detects genes with local synteny disruption and, vice versa, appearance of genes with specific local synteny. It examines the co-localization of homologous genes and counts the number of their copies. The method provides for flexible definition of detecting conditions and different forms of local synteny. Our algorithm outputs rather short gene lists, and quite similar lists for a wide range of parameters. Thus, we identified several genes that present only in fishes, amphibians and reptiles and revealed the genes, which demonstrated an increased expression during regeneration of tails and hindlimb buds in the model organisms, the *Xenopus laevis* tadpoles. We found out that one of these genes encodes a membrane protein, which is strongly up-regulated already at the 1st day of regeneration predominantly in the wound epithelium. As we demonstrated, this gene regulates the body appendages regeneration and also the telencephalic and eye development. We named the protein encoded by the revealed gene *c-Answer*, after cold-blooded Animals specific wound epithelium receptor-like protein. We suppose that the loss of *c-Answer* although resulted in a decrease of the regenerative capacity in birds and mammals, could be fixed by natural selection because the loss of this gene might provide new opportunities for the forebrain evolution. On the whole, local rearrangement of gene synteny is a likely driving force in many aspects of forebrain and species evolutions.

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P.2.1-017
Fluorescently labeled isopentenyladenine is a new tool for cytokinin receptor domain mapping

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Signaling of cytokinins, important plant hormones, is initiated through their perception by histidine-containing kinases (HKs). Recently, GFP-fused Arabidopsis HKs were found to be massively localized to the membrane of endoplasmic reticulum, however, their previously believed localization and function in plasma membrane has still not been disproved. Cytokinins structurally based on C6-substituted purine belong to a class of plant hormones that play important roles in many aspects of plant growth and development. To gain better insight into the dynamics of cytokinin receptor localization within the cell we developed series of cytokinin fluorescent probes. To this end, isoprenoid cytokinin N6-isopentenyladenine (iP) was accompanied with selected spacers in C2, C8 and N9 position of the adenine moiety and fluorescently labeled with nitrobenzoxadiazole (NBD) fluorescent label. The ligand properties of iP-derived probes were first assessed *in vitro* with Arabidopsis cytokinin receptors (AHK3 and AHK4) in a bacterial receptor test where the competition of a cytokinin fluoroprobe with radiolabeled tZ was measured. Although the structural changes within the fluorescent probes led mostly to significant loss of the biological activity, some probes with N9 substitution were still able to interact with the receptor binding site as revealed by our ligand binding studies. Thus, NBD-labeled iP derivatives seem like a promising tool in rapid staining procedures for visualization of the cytokinin receptor pool inside the cell. *In planta* experiments revealed that these compounds were transported to the cell cytosol and the signal was associated with several subcellular structures, most importantly with the endoplasmic reticulum, which is probably the intracellular site for hormonal cross-talks between cytokinin and other plant hormones.

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P.2.1-018
The effect of high cholesterol diet on scavenger receptor expression and fatty acid profile in cardiac tissue

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Hypercholesterolemia plays an important role in the progression of cardiovascular diseases (CVD) which is the major cause of death worldwide with highest mortality and morbidity rates. Excessive cardiac remodeling conditions result in various cell

death mechanisms that cause the loss of function in cardiomyocytes followed by heart failure. Scavenger receptors (SRs) are classified in 10 families (Classes A-J) of membrane-bound receptors which are bind and internalize modified low-density lipoprotein (oxidized, acylated and carbamylated). Modified LDL stimulated activation of intracellular signaling pathways might leads an increase in lipid accumulation, apoptosis, inflammation, cell adhesion and migration.

The aim of our work; is to investigate if high cholesterol diet mediated oxidative stress affects SR expression, fatty acids profile and various transcription factors that might be related with heart failure. In this purpose we have measured MDA levels in heart tissue by LC-MS/MS, while fatty acid profiles were determined by GC-MS. Also, mRNA expressions of well-identified SRs (SCARA3, SRB1, CD36, CD68, LOX1, SRF1, SRI, SRG) and following transcription factors (LXR, NRF2, PPAR, SREBP, ABCA1) were measured by qPCR in addition to the protein levels of transcription factors (SREBP1c, SREBP2, LXR, PPAR-γ) that regulate modified lipid-scavenger receptor signaling pathways in cardiac tissue evaluated by western blotting. The results will be discussed.

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P.2.1-019
Influence of glucose concentration on Escherichia coli FOF1-ATPase and hydrogenase 4 (hyf) enzymes activities

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Escherichia coli produces molecular hydrogen (H_2) during fermentation of different carbon sources (glucose, glycerol): four hydrogenase (Hyd) enzymes are involved in this process. An interaction of Hyds with the FoF_1-ATPase and potassium transporter TrkA was suggested. It has been shown that Hyd-4 (*hyf*) is active at pH 7.5 and sensitive to the FoF_1-ATPase inhibitor *N, N'*-dicyclohexylcarbodiimide (DCCD). Moreover, H_2 producing activity of Hyd-4 and its dependence on glucose concentration at pH 7.5 was proposed.

In the present study ATPase activity and its relationship with Hyd 4 was investigated upon utilization of different concentrations of glucose by *E. coli* BW25113 wild type parental strain (PS) and JRG3621 (*hyfB-R*) mutant (MS).DCCD-sensitive ATPase activity of PS during 0.2% glucose fermentation was stimulated $\sim$1.4 fold by addition of 100 mM K^+ at pH 7.5. The ATPase activity of MS was lowered 1.5-fold ($P \leq 0.025$) compared to PS in K^+-free medium, and slightly increased by K^+. DCCD inhibited ATPase activity of both PS and MS was $\sim$5-fold ($P < 0.02$) and $\sim$7-fold ($P < 0.01$), respectively. In contrast to 0.2% glucose, DCCD-sensitive ATPase activity of PS was $\sim$1.2-fold higher upon growth on 0.8% glucose in K^+-free medium. PS DCCD-sensitive ATPase activity was lowered in K^+-containing medium compared to K^+-free medium. Whereas, at pH 6.5, both PS and MS demonstrated lower ATPase activity in K^+-free and K^+-containing media during 0.2% glucose fermentation. K^+ has increased the ATPase activity of PS only. In comparison to PS, the ATPase activity of MS was stimulated in the presence of K^+ in 0.8% glucose fermenting cells.The results support the idea that K^+ increases ATPase activity of PS only at glucose limited conditions at both pH 7.5 and pH 6.5. Probably, interaction between the FoF_1-ATPase and Hyd-4 depends on glucose concentration.

P.2.1-020
Redox modification of Na,K-ATPase changes its receptor function under hypoxia

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Na,K-ATPase maintains the homeostasis of Na+ and K+ in animal cells and it is the receptor for cardiotonic steroids, in particular, ouabain. One of the main ways of signal transmission upon binding of ouabain is the activation of Src kinase. Under hypoxia and ischemia, the activity of Na,K-ATPase is decreased due to glutathionylation of regulatory cysteine residues, but it remains unknown how the receptor function of the enzyme changes at low oxygen level. In this study we have estimated the effect of hypoxia on Na,K-ATPase receptor function using SC-1 murine fibroblast cell line. We have shown that under conditions of hypoxia and ischemia the cytotoxic effect of ouabain on cells decreases. At 20% of oxygen, ouabain causes dose-dependent growth of ROS. Under conditions of acute hypoxia, the level of ROS is also increased. However, we revealed that at the presence of ouabain under hypoxia, the production of ROS induced by hypoxia is reduced. The similar effect was observed for Src kinase activation. At 20% of oxygen, ouabain treatment of the cells leads to Src kinase activation. Under conditions of acute hypoxia, Src kinase is also activated. At the same time, we found the addition of ouabain under hypoxia results in decrease of Src activating phosphorylation. We obtained that, possibly due to the growth of ROS, ouabain treatment, like incubation under hypoxia, causes the increase in glutathionylation of the Na,K-ATPase. According to our modeling data, glutathionylation of Cys 458-459 will disturb the interaction of Na,K-ATPase and kinase domain of Src which could result in Src kinase activation. So, Na,K-ATPase glutathionylation may be a reason for Src kinase activation at hypoxia. Thus, our results indicate that under hypoxia the receptor function of Na,K-ATPase changes, cells response to cardiotonic steroids in a different way, which should be taken into account in clinical practice. The study was supported by Russian Science Foundation (grant #14-14-01152).

P.2.1-021
Variety of functional complexes of calcium-transporting and calcium-regulated channels in mammalian cells

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Stretch-activated calcium-permeable channels (SACs), whose activity could be evoked by plasma membrane deformation are the most widespread type of mechanosensitive channels in eukaryotic cells. Calcium influx via SACs is implicated into different membrane and intracellular signaling processes. Identification of calcium-dependent subcellular targets, whose activity is controlled by localized calcium entry due to SAC activation is a valuable tool to search for new signaling pathways involved in cellular mechanotransduction. Here, with the use of single channel patch clamp technique we report that in mammalian cells of different origin stretch-induced calcium entry could control various calcium-dependent molecules, particularly calcium-activated potassium (KCa) channels of small (SK), intermediate (IK) and big (BK) conductance. In the same time, biophysical properties of SAC currents are similar in all cell lines tested and correspond to Piezo channels recorded under same ionic conditions. In

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3T3B-SV40 transformed mouse fibroblasts and MCF-7 human breast carcinoma cells SAC activation leads to stimulation of SK currents of 9-11 pS single channel conductance. In human myeloid leukemia K562 cells SAC induced IK channel activation with conductances of 30–40 pS. Importantly, SAC channels in in mesenchymal stem cells are functionally coupled with BK channels (80–100 pS) but not with SK channels whose activity could be recorded in the same patch. In sum, we found that native SACs can act as activators of different types of KCa channels. Our results clearly demonstrate single channel interactions indicating that local changes of calcium concentration due to SAC activity could control status of various calcium-dependent molecules in living cells.

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P.2.1-022
Proteolytic activation of amiloride-insensitive ENaC channels in human leukemia cells

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Na^+ concentration is tightly regulated in cells, extracellular fluid and blood. Non-voltage-gated sodium channels were identified as one of the main Na^+-transporting pathways in cells of blood origin, including leukemia-lymphoma cell lines. Functional expression of amiloride-insensitive ENaC channels in human leukemia K562 cells was demonstrated using RT-PCR, immunofluorescent staining and electrophysiological assay. To date, all known regulatory pathways of ENaC-like channels in leukemia cells are directly coupled with actin cytoskeleton rearrangements. Physiologycal mechanisms modulating Na^+ permeability in blood cells remain to be elucidated. Proteolytic cleavage in extracellular domain was reported to be critical for canonical ENaC function in renal epithelia. Here, we tested the effect of serine protease trypsin on membrane currents in K562 cells which provide a unique model to analyze single channel behaviour in whole-cell patch-clamp experiments. We found that Na^+ channel activity drastically increased in response to extracellular application of trypsin (5 μg/ml). An involvement of proteolytic activity of trypsin in channel opening was confirmed in whole-cell experiments with soybean trypsin inhibitor (SBTI): trypsin in the presence of SBTI failed to have a stimulatory effect on ENaC-like channels in K562 cells. Biophysical properties of Na^+ channels activated by proteolytic cleavage or by actin disruptor cytochalasin D were practically identical; unitary conductance was 15 pS. Importantly, stabilization of F-actin with phalloidin did not prevent trypsin-induced channel activation. Single channel analysis clearly demonstrates that trypsin-induced currents did not blocked by amiloride and its derivative benzamil, known inhibitors of renal ENaC. Our observations imply common extracellular regulatory mechanism for canonical ENaCs and amiloride-insensitive ENaC-like channels in leukemia cells. This work was supported by RFBR 15-04-02950, 16-04-00467.

P.2.1-023
Novel calmodulin and S100A1 binding site in distal TRPM4 N-terminus

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Transient receptor potential (TRP) channels are calcium-permeable cation channels that are involved in a wide variety of physiological processes, e.g. thermosensation, mechanosensation, taste and vision. The membrane topology of TRP channels indicates six transmembrane domains flanked by long N- and C-termini that contain binding sites for ligands. TRPM4 is a member of TRP family, which plays a key role in calcium-activated signalling cascades involved in the cardiac conduction, immunity response or insulin secretion. Several conserved TRPM4 ligand binding domains modulating permeability of TRPs, which are typically present at the intracellular N- and C-termini, have been identified. The most common TRPM4 channel modulators include calcium binding proteins (CBP) calmodulin (CaM), S100A1; ATP and phosphatidylinositol phosphates. Here, we present two novel binding sites for CaM and S100A1 localized in distal part of TRPM4 N-terminus. Identification of ligand binding sites in TRPM4 and determination of the amino acid sequence contributing to protein-protein interactions were performed using fluorescence methods. Data obtained from fluorescence experiments led to the conclusion that the TRPM4 binding domains for calmodulin and S100A1 are overlapped. These results are also supported by De Novo molecular models of the complexes conforming that the interactions are formed by positively charged (K271, R273, R274) and hydrophobic (L263, V270, L276) residues of TRPM4. Taking together, our data provides a new potential mechanism for TRPM4 regulation. Further insights into TRPs modulation may allow the treatment of human diseases associated with TRP channel regulation disorders. Supported by grants GACR 17-04236S and GACR P-304/12/G069.

P.2.1-024
The human facilitative glucose transporter GLUT12: expression and regulation in Caco-2 and 3T3-L1cells

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The human facilitative glucose transporter GLUT12 (SLC2A12) was isolated from the breast cancer cell line MCF-7 by its homology with GLUT4. GLUT12 is expressed in human crude membranes of adipose tissue, small intestine and skeletal muscle, but its function is still unknown. We have previously demonstrated in *X. laevis* oocytes expression system, that hGLUT12 can transport α-methyl-glucoside (αMG), a specific SGLT substrate, and that this transport is enhanced by Na^+. GLUT12 substrate selectivity is: D-glucose>αMG>2-deoxy-glucose>D-galactose>D-fructose. The aim of the present work was to investigate the expression and regulation of GLUT12 in Caco-2 cells, a human model of enterocytes, and in 3T3-L1 cells, a mouse model of

adipocytes. We performed αMG-radiolabeled uptake measurements and Western blot analysis. We also studied GLUT12 expression by immunohistochemical methods in human and murine tissue samples. In small intestine, GLUT12 is located in the perinuclear region and the apical cytoplasm, below the brush border membrane (BBM), of human duodenal enterocytes. It is also expressed in the apical membrane of Caco-2 cells. Insulin and TNF-α increase αMG uptake by stimulation of translocation of GLUT12 to the BBM. Activation of PKC and AMPK, and inhibition of ERK also up-regulates αMG uptake and GLUT12 expression in the apical membrane. All the sugars substrates induce translocation of GLUT12 to the BBM. Interestingly, sorbitol increases αMG uptake without modifying the transporter expression in the membrane. GLUT12 is also found in the perinuclear region of mouse adipocytes and expressed in protein homogenate of 3T3-L1 cells. In these cells, insulin and TNF-α increase αMG uptake, whereas leptin and adiponectin decrease it. As in Caco-2 cells, activation of AMPK and inhibition of ERK increase αMG uptake. These data demonstrate the functional activity of GLUT12 in small intestine and adipose tissue, opening new perspectives to investigate its role in the organism.

P.2.1-025
Sterol-binding protein Ysp2p contributes to the resistance of Saccharomyces cerevisiae cells to various stresses

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Ysp2p belongs to the evolutionarily diverse family of endoplasmic reticulum (ER) membrane proteins with StART-like lipid transfer domains called Lams. Ysp2p and its homologs Ysp1p, Sip3p and Lam4p are localized at ER-plasma membrane (PM) contact sites distinct from those occupied by the known ER-PM tethers. There is some evidence that Ysp2p, Ysp1p and Sip3p transport ergosterol from PM to ER.

Here we showed that Ysp2p is necessary for the adaptation of *S. cerevisiae* cells to the various stresses. The deletion of *Δysp2* leads to increased sensitivity to hyperosmotic stress, heat shock, drying and freezing. *Δysp2* is more sensitive to the antimycotic miconazole and has no pronounced sensitivity to other antimycotics, such as clotrimazole, fluconazole or ketoconazole. Overexpression of *YSP2*, on the contrary, leads to an increase in the cell resistance to hyperosmotic stress and miconazole. We studied the combined effect for ergosterol biosynthesis genes deletion (*Δerg2-6*) with the deletion of *Δysp2* or its overexpression and found genetic interaction between them in terms of hyperosmotic stress and amiodarone resistance (Ysp2p was initially selected as providing resistance to ion channels blocker - drug amiodarone). Since PM distribution of ergosterol is uneven, we investigated the possibility of Ysp2p colocalization with the lipid rafts proteins Pma1p, Can1p and Pdr5p and did not find any.

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P.2.1-026
Short distance cell-to-cell communication in response to wound stress in Arabidopsis root

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Plants during their entire lifetime are opposed to various threats resulting in tissue damage, such as physical wounding, herbivore feeding, or crushing by animals. During attack plants adapt to stresses by recognizing biotic, abiotic and physical factors and adequately quickly respond to it, by orchestrating specific signaling pathways. However, the mechanisms by which these signals are perceived by cells and how the signal is further transmitted from one cell to another for local and systemic signaling is still largely unknown. In the aerial tissues, plants evolved long distance communication system, from leaf-to-leaf as response to wound signaling, which lead to the distal production of jasmonates mediated by electro potential changes (Mousavi *et al.,* 2013). In our work we focused on short cell-to-cell signal transmission upon nematodes invasion and single cell laser ablation (mechanical wound) in the root of *Arabidopsis thaliana*. We demonstrate that physical wounding caused by single cell laser ablation, which mimics nematode behavior during feeding, elicit surface potentials changes depending on ion channels and ROS production. These changes turn on the local production of ethylene as a potent regulator of wound responses and deterrence of nematode feeding. Our observations provide insights into the distinct mechanisms of short-distance cell-to-cell wound signaling in roots, allowing cells to rapidly spread information among neighbors in response to local stressors physical wounding caused by single cell laser ablation and nematode feeding elicit surface potentials changes depending on ion channels and ROS production.

Reference

Mousavi SA *et al*., (2013). Nature. 500(7463):422–6.

P.2.1-027
Dual action of the Gαq-PLCβ-PI(4,5)P2 pathway on TRPC1/4 and TRPC1/5 heterotetramers

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The transient receptor potential canonical (TRPC) 1 channel is widely distributed in mammalian cells and is involved in many physiological functions. TRPC1 is primarily considered a regulatory subunit that forms heterotetrameric TRPC1/4 and TRPC1/5 channels to modify the pore properties of TRPC4 and TRPC5 and their activation by $G\alpha_q$-coupled receptors. Here, we reveal that the self-limiting regulation of the heterotetramers by the $G\alpha_q$-PLCβ pathway is dynamically mediated by $PI(4,5)P_2$. We recorded channel activity and plasma membrane $PI(4,5)P_2$ when manipulating $G\alpha_q$ activity and $PI(4,5)P_2$ levels to conclude that, following G-protein coupled receptor (GPCR) activation, $G\alpha_q$ directly binds to TRPC1/4 and TRPC1/5 channels, resulting in channel gating. Simultaneously, $G\alpha_q$-coupled PLCβ activation results in the breakdown of $PI(4,5)P_2$. Dissociation of $PI(4,5)P_2$ from the channels inhibits the activated currents. The subsequent increase in cytoplasmic Ca^{2+} due to Ca^{2+} release from the ER and activation of PKC resulted in a second phase of channel inhibition.

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P.2.1-028
Regulation of TRPC4, TRPC5 homotetrameric and TRPC1/4, C1/5 heterotetrameric channel activity by PI(4,5)P2 hydrolysis

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Transient receptor potential canonical (TRPC)4 and TRPC5 are subfamily of TRPC channels, known to be modulated by Gq-PLC pathway. Since phosphatidylinositide 4,5-biphosphate (PI(4,5)P_2) maintains TRPC4 and TRPC5 channels, Gq-PLC pathway inhibits channel activities through the hydrolysis of PI(4,5)P_2. The effects of PI(4,5)P2 on TRPC4, TRPC5 homotetrameric and TRPC1/4, TRPC1/5 heterotetrameric channels were not known. Thus we investigated the difference in PI(4,5)P_2 sensitivity not only between the channel types but also between homomers and heteromers. First, by using a voltage-sensing phosphatase(DrVSP), we show that PI(4,5)P_2 dephosphorylation robustly inhibited not only TRPC4α, C4β, C5 homotetramer current but also TRPC1/4α, C1/C4β, C1/C5 heterotetramer current which were induced by Englerin A(-) (EA). Secondly, we used step pulse to observe the sensitivity of PI(4,5)P_2 dephosphorylation. Applying depolarizing step pulses decreased currents by activates DrVSP when channels and VSP were co-expressed. The sensitivity to PI(4,5)P_2 dephosphorylation of homotetramer followed by TRPC5 > TRPC4α > TRPC4β. Forming heterotetramers with TRPC1 also ranged same as homotetramers, but interestingly the sensitivity curve converged and showed only mimic difference between the channels. Thirdly, neutralization of basic residues, K491, H501 in cytoplasmic domain between TM4-5, K518 in TM5 and K636 in TRP box of TRPC4 channel showed reduced FRET with pleckstrin homology (PH) domain, which binds PI(4,5)P_2. And these sites show two kinetically distinct effects independently; regulate voltage sensitivity that affects PI(4,5)P_2 affinity to channel and change in current amplitude which cause to be nonfunctional or dysfunctional. In conclusion, our results indicate a fundamental role for PI(4,5)P_2 in regulating TRPC1/4 and C1/5 heterotetramer activity as well as TRPC4, C5 homotetramer.

P.2.1-029
Heterologous expression of bacteriorhodopsin from *Halobium salinarum*

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Bacteriorhodopsin from *H. salinarum* (HsR) is a 26 kDa light-activated integral membrane protein that can transports proton across the cell membrane of the archaea. For decades, HsR has been in focus of numerous basic and applied research and became a general model for membrane protein expression, folding, crystallization, and X-ray crystallography. HsR is abundant in the natural source and the wild-type protein can be easily obtained. Producing the much required HsR mutants using the natural host is however laborious, time, and resource consuming. A number of attempts to express HsR in *E. coli* have been reported but non resulted in a high-level of a functional protein. We have analyzed the predicted structure of HsR mRNA and found a putative stem structure near the ribosome binding site. Two silent mutations in the region, C9A and G12A, allowed a 28% reduction of the stem stability. To optimize a heterologous translation further and compensate for the *H. salinarum* codon bias we utilized pSCodon expression vector providing low abundant in *E. coli* tRNAs. To favor lipid membrane integration and correct protein folding we induced HsR expression at 18 °C. This approach allowed us to purify under non-denaturing conditions few milligrams of wild type HsR as well as V49A, D85N, and D96N mutants from a liter of culture. The high crystallographic quality of 3D crystals obtained from the purified proteins confirms that the produced in *E. coli* HsR closely resembles the native protein from the natural source and that the presented approach for expression of HsR and its mutants is suitable for all scientific research and industrial applications.

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P.2.1-030
Microbial rhodopsin from radioresistant Deinococus Thermus discovers unusual properties

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Bioinformatics search has shown that exist about 7000 unstudied microbial rhodopsins (Ushakov et al., FEBS Journal, V.283 Suppl.1, Sept.2016). They are interesting not only for molecular mechanisms of ion transport which is of fundamental interest, but as a potential optogenetics tools.

Gene encoding one of them belongs to the *Deinococus Thermus*. This microorganism is highly resistant to ionizing radiation (150 kGy or 1.5 Mrads, more than 100-fold as compared to *E. coli*), to temperature drops and to desiccation. These give us reasons to expect new and interesting properties of the *Deinococus Thermus* rhodopsin (further mentioned as VR1). It differs from the bacteriorhodopsin (BR) of *H. salinarum* in several amino acids (numbering according to the nomenclature of BR): D96A and D216P. These replacements must cause big differences in ion selectivity and/or conductivity. Pro in amino acid chain usually makes it rigid, side chain small volume compared to Asp may led to more volume in a cavity. At the position 96 are the similar changes: a short hydrophobic side chain of Ala instead of a long charged side chain of Asp. So, two big negatively charged Asp are replaced by two small uncharged. These may lead to better dissociation of a transported ion.

The DNA optimized for overexpression of VR1 with His-tag was inserted into pET39 plasmid. VR1 expression in *E. coli* induced by IPTG and autoinduction was optimized. The best outcome of folded protein in membrane fraction appears at 37°C in BL2(DE3) strain producer. Membrane faction with VR1 was soluble in no less than 2% N-Lauroylsarcosine. Protein was purified and its' spectrum showed two absorption peaks: at 560 and 410 nm, which can be explained as the presence of both M- and L-forms of this *retinal* protein simultaneously. The light-driven functions of this new protein are not determined yet and father experimental study is planed. This work is supported by 16-15-00242 RSF.

P.2.1-031
The major *E. coli* MDR efflux pump AcrAB-TolC does not require AcrZ protein for expelling the novel highly effective antibiotic SkQ1 out of bacterial cells

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Mitochondria-targeted antioxidants are known to alleviate mitochondrial oxidative damage that causes a variety of diseases. We have found that SkQ1, a decyltriphenyl phosphonium cation conjugated to a quinone moiety, exerts a strong antibacterial effect on Gram-positive *Bacillus subtilis*, *Mycobacterium sp.* and *Staphylococcus aureus* and Gram-negative *Photobacterium phosphoreum* and *Rhodobacter sphaeroides* at submicromolar and micromolar concentrations. Experiments with the potential-sensitive dye DiS-C3-(5) have shown that SkQ1 causes a decrease in the membrane potential of *B. subtilis* in the minute time scale at submicromolar concentrations, and a complete collapse of the bacterial membrane potential at micromolar concentrations. SkQ1 exhibits much lower antibiotic activity towards *Escherichia coli* obviously due to the presence of the highly effective multidrug resistance pump AcrAB-TolC. *E. coli* mutants lacking any of AcrAB-TolC transporter proteins display similar SkQ1 sensitivity, as *B. subtilis*. Mutants lacking AcrZ, a small membrane protein, associated with the multidrug efflux pump AcrB, are sensitive to many, but not all, of the antibiotics transported by AcrAB-TolC. By applying SkQ1-dependent bacterial growth suppression screening, we have shown that AcrZ is not required for removal of SkQ1 out of cells, and AcrABZ-TolC complex is not needed for removing SkQ1. It can be also concluded that amino acid residues of AcrZ are not involved in the formation of the binding site for SkQ1 on AcrAB-TolC.

P.2.1-032
Structure-function analysis of coccolithophore voltage gated proton channel

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Voltage gated ion channels transport various ions through cell membranes and have multitude functions in the cell. These channels contain six transmembrane helices: helices 1-4 comprising the voltage sensor and helices 5-6 forming the pore. Voltage gated proton channels (Hv1) are unique members of this family since they harbor only four transmembrane helices and are lacking a pore domain. Here we focus on Hv1 from the coccolithophore *Emiliania huxley*i (Eh-Hv1). Coccolitophores are unicellular eukaryotic phytoplankton. They produce calcium carbonate plates termed coccoliths, which have an important role as oceanic carbon sink. CO_2 emissions are predicted to influence calcifying organisms by altering ocean water pH and changing the saturation state of calcium carbonate. During calcification, H^+ ions are formed and Eh-Hv1 participates in pH homeostasis. Thus unravelling the properties of Eh-Hv1 structure and function may facilitate our knowledge on calcification and on the effect of CO_2 emissions on coccolitophores. Human and Eh-Hv1 share similar electrophysiological properties and homologous structures. Interestingly, Eh-hv1 contains a long extracellular loop (which is absent in the human homolog), which is predicted to be globular. One remarkable feature of this loop is the large number of histidines which may suggest that this domain is involved in pH sensing or metal binding. In this work we aim to determine the atomic resolution structure of the full length Eh-hv1. Using Fluorescence size exclusion chromatography we were able to screen different constructs of the full-length channel and various detergents to find the best conditions for channel purification. Hitherto we successfully expressed and purified the full-length channel from *s. cerevisiae* and currently we continue with optimizing the purification protocol and establishing a liposome reconstitution system for functional analysis of channel currents and gating associated conformational rearrangements.

P.2.1-033
Insulin receptor-related receptor (IRR): functional and structural studies

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Insulin receptor-related receptor (IRR) is a member of the insulin receptor (IR) family that works as an extracellular alkali sensor. IRR shows highly specific tissue distribution, with highest concentration in the kidney and lower levels in the stomach and pancreatic alpha- and beta-cells of islet. Recent reports indicate that the pancreatic duct system containing the alkaline juice may adjoin the endocrine cells of pancreatic islets. Here, we show that those islet cells that are in contact with the excretory ducts are also IRR-expressing cells. Using pancreatic beta cell line MIN6 we found activation of endogenous IRR but not of IR was detected under alkali treatment that could be inhibited with linsitinib, a synthetic inhibitor of the IR minifamily receptor tyrosine kinases. The IRR autophosphorylation correlated with pH-dependent linsitinib-sensitive activation of insulin receptor substrate 1 (IRS-1). The alkaline medium but not insulin also triggered actin cytoskeleton remodeling in MIN6 cells that was blocked by pre-incubation with linsitinib. We propose that the activation of IRR by alkali is a component of a local loop of signaling between the exocrine and endocrine parts of the pancreas.

The pH-sensing property of IRR is defined by its extracellular region and involves multiple domains. To get a structural insight into IRR function, we generated a panel of monoclonal antibodies against the human IRR ectodomain. One of them named 4C2 recognized epitope in the second and third fibronectin type-III domains and was able to inhibit alkali-induced IRR activation. The other one named 4D5 bound to the first fibronectin type-III domain of IRR. Treatment of IRR-expressing cells with 4D5 resulted in IRR activation at neutral pH; thus, to the best of our knowledge, 4D5 antibody represents the first known IRR agonist of the protein nature.

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P.2.1-034
Study of structure and binding kinetics of human adenosine A2A receptor with novel ligands

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This project focuses on structural and functional studies of the human adenosine-binding A2A receptor coupled to G protein (GPCR). Adenosine receptors are membrane receptors of the

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GPCR class, activated by the endogenous nucleotide adenosine. There are several types of adenosine receptors - A1, A2A, A2B, A3, which are involved in the regulation of various physiological processes in the human body, including regulation of inflammation and immune response, stimulation of neurotransmitter ejection, and regulation of heart rate and blood flow.

In particular, it has been shown that the use of selective antagonists to the A2A receptor can potentially be useful in the treatment of Parkinson's disease. We have chosen the adenosine A2A receptor as an object of research in view of the urgency of its research as a target for the development of drugs against sepsis and neurodegenerative diseases, and a known technique for its preparation in a relatively highly stable state and its ability to give ordered crystals. Despite the fact that high-resolution structures with ligands UK432097 and ZM241385 for this receptor are known (W. Liu, E. Chun, AA Thompson, P. Chubukov, F. Xu, V. Katritch, GW Han, CB Roth, LH Heitman, AP IJzerman, V. Cherezov and RC Stevens (2012) Structural basis for allosteric regulation of GPCRs by sodium ions, Science 337: 232–236; F. Xu, H. Wu, V. Katritch, GW Han, KA Jacobson, Z.-G. Gao, V. Cherezov and RC Stevens (2011) Structure of an agonist bound human A2A adenosine receptor, Science 332: 322–327), the structure of the A2A receptor remains unknown in complex with various other known ligands, including promising candidates for medicines. We also want to investigate the kinetics of the interaction of these ligands with A2A by surface plasmon resonance and microscale thermophoresis using nanodisks. In this study we report our efforts in this research directions.

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P.2.1-035
Expression of the epithelial sodium channel (ENaC) and CFTR in the male reproductive system

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Mammalian testes have two major functions, to produce spermatozoa and androgens. The production of spermatozoa starts in the germ cells of the seminiferous tubules (ST). Whereas, androgens are produced in interstitial Leydig cells. The process of spermatozoa production is dependent on the maintenance of ST fluid composition. Moreover, fluid movement is essential for the transport of sperm towards the epididymis. Na^+ and Cl^- ions are major determinants of body fluid osmolarity and influence fluid movement in the ST. In high-resistance epithelia, ENaC plays a major role in Na^+ transport. Therefore, in this study, we examined the sites of localization of ENaC and CFTR (CF transmembrane conductance regulator) in sections of mouse and rat testes by immunofluorescence-based confocal microscopy and western blot analysis. To identify the stage of spermatogenesis in the ST, and to verify the structural integrity of the tissue sections, we also examined F-actin localization using fluorescent phalloidin. Acrosomes of sperm were visualized using wheat-germ-agglutinin-FITC. Leydig cells were visualized by anti-adrenodoxin (mitochondrial $P450_{scc}$ system electron transfer protein). In overview scan images, actin fluorescence could clearly identify stages of spermatogenesis. ENaC was found to be expressed in the seminiferous tubules but absent in interstitial regions. The extent of ENaC expression was associated with stage of spermatogenesis. CFTR was observed only in certain types of cells present near the basement membrane of the tubules. In the caput, corpus, and cauda regions of the epididymis and the vas deferens, ENaC was localized on the apical membrane, and in interstitial muscle cells. The widespread localization of ENaC in the ST, and the specific high-level localization of ENaC in the epididymal and vas deferens luminal membranes, suggest that ENaC has a major role in the maintenance of ion transport and hence fluid movement in all parts of the male reproductive system.

P.2.1-036
Crystallization trials of human endothelin receptor B

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Endothelin receptors are important members of G-protein coupled receptor (GPCR) class. Receptors of this class share common secondary structure of transmembrane part and represent amazing diversity of signals they can respond to. All GPCRs are famous for their pharmacological significance - they comprise about 40% of all modern marketed drugs. In this regard, Endothelin receptors are GPCRs of especial interest because they play important role in blood pressure regulation. It is not a secret that many elderly people suffer from high blood pressure: in the USA 1 out of 3 adults has high blood pressure and another third of population has prehypertension - blood pressure numbers higher than normal, but not yet in the high blood pressure range. That is why drug development against this disease is of tremendously high importance.

For crystallization trials we developed different genetically engineered constructs. In order to stabilize ETB receptor and create crystal contacts in the future we introduced a compact soluble protein, apocytochrome b564RIL (BRIL), in the third extracellular loop of the receptor or at the N-term. BRIL is known to be an effective crystallization driver for GPCRs. Also all constructs have different truncations of Cterm. Expression level and quality for these created constructs was determined by flow cytometry and confocal microscopy. Homogeneity was studied using SEC. Thermal stability of the protein in the presence and absence of ligands was measured by the analysis of melting in the presence of CPM dye. Finally, the mobility of the receptor in Lipid Cubic Phase (LCP) at many different conditions was probed by the LCP-FRAP (Fluorescence Recovery After Photobleaching) assay. These tests allowed us to determine effectiveness of various constructs for crystallization. The best constructs showed good SEC, thermal stability, mobility in LCP and expression level. This work was supported by the Russian Science Foundation (project no. 16-14-10273).

P.2.1-037
A novel mouse pacemaker cell mathematical model to study autonomic nervous system regulation of the beating rate and aging impairment

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Cardiac diseases affect millions of people every year and their prevalence increases with aging. Research has associated a number of these conditions with changes in heart rate and specifically with the failing of sinoatrial node pacemaker cell (SANC) mechanisms. Experimental results together with biophysical mathematical models reveal the complex interaction between ion channels,

signaling pathways and the resulting action potential (AP) firing rate. We developed a new mathematical model that simulates the pacemaker function of single mouse SANC. The model includes the description of membrane and intracellular mechanisms as well as the description of brain receptor activation pathways and post translation modification signaling cascades. The effects of aging were simulated by varying key membrane and sarcoplasmic reticulum (SR) proteins. The basal model predicts that cycle length (CL, 235 ms), maximum depolarisation potential (MDP, −65.3 mV), the action potential duration at 50% of repolarization (APD50, 55 ms) and maximal dV/dt (8.9 V/s) to fall within the experimental range of mouse AP. The action potential firing rate increases under adrenergic stimulation (+30%) and decreases under cholinergic stimulation (-18.4%). The model showed how AC-cAMP-PKA signaling crosstalk autonomic activity to SANC function. The change in the PKA activity level will modulate the activation of critical membrane and SR proteins and changes in the cAMP level will modify the I_f activation curve. The model also predicted that PLB phosphorylation is a dominant mechanism mediating between autonomic receptor stimulation and SANCs function. In the aging simulations, we showed that both M and Ca^{2+} clocks contribute to the deterioration of basal pacemaker function in advanced age. This conclusion is in agreement with experimental data from mouse SAN tissue and pacemaker cells.

P.2.1-038
Detergent free isolation of the CGRP receptor using styrene-maleic acid co-polymer

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Calcitonin gene-related peptide (CGRP) and its receptors are expressed in the central and peripheral nervous system and have emerged as important drug targets for migraine. The CGRP receptor is a heterodimeric complex of a family B G protein-coupled receptor (GPCR) the calcitonin-like receptor (CLR) and an accessory protein receptor activity-modifying protein 1 (RAMP1). Better understanding of this receptor complex will assist in drug development. Structural and biochemical characterisation of receptors often requires their removal from the native cell membrane. Detergents are widely used for this purpose, but do not accurately replicate the native membrane which can lead to the denaturation and aggregation of detergent solubilised membrane proteins. Extracting proteins direct from the membrane into styrene maleic acid co-polymer lipid particles (SMALPs) is a novel approach to solubilising membrane proteins that avoids the use of detergent. This allows the preservation of the native lipids surrounding the membrane protein improving stability. We have expressed the CGRP receptor in HEK293 cells and solubilised it into SMALPs then purified using affinity chromatography. Preliminary experiments have demonstrated that this method can be used to isolate functional heterodimeric GPCR complexes. SMALP solubilised receptor complexes have the potential to be used for multiple purposes including electron microscopy, x-ray crystallography and mass spectrometry.

P.2.1-039
Hypothalamic GLUT2 inhibition impacts feeding behavior

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Glucose is a key modulator of feeding behavior both at peripheral tissues and the central nervous system, where it is known to modify neuropeptide expression involved in hunger/satiety signals. In the brain, glucose sensing occurs in the hypothalamus and relies on the presence of glucose transporter 2 (GLUT2), whose expression has been detected on glial cells lining the third ventricle (3V), known as tanycytes. These cells are in contact with cerebrospinal fluid and in close proximity with arcuate nucleus (AN) neurons that regulates food intake. This study aims to clarify the role of tanycytes in feeding behavior using injections of adenovirus encoding a shRNA against GLUT2 (Ad-shGLUT2) into the 3V of rats. This method allows specific transduction of tanycytes and GLUT2 inhibition. Through real time PCR and Western blot we determined a decrease in GLUT2 expression levels on animals treated with Ad-shGLUT2, compared with control groups treated with adenovirus encoding a shRNA for β-galactosidase (Ad-shβgal). Neuropeptide expression in response to intracerebroventricular glucose in GLUT2 *knock down* was measured using real time PCR, observing a loss of response. Feeding behavior in GLUT2 *knock down* rats was evaluated in a fast-feeding cycle (24 h/24 h). GLUT2 *knock down* showed an increase on food intake and body weight suggest an inhibitory effect on satiety. In summary, our results show GLUT2 inhibition on tanycytes produces a disruption of the hypothalamic glucose sensing mechanism that alters feeding behavior.

P.2.1-040
Cold stress-induces changes in protein and carbohydrate level, and mitochondrial metabolism in tropical insect Gromhadorhina coquereliana

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Environmental stress affects many aspects of biological function of ectotherms. Even rare exposures to temperature variations lead insect to the evolution of some protective biochemical and physiological mechanisms. In our study, we studied changes in tropical insect *Gromphadorhina coquereliana* triggered by cold stress. We examined the effect of short-term (3 h) and long-term (8 h) cold on biochemical parameters, mitochondrial activity, and the level of heat shock proteins (HSPs) and aquaporins (AQP) in fat body tissue, a physiological analogue of mammalian liver and cytological counterpart of brown fat. Following of short and long cold exposure we observed a significant upregulation of HSP70 and differentiated changes in AQP level. Upon 3 h of cold stress the level of aquaporins lowered, whereas 8 h of cold duration elevated AQP expression. Moreover, as the response to 8 h cold stress we observed increase in the level of glucose and polyols. In fat body mitochondria, the oxygen consumption of resting state (state 4) increased and respiration rate of phosphorylating state (state 3) decreased after cold exposure compared to control conditions. The activity of uncoupling protein (UCP) and production of superoxide anion in mitochondria isolated from fat body of insects exposed to cold stress were significantly higher.

Above results suggest that insect fat body by engagement of different routes and mechanisms plays an important role in

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protecting tropical insects from cold stress. Moreover, stimulation of UCP activity may indicate a themogenic role of fat body in insect tissue upon cold exposure.

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P.2.1-041
Towards the understanding of the Toll-like receptor activation mechanism: predicted juxtamembrane cytoplasmic region is in fact a part of the long transmembrane domain

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Toll-like receptors (TLRs) play a critical role in innate immunity as the first line of host defense. The medical and biological significance of the TLR signaling is obvious, since the disregulation of the TLR system causes various autoimmune diseases and septic shock, and some therapeutic strategies targeting TLRs have already emerged. Despite a lot of biochemical and structural data (to date, more than 40,000 articles are found in PubMed upon the "TLR" request) the detailed mechanistic understanding of signal transduction remains illusive. TLRs are type I integral membrane proteins with an N-terminal extracellular ligand-binding domain, a single-span helical transmembrane (TM) region and a C-terminal cytoplasmic signaling domain. Most of the structural data are available for the extra- and intracellular domains. The only model of the dimeric full-length TLR3 receptor in the active state was build but the conformations of the TM domain and juxtamembrane regions are still unclear. Here we present the spacial structure of the TM and juxtamembrane parts of human TLR4 receptor using solution NMR spectroscopy in a variety of membrane mimetics, including phospholipid bicelles. It is thought that TM domain contains a typical stretch of approximately 20 uncharged residues, but we show that thejuxtamembrane hydrophobic region of TLR4 is helical and is a part of long TM α-helix. Also we found the dimerization interface of the TM domain and claim that all TLRs are characterized by relatively long (32–35 residues) TM helices with charged aminoacids quite deep inside the membrane. This fact may be relevant for the functioning of the receptors and give a new insights into the TLRs activation mechanisms.

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P.2.1-042
The importance of potassium channels and NO/cGMP signaling pathway in the mechanism of relaxing effect of pinacidil on isolated rat uteri

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Pinacidil is antihypertensive agent, which can relax various smooth muscles. To elucidate the mechanism of the action of pinacidil, isolated rat uteri were treated with increased doses of pinacidil in the presence of: methylene blue - guanylate cyclase (cGMP) blocker, tetraethylamonium (TEA, 6 mM) - inhibitor of BK_{Ca} channels and a suppressor of two types of voltage-gated K^+-channel currents, 4-aminopyridine (4-AP, 1 mM) - inhibitor of voltage-gated K^+-channels and glibenclamide (GLB, 2 μM) - selective ATP-sensitive potassium channel blocker. Uteri were isolated from virgin Wistar rats (180–220 g) and suspended in an isolated organ bath chamber containing De Jalon solution, aerated with 95% O_2 and 5% CO_2 at 37°C. Uteri were allowed to contract spontaneously or in the presence of Ca^{2+} (6 and 12 mM) or acetylcholine (ACh). Pinacidil caused concentration-dependent inhibition of spontaneous and Ca^{2+}-induced utrine activity and the relaxing effect of pinacidil depended on the calcium concentration in the medium. Furthermore, inhibitory effect was significantly stronger for spontaneous comparing to Ca^{2+}-induced contractions. Pinacidil exerted the weakest relaxant effect on acetylcholine induced contractions. The results of pretreatment with GLB suggest that the mechanismof the action of pinacidil does not lead to K_{ATP} channels opening. However, opening of BK_{Ca} and voltage-dependent Ca^{2+} channels had some role, but to varying degrees, in both spontaneous and calcium-induced uterine contractions. Our results provide additional confirmation of the dominance of the NO/cGMP signaling pathway in the mechanism of pinacidil relaxing effect, since the presence of methylene blue significantly antagonized its effect. These results indicate that pinacidil could be a potential tocolytic drug.

P.2.1-043
Dynamics of membrane potential in living cells

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In order to avoid a rise in osmotic pressure every living cell is continually adjusting its concentrations of membrane-permeable ions. As a result the membrane potential fluctuates around its resting value.

Understanding the dynamics of these membrane potential fluctuations will help us to understand cell-to-cell interactions, provide a realistic model of action potential generation and, possibly, find the conditions to evoke oscillations in non-excitable cells. While the average membrane potential can be to great extent be predicted by the well-known Goldman-Hodgkin-Katz equation [1], fluctuations have not been studied to date. Recordings are usually made under voltage-clamped conditions, where the voltage fluctuations cannot be recorded or understood. In free-running voltage, whole-cell, patch-clamp experiments [2], voltages in non-excitable cells were recorded for 7-10 min. Cells were divided into 3 cohorts based on their dominant permeability. Recordings were made in the resting state and altered extracellular concentrations of $K^+/Na^+/Cl^-$, or with added intracellular Ca^{2+} ions, or ATP, or both.

We present a systematic analysis of fluctuations in membrane potential including standard deviations, average amplitude, time correlations and spectral power (obtained with Fourier and wavelet transforms). While the power spectra do not indicate clear peaks in well-defined frequency bands, temporal correlations are present in the time series. The amplitude of fluctuations is to great extent determined by electrochemical gradients of ions; but is also significantly increased after adding intracellular Ca^{2+} or ATP.

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P.2.1-044
The significance of the modulation of potassium channels and nitrogen monoxide in the mechanisms of relaxant effect of amifostine

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In our earlier work, we showed that relaxing effect of a radioprotector amifostine (WR-2721) on the isolated rat uterus probably occurs through stimulation of beta-adrenergic receptors. In the present work we studied the role of potassium channels and NO in its relaxation effect. Increased concentrations of amifostine were applied on uteri isolated from virgin Wistar rats (180–220 g) and suspended in an isolated organ bath chamber containing De Jalon's solution, aerated with 95% O_2 and 5% CO_2 at 37°C. Uteri were also pretreated with methylene blue (guanylate cyclase, NO/cGMP, blocker) and potassium channel blockers: glibenclamide (GLB – 2 mM), 4-aminopyridine (4AP – 1 mM) and tetraethylammonium (TEA – 3 mM). Amifostine caused concentration-dependent inhibition of spontaneous and Ca^{2+}-induced uterine activity and its effect did not depend on the type of the uterine activation. GLB, 4AP and TEA to a certain extent antagonized the inhibitory effect of amifostine on both types of uterine activation. On an equimolar basis GLB exhibited the strongest antagonistic effect. Obtained results suggest that the mechanism of amifostine action is related to the opening of K_{ATP} channels. However, the opening of BK_{Ca} and voltage-dependent Ca^{2+} channels also had a role, but to varying degrees that depend on the type of uterine activation. Our results also indicate that in the mechanism of amifostine activity other factors are involved such NO/cGMP signaling pathway, but only on the type of calcium activated uterus, since the presence of methylene blue significantly antagonized this effect. Radioprotector WR-2721 exhibits direct pharmacological effects, which can be a basis for extending the indication of its use.

P.2.1-045
The prediction of essential donors and acceptors involved in proton transfer through the D-channel of cytochrome c oxidase

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Cytochrome *c* oxidase is the terminal enzyme complex of the respiratory chain present both in mitochondrial and cytoplasmic membranes. This enzyme catalyzes the reduction of molecular oxygen to water, which is carried out in its binuclear center. Cytochrome *c* oxidase provides electron transport coupled with proton pumping through the membrane, which leads to an energy store in the form of the transmembrane proton electrochemical gradient. The precise mechanism of the process of proton transport is still under discussion remaining a key issue of biochemistry. Special attention is focused on the D-channel of cytochrome *c* oxidase supplying four pumped protons and at least two substrate ones to the opposite side of the membrane and to the active site of the enzyme respectively. In our model the proton channel is represented as a set of probable donors and acceptors, which are conserved amino acid residues and the chain of water molecules. Transition intensities used for stochastic simulation modeling are obtained according to Marcus theory of proton transfer. Application of stochastic approach allows us to determine both the key residues and the water molecules forming the main transduction pathway. Analysis of the simulation results shows that Asn-207, Asn-121 and the water molecules 2052, 2083 and 2037 do not take part in the proton transport. It is assumed in our model that the entrance amino acid residue Asp-132 is directly protonated only from the mitochondrial matrix. Thus, the dependence of the proton transfer rate on the matrix pH shows that the rate reaches the value of $7.7 \cdot 10^3$/s at pH below 7.5. The influence of pK_a shift on the rate of proton transport observed for Tyr-33 allows us to suppose its possible participation in the process of triggering direction of proton transitions. In the case of the conserved amino acid residues Asp-132 and Glu-286 it was shown that their pK_a shifts did not affect the rate value of the proton transfer through the D-channel.

P.2.1-046
The role of potassium channels, calcium and nitrogen monoxide on the amplitude and frequency of rat uterine contractions after treatment with magnesium sulfate

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Magnesium sulfate ($MgSO_4$) is used as a saline laxative, tocolytic agent and functional calcium channel antagonist. However, precise mechanism of its action is still unknown. The aim of this study was to examine possible role of potassium channels, calcium and NO/cGMP pathway in the relaxation mechanism of $MgSO_4$. The uteri were isolated from female Wister rats, incubated for about 30 min in water-bath with De Jalon's solution at 37°C and oxygenated with 95% of O_2 and 5% CO_2. Both spontaneous and Ca^{2+}-induced (6 and 12 mM) active uteri were treated with increased cumulative doses of $MgSO_4$ (0.1 – 30 mM). In order to analyze the role of different subtypes of potassium channels, uteri were pretreated with glibencamid (GLB), tetraethylammonium (TEA), 4-aminopyridine (4-AP) and barium chloride ($BaCl_2$) as a potassium-selective ion channel blockers. Also, methylene blue (MB), a guanylat cyclase inhibitor, was used in order to examine possible role of NO/cGMP pathway. Our results confirm $MgSO_4$ dose-related inhibition of uterine activity. However, pretreatment with GLB, TEA, 4-AP, $BaCl_2$ and MB did not antagonize, even increased the amplitude of contractions, but to varying degrees. According to our results, potassium channels (K_{ATP}, K_v, K_{ir}) have a role in the maintaining the frequency of uterine contractions. Signalling pathway of NO/cGMP system does not play a significant role in the mechanism of action of $MgSO_4$, since pretreatment with MB failed to prevent $MgSO_4$ induced inhibition of contractile activity. Taken together, it seems

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that the main mechanism of inhibitory action of $MgSO_4$ is as a calcium channel blocker. However, future research needs to specify other aspects of NO influence on the effect of $MgSO_4$.

P.2.1-047
Optimal isotope-labeling schemes for fast NMR analysis of membrane proteins with limited stability. Application to isolated voltage-sensing domains of K+ and Na+ channels

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Voltage-gated K+ and Na+ channels (VGICs) are involved in a wide range of physiological phenomena, including the excitability of cardiac, muscle and neuronal cells, and secretion of hormones and neurotransmitters. VGICs have modular structure and contain five membrane domains: four voltage-sensing (VSD) and one pore domain. The VSDs of the different channels possess unique ligand-binding sites, thus representing attractive targets for drug development. The modular organization of VGICs gave an idea for NMR structural studies of the isolated VSD of human Kv2.1 channel and first VSD of human Nav1.4 channel. However, these studies were hampered by limited stability of the VSDs samples in the membrane mimicking environment, associated with the proteolysis and aggregation of the domain molecules.

To solve this problem, the 'fast' strategy for backbone resonance assignment based on selective ^{13}C,^{15}N-isotope labeling in cell-free expression systems was used. Selective labeling in combination with the simplified NMR spectra acquisition provided information about the residue types in each dipeptide. To collect maximum information from as few NMR samples as possible we developed a program 'CombLabel' that calculates the optimal labeling schemes using protein sequence and stock of available labeled amino acids as input. The calculated price-optimal labeling schemes for each of the VSDs (~150 residues) contained five different samples. Analysis of the 2D ^{1}H,^{15}N-TROSY and HNCO spectra measured for each sample in combination with the limited data from standard triple-resonance NMR spectra resulted in assignment of ~70% and 50% of backbone resonances for Kv2.1 and Nav1.4 VSDs, respectively. The secondary structure and backbone dynamics of VSDs were characterized. The obtained sequence coverage is sufficient to study VSDs interactions with various ligands.

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P.2.1-048
GnRH induces ERK-dependent bleb formation in gonadotrope cells, involving recruitment of members of a GnRH receptor-associated signalosome to the blebs: possible role in cell migration

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We have previously described a signaling complex (signalosome) associated with the GnRH receptor (GnRHR). We now report that GnRH induces bleb formation in the gonadotrope-derived LβT2 cells. The blebs appear within ~2 min at a turnover rate of ~2–3 blebs/min and last for at least 90 min. Formation of the blebs requires active ERK1/2 and RhoA-ROCK but not active c-Src. Although the following ligands stimulate ERK1/2 in LβT2 cells: EGF>GnRH>PMA> cAMP, they produced little or no effect on bleb formation as compared to the robust effect of GnRH (GnRH>PMA>cAMP>EGF), indicating that ERK1/2 is required but not sufficient for bleb formation possibly due to compartmentalization. Members of the above mentioned signalosome are recruited to the blebs, some during bleb formation (GnRHR, c-Src, ERK1/2, FAK, paxillin and tubulin), and some during bleb retraction (vinculin), while F-actin decorates the blebs during retraction. Fluorescence intensity measurements for the above proteins across the cells showed higher intensity in the blebs vs. intracellular area. Moreover, GnRH induces blebs in primary cultures of rat pituitary cells and isolated mouse gonadotropes in an ERK1/2-dependent manner. The novel signalosome-bleb pathway suggests that as with the signalosome, the blebs are apparently involved in cell migration. Hence, we have extended the potential candidates which are involved in the blebs life cycle in general and for the GnRHR in particular.

CRISPR and RNA processing and regulation

P.2.2-001
Feedback mechanisms controlling the plant nonsense-mediated mRNA decay (NMD) pathway

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic RNA surveillance mechanism that down-regulates aberrant transcripts, including mutated transcripts with pre-mature termination codons (PTCs) as well as transcripts derived from pseudogenes, transposable elements, and aberrant mRNA-like non-coding RNAs. Moreover, NMD controls the levels of many normal mRNAs with cis-elements that can be recognized as PTCs. These elements include features that are abundant in normal mRNAs, such as upstream open-reading frames (uORFs) and long 3′ untranslated regions (3′ UTRs). Considering its major impact on the eukaryotic transcriptome, it is of importance to understand how NMD itself is regulated. We found that the *Arabidopsis thaliana* NMD factor UPF3 is feedback regulated by NMD at multiple levels. This feedback control is mediated by the long 3′ UTR of *UPF3* and by other regulatory elements of this gene. We showed that a delicate balancing of *UPF3* expression by this feedback loop plays a crucial role in NMD regulation in plants. We also found that UPF3 plays a role in plant response to salt stress. *UPF3* expression is induced by salt stress, and the balanced expression of this gene is essential for coping with this stress. These findings demonstrate the physiological significance of proper NMD balancing.

P.2.2-002
The cancer-associated U2AF35 470A>G (Q157R) mutation creates an in-frame alternative 5′ splice site that impacts on splicing regulation in Q157R patients

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Recent work has highlighted frequent mutations in spliceosome components to cause or contribute to malignant transformation.

A prominent example is U2AF35, for which cancer-associated missense mutations in two zinc-finger (ZnF) domains have been identified. While most analysis have focused on the S34 mutants, little is known about the Q157 substitutions within the second ZnF. Interestingly, we find that the c.470A>G mutation not only leads to the Q157R substitution, but also creates an alternative 5′splice site (ss). Usage of this alternative 5′ss, which also happens in c.470A>G patients, results in the deletion of four amino acids (Q157Rdel) within the second ZnF, leading to changed specificity for U2AF35 target exons. Additionally we show varying responsiveness of individual splicing targets to Q157R and Q157P variants in a knockdown complementation assay with quantitative read-out. This is consistent with distinct alternative splicing patterns in Q157R and Q157P patients, which is dependent on splice site sequence and in several cases likely caused by the Q157Rdel protein. Our data supports the notion that disease formation is caused by missplicing of different targets in Q157R and Q157P patients and furthermore emphasizes the importance to explore missense mutations beyond altered protein sequence.

P.2.2-003
ARGONAUTE1 acts in a close relation to chromatin and is involved in alternative splicing regulation in *Arabidopsis thaliana*

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Arabidopsis thaliana ARGONAUTE1 (AGO1) protein predominantly localizes and acts in the cytoplasm as a component of the RISC complex, and takes part in a mRNA target recognition and cleavage or translation inhibition. Recently, we found that AGO1 localizes also in the nucleus and acts co-transcriptionally, affecting *MIR161* and *MIR173* genes expression. In salt stress conditions AGO1 interacts or stay in close proximity with *MIR161* and *MIR173* causing premature termination of transcription by RNA Polymerase II (RNAPII).

Arabidopsis in not the only one organism for which new nuclear function for ARGONAUTEs have been recently described. Much more is known about AGO1/2 functions in the nucleus for human and animal cells. Chromatin associated AGO1/2 interacts with histones, chromatin remodelers, H3K9 metyltransferases and histone deacetylases as well as with many of RNA-binding proteins and splicing factors. Moreover, AGOs modulate elongation rate of RNAPII and affect alternative splicing in agreement with the kinetic model of splicing regulation. Additionally, the recruitment of AGO1/AGO2 results in increased level of H3K9me3 which slow down RNAPII processivity on alternative exons.

Conducted immunoprecipitation of *Arabidopsis* AGO1 containing protein complexes and mass spectroscopy analysis indicated several very interesting proteins like: transcription elongation factors, splicing factors, chromatin remodelers and histones.

Our studies shows that also in plants AGO proteins are involved in alternative splicing regulation. We tested several splicing events in the *A. thaliana* mutant *ago1-36*, in control and salt stress conditions, and we found that many of them been affected. Moreover, using AGO1 RNA-IP we proved that AGO1 interacts with alternative exons affected in mutant plants.

Additionally, for several genes undergoing alternative splicing regulation, we found changes in nucleosome position and AGO1 interaction with chromatin.

P.2.2-004
Investigation of the role of CDC5L phosphorylation during spliceosome assembly in controlling alternative splicing and cell viability

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Cell division cycle 5-like protein (CDC5L) is involved in cell cycle control and is a major component of the spliceosomal Prp19/CDC5L complex. During the transition of the spliceosome from the catalytically active B* complex to the C complex, CDC5L gets phosphorylated at various serine/threonine residues comprised in exon 9 and exon 10. This suggests that these modifications are involved in controlling compositional and structural rearrangements of the spliceosome during catalytic activation. To examine the role of these phosphorylations in the activation of the spliceosome, we used CRISPR/Cas9 to generate a HEK293 cell line lacking CDC5L exon 9 and 10. First, we determined the impact of this deletion on cell growth and viability using flow cytometry analyses. Our results indicate that the deletion of CDC5L exon 9 and 10 reduces the growth of HEK293 cells, but as the cells are viable, these exons are not strictly required for cell viability. Analysis of the splicing pattern of CDC5L responsive exons revealed increased exon skipping in the mutant cell line, suggesting reduced splicing efficiency. The splicing reaction is not completely blocked in the mutant cell lines, suggesting that CDC5L phosphorylation during the spliceosome assembly cycle controls alternative rather than constitutive splicing. Further studies will focus on the functional role of individual phosphorylation sites in CDC5L, e.g. by using rescue experiments with proteins harboring point mutations of the respective residues. This will reveal fundamental insights into spliceosomal activation and the molecular connection to the defect in cell growth. Additionally, by using a clonal CRISPR/Cas9 cell line we can obtain large quantities of homogeneous spliceosomes containing CDC5L lacking exon 9 and 10. Based on this, we want to investigate compositional and structural changes and interactions during the activation of the splicing machinery by using cryo-EM analyses.

P.2.2-005
Genetic constructs creation for targeted meiotic recombination in *Arabidopsis thaliana*

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The directed meiotic recombination could significantly increase the selection material diversity and give the new and more effective method for plant breeding. It is known that in most organisms, including plants, the meiotic recombination starts from the initiation of double strand breaks (DSB) catalyzed by SPO-11 protein. To date however, the particular sequences for DSB are not determined. In this study, we develop a Crispr-Cas9-based tool for targeted DSB by fusing the dCas9 endonuclease with inactive catalytic domain and SPO-11 protein under the control of different meiotic promoters. Using short complementary sgRNA for definite locus, dCas9 would guide SPO-11 protein towards the DNA sequence of interest, where recombination has to be occurred. To design this genetic construction, we used Golden Gate Cloning method and Crispr/Cas9 plasmids, kindly provided by prof. H. Puhta. For an accurate estimation of the

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recombination frequency, the study of relatively large population of individuals is required. We will use *Arabidopsis thaliana* special lines (Copenhaver G.P., 1998) to simplify the recombination detection and designed structures verification. These are the ecotype Columbia fluorescent tagged lines (FTLs) with a system of fluorescent markers, which would allow us to estimate the direction of recombination in gametophyte. The comparison of recombination frequency in hybrid between FTLs, carrying the genetic constructs, as well as in non-transformed control, makes feasible the estimation of recombination intensity at a particular locus and would allow to determine whether these genetic constructions promote its increase.

P.2.2-006
Target recognition by Cascade complexes bearing altered length crRNA

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CRISPR with associated Cas genes comprise an adaptive immunity system in prokaryotes. In CRISPR systems foreign nucleic acids are targeted in RNA-dependent sequence specific manner. In Type I-E CRISPR-Cas systems, invading DNA is detected by a multiprotein surveillance complex called Cascade. Guided by crRNA Cascade complex binds to DNA target, if PAM sequence is present, and forms a structure, called R-loop. Once stable "locked" R-loop is formed, the DNA target degradation is performed by Cas3 nuclease-helicase. Recently, it was shown that multimodal nature of I-E type Cascade complex accommodates crRNA of altered length.

Here, we investigated a molecular mechanism of the target recognition by altered Cascade complexes from *S. thermophilus* with shortened (−18, −12, −6 nt) and extended crRNAs (+6, +12, +18, +24 nt). By native mass spectrometry, we showed that +12 and −12 complexes with altered crRNA have different stoichiometry: an additional copy of Cas7 protein is bound/removed every 6th nt and a copy of Cse2 is bound/removed every 12th nt. The target recognition was monitored by single molecule tweezer technique. All complexes formed shortened/extended R-loop structures corresponding to the crRNA length. However, the stability of the R-loop or the plasmid cleavage rate was not correlated with the length of the crRNA.

P.2.2-007
Development of synthetic regulators of gene expression in human cells based on nascent non-coding RNAs

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Non-coding RNAs are involved in numerous processes of gene expression regulation: from post-transcriptional modification and alternative splicing to regulation of transcription and translation levels. One class of regulatory non-coding RNA is small nucleolar RNAs (snoRNAs), which participate in post-transcriptional modification of ribosomal and nuclear RNA in eukaryotes. Earlier, synthetic snoRNAs have been successfully applied for modulation of alternative splicing of the target gene in human cells. Here, we constructed and obtained synthetic analogues of box C/D snoRNA targeted at 2'-O-methylation of various nucleotides of 18S and 28S rRNAs in human cells. Transfection of human adenocarcinoma cells with synthesized analogues demonstrated increase in modification level of the nascent sites of 2′-O-methylation in human rRNA. Further application of analogues containing modified monomers reduced non-specific effects on human cells, including cellular immune response and cytotoxic influence. There have been also data published on the involvement of transcripts associated with promoter regions of various genes in regulation of self-gene expression at transcriptional level in mammalian cells. It was shown that synthetic analogues of promoter-associated RNA are capable of inducing gene expression. In our study, we synthesized long RNA molecules complementary to the promoter region of the model gene eGFP encoded in the pEGFP-N1 expressing vector. Transfection of human MCF-7 line cells expressing plasmid carrying target gene under CMV promoter with synthetic RNAs showed 2-fold increase in the level of eGFP transcript. Thus, modulation of human gene expression is possible at various stages with implementation of synthetic analogues of regulatory RNA.

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P.2.2-008
New role of a DRB1 (HYL1) in an RNA metabolism

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MicroRNAs (miRNAs) are short (21–24 nt) RNA molecules that control gene expression at the posttranscriptional level by cleavage of mRNA targets or by inhibition of their translation. All plant miRNA genes (MIRs) are transcribed exclusively by RNA polymerase II (RNAPII), and primary MIR transcripts (pri-miRNAs) must be precisely processed to produce mature miRNAs. HYL1 (DRB1) beside SE (Serrate) and DCL1 (Dicer Like 1) is one of main component of plant Microprocessor. This dsRNA binding protein is required for the precise cleavage of miRNA/miRNA* duplexes from the stem of pre-miRNA by DCL1. Additionally, it was shown that the HYL1 protein needs to be dephosphorylated by a CPL1 protein that is also able to dephosphorylate a CTD of the RNAPII at serine 5 residue. These observations prompted us to investigate if a HYL1 protein is involved in the transcription of *MIR* genes.

Using *Arabidopsis* lines caring GUS under *MIR393a* and *MIR393b* we found lower GUS activity in the *hyl1-2* background than in WT. IT indicates that HYL1 might be important for proper transcription of these genetics constructs. By ChIP-seq, we found RNAPII pausing on several others *MIR* genes, mainly in the hairpin coding region, what supports hypothesis about co-transcriptional miRNA biogenesis in *Arabidopsis*. What is more important, we found prominent changes in RNAPII distribution in *hyl1-2* for many protein coding genes, especially at 3′UTRs and at the region downstream of the genes. Moreover, RNA immunoprecipitation using HYL1:HA line proved that HYL1 interacts with transcripts encoded by RNAPII affected genes in *hyl1-2*. By 3′RACE technique we proved strong heterogeneity in poli(A) sites for selected HYL1-bound transcripts. Finally, qPCR have shown downregulation of HYL1-bound transcripts in *hyl1-2* background. All this data, taken together, indicate that HYL1 is not only involved in miRNA biogenesis but play important role in transcription regulation of many protein coding genes.

P.2.2-009
The mechanism of mod(mdg4) locus trans-splicing

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Most eukaryotic messenger RNAs are capped, spliced, and polyadenylated via co-transcriptional processes that are coupled to each other and to the transcription machinery. Coordination of these processes ensures correct RNA maturation and provides for the diversity of the transcribed isoforms. RNA processing is a chain of events in which the completion of one event is coupled to the initiation of the next one. This co-transcriptional mechanism of mRNA processing preserves forming of chimeric non-productive mRNAs. Unlike typical cis-splicing, trans-splicing joins exons from two separate transcripts to produce chimeric mRNA, which represents a unique case of functional diversification. Following the transcription logic trans-splicing is harmful process that can produce a lot of wrong products and as a rule is blocked in most organisms. Trans-splicing occurs in lower eukaryotic trypanosomes and nematodes, provides peculiar 5′-end processing and doesn't affect coding regions. The most confirmed case of trans-splicing between protein coding exons is the *mod(mdg4)* locus of *Drosophila melanogaster* that contains several transcription units encoded on both DNA strands. *Mod (mdg4)* produces at least 31 splicing isoforms that share four common 5′-exons. We show that RNA processing rules should be broken to provide trans-splicing. We identified non-canonical transcription termination that occurred within intron of *mod (mdg4)* gene and mapped regions essential for trans-splicing. This events are coupled with RNA polymerase II pausing. Our data shows that trans-splicing of *mod(mdg4)* locus is implemented by new uncharacterized mechanism.

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P.2.2-010
Study of posttranscriptional regulation of ITSNs by microRNAs

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ITSN1 and *ITSN2* are the genes encoded adaptor proteins with multiple isoforms participating in clathrin-mediated endocytosis (CME), MAPK signaling and reorganization of actin cytoskeleton. Changes in ITSNs expression can lead to different neurodegenerative disorders and cancers. To date little is known about regulation of ITSN genes on posttranscriptional level. The aim of our work was to predict and experimentally confirm target sites for microRNAs that could potentially regulate ITSNs expression and identify the most abundant predicted microRNAs for CME and related pathways. Using 8 web servers we analyzed 3′UTRs of short and long isoforms of human ITSN mRNAs and found conservative target sites for miR-34, miR-19, miR-129, miR-103/107, miR-194, miR-181 and miR-30 in 3′UTR of *ITSN1-S*, predicted by 5 servers, miR-203 predicted by 5 servers for *ITSN1-L*, and miR-153, miR-148/152, miR-27, miR-144 and miR-128 predicted by 5 to 6 servers for *ITSN2-L*. To elucidate potential impact on CME, MAPK signaling and actin cytoskeleton regulation by these miRNAs we performed enrichment analysis by Diana-MirPath server and found that miR-34, miR-19, miR-103/107, miR-181, miR-30 and miR-148 were highly enriched for all analyzed pathways. Since CME is tightly connected to actin cytoskeleton reorganization and caveolin-mediated endocytosis, we have searched for the common microRNAs and found that miR-106b, miR-17, miR-30, miR-20, miR-19, miR-130, miR-124, miR-93, and miR-133 may regulate numerous genes in three abovementioned processes. To confirm *ITSN1-S* regulation by antitumor microRNA miR-181a we cloned 3′UTR of *ITSN1-S* into luciferase reporter vector, transfected HEK293 cells by this construction and miR-181a and obtained up to 40% decrease of expression of *ITSN1-S* 3′UTR-bearing plasmid. These data may confirm miR-181 target site in 3′UTR of *ITSN1-S* mRNA but needed additional research. Thus, we established what microRNAs may be important for CME and related processes.

P.2.2-011
Nanoparticle-mediated delivery of the CRISPR/Cas9 system components into plant cell for genome editing

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Rapid advances in nanotechnology have led to the development of innovative applications based on nanoparticles (NP, ultrasmall particles with sizes ≤100 nm). Development of applications in the field of basic and applied plant sciences promises no less revolutionary results as in biomedicine. NPs have a number of unique properties associated with their ultrasmall size and exhibit many advantages compared with existing plant biotechnology platforms for delivery of proteins, small RNAs, RNA and DNA in the plant.

The main aim of this work was to develop new effective NP-mediated technology for delivering the CRISPR/Cas9 system components for genome editing in plants. To identify most appropriate nanoplatforms which are best suited for this purpose, we developed two types of NP platforms: gold NPs (AuNPs), magnetic NPs of zerovalent iron (MNPS). These NPs were loaded with fluorescent model proteins (GFP, FITS labeled BSA) and fluorescently labeled small RNAs [tRNA and single guide (sg) RNA (RNA component of the CRISPR system) labeled with the Cy3 fluorescent probe].

The efficiency of particle penetration was determined by measuring fluorescence pattern and intensity using fluorescence and confocal microscopy. The best performance in delivering cargo molecules (both proteins and RNA) into plant cells has been exhibited by AuNPs.

Therefore, to elucidate if nanoparticles can be used for delivery of the CRISPR system components into plant cells and consequent genome editing, we functionalized AuNPs with Cas9 (RNA-guided DNA endonuclease) and sgRNAs designed to knock out plant genes encoding coilin and phytoene desaturase (PDS). The resultant NPs were biolistically bombarded into apical meristems and axillary buds of the potato plants. The genome editing events (coilin and PDS genes knock out) were confirmed using the NGS of the corresponding genes.

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P.2.2-012
Optimization for guide RNA design for effective CRISPR/Cas9 gene editing

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The CRISPR/Cas9 system consists of clustered regulatory short palindromic repeats combined with associated genes to form a prokaryotic immune system which is able to adapt and sustain resistance against malicious plasmids and viruses. Due to addressing simplicity CRISPR/Cas9 has become very handful and popular technique to mutate genomes of various organisms (point, insertion or deletion mutations). One of the most important stages in the development of an application for genome editing using CRISPR/Cas9 is the design of guide RNAs (gRNAs). The aim of this work was to develop applications for effective cleavage of DNA sequences encoding coilin and phytoene desaturase (PDS) genes (*Solanum tuberosum*). We synthesized five single guide RNAs (sgRNAs) for coilin gene based on bioinformatics prediction and only one of them demonstrated the cleavage activity *in vitro*. Of six sgRNAs synthesized for PDS gene five were active. In order to optimize the efficiency of sgRNA sequence for coilin gene cleavage, we designed ten new gRNAs with subsequent substitutions of pairs of degenerate nucleotides. It was found that the replacement of sgRNA nucleotides from 12 to 16 by degenerate nucleotides led to increase of *in vitro* cleavage efficiency. In addition, we tested the effect of the nucleotide sequence downstream of the PAM site on the cleavage specificity and efficiency. For this purpose, four genetically engineered constructs were prepared in which six nucleotides downstream of the PAM site were changed to six G, C, A or T, respectively. The cleavage efficiency was evaluated by fragment analysis in agarose gel and qPCR. The obtained data will be discussed in the context of the possible application for genome editing technologies.

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P.2.2-013
Therapeutic genome editing and its potential enhancement through CRISPR guide RNA modifications

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Hematopoietic stem cell transplant (HSCT) is a treatment option for monogenic diseases of the immune system. While allogeneic HSCT can be curative for these diseases, there remain significant limitations to this approach such as graft-versus-host disease and graft rejection. Autologous gene therapy using viral vectors containing a corrective transgene is being developed for some of these disorders, most successfully for adenosine deaminase deficiency. However, for other immune disorders, the transgene needs to be expressed in a precise, developmental and lineage specific manner to achieve functional gene correction and to avoid the risks of cellular transformation. In contrast to using viral vectors to deliver transgenes in an uncontrolled fashion, we are working towards using genome editing by homologous recombination (HR) to correct a disease-causing mutation by precisely modifying the genome of hematopoietic stem cells. CRISPR/Cas-mediated genome editing relies on guide RNAs to direct site-specific DNA cleavage mediated by the Cas endonuclease. We chemically synthesized single guide RNAs (sgRNAs) targeting three different genes (IL2RG, HBB, and CCR5), alongside sgRNA variants containing modified nucleotides. We show that sgRNAs containing modified nucleotides dramatically enhance genome editing compared to unmodified sgRNAs. When co-delivering chemically modified sgRNAs and Cas9 mRNA we observe ~70% in/del frequencies in human primary T cells, and ~40% in hematopoietic stem and progenitor cells. In addition, we present a CRISPR/Cas9 gene-editing system that combines Cas9 ribonucleoproteins and adeno-associated viral vector delivery of a homologous donor to achieve HR in hematopoietic stem cells. Collectively, these studies outline a CRISPR-based methodology for targeting hematopoietic stem cells by HR to advance the development of next-generation therapies for monogenic disease of the immune system.

P.2.2-014
A real time, single molecule view of transcription in living human cells

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Transcription is a fundamental step in gene expression. However, its dynamics are incompletely characterized in single living cells. To address this question, we developed an improved RNA tagging system using the MS2-binding protein fused to GFP. The reporter consisted of a HIV-1 derived vector tagged with 128 MS2 stem loops, allowing a quantitative, single molecule view of transcription in real time. Briefly, by measuring the intensity of the transcription site, we could quantify the number of mRNA molecules synthesized at any given time. We found that HIV-1 is transcribed by groups of closely spaced polymerases referred to as polymerase convoys, where 10–20 polymerases move synchronously through the gene with the same speed. Furthermore, the activity of the HIV promoter fluctuated stochastically on two time scales, minute and sub-hour, and we refer to this phenomenon as multiscale bursting. The slow and rapid fluctuation were controlled by different factors: were (i) TBP/TATA-box interaction for the sub-hours level; (ii) Mediator complex for rapid fluctuations and convoy formation. On the basis of these findings, we studied how the promoter architecture controls the dynamics of of transcription. We produced cell lines where transcription of the reporter was driven by six different cellular promoters, which differ greatly by their expression level, pausing index and promoter sequences. Using a similar imaging method method, we are calculating the kinetic parameters that precisely describe their transcriptional behavior.

P.2.2-015
Assaying the order of events in cells through CRISPR/Cas9

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Cell is a complex environment composed of several pathways and systems that intricately together determine a phenotype. For this reason is difficult to assess the order of events leading to a specific phenotype. Usually it is completely unknown in which order these events occur and which event comes first. Currently, the only feasible manner is to interrogate each event individually that permits to visualise what is instantaneously happening in the cell or to study at the end of a process which are the events that occurring in the cell during a long period of time. Our goal is different: we want to understand also, in which orders these modifications occur before getting the phenotype. There are many examples in the literature of research groups that are focusing to turn the cell in a computer, reordering all the events that happen in the cell, but any of these papers are developed on mammalian cells. This is the reason why I designed an assay to obtain *a posteriori* the order in which a series of events have occurred in a mammalian system. The assay exploits the Cas9/sgRNA system to induce recombination of an artificial DNA cassette, which bears barcodes interspersed among Cas9/sgRNA target sequences. The Cas9 is targeted to the cassette by different sgRNAs, individually linked to specific events. Thus, upon induction of the sgRNAs, the onset of double-strand breaks on the cassette will induce its sequential recombination, and the presence of the barcodes in the terminally recombined cassette is determined by the order in which the sgRNAs have been induced. At the same time, we are assessing the system on real biological processes like transcription factor activation of two different promoters in iPS cells and differentiation. The activation of two different promoters by the action of two transcription factors, lead the expression of cas9 specific for both the targets: sequencing the recombined cassette it is possible to understand which promoter is activated first.

P.2.2-016
A sequence element enriched in Alu repeats drives nuclear localization of long RNAs in human cells

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Long noncoding RNAs (lncRNAs) are emerging as key players in multiple cellular pathways, but their modes of action, and how those are dictated by sequence remain elusive. While lncRNAs share most molecular properties with mRNAs, they are more likely to be enriched in the nucleus, a feature that is likely to be crucial for function of many lncRNAs, but whose molecular underpinnings remain largely unclear. To identify elements that can force nuclear localization we screened libraries of short fragments that tiled across nuclear RNAs and were cloned into the untranslated regions of an efficiently exported mRNA. The screen identified a short sequence derived from Alu elements and found in many mRNAs and lncRNAs that increases nuclear accumulation and reduces overall expression levels. Measurements of the contribution of individual bases and short motifs to the element functionality identified a combination of RCCTCCC motifs that are bound by the abundant nuclear protein HNRNPK. Increased number of RCCTCCC motifs and HNRNPK binding are predictive of substantial nuclear enrichment in both lncRNAs and mRNAs, this mechanism is conserved across species, and adoption of Alu and B1 elements has contributed to the divergence of subcellular distribution of mRNAs, which is otherwise very well conserved between species. Our results thus detail a novel pathway for regulation of RNA accumulation and subcellular localization that preferentially act on lncRNAs that are less depleted of Alu elements than mRNA.

P.2.2-017
Comparison of different strategies for siRNA delivery against influenza A virus

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Human influenza A virus (IAV) constitutes a danger to humanity as evidenced by massive epidemics and pandemics. Modern antiviral agents are limited in their effectiveness. The RNA interference method (RNAi) for gene silencing is the most promising antiviral therapeutic approach. Despite the great potential of RNAi technology, the main challenge is an old one: safe and efficient delivery. Our research group has evaluated and compared several delivery systems for intracellular delivery of siRNAs against influenza virus infection. A commercial lipoplex system (Lipofectamine RNAiMAx, Lip), polyplex-based systems (polyethyleneimine, PEI, and chitosans, Ch), and hybrid microcapsules were studied.

To evaluate the antiviral efficiency of the aforementioned carriers using a standard IAV strain (A/PR/8/34 H1N1), we selected six siRNAs targeting the conserved regions of IAV nucleoprotein (NP). Cells were transfected with Lip and PEI, and the medium was changed after 24 h. Next, they were infected with IAV (0.01 moi). The antiviral activities were evaluated by measuring cytopathic effects on cell monolayers and by hemagglutination assay of supernatants. It was shown that three siRNAs were the most effective in both the Lip and PEI systems.

In preliminary experiments, we found that PEI and Ch-polyplexes induce a 4–8 fold reduction in viral titer. NP concentrations were 40% lower than in controls. Following that, we studied three main siRNAs encapsulated in hybrid micro-sized containers. A 4–8 fold decrease of viral titer was shown. A 90% or more decrease in NP level was observed in comparison to controls. Dose-dependent effects were demonstrated. The antiviral activity of our microcapsules containing siRNAs was shown for two IAV subtypes (H1N1 and H5N1) in comparison with Oseltamivir's effectiveness. These findings reveal the potential of RNAi as a therapeutic approach in influenza virus infection.

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P.2.2-018
In vivo targeted mutagenesis via CRISPR/Cas9 and TALEN in zebrafish enables rapid screening of candidate rare diseases genes

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Discovery of transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) system enabled *in vivo* targeted genome editing to be realized at high precision and efficiency. In zebrafish, generation of knockout alleles is rapid as homozygous mutant fish can be identified in only two generations. Traditionally used gene knockdown technologies such as morpholinos are now replaced by genome editing tools. In Turkey high incidence of rare disorders due to consanguinity provides an important source for the identification of novel candidate genes and variants in families by using homozygosity mapping and exome sequencing approaches. Our aim is to implement a rapid and easy methodology owing to create knockout alleles of candidate disease-related genes in zebrafish. These models enable us to test the effects of loss-of-function of these genes in the tissue/tissues that are affected by the disease in human. To this end we generated knockout alleles in four different genes by using TALEN or CRISPR/Cas9 technologies and tested mutagenesis rate by high-resolution melt analysis and sequencing. Mutagenic efficiency and germline transmissibility was further assessed by screening for F0 mutant fish and F1 offspring. We found that mutagenesis, mosaicism and transmissibility rates vary greatly depending on the targeted gene. Both mutagenic efficiency and transmission were found to be significantly higher (up to 100% transmission) in mutants generated by using CRISPR/Cas9 as compared to TALEN. As a result we are able to rapidly generate heterozygous and homozygous knockout alleles in zebrafish by using CRISPR/Cas9 or TALEN. This methodology allows us to rapidly screen for novel genes related to rare genetic disorders. In the future we aim to create patient-specific mutant lines in order to understand the molecular basis of rare genetic disorders studied in our University.

P.2.2-019
Unraveling cis-regulatory elements by mapping structural changes in mRNAs

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mRNA molecules are generally thought to be messengers of genetic information in the cell. Stretches of RNA that are complementary in sequence have a propensity to pair, forming elements of secondary structure within RNA molecules. Although these structures will exist in every mRNA molecule, the role they play in gene regulation is not well understood. Currently two techniques are available to profile the cell RNA structure, *in-vivo,* in an unbiased manner. We applied one of those techniques, DMS-seq, for probing the human mRNA structure in primary foreskin fibroblasts (HFFs) along human cytomegalovirus (HCMV) infection. As a proof of concept, using DMS-seq, we managed to predict the already solved human 28S rRNA structure with high accuracy. Using our data, we are able to show for the first time *in-vivo*, that human coding sequences (CDSs) are less structured relative to UTRs. Additionally, we provide systematic *in-vivo* evidences for unwinding of the mRNA by the ribosomes during translation. Intriguingly, we also found structural changes in human CDSs around the start and stop codon, and also in 3′UTRs. The combination of accurate measurements of translation regulation and mapping changes in mRNA structure along a dynamic process can be used as a platform for deciphering *cis*-regulatory elements that control gene expression in various cell types, organisms and biological processes.

P.2.2-020
Characteristics of human lncRNA transcripts across cell lines

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Long non-coding RNAs (lncRNAs) are a class of intensely studied, yet enigmatic molecules that make up a substantial portion of the human transcriptome. Motivated by our preliminary studies and published data, we are investigating the idea of lncRNAs regulating gene expression levels by coming into direct interactions with mate mRNAs or pre-mRNAs. The molecular mechanisms that we are taking into consideration include splicing modulation by masking splicing signals on a pre-mRNA molecule, triggering RNA editing by forming dsRNA regions and abrogation of miRNA functions by masking their target sites. By means of bioinformatics analysis of large-scale transcriptomic data from ENCODE and our sequencing data, such as RNA-Seq and 4SU-Seq, we performed lncRNA search, followed by functional studies, leading us to a number of promising candidates, some of which now undergo experimental check. In particular, we identified a subset of lncRNAs being expressed from an antisense strand of retrocopies, therefore able to base-pair with their parental genes and affect their expression and processing. We also found that lncRNA-mediated alternative splicing might be responsible for coding <=> noncoding shift in production of splicing isoforms, with potential functional implications. Finally, by comparing 154 *ab initio* assembled transcriptomes from RNA-Seq data of different origin (PolyA+/PolyA-, nuclear/cytoplasmic fraction etc.) we reached the conclusion that lncRNAs themselves as well as effects of their functionalities might be among major contributors to the observed diversification of transcriptomes.

P.2.2-021
CRISPR ribonucleoprotein tools for genome editing

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CRISPR (clustered, regularly interspaced, short palindromic repeats) is a form of adaptive immunity found in bacteria, which is being developed into a highly-specific mammalian genome editing tool. The potential applications of this technology are restrained by off-target effects, which could cause unwanted genome edits of great consequence in delicate biological experiments. Directly transfecting/nucleofecting CRISPR ribonucleoproteins (RNP) has the potential to greatly strengthen and expand the possible applications of this technology through decreasing the risks associated with off-target effects. Here, we show the recent progress in our continuous efforts to select and optimize novel programmable endonucleases in conjunction with advanced synthetic RNA design and transfection reagents as efficient and precise genome editing tools.

P.2.2-022
A quality control mechanism within the endogenous pre-mRNA processing machine – the supraspliceosome

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The endogenous spliceosome assembles individually Pol II transcripts in a huge (21 MDa) and dynamic complex - the supraspliceosome - that package pre-mRNA transcripts of different sizes and number of introns into complexes of a unique structure, indicating their universal nature. It coordinates pre-mRNA processing in the nucleus including splicing, alternative splicing, 5′ and 3′ end processing, RNA editing and processing of intronic short ncRNAs. It also harbors a pre-mRNA quality control mechanism termed Suppression of Splicing (SOS). Human introns harbor numerous 5′ splice site sequences that are not used for splicing under normal growth conditions (latent splice sites), but are activated in stress. In most cases, activation of splicing at these sites (latent splicing) would lead to mRNA with premature termination codon (PTC). Removal of the PTC results in latent splicing. We demonstrated that the lack of latent splicing is due to the quality control mechanism SOS, and not due to degradation of potential latent mRNA harboring a PTC. Importantly, the initiation codon is required for SOS, and the initiator-tRNA (ini-tRNA), which is associated with the supraspliceosome, is an essential factor required for SOS regulation, independent of its role in translation. To identify proteins associated with ini-tRNA in the nucleus, we used affinity purification of proteins directly associated with ini-tRNA there. We UV crosslinked biotinylated-^{32}P-labelled ini-tRNA in *Xenopus Laevis* oocytes and affinity purified the bound proteins using streptavidin magnetic beads. A number of proteins were found associated with ini-tRNA in the nucleus and not in the cytoplasm, representing nuclear components that interact directly and specifically with ini-tRNA, as they were chased by cold ini-tRNA and not by cold elongator-tRNA. Using mass spectrometry, we identified these putative SOS factors that interact directly with ini-tRNA in the nucleus, opening the way to decipher the SOS mechanism.

P.2.2-023
A selected set of mature microRNAs is associated with the endogenous spliceosome

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MicroRNAs (miRNAs) are short ncRNAs that via base-pairing with target mRNAs negatively regulate expression and translation in healthy and diseased tissues. Most studies focus on the role of miRNAs in the cytoplasm. However, emerging evidence suggests a function for miRNAs also in the cell nucleus. In this study, we investigate short RNAs from human HeLa cells and characterize the composition of miRNAs that are associated with the endogenous spliceosome. This complex is a huge (21 MDa) dynamic machine that participates in various aspects of coordinating pre-mRNA processing in the nucleus. Following isolation of the endogenous spliceosome from cell nuclei, the associated small RNAs (<200 nt) were extracted and subjected to next generation sequencing, and about 450 types of pre-miRNAs were identified. These pre-miRNAs are enriched with intronic localization compared with the genomic distribution of cytoplasmic miRNAs. Surprisingly, the majority of the identified sequences represent mature miRNAs. These mature miRNAs are most likely shuttled back to the nucleus after completing maturation in the cytoplasm. For 30 of 215 mature miRNAs, we found a significant over-representation in the spliceosome compared to the cytoplasm. In addition, we identified 21 miRNAs that could not be identified in the cytoplasm, but are abundant in the spliceosome. Six out of these 21 miRNAs overlap the first non-coding exon of their host gene. Interestingly, for 12% of the detected spliceosomal mature miRNAs, the relative abundance of sequences associated with the 5′p relative to 3′p miRNA, markedly differs from that in the cytoplasm. For example, we identified a high level of 3′p hsa-mir-151 miRNA, while it is the 5′p miRNA that dominates at the cytoplasm. Our results imply an unexplored function for miRNAs at the supraspliceosome in regulating pre-mRNAs processing.

P.2.2-024
Developing a CRISPR/Cas9 screening platform for Chinese hamster ovary cells

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Chinese hamster ovary (CHO) cells are the most common mammalian cell line used for producing bio-therapeutic proteins and serve as the expression system of choice for most best-selling biologics. Although these cells are widely used, the genetic bases underlying desirable phenotypes remain difficult to elucidate. CRISPR/Cas9-mediated genome engineering has proven effective in CHO cells in smaller formats, yet rational target identification on a high throughput level remains a bottleneck. CRISPR screening methods have been used in human cancer cell lines to identify novel therapeutic targets but their efficacy in CHO cells for identifying targets for improved cell behavior has not yet been demonstrated. Here we develop a CRISPR/Cas9 knockout screening platform in CHO cells, enabling high throughput target discovery under diverse selection pressures.

We have designed a library comprising ~16,000 gRNAs against ~2500 metabolic targets using the CHO-K1 genome and genome scale model of CHO cell metabolism. The library was used to generate a pool of cells each expressing a single gRNA. As a proof of concept, we have independently subjected the pool of cells to strong and weak selection pressures to identify genes that influence cell growth and product quality. We will discuss the unique challenges encountered in translating CRISPR/Cas9 screens to CHO cells – including technical optimizations during generation and validation of the library that are necessary to ensure the accurate identification of targets. Furthermore, we will present preliminary results from our initial selection experiments.

P.2.2-025
Evaluation of plant miRNAs abundance in human breast milk

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Breast milk is perceived as required for optimal growth and development of infant. Apart alimentary substances it contains many important bioactive compounds, including endogenous miRNAs. These regulatory molecules were identified in various mammalian biological fluids and were shown to be mostly

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packed in exosomes. Recently, it was revealed that plant, food-derived miRNAs are stably present in human blood and regulate expression of specific human genes. Since then scientific community has focused its efforts to contradict or confirm mentioned discovery. With the same intention, the qRT-PCR experiments were performed to evaluate the presence of 5 plant, food-derived miRNAs (miR166a, miR156a, miR157a, miR172a and miR168a) in breast milk (whole milk and exosomes) of healthy volunteers. In whole milk samples, all examined miRNAs were identified, in exosomes the presence of only 2 of them was confirmed. The plant miRNA concentration in samples ranged from 4 to 700 fM. Several appropriate and careful controls were also included in the experiments to eliminate any possibility of false positive results. To supplement described results, bioinformatics analysis was carried out to evaluate the potential influence of these 5 plant miRNAs on infant's organism. The *in silico* human target prediction and functional annotation have revealed that identified molecules may regulate some important biological processes. Described results support our previous findings based on *in silico* analysis (Lukasik *et al.,* Plos One 2014; 9(6):e99963). The presented study provides further evidence that plant, food-derived miRNA molecules are abundant in body fluids of healthy humans. This is a big step towards the discovery of plant miRNAs new properties, potential roles in living organisms and their applicability.

P.2.2-026
The HCMV long non coding RNA 4.9 is important for viral DNA replication

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Viruses are known for their extremely compact genomes and the Human Cytomegalovirus (HCMV) hardly contains any intergenic regions. Nonetheless, four long intergenic non-coding RNAs (lincRNAs) have been previously identified in the HCMV genome. Although these lincRNAs accumulate to extremely high levels, their functions are largely unknown. Here we show that during lytic infection, the HCMV encoded lincRNA *RNA4.9* localizes to the viral nuclear replication compartment and its depletion restricts viral DNA replication and viral growth. We further demonstrate that *RNA4.9* is not directly involved in DNA replication, as it does not co-localize with nascent viral DNA and its localization to sub-nuclear domains precedes that of the viral DNA polymerase. Instead, we present evidence that *RNA4.9* is important in the generation of the pre-replication structures, indicating that it might be required for the nucleation of the replication compartment.

P.2.2-027
RNA editing in bacteria recodes multiple proteins and regulates an evolutionarily conserved toxin-antitoxin system

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Adenosine (A) to inosine (I) RNA editing is wide-spread in Eukaryotes. In Prokaryotes however, A-to-I RNA editing was only reported to occur in tRNAs, but not in protein coding genes. By comparing DNA and RNA sequences of Escherichia coli, we show for the first time that A-to-I editing occurs also in prokaryotic mRNAs and has the potential to affect the translated proteins and cell physiology. We found 15 novel A-to-I editing events, of which 12 occurred within known protein-coding genes where they always recode a tyrosine (TAC) into a cysteine (TGC) codon. Furthermore, we identified the tRNA adenosine deaminase A (tadA) as the editing enzyme of all these editing sites, thus making it the first identified RNA editing enzyme that modifies both tRNAs and mRNAs. Interestingly, several of the editing targets are self-killing toxins that belong to evolutionarily conserved toxin-antitoxin pairs. We focused on hokB, a toxin that confers antibiotic tolerance by growth inhibition, as it demonstrated the highest level of such mRNA editing. We identified a correlated mutation pattern between the edited and a DNA hard coded Cys residue position in the toxin, and demonstrated that RNA editing occurs in hokB in two additional bacterial species. Thus, not only the toxin is evolutionarily conserved but also the editing itself within the toxin, is. Finally, we found that RNA editing in hokB increases as a function of cell density and enhances its toxicity. Our work thus demonstrates the occurrence, regulation and functional importance of RNA editing in bacteria.

P.2.2-028
Development of novel antibodies for detection and analysis of CRISPR-Cpf1 RNA-guided endonucleases

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CRISPR (clustered, regularly interspaced, short palindromic repeats) adaptive immunity found in bacteria is being recruited into a highly-specific mammalian genome editing tool. Depending on the architecture of the effector-CRISPR RNA (crRNA) interference module, different CRISPR-Cas systems could be assigned into two classes: Class-1 systems of multi-subunit complex, such as Cascade, and Class-2 systems of single enzyme, such as Cas9.

Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) belongs to Class-2 type V CRISPR-Cas endonuclease system. Cpf1 comprises several differences from Cas9 protein, including cleavage with 5′ overhangs, a shorter guide RNA and a longer distance between the seed sequence and cleavage site. AsCpf1 (Cpf1 from *Acidaminococcus sp.* (strain BV3L6)) and LbCpf1 (Cpf1 from *Lachnospiraceae bacterium* ND2006) are the two representatives of the Cpf1 family which were demonstrated to mediate efficient genome editing in HEK293FT cells with improved results compared to SpCas9.

Herein we describe the development of both anti-AsCpf1 and anti-LbCpf1 mouse monoclonal antibodies. These antibodies specifically recognize AsCpf1 and LbCpf1, respectively, and do not cross-react with each other. The antibodies are shown to be implemented in several important immunochemical techniques, including Immunoblot, Immunofluorescence and Immunoprecpitation. Thus we believe it will serve as a useful tool for genome editing research, including detecting and monitoring Cpf1- transfected cells, Cpf1 protein analysis and RNA/DNA interactions.

P.2.2-029
Tumor-related abnormalities in miRNAome can lead to the RNA editing enhancement

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ADAR (adenosine deaminase acting on RNA)-mediated RNA editing takes place in many cancer types and correlates with tumor progression, invasion potential, therapeutic resistance and poor prognosis. Our research aims to identify in what way the shifts in miRNA expression profile can facilitate ADAR expression and contribute thereby to RNA editing abnormalities. MiRNA targets within gene transcripts were predicted *in silico* using the TargetScan software. High-conservative targets of miRNAs miR-1/206 and miR-17-5p along with numerous non-conservative targets of miRNAs miR-15/16, miR-22, miR-33, miR-125, miR-133a, miR-140, miR-143, miR-199 and miR-204 were revealed in *ADAR1* gene transcript. *ADARB1 (ADAR2)* gene transcript carries high-conservative targets of miRNAs miR-199 and miR-218, conservative targets of miRNAs miR-320, miR-340 and miR-599 as well as non-conservative targets of miRNAs miR-15/16, miR-17-5p, miR-22, miR-124, miR-125, miR-133a, miR-140, miR-141/200a, miR-143, miR-148/152, miR-216b and miR-302. Down-regulation of these miRNAs is characteristic to the cancer cells and usually correlates with tumor grade. We suppose that the miRNA down-regulation can allow overexpression of *ADAR1* and *ADAR2* genes. MiRNAs, which hyperexpression is essential for cancer cells, can also silence *ADAR1* and *ADAR2* genes - high-conservative site of miRNA miR-365 along with non-conservative sites of miRNAs miR-27, miR-181 and miR-183 were found in *ADAR1* gene transcript, whereas high-conservative targets of miRNAs miR-150 and miR-181 as well as non-conservative targets of miRNAs miR-18, miR-19, miR-21, miR-23, miR-183, miR-210, miR-373/520 were revealed in *ADAR2* gene transcript. Nevertheless, sites of hyperexpressed miRNAs are less abundant than sites of down-regulated miRNAs, especially in *ADAR1* gene transcript. This circumstance may explain why ADAR1 is the primary contributor to RNA editing in cancer cells.

P.2.2-030
Two group I introns in bacterial flagellin gene and homing endonuclease gene which located downstream of the flagellin gene from thermophilic Geobacillus sp. Kps3

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Bacterial flagellum is a rotary motility device that consists of three parts: a basal body, a hook, and a long helical filament. The flagellar filament is composed of a single protein subunit, flagellin, which is abundantly, expressed when flagellar filament is elongated. The self-splicing group I introns were identified in flagellin genes of thermophilic *Geobacillus* species, which interrupt highly conserved 3′ region. These group I introns can be spliced at wide range temperature. *In vitro* splicing efficiency was increased with temperature, and the most efficient splicing was observed around the optimal growth condition of these thermophiles, 60-70°C. We recently found the presence of two self-splicing group I introns (a and b), expression of two types of flagellin proteins, and formation of the flagellar filaments in the gene encoded flagellin in thermophilic *Geobacillus* sp. Kps3. In addition, free-standing homing endonuclease gene (HEG) was found in downstream of the flagellin gene. Homing endonuclease (HEase) is a restriction enzyme which recognizes non-palindromic DNA sequence (14 – 40 bp). HEG is usually encoded within their group I or group II introns, and rarely located at the outside of the introns as an independent open reading frames (free-standing). HEG play an important role in the beginning of the intron transition (intron homing). It is known that many of the introns are found in archaea and eukaryotes. However bacterial introns are mostly found in non-protein coding genes, such as tRNA or rRNA. We analyzed the purified HEase to elucidate the function in detail. We found that the HEase catalyzes double-strand break of the gene encoded flagellin whose two introns were eliminated. Furthermore, we revealed that cleavage site of the HEase is 5′-TGGCTCAA↓GCCAACCA-3′ which located downstream of the insertion site of intron b.

P.2.2-031
Analysis of RNA editing regulation and function *in vivo*

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Since its discovery, RNA editing has been investigated from different perspectives in many laboratories around the world. A huge amount of information was revealed, however, many basic questions on the function and regulation of RNA editing remain unresolved. For example: 1. It is still unknown if this process is developmentally dependent or tissue specific in human and in *C. elegans*. 2. Who are the regulators of the RNA editing process? 3. Although many editing sites were found, their connection to the phenotypes is still unknown. In this work, we aim to answer some of these questions.

To study RNA editing *in vivo*, we designed a system that enables to visualize when RNA editing occurs, in which tissues and developmental stage. The system includes RNA editing dependent expression of GFP and mCherry. The main goal of this system is to indicate whether the RNA editing process is tissue specific and/or developmental stage dependent. In addition, I will show how we plan to use the system to perform RNAi and mutagenesis screens to find genes that are involved in regulation of RNA editing. ADARs are mainly localized in the nucleus, however there are some indications that they are also localized and perhaps function in the cytoplasm. In this work I will correlate between ADARs localization and RNA editing function. In addition, we will determine if editing sites in non-coding regions such as 3′ UTR affect the target protein expression and functionality using immunohistochemistry and genetic methods. This work can lead to better understanding of RNA editing biological role and functionality.

Mechanisms for protein homeostasis

P.2.3-001
Ubiquitin dynamics during *Drosophila* development

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Ubiquitin is an evolutionarily conserved short polypeptide that, as a posttranslational protein modifier, plays critical roles in most intracellular processes. Ubiquitin modification occurs through the process of ubiquitylation, in which single or multiple ubiquitin

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moieties bind covalently to target proteins. Such ubiquitin attachments are reversed in the process of deubiquitylation. Due to the reversible nature of ubiquitin modification, the ubiquitin pool of the cells is divided into distinct fractions that include free monoubiquitins as well as covalently linked mono- and polyubiquitin-protein conjugates. These ubiquitin forms reach a dynamic intracellular equilibrium in which the availability of free monoubiquitins appears to be essential for normal cell physiology. Precise measurement of the ubiquitin pool and the ratio of free versus conjugated ubiquitin forms is an important step in the study of the ubiquitylation machinery. Recently, a quick, sensitive and simple immunoassay was developed for simultaneous determination of total, as well as free and conjugated ubiquitins in mice from whole protein extracts by densitometric analysis of Western blots. In the assay, endogenous deubiquitinating enzymes (DUBs) present in the lysates process all conjugated ubiquitins to monoubiquitins, therefore the total ubiquitin content of cell lysates can be determined in the form of monoubiquitins. The free monoubiquitin fraction in turn is determined from similar lysates supplemented with a DUB inhibitor. Appropriate samples of these lysates are Western blotted together with ubiquitin standards that permit the quantification of the different ubiquitin fractions by densitometric analysis. After adapting this assay to *Drosophila*, we determined the total, free monoubiquitin and conjugated ubiquitin concentrations in different developmental stages and in various tissues of *Drosophila melanogaster*. Our data demonstrate the highly dynamic nature of the ubiquitin equilibrium.

P.2.3-002

Withdrawn

P.2.3-003
Differential effect of SUMO1 and SUMO3 on PKR localization and activation

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Double-stranded RNA (dsRNA)-dependent protein kinase (PKR), is a serine/threonine kinase that exerts its own phosphorylation and the phosphorylation of the a subunit of the protein synthesis initiation factor eIF-2a. Lysine residues Lys-60, Lys-150, and Lys-440 were previously identified SUMOylation sites in PKR and the triple PKR-SUMO deficient mutant was shown to have reduced PKR activity. There are five SUMO family members, the most studied being SUMO1 and two highly homologous paralogs, SUMO2 and SUMO3. We report that SUMO1 and SUMO3 exert differential effects on endogenous PKR localization and activation. Indeed, SUMO1 expression did not alter PKR localization and resulted alone in PKR and eIF-2α activation with enhanced phosphorylation of PKR and eIF2a upon viral infection or dsRNA transfection, whereas, SUMO3 expression induced the transfer of PKR from the cytoplasm to the nucleus and annihilated their activation. In addition, encephalomyocarditis virus (EMCV) enhanced PKR conjugation to SUMO1 and SUMO3 but only SUMO3 expression accelerated a caspase-dependent EMCV-induced PKR degradation that occurs in the nucleus. Furthermore, the higher EMCV-induced PKR activation by SUMO1 was correlated with an inhibition of EMCV. Importantly, PKR activation by SUMO1 in the absence of viral infection suggests a novel mechanism for PKR activation and the shift of PKR to the nucleus by SUMO3, may shed a new light on the possible nuclear PKR functions independently of its activation.

P.2.3-004
Plant protease phytaspase: overtaking targets of various locations

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A search for protein targets of phytaspase, a plant cell death-related aspartate-specific protease was undertaken. By testing proteins of plant and plant pathogen origin encompassing a putative phytaspase cleavage site for susceptibility to phytaspase-mediated hydrolysis, a set of phytaspase targets was revealed. Due to exclusive specificity of the enzyme, phytaspase was shown to introduce a single or a double break in the target proteins leading to their specific fragmentation/processing. Being synthesized as an inactive precursor protein, phytaspase is constitutively and autocatalytically activated and secreted into the apoplast (intercellular space). However, upon application of programmed cell death-inducing stresses, phytaspase is rapidly relocalized back into the cell. We present evidence that, consistent with this behavior, phytaspase is able to find its targets at various locations: within the secretory pathway, outside the cell, and inside the cell upon the induction of cell death. Furthermore, wounding of plant tissues with concomitant damage of the plasma membrane provides the enzyme with an additional opportunity to get access to intracellular proteins. Accordingly, our data show that phytaspase-mediated protein fragmentation is essential for systemic wound response in plants. We will also discuss a possibility that phytaspase ingested with plant food could find its targets at a quite unexpected place, within the gastrointestinal tract of animals. We show that fragmentation of the phytaspase targets can lead to their inactivation due to detachment of a functionally important domain. However, phytaspase cleavage can cause activation of its targets as well, as exemplified by the phytaspase-mediated processing of precursor proteins of plant peptide hormones. Our study thus suggests that in plants like in animals, cell death proteases may possess multiple functions.

P.2.3-005
Involvement of methyl-DNA binding protein Kaiso in environmental stress response

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Kaiso is a member of BTB/POZ zinc finger family, involved in oncogenesis, cell cycle control, apoptosis and WNT signaling. Previously, we demonstrated that Kaiso repression properties depend on posttranslational modification SUMOylation. Various stress treatments including hypoxic, hyperosmotic etc revealed that only hyperosmotic stress influence on Kaiso deSUMOylation. The purpose of this study was determination of Kaiso involvement in hyperosmotic response *in vivo*, using different knockout models.

First, we found that Kaiso is expressed in distal tubule cells of kidney. Previously determined pattern of Kaiso expression in epidermis, corneal, intestinal, bladder, along with kidney cells implies the possibility of its participation in hyperosmotic response, since all these tissues may contact physiological fluids with high osmolality (Shumskaya et al, 2015). We confirmed involvement of Kaiso in hyperosmotic stress response by salt loading experiments using Kaiso knockout mice. To find

potential Kaiso targets in response to hyperosmotic treatment we obtained Kaiso knockout HEK293 cells via CRISPR/CAS9 genome editing.

Second, it was shown that the hyperosmosis facilitates somatic cell reprogramming by p38 activation that lead to reduction of DNA methylation level (Xu et al, 2013). Using this model system we found enhanced iPSc reprogramming efficiency of Kaiso knockout MEF cells comparing to wild type cells. We demonstrated that the reprogramming of somatic cells under hyperosmotic stress undergoes much more efficiently in the MEF wild type cells, while Kaiso knockout MEF cells were not sensitive to salt addition. Thus, we propose Kaiso novel role in homeostasis regulation *in vivo*.

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P.2.3-006
Hemoglobin conversion to the membrane-bound state by the action of NO donors at carbonyl stress conditions

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Hemoglobin (Hb) in intact erythrocytes can exist in both soluble and membrane-bound states. Hb is usually binding to the membrane through the cytoplasmic domain of so-called trans-membrane protein Band-3. Nitric oxide (NO) can affect Hb-membrane binding. The goal of the work was the comparative study of effects caused by NO donors at the presence of methylglyoxal (MG) modeling carbonyl stress on erythrocytes, including formation of MBHb at these conditions. The new spectrophotometric technique was elaborated for estimating MBHb possible to spot 0.05% of this form. Erythrocytes from heparinisated blood of "Wistar" line rats were used. Addition of $NaNO_2$, Cys-NO and GS-NO to suspension of erythrocytes resulted in changes of MBHb concentration ([MBHb]). $NaNO_2$ gave the largest amplitude of [MBHb] changes what can be result of the formation of free-radical products in the system (oxyHb/$NaNO_2$). MG provoked the dose-dependent increase of [MBHb]. [MBHb] had the negative correlation with the amount of reduced SH-groups of membrane proteins. NO metabolites had different effects on erythrocytes in presence of MG: $NaNO_2$ decreased [MBHb] by 50%, Cys-NO by 20%, but GS-NO insufficiently increased [MBHb]. The effect of nitrosothiols could be explained by the formation of free-radical products in the system (MG/NH3-R/RS-NO) which induced lipid and protein oxidation processes and caused Hb binding with membrane. Hemolytic stability depended on [MBHb]: even small [MBHb] fluctuations resulted in level of erythrocytes hemolysis. Forming of MBHb in definite concentration levels can have physiological significance as a part of adaptive mechanism for changes of internal and external conditions, but strict Hb binding to the membrane (i.e. as result of oxidative processes) destabilizes membrane resulting in hemolysis and Hb withdrawal to vessels.

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P.2.3-007
The many facets of proteostasis remodeling: dietary restriction can regulate proteostasis collapse indepednet of the reproductive system

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Protein folding and clearance networks sense and respond to the expression of misfolded proteins and maintain the health of the organism. However, early in adulthood of *Caenorhabditis elegans*, gonadal-signaling remodels somatic protein homeostasis (proteostasis) with strong consequences to protein quality control functions later in life. Given that environmental conditions, such as food availability and temperature, impact reproductive success, we asked whether proteostasis remodeling would be affected by conditions of dietary restriction and if so, how would the crosstalk between environment and reproduction affect somatic quality control systems? To address this question, we used the *eat-2* genetic model for dietary restriction to examine how modulating food intake affects proteostasis. We found that *eat-2* animals maintained their heat shock response activation and basal proteostasis functions during. Moreover, dietary restriction was also able to improve proteostasis of gonad-less (*gon-2*) or germline-arrested (*glp-1*) mutant animals, and was thus independent of the reproductive system. Comparing the basal and stress activation functions of germline-arrested animals with dietary restricted animals demonstrated improved but district proteostasis capacities that correlated with different protein folding and clearance networks. These data suggest that at the transition to adulthood both environmental conditions and GSC competence are weighed to determine reproductive potential and the mode of somatic proteostasis.

P.2.3-008
Ectopic F1-ATPase is expressed in renal brush-border membranes

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The main organ involved in small protein catabolism is the kidney. Protein reabsorption takes place mainly in the proximal tubules and numerous studies revealed the receptor-mediated endocytosis as the main mechanism responsible for protein retrieval from primary urine. Two receptors megalin and cubilin, assisted by a chaperone protein amnionless, have been shown to cooperate in the proximal tubular reabsorption of filtered proteins. However, there is evidence that cubilin depletion does not inhibit reabsorption of certain ligands, e.g. transferrin and apolipoprotein A-I. Therefore, existence of an alternative mechanism for protein uptake has been postulated. Recently, clathrin-independent endocytosis, involving ectopic F1-ATPase, was identified to be responsible for protein uptake in hepatocytes. Moreover, the protein has been shown to be a high-affinity receptor for some cubilin ligands, including apoA-I and hemoglobin. In the present study we aimed to elucidate whether expression and activity of the receptor can be detected in the apical membrane of the kidney proximal tubule.

Cortical brush-border membranes (BBM) were isolated by magnesium precipitation and differential centrifugation. The preparation consisted of highly enriched plasma membrane vesicles and was free from mitochondrial contamination. Blue native PAGE (BN-PAGE) was used for one-step separation of membrane protein complexes. Gels were either stained for ATPase activity or transferred to PVDF membranes for native immunoblotting.

The study revealed both expression and activity of F1-ATPase in the BBM preparation. The results may be important for further research into molecular mechanisms responsible for the protein uptake in the proximal tubule, and thus for better understanding of pathophysiology of the proteinuric kidney. Functional significance of the receptor expression in the kidney remains to be elucidated in further studies.

Organelle Biogenesis and Dynamics

P.2.4-001
The guided entry of tail-anchored proteins machinery is involved in mis-targeting of mitochondrial proteins to the endoplasmic reticulum

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Tail-anchored (TA) proteins are anchored to their corresponding membrane via a single transmembrane domain (TMD) at their C-terminal region. Therefore, they must be targeted to their final intracellular location by a post-translational process. In *S. cerevisiae*, the targeting of most TA proteins to the ER is mediated by the guided entry of TA proteins (GET) pathway. On the other hand, the targeting process of TA proteins to mitochondria is only partially elucidated. Although the targeting of proteins to the right organelle is tightly controlled, some mitochondrial proteins are mis-targeted to the ER upon their overexpression or when their targeting signal is masked. The mechanism of this erroneous sorting is still unknown. In this study we analysed the potential involvement of the GET machinery in mis-targeting of three mitochondrial outer membrane proteins: (i) the single-span protein Mim1, (ii) the multi-span protein Mcp3, which harbours one TMD very close to the C-terminus and mitochondrial targeting signal at the N-terminal region, and (iii) the mitochondrial isoform of the mammalian protein cytochrome b5. The mis-localization of these proteins as analysed by subcellular fractionation and fluorescence microscopy, was significantly reduced in cells lacking GET components. This suggests that the GET machinery can, in principle, recognizes also mitochondrial factors and non-canonical TA proteins. Under normal conditions, the balance between ER and mitochondrial targeting is towards the last one, but if the mitochondrial pathway is impaired or becomes saturated, the GET machinery has the chance to direct the protein to the ER membrane.

P.2.4-002
Characterization of a de novo heterozygous DNM1L mutation and evaluation of small molecules in patient's fibroblasts

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An emerging class of mitochondrial diseases is characterized by mutations in nuclear genes that participate in the mitochondrial dynamic processes. One of these genes is the *DNM1L* gene, which encodes Drp1 a key component in the mitochondrial fission process. To date, eight studies (including the present) reported an association between several mutations in the *DNM1L* gene and developmental defects. In current study, we aimed to characterize the pathophysiology of a novel *de novo* *DNM1L* c.1084G > A (p.G362S) mutation in a patient with microcephaly and pain insensitivity, to prove its pathogenicity, and explore potentially beneficial small molecules. The patient's fibroblasts displayed decreased complex IV activity, decreased ATP production, impaired growth on galactose, elevated reactive oxygen species (ROS) and abnormal mitochondrial morphology and distribution but normal peroxisomal staining. Overexpression of the mutated gene in normal fibroblasts revealed a markedly aberrant mitochondrial morphology and distribution. We evaluated six small molecule compounds on the patients' fibroblasts observing significant positive effects of bezafibrate, idebenone and resveratrol which led to increased ATP production and growth on galactose, and normalized mitochondrial morphology. The positive effect is, in part, mediated by increased mitochondrial biogenesis. The current study confirmed the pathogenicity of the mutation, and suggests possible therapeutic intervention.

P.2.4-003
Downregulation of the cytoskeletal protein Zyxin leads to activation of stem cell markers in the Xenopus laevis and zebrafish midneurula embryos

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Zyxin is a low-abundant LIM-domain containing protein that binds to alpha-actinin and initiates nucleation and assembly of actin filaments but is also able to enter cell nuclei and interact with proteins involved in the transcription machinery. Due to such ambivalence, Zyxin is a good candidate to be a mediator that couples, cell morphogenetic movements with gene expression, during embryogenesis In the present work we compared by high-throughput sequencing transcriptomes of the axial explants derived from the wild type and anti-Zyxin morpholino injected midneurula stage Xenopus embryos. As a result of the subsequent bioinformatic analysis of the differentially expressed genes, we have established that downregulation of Zyxin functioning leads to suppression of the pathways responsible for the neural and skeletal muscle differentiation, but enhances the expression of POU 5F3 family stem cells markers. By using another model organism, the fish Danio rerio, we demonstrated that Zyxin suppression also leads to the activation of the stem cell markers in the fish embryos.

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P.2.4-004
The nuclear-encoded plant pentatricopeptide protein MEF31 is involved in RNA editing at two near sites in the mitocondrial transcript encoding subunit C of the twin-arginine translocation pathway

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RNA editing is one of the major post-transcriptional RNA maturation events in plant mitochondria and chloroplasts. In flowering plants, about 40 chloroplast and more than 400 mitochondrial Cs are deaminated to Us, most changes occurring in mRNA coding regions and changing their coding potential. A region between −20 or −25 and +6 relative to the C to be edited is generally sufficient and necessary for editing, and the region upstream of the edited C in these *cis* elements is recognized by specificity factors belonging to the largest protein family in angiosperms: the pentatricopeptide repeat (PPR) proteins. Here we describe the novel mitochondrial editing factor 31, an E-PPR protein involved in editing at two sites in the same transcript, which encodes subunit C of the twin-arginine translocation (tat) pathway. MEF31 is required for C581 editing, which changes a Pro CCA codon to a Leu CUA codon, and application of a recently proposed amino acid code for modular RNA recognition by PPR proteins shows that MEF31 likely directly targets the C581 *cis* sequence. Our data also demonstrates that MEF31, although not absolutely required, influences C586 editing. MEF31 probably acts by modifying the C586 *cis* sequence through editing at site C581, increasing its affinity for a putative unknown second PPR protein. A model for MEF31 action on the *tatC* transcript will be presented.

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P.2.4-005
Vesicular elongator regulates axonal transport via a-tubulin acetylation

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The Elongator complex (ELP1-ELP6) regulates distinct cellular processes, including transcription, translation and post-translational modifications. A growing body of evidence supports an emerging role for mutations that disrupt the activity of the Elongator complex role in various pathologies characterized by synaptic defects, ranging from familial dysautonomia, amyotrophic lateral sclerosis, epilepsy, to intellectual disabilities and autism.

Recent findings have shown that loss of Elongator activity correlates with a reduction in a-Tubulin acetylation and that ELP1 co-localizes with synaptic vesicle markers. Here, we aimed to integrate these findings in order to decipher whether vesicular Elongator could control axonal transport. To this end we applied a multidisciplinary approach *in-vitro* and *in-vivo* using biochemical, proteomic, electron microscopy and live imaging analyses of human, mice, and Drosophila models. Our results demonstrate that 1) ELP1 is required for Elongator complex recruitment to axonal vesicles; 2) Elongator transport defects are associated with reduction in microtubules acetylation and could be rescued by inhibition of a-Tubulin deacetylase HDAC6; 3) brain vesicles derived from *Elp3* cKO mice show lower acetylation activity of a-Tubulin in cell free *in-vitro* assay. Altogether these results uncover the mechanism through which Elongator controls axonal transport and places axonal vesicles as predominant drivers of a-tubulin acetylation. *These authors contributed equally to the work

P.2.4-006
Systematic identification and functional characterization of contact site resident proteins in *Saccharomyces cerevisiae*

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Contact sites are areas of close apposition between organelle membranes that enable the transfer of lipids, metabolites and small molecules in an efficient manner. In recent years, several new membrane contact sites have been described and the tethering proteins that hold the two membranes together have been identified. Despite these advances we are still far from having a deep understanding of the function and regulation of most contact sites. To mechanistically characterize a contact site it is essential to know its entire repertoire of resident proteins yet very few proteins that are enriched in any contact site have, to date, been described. To systematically characterize the proteome of contact sites we have decided to utilize a panel of split fluorescence sensors for a diversity of contact sites in *Saccharomyces cerevisiae*. With this sensor one part of a fluorophore is fused to the outer membrane of one organelle while the second is fused to another organelle's membranes. If a contact site is present between both membranes, a fluorescent signal is emitted. We have taken sensors for 15 contacts between several organelles such as mitochondria, peroxisomes, lipid droplets, vacuoles, the plasma membrane and the endoplasmic reticulum, and crossed these split-tagged strains with a novel library of mCherry tagged yeast proteins. By analyzing co-localization events we have discovered a large number of new, previously unappreciated, contact site residents. Following up on these proteins, especially those that are conserved to humans, should

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give us a fresh look at the diversity of functions performed at contact sites and how this affects eukaryotic physiology.

P.2.4-007
Role of mitochondria in inflammatory processes in endothelial cells

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In endothelial cells glycolysis is the major source of ATP. For this reason the role of mitochondria in endothelial cells for a long time were overlooked. Recently it was discovered that mitochondria in endothelial cells, function as sensing organelle of environmental signals to signalling cellular responses. An important modes of mitochondrial signalling in endothelial cells are the regulated production of ROS, autophagy, calcium homeostasis, and apoptosis. It is now clear that mitochondria are important in endothelial physiology and pathophysiology. It is also well documented that inflammation is a key factor that accelerates the onset of atherosclerosis. In our study we have investigated the role of endothelial cells mitochondria in regulation of inflammatory responses, ROS, and NO production. As a model of mitochondrial dysfunction endothelial cells EA.hy 926 modified with 2′,3′-dideoxycytidine (ddC) were used. Inflammation was stimulated with TNF-α in dose and time dependent manner. The ROS and NO synthesis were measured using fluorescence dyes methods. ICAM-1 expression were detected using fluorescence techniques at protein level and real time PCR at mRNA level.

We demonstrated that endothelial cells EA.hy 926 cultured in the presence of ddC were entirely lacking of oxygen consumption on basal level which was not stimulated by FCCP. The level of ROS production was significantly higher in ddC treated cells in comparison to control conditions as measured with DCF and mitoSOX. The inner mitochondrial membrane potential $\Delta\Psi$ was lower in ddC treated cells than in control cells as measured with JC-1 fluorescence method. The TNF-α stimulated ICAM-1 synthesis, as a marker of inflammation, was lower in ddC treated EA.hy 926 cells on protein and mRNA levels.

The results suggest that mitochondria are potent target in modulation of inflammatory processes in endothelial cells.

Study was supported by the National Science Centre, Poland; Grant number: 2015/19/B/NZ3/02302

Integrated Structural Biology for Innovative Translational Research

P.2.5-001
Investigation of quaternary structure of natural killer cell receptor: ligand complexes by super resolution fluorescence microscopy

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Natural killer (NK) cells possess a unique ability to recognize and induce death of tumor and virus-infected cells without a prior antigen sensitization. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell. Human natural killer receptor protein 1 (NKR-P1; gene *klrb1*) and its physiological binding partner lectin-like transcript 1 (LLT1; gene *clec2d*) are representatives of the NK cell receptor C-type lectin-like family. The inhibition of NK cell immune response facilitated by the interaction between NKR-P1 expressed on NK cells and LLT1 on the target cell is regarded as a primary function of these receptors. Previous research showed that this signalization pathway is exploited by glioblastoma tumor cells, which dampen the NK cell immune response by overexpression of LLT1; furthermore, LLT1 was recently suggested as a biomarker for B-cell non-Hodgkin's lymphoma and implicated in other cancerous manifestations. However, besides the NK cells, LLT1 is also present on circulating T and B cells and on antigen presenting cells; and NKR-P1 is also expressed by NKT and T cells. Interestingly NKR-P1 is considered as a marker for all Th17 and some Tc17 cells, and could play a role in the homing and transendothelial migration of these lymphocytes into immunologically privileged sites. Therefore, it is not surprising that relationship between NKR-P1 and LLT1 positive cells is more and more being implicated with diseases connected with immune malfunctions – e.g. multiple sclerosis, rheumatoid arthritis or Crohne's disease. Here we present the results of our efforts to elucidate the biological reality of NKR-P1:LLT1 complex's quaternary structure utilizing insights provided by soluble forms protein crystallography and super resolution fluorescence microscopy of full length receptors on biological membrane.

P.2.5-002
Gaining molecular level information on the cellular copper cycle by EPR spectroscopy

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In the last couple of years, my lab has been exploring the cellular copper cycle in eukaryotic and prokaryotic systems using Electron Paramagnetic Resonance (EPR) spectroscopy.

While most of the proteins involved in the copper cycle are believed to be known, as well as some of the crystal structures, there is still lack of information on the kinetic and the transfer mechanism of the copper in the cellular environment. Since dysfunction of the copper regulation system can lead to neurological diseases and to the cell death, it is essential to understand every little detail in the copper cycle to be able to control it according to specific needs. EPR has become a powerful tool for studying complex dynamic biological systems since it is not limited to the protein size and does not require crystallization. Hence, the biological system can be studied in solution, lipids, and even the cellular environment. Pulsed EPR spectroscopy can provide nanoscale type of information by measuring the dipolar coupling between paramagnetic centers in the range of 2.0–8.0 nm. With the progress of the pulsed EPR methods, several computational programs have been developed to correlate between the distance distribution functions derived from the EPR and the structure of the studied system. In our group, we are applying various EPR measurements together with computations, biochemistry experiments, CD and NMR to identify the copper binding sites, as well as to understand how one protein in the cycle coordinated to another protein to transfer the metal ion. We target the conformational changes that occur in each protein, and we aim to gain also kinetic data on the transfer mechanism. Here, I will present our results regarding the copper cycle from the blood carrier protein, human serum albumin (HSA), through the Ctr1 copper transporter, and to the metallochaperone Atox1.

P.2.5-003
Isolated ABD domain of human glycyl-tRNA synthetase can bind enterovirus IRES

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The studying of viral IRES is of great interest. Cap-independent translation initiation is in the focus of studying, both for the fundamental and applied science. Nevertheless, there is still no clear understanding of the processes occurring in the course of translation initiation on the viral IRES. Due to the fact that for the I type IRES additional regulator factors (ITAF) are necessary the translation initiation mechanism for this type of IRES isn't clear. The full set of ITAF isn't known yet. One of them is a human glycyl-tRNA synthetase (hGARS). It binds specifically to the mRNA in the region of domain V of poliovirus IRES which imitate glycyl-tRNA anticodon hairpin. Such binding leads to significant stimulation of poliovirus mRNA translation directed by IRES.

We suggest that the specific interaction of hGARS with enterovirus IRES can be used as the target for the antiviral drugs developing. The cytoplasmic form of hGARS consists of three domains: WHEP, core and the anticodon binding domain (ABD). It was shown that the protein without the WHEP domain has the same ability to stimulate translation as the full-length enzyme. But the ABD-domain excision leads to the loss of the ability to enhance the translation initiation. To better understand what role the ABD-domain plays in the poliovirus mRNA binding earlier we obtained the isolated ABD-domain. Unfortunately it was very prone to aggregation and doesn't bind viral mRNA. Now we've made a genetic construct of a shortened version of this domain, which should be less aggregated. We've developed a scheme for purification of this protein and checked its ability to form a specific complex with enterovirus IRES fragment. In contrast to the first variant the shortened version of ABD-domain binds enterovirus mRNA. However, the complex should be formed immediately after the protein isolation, since over time it starts to aggregate too.

This work was supported by Russian Scientific Foundation (No.15-14-00028).

Education, Training, and Career Planning in Molecular Life Sciences

P.Edu-001
Sharpening data analysis skills in the next generation of life scientists

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Trees, rivers, and the analysis of next generation sequencing (NGS) data are examples of branching systems so ubiquitous in nature. Indeed, numerous types of NGS applications (i.e., variation detection, ChIP-seq, RNA-seq) share the same initial processing steps (quality control, read manipulation and filtering, mapping, post-mapping thresholding etc.) making up the trunk and main branches of this tree. Each of these main branches subsequently gives off smaller offshoots (variant calling, RNA-, ChIP- and other "seqs"), that, in turn, split further as analyses become focused towards the specific goals of an experiment. As we traverse the tree, the set of established analysis tools becomes increasingly sparse and it is up to an individual researcher to come up with statistical and visualization approaches necessary to reach the leaves (or fruits) representing conclusive, publishable results. In order to do this researchers must be well versed in the model scientific computing approaches, which is an enormous challenge for life scientists. Due to the rapidly increasing volume of biological data from sequencing, imaging, and other technologies, data processing needs in the Life Sciences are now on par with physical, mathematical, and engineering disciplines. Importantly, the distributed nature of data generation in biology makes this situation even more challenging. Today one can hardly find a research institution without multiple high-throughput sequencing machines, and we often hear about a "data crisis" in biology. Yet despite the fact that biology is equal or even superior to physical sciences in the terms of data production capacity it is dramatically lagging behind in terms of data analysis capacity. At galaxyproject.org we are developing new approaches for enabling life scientists to perform complex analyses from raw datasets to publication-ready results. In this presentation I will discuss novel resources for developing data analytics skills in life scientists.

Tuesday 12 September 13:00–15:00

Protein Dynamics and Interactions

P.1.3-001
Domain-swapped dimeric cyanovirin-N with and without disulfide-bridges differ in binding to influenza glycoprotein hemagglutinin

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Specificity and high affinity are usually the prerequisite for a protein to function in an Enzyme-Linked Immunosorbent Assay (ELISA). Consequently, anti-allergenic serum, for example, from mostly adult and aged patients with diminished or no allergic symptoms, can sometimes not be tittered against detectable levels of allergens in blood as these sera are considered to comprise broadly binding, or broadly neutralizing, antibodies.

We monitor binding of antiviral cyanovirin-N (CV-N) to influenza hemagglutinin and Ebola glycoprotein (GP 1,2) by using a dimeric CV-N molecule designed to function with two carbohydrate-binding sites but with a reduced number of natural disulfide bridges. We call these types of lectins "pseudo-immunoglobulin" and hypothesize that CV-N's flexibility in binding versus its conformational stability will broaden the binding to complex and variable high-mannose moieties exposed on enveloped viruses.

Concentration-endpoint titration curves with limitations in quantitative analysis revealed a spectrum of signals reflecting various numbers of binding sites. The lectin is recombinantly expressed in Escherichia coli, designed as domain-swapped dimeric CV-N molecule, and either attached to a protein stabilizing Small-Ubiquitin-like Modifier (SUMO)-tag without expressing disulfide bridges, or with disulfide bridges in the periplasm. Binding signals in the search for computationally designed high-affinity CV-N mutants will even be precise at the maximum difference to structure-based design calculations. Surface Plasmon Resonance and ELISA revealed binding kinetics between domain-swapped dimeric CV-N (with and without disulfide-bridges) to influenza glycoprotein hemagglutinin in the nanoMolar range.

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Regardless of CV-N's given *in vivo* redox-environment structural homologs of similar proteins which stimulate immunoregulatory intracellular anti-inflammatory protein cascades would be interesting to explore?

P.1.3-002
The role of RepoMan in chromosome-dependent microtubule nucleation

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The assembly of a mitotic spindle that makes stable contacts with chromosomes is crucial for accurate cell division. Both the centrosomal and chromosomal pathway of microtubule nucleation contribute to the efficient build-up of the bipolar spindle. The growth of microtubules starts at the γ-tubulin ring complex (γ-TuRC), where γ-tubulin serves as a template for α-/β-tubulin dimers to polymerize. Here, we investigated the role of RepoMan, a chromosome associated protein, in spindle assembly. Using immunofluorescence, we show that a RepoMan knockdown leads to less spread chromosomes in prometaphase. This effect is dependent on the stability of microtubules as shown by nocodazole washout and taxol stabilization assays. This effect is due to the misregulation of the chromosomal pathway of microtubule nucleation since a knockdown of RepoMan leads to a decrease in the size of chromosomal microtubule asters. Using BioID and the newly developed Split-BioID technique, we found that RepoMan interacts with several regulators of spindle stability and α-, β- and γ-tubulin itself. Furthermore, we found that RepoMan co-immunoprecipitates with importin in a RanGTP dependent manner, just like spindle assembly factors such as TPX2, NuSAP1 and Kid. We propose a model where RepoMan regulates the activity or localization of one or more of these newly identified interactors. This can explain RepoMan's stabilizing effect on chromosome-dependent microtubules in prometaphase.

P.1.3-003
Amino acid substitution equivalent to human chorea-acanthocytosis I2771R in yeast Vps13 protein affects its binding to phosphatidylinositol 3-phosphate

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The rare human disorder chorea-acanthocytosis (ChAc) is caused by mutations in hVPS13A gene. The hVps13A protein interacts with actin and regulates the level of phosphatidylinositol 4-phosphate (PI4P) in membranes of neuronal cells. Yeast Vps13 is involved in vacuolar protein transport and, like hVps13A, participates in PI4P metabolism. Vps13 proteins are conserved in eukaryotes, but their molecular function remains unknown. One of the mutations found in ChAc patient causes amino acids substitution I2771R which affects the localization of hVps13A in skeletal muscles. To dissect the mechanism of pathogenesis of I2771R, we created and analyzed a yeast strain carrying the equivalent mutation. Here we show that in yeast, substitution I2749R causes dysfunction of Vps13 protein in endocytosis and vacuolar transport, although the level of the protein is not affected, suggesting loss of function. We also show that Vps13, like hVps13A, influences actin cytoskeleton organization and binds actin in immunoprecipitation experiments. Vps13-I2749R binds actin, but does not function in the actin cytoskeleton organization. Moreover, we show that Vps13 binds phospholipids, especially phosphatidylinositol 3-phosphate (PI3P), via its SHR_BD and APT1 domains. Substitution I2749R attenuates this ability. Finally, the localization of Vps13-GFP is altered when cellular levels of PI3P are decreased indicating its trafficking within the endosomal membrane system. These results suggest that PI3P regulates the functioning of Vps13, both in protein trafficking and actin cytoskeleton organization. Attenuation of PI3P-binding ability in the mutant hVps13A protein may be one of the reasons for its mislocalization and disrupted function in cells of patients suffering from ChAc.

P.1.3-004
A strategy to identify protein interactions in situ specifically and sensitively

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To be in situ, the protein complexes were crosslinked by high concentration formaldehyde for a short time for partial crosslinking.

To be specific, the protein complexes were collected by immunoprecipitation with antibody for targeted protein and the all complexes were separated by SDS-PAGE without de-crosslinking. The gel was then sliced based on molecular weight. Proteins in each slices were identified by mass spectrometry. To make sure the identified proteins were true ligands, the target protein must also be identified in the same slice as its ligands. The ligands must be identified in slices for the experimental group but not in the corresponding control slices. Only proteins that appear in the range of molecular weights equal to or greater than the sum of the proteins' theoretical molecular weights, together with the target, were considered true ligands.

To be sensitive, the SDS-PAGE gel containing protein complexes was sliced without staining. The sensitivity become the sensitivity of mass spectrometry instead of the staining technique.

In this study, using albumin interactome as an example, 5 s of cross-linking with 10% formaldehyde was achieved with human blood. Thirty five ligands for albumin were identified. Comparison with four major previous studies of the albuminome revealed that 68.57% of the 35 ligands identified in our study were also identified in these other studies.

P.1.3-005
Structural characterization of human protein kinase ASK1 and its interaction with thioredoxin

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase (MAPK) cascade, part of the stress-activated intracellular signaling pathways in eukaryotes. The activity of ASK1 depends on various stimuli, including oxidative stress (presence of reactive oxygen species ROS), endoplasmic reticulum (ER) stress, presence of lipopolysaccharide molecules (LPS) or influx of calcium ions. The regulation of ASK1 has a strong influence in pathogenesis of several diseases, the excessive activation of human ASK1 is associated with cardiovascular diseases, neurodegenerative disorders, infectious and inflammatory diseases, tumorigenesis, diabetes.

The activity of ASK1 is regulated by its interaction with several proteins, including two physiological inhibitors, mammalian thioredoxin (TRX) and the 14-3-3 protein. The inactive form of ASK1 is stabilized by complex formation with TRX and 14-3-3, however the explicit mechanism of this interaction and inhibitory effects of these two proteins are unclear due to the absence of structural data. The understanding of ASK1 structure is critical for any intervention into its regulation and the controlled inhibition of ASK1 molecule might be important for a treatment of many different diseases.

We have previously shown that the thioredoxin-binding domain of ASK1 (ASK1-TBD) is a relatively compact rigid and monomeric domain with slightly assymetric shape. It forms with TRX under reducing conditions well defined and stable complex with 1:1 stoichiometry with a large binding interface.

Here we present a structural model of ASK1-TBD in both reduced and oxidized conditions and characterize the interaction of ASK1-TBD with TRX based on sparse NMR data, homology modelling, small-angle x-ray scattering (SAXS) data and high-resolution mass spectrometry.

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P.1.3-006
Functional analysis of a putative ceramide sensor involved in mitochondrial apoptosis

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Ceramides are essential but potentially lethal precursors of sphingolipids. Consequently, cells must monitor their ceramide levels closely to avoid killing themselves during sphingolipid biosynthesis. How this works is poorly understood. We previously identified sphingomyelin synthase-related protein SMSr, an ER-resident ceramide phosphoethanolamine synthase, as critical regulator of ER ceramides and suppressor of ceramide-induced mitochondrial apoptosis in cultured cells. Using RNAi approaches, we showed that SMSr-mediated ceramide homeostasis relies both on the enzyme's catalytic activity and its N-terminal sterile alpha-motif or SAM domain. The importance of SMSr as negative regulator of mitochondrial apoptosis is further emphasized by our recent finding that SMSr itself is a target of the apoptotic machinery and looses its SAM domain upon proteolytic cleavage by caspases. Chemical cross-linking studies and single molecule photo-bleaching analysis revealed that SMSr-SAM drives self-assembly of the enzyme into ER-resident oligomers and that SMSr oligomerization is critical for retaining the enzyme in the ER. To further dissect the mechanism by which SMSr controls ceramide levels in the ER, my ongoing work focuses on: i) defining what cellular signals influence SMSr oligomerization; ii) identification of SMSr binding partners through advanced approaches in membrane proteomics; iii) application of a CRISPR-engineered cell-line in which SMSr can be acutely inactivated upon drug-induced proteolysis.

P.1.3-007
Glycosylation changes of fibrinogen in relation to aging and possible influence on fibrinogen activity

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Fibrinogen is a coagulation factor whose concentration may increase with aging. Aging is often accompanied by structural and functional changes of proteins due to altered glycosylation and/or oxidation. The aim of this study was to investigate changes of fibrinogen isolated from plasma from 70 healthy persons, 21 to 83 years old. Degree of fibrinogen oxidation was determined by measuring corbonyl groups using DNPH method and immunoblotting with anti-DNPH antibody. Glycosylation profile of fibrinogen was analysed using high-throughput lectin-based glycoprotein microarray, employing 15 biotinylated lectins and fluorophore-labelled streptavidin. The activity of fibrinogen was investigated by determining clotting features (clotting time, speed and maximal clot density of fibrin) and reactivity with its binding partner insulin-like growth factor binding protein 1 (IGFBP-1), which acts as wound-healing agent. Results have shown that fibrinogen molecules in healthy older persons are more prone to oxidation, but the change is not significant compared to younger individuals. On the other hand, fibrinogen is additionally glycosylated during ageing and all tested lectins exhibited positive correlation with age. The highest correlation was seen with GSL, NPL, MAL-I, GNL and HHL lectins, specific for N-acetylgalactosamine, N-acetylglucosamine, α-1,3 and α-1,6 mannose residues. Fibrinogen clotting time and maximal clot density of fibrin are also positively correlated with ageing. Reactivity of fibrinogen with IGFBP-1, however, seems to be unaffected by ageing. According to these results, the structure of fibrinogen changes with age, especially glycosylation pattern, but additional experiments are needed to resolve how significant these changes are for the coagulation process.

P.1.3-008
Solution structure of human STARD1 protein and its interaction with fluorescently-labeled cholesterol analogues with different position of the NBD-group

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Intracellular cholesterol transfer to mitochondria, a bottleneck of adrenal and gonadal steroidogenesis, relies on the functioning of the steroidogenic acute regulatory protein (StAR, STARD1), for which many disease-associated mutations have been described. Despite significant progress in the field, the exact mechanism of cholesterol binding and transfer by STARD1 remains debatable, and the solution conformation of STARD1 is insufficiently characterized, partially due to its poor solubility. Although cholesterol binding to STARD1 was widely studied by commercially

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available fluorescent NBD-analogues, the effect of the NBD group position on binding remained unexplored. Here, we analyzed in detail the hydrodynamic properties and solution conformation of STARD1 and its interaction with cholesterol-like steroids bearing 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) group in different position, namely 22-NBD-cholesterol (22NC), 25-NBD-cholesterol (25NC), 20-((NBDamino)-pregn-5-en-3-ol (20NP) and 3-(NBDamino)-cholestane (3NC). The small-angle X-ray scattering (SAXS)-based modeling and docking simulations show that, apart from movements of the flexible Ω1-loop, STARD1 unlikely undergoes significant structural rearrangements proposed earlier as a gating mechanism for cholesterol binding. While being able to stoichiometrically bind 22NC and 20NP with high fluorescence yield and quantitative exhaustion of fluorescence of some protein tryptophans, STARD1 binds 25NC and 3NC with much lower affinity and poor fluorescence yield. In contrast to 3NC, binding of 20NP leads to STARD1 stabilization and increases the NBD fluorescence lifetime. Remarkably, in terms of fluorescence response, 20NP outperforms commonly used 22NC and is recommended for future studies. Our study benefits from state-of-the-art techniques and revisits the results of the STARD1 research over the last 20 years, revealing important novel information.

P.1.3-009
Functional and structural implications of phosphorylated cytochrome *c*

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Cytochrome *c* (C*c*) plays a pleiotropic role of in cell life and death. It undergoes phosphorylation at Thr28, Ser47 and Tyr48 *in vivo* and, indeed, phosphorylation of Tyr48 of C*c* relates to several human diseases. However, its analysis is challenging because the low yield of purification of phosphorylated C*c* from cell extracts. Further, C*c* specific kinases remain unknown, so best approaches resort to mutations that mimic targeted phosphorylation. Here, we have replaced Thr28 and Ser47 by Asp[1]. Additionally, Tyr48 phosphorylation has been mimicked by the non-canonical amino acid *p*-carboxymethyl-*L*-phenylalanine (*p*CMF), which retains essential features of the aromatic residue. Noteworthy, the Y48*p*CMF mutation significantly destabilizes the Fe-Met bond in the ferric form of C*c*, lowering the pK_a value for the alkaline transition of the heme-protein to physiological pH[2]. The negative charges at positions 28 and 48 decrease the midpoint redox potential value by *ca.* 30 and 60 mV, respectively. Moreover, they lower the affinity towards the distal site of cytochrome c_1 in complex III[1,2,3]. In contrast, the phosphomimic variants at positions 28, 47 and 48 donate electrons to cytochrome *c* oxidase more efficiently than the wild-type species. In addition, acidic groups at positions 28, 47 and 48 increase the peroxidase activity of C*c*, associated to the ability of C*c* to leave the mitochondria and reach the cytoplasm. Regarding the role of C*c* in apoptosis, a negative charge at position 48 and any modification of residue 47 hinder its ability to trigger caspase-3 activation[1,2,3]. In summary, phosphorylation of C*c* at particular positions modulates essential functions of the hemeprotein in cell life and death.

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P.1.3-010
Short peptides binding to Fc region of IgG as capturing ligands in affinity chromatography

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In the past 20 years, affinity chromatography based on bacterial immunoglobulin (Ig)-binding proteins such as staphylococcal protein A and streptococcal protein G have been adopted as the capture step of choice by majority of industrial antibody manufacturers despite initial misgivings related to relatively high costs, limited ligand stability at column cleaning and sanitization conditions, and concern over the clearance of leached ligands. Short peptides represent potential alternative affinity ligands to natural immunoglobulin-binding proteins as they display higher stability and are less expensive to produce. Moreover, the expected lower binding affinity for immunoglobulins should allow elution from peptide affinity media under milder conditions, preserving antibody structure and function. The aim of our research was to identify novel short peptide ligands for the Fc region of human IgGs.

We have screened three commercially available phage display libraries of random cyclic and linear peptides for binding to the human Fc region in solution using an optimized biopanning approach. Five selected non-homologous linear peptides were shown to specifically interact with different subclasses of immunoglobulins as verified by a set of phage ELISA assays. Individual phage-displayed peptides were able to recognize specific subclasses of IgG. The highest-affinity peptide (12L-19Fc), which competed for Fc binding with protein A, was subjected to mutagenesis studies. The results revealed the minimal binding motif and the minimal length of the peptide ligand. Binding characteristics of the minimized peptide were further analysed using SPR biosensor. The peptide was immobilized onto a stationary phase to produce affinity matrix and its binding characteristics were evaluated.

P.1.3-011
Erythrocytes ghost receptors interaction with disintegrin investigated by surface acoustic waves technique

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The integrin - mediated binding is important for the metastatic dissemination of different types of cancer cells. Snake venom disintegrins obtustatin and echistatin are potent, irreversible and selective inhibitors of α1β1 and αvβ3 integrins respectively. Aiming to describe the structural requirements of disintegrins for membrane-target recognition, the affinity of these specific binding have to be elucidated, being this topic an issue of extremely importance for the human health. Obtustatin is the shortest disintegrin yet described, containing only 41 amino acids. It contains a similar pattern of cysteines to the short disintegrin echistatin but contains the sequence KTS rather than RGD in its active site loop. To confirm molecular recognition of disintegrins by their substrates, a surface acoustic wave-biosensor was applied. The human erythrocyte ghost cells were immobilized at the sensors to allow for detection of kinetic binding constants of disintegrins compared to GUVs surface. Obtustatin binds to erythrocyte ghost membrane with affinity in mid-nanomolar range (2.32×10^{-7} M), and of echistatin in the low micromolar range,

which clearly indicates specific molecular recognition for both disintegrins, but the higher response for obtustatin. The data provide evidence for a direct confirmation of disintegrin binding to erythrocyte ghost membrane and thus, contribute to prove the presence of integtrins in the red cell membranes earlier neglected.

P.1.3-012
Investigation of the cleavage mechanism and specificity of 2A protease of genetic group B Rhinoviruses

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Rhinoviruses, causing upper respiratory infections, are a major burden to human health. With an average of 2–3 in adults and up to 12 infections in children per year, the common cold has a heavy impact on economy. For immunocompromised people, elderly and other risk groups, upper airway infections can have a severe impact. Nevertheless there is no effective vaccine or treatment against rhinoviruses, which are comprised of nearly 200 genotypes, divided into 3 groups A, B and C. All rhinoviruses encode the 2A protease, which is important for self-processing and protein cleavage to shut down host-translation. Surprisingly, $2A^{pro}$ sequence variation between different types of rhinoviruses is high. In an attempt to investigate differential substrate recognition by picornaviral 2A proteases, we undertake the effort to obtain the first structure of a group B rhinoviral 2A protease. Understanding structure and function of this cysteine protease will push the search for an antiviral drug or vaccine against rhinoviruses.

P.1.3-013
The neuronal S100B cytokine targeting amyloid-β aggregation in Alzheimer's disease

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Insoluble β-amyloid peptide (Aβ) deposits formed in the synaptic cleft and neuroinflammation are consistent features in Alzheimer's disease (AD) and strong candidates for the initiation of the neurodegeneration process. S100B is one of the most abundant pro-inflammatory proteins which is up regulated in AD and is found associated with senile plaques. S100B is a small dimeric protein whose structure and functional regulatory interactions with other proteins are modulated by calcium-binding through EF-hand motifs and by zinc-and copper-binding to the dimer interface. These facts and our recent observation that S100 proteins have intrinsic β-aggregation propensity [1] have prompted us to investigate the impact of S100B on Aβ fibrillation. Here we report the co-aggregation phenomena involving S100B and Aβ and the development of nanobodies targeting S100B. Our studies that combine biochemical, biophysical and structural approaches indicate that S100B is a new key modulator of Aβ42 aggregation. We found that S100B forms a complex with monomeric Aβ as shown by NMR and ITC, delaying the formation of ThT-binding Aβ oligomers, a finding corroborated by TEM imaging. Analysis of Aβ aggregation kinetics and subsequent data fitting elicited quantitatively the mechanisms involved. With this approach, we expect to generate knowledge that will translate into the potential use of S100B as a new druggable target to prevent or ameliorate inflammation in neurodegeneration.

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P.1.3-014
New splice-forms of TDRD7 protein mutated in cataract's and glaucoma's role in mTOR-pathway

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Ribosomal S6 kinases (S6K) are important players in cellular PI3K/mTOR signalling network, deregulation of which has been associated with methabolic disorders, inflammation and cancer. Previously we had identified a novel binding partner of S6K1 – TDRD7 (Trap). TDRD7 is a scaffold protein detected in complexes involved in the regulation of cytoskeleton dynamics, mRNA transport, protein translation, non-coding piRNAs processing, transposons silensing. It was reported recently that mutations in human TDRD7 result in cataract and glaucoma formation, defined by elevated intraocular pressure (IOP) and optic nerve damage.

The aim of current project was to study S6Ks interplay with TDRD7.

Bioinformatical analysis of TDRD7 sequence revealed the presence of potential phosphorylation sites of S6K2. Using *in vitro* kinase assay, we have demonstrated that recombinant S6K2 phosphorylate 3 from 5 fragments of TDRD7. Formation of S6K2-TDRD7 complexes *in vivo* was further confirmed by co-immunoprecipitation using anti-S6K2 and anti-TDRD7 antibodies generated previously in HEK293, HEPG2 and rat brain lysates.

Purified domains of TDRD7 were used as antigens for mouse immunizations and generation of monoclonal antibodies. The generated anti-TDRD7 antibodies allow us to find several new TDRD7 isoforms in HEK293.

Confocal microscopy studies suggest possible co-localization of S6K1/TDRD7 and S6K2/TDRD7 within perinuclear region in HEK293, HEPG2 cells and in soma of primary rat hippocampal neurons. And finally, we have detected that C-terminal synthetic peptides of S6K2 with methylated Arg interfere with TDRD7 from HEPG2 lysates. The physiological characteristics of S6K2-TDRD7 interaction and the role of this complex formation need further investigation.

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P.1.3-015
Mapping binding landscapes of different trypsin/BPTI complexes by experimental and computational methods

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Molecular interactions in protein-protein complexes have been studied for a long time. Yet, it remains unclear how the interplay between these interactions produces such large differences in PPI binding affinities. This study aims at mapping binding landscapes of four homologous complexes between trypsin-like proteases and their inhibitor BPTI. While structurally very similar, the four complexes span more than nine orders of magnitude in binding affinity. To map the binding landscapes for these interactions, we constructed a library of BPTI mutants containing all possible single mutations in the BPTI binding interface. Using the yeast surface display technology, we sorted the BPTI single mutants into four affinity groups when interacting with each of the four target proteases. The BPTI populations having high-, WT-like-, lower- and the lowest-affinity for each protease were collected and sequenced with the next generation sequencing. The frequency of each variant in each population was used to create comprehensive binding landscapes of BPTI interacting with four proteases. The generated binding landscapes correlated well with our computational predictions and with already published binding energy values for some purified BPTI mutants. In each protease/BPTI complex, we saw a particular pattern of hot- spots (i. e. positions where all mutations lead to affinity decrease) and cold-spots (i. e. positions where most mutations lead to affinity increase) and were able to analyze the conservation pattern of these hot and cold spots between homologous complexes. Our results bring invaluable insights on evolution of protein-protein interactions and facilitate design of novel protein-based therapeutic molecules.

P.1.3-016
Thermally stable and thermally sensitive regions of short-chain alcohol dehydrogenases determined by molecular dynamics method

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Protein mechanical rigidity secures stability and high-temperature functionality of thermophilic proteins. In this work, we challenge thermally stable and thermally sensitive regions of short-chain alcohol dehydrogenases (sADHs) adapted to different thermal environments and factors of their thermo stability at different temperatures (300 and 358 K) using molecular dynamics method. Enhanced protein flexibility and high temperature stability can coexist in the apo hyperthermophilic variant (TsAdh319 from archaeon Thermococcus sibiricus with an optimal growth temperature of 358 K, pdb id 3TN7). Here we focus on the holo states of hyperthermophilic TsAdh319 and homologues (TM0441 from Thermotoga maritima with an optimal growth temperature of 353 K, pdb id 1VL8 and SR from Gluconobacter frateurii with an optimal growth temperature of 303 K, pdb id 3AI2). The proteins have 30% sequence identity, share the same catalytic mechanisms and have a highly similar well organized tertiary structure (RMSD between 1.02–1.60 A). To study the dynamics of the TsAdh319, TM0441 and SR, we performed Molecular Dynamics (MD) simulations of 35-ns duration for each sADHs structure. The root mean square fluctuations (RMSFs) were computed for the proteins' Ca atoms from the last 15 ns of the equilibrated MD simulations. As expected, the most conformationally labile parts of the sADHs are found between the secondary structure elements, especially on the solvent-accessible protein surface. The comparison of RMSFs of the atoms in thermophilic sADHs at 300 and 358 K revealed an existence of stable residues at 358 K. This residues form the Nucleus of Rigidity in thermophilic sADHs, which is surrounding the active center. The results of our studies suggest that the existence of the Nucleus of Rigidity is a crucial factor for the stability of TsAdh319.

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P.1.3-017
From magnetosome membrane invagination to transport – the dual role of MamB in magnetosome formation

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Magnetotactic bacteria (MTB) are a unique group of prokaryotes that synthesize specialized organelles, magnetosomes, for magnetic navigation. In the MTB model, *Magnetospirillum gryphiswaldense* MSR-1, magnetosomes consist of membrane-enclosed magnetite (Fe_3O_4) nanoparticles that are aligned into a single coherent chain by dedicated cytoskeletal structures. The formation of these unique organelles is a complex process that comprises several key steps that are governed by magnetosome-associated proteins. One of the most important proteins during this process is the cation diffusion facilitator (CDF) family member, MamB. MamB was shown to be required for magnetosome membrane invagination from the cytoplasmic membrane and was implicated in magnetosome-directed iron transport but its precise role remained elusive. In this study, we employed a multi-disciplinary approach to define MamB's role during magnetosome formation. Using site-directed mutagenesis complemented with structural analyses, fluorescence microscopy, and cryo electron tomography we show for the first time that MamB is an active magnetosome-directed transporter with two distinct, essential functions. First, MamB is required for magnetosome vesicle formation. Second, MamB's transport activity is essential for magnetite nucleation but not required for vesicle formation. Furthermore, we determined the crystallographic structure of MamB cytosolic terminal domain and showed that it shares similarities with the cation diffusion facilitator protein family.

Additionally, we provide evidence that magnetosome vesicle growth and chain formation are independent of magnetite nucleation and magnetic interactions, respectively. Together our data indicate that MamB is an essential bifunctional protein that has two distinct roles in magnetosome formation.

P.1.3-018
A new approach to recurrent pregnancy losses: anti-cardiolipin-specific peptide screening with phage display technique

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Recurrent pregnancy losses (RPL) can be seen for variety of reasons. Antiphospholipid syndrome (APS) which is a thrombosis problem that occurs in the first and second trimesters, causing cell membrane breakdown and unwanted blood clotting, was focused on as one of these reasons. Is estimated that APS is responsible for approximately 10–15% of recurrent miscarriages while causing endometrial blood vessels to clot and fall in early gestation. Anticardiolipin antibody is one of the most studied biomarkers for this syndrome, yet it does not have sufficient safety in ELISA diagnosed method, definite outcome or a significant possibility of disease. Phage display technology is a very powerful method for identifying specific peptides (proteins or antibodies) that target proteins, cells, or any molecule of interest with simply panning-washing-elution steps. In this study, a PhD-12 phage display peptide library will be incubated with immobilized Anticardiolipin antibodies for 5 biopanning cycles, and obtained phage clones will be sequenced to have information on eluted individual peptides. In subsequent steps, binding levels of each eluted phage clones will be characterized by phage tittering, competitive inhibition, ELISA and immunofluorescence microscopy experiments. Selected anticardiolipin antibody specific peptides will be candidate biological marker molecules that can be used for the examination of the possibility of RPL before planning the pregnancy, to prevent possible pregnancy losses and decrease the treatment-diagnosis process, with increased diagnostic quality of the syndrome.

P.1.3-019
Catalytically competent novel 3D structure of phenylalanine ammonia-lyase reveal key insights into ligand binding dynamics

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Aromatic amino acid ammonia-lyases and 2,3-aminomutases contain the post-translationally formed prosthetic 3,5-dihydro-4-methylidene-5H-imidazol-5-one (MIO) group. The so-called MIO-enzymes may be used for the stereoselective synthesis of enantiopure α- or β-amino acids, making these chemical processes more environmentally friendly and more affordable. Even though, a number of structures are available in the PDB for MIO enzymes, the only structure for a eukaryotic phenylalanine ammonia-lyase (PAL) (PDB:1W27) is in catalytically inactive conformation, due to the "loop-out" conformation of catalytically important Tyr-loop. Lack of protein structure in a catalytically competent conformation impeded understanding the PAL mechanism and the key reasons for the difference in lyase and mutase activity. Our recent study characterized novel amino phosphonic acid inhibitors of MIO enzymes. Enzyme kinetic measurements and isothermal titration calorimetry demonstrated that addition of a methylidene substituent to the β-carbon atom of the phosphonic acid analogue of the natural substrate, l-Phe, enhanced binding (K_d = 40 nM) and reversed the enantiopreference of the enzyme. Here we provide an in-depth structural and mechanistic analysis of *Petroselinum crispum* PAL, based on our presently determined crystal structure complexed with the methylidene substituted phosphonic acid inhibitor at 1.5 Å resolution. Our strategy in using the very strong binding, mirror image enantiomer was essential to growing well diffracting crystals. Unlike the currently available crystal structure, in this new structure the catalytically essential Tyr-loop is in a catalytically competent "loop-in" conformation. The high resolution enabled straightforward molecular dynamics studies of the entire tetrameric protein. Random Accelerated Molecular Dynamics investigated ligand binding pathways, and loop dynamics related to the binding process.

P.1.3-020
CEP55 contains two new NEMO-like ubiquitin domains which play different roles in cytokinetic abscission

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The CEP55 protein (Centrosomal protein 55 kDa) critically regulates the final step of cell division termed cytokinetic abscission. Even though the importance of ubiquitin signaling has been previously described in abscission, its precise role is still unknown. We report herein that CEP55 contains two NEMO-like ubiquitin binding domains (UBD) NOA and ZF, which regulate its function in a different manner. *In vitro* studies of isolated domains showed that NOA adopts a dimeric coiled-coil structure, while ZF is based on a UBZ scaffold. Quantitative binding studies pointed out that NOA selectively binds to M1 poly-ubiquitin chains, whilst ZF does not discriminate between mono-ubiquitin, M1, and K63 chains. Based on modeling, we also generated mutants either of NOA or ZF domains unable to bind to ubiquitin. Interestingly, CEP55 knock-downed HeLa cells reconstituted with the full-length CEP55 ubiquitin binding defective mutants - containing mutations either in NOA or ZF domains – display severe abscission defects. Furthermore, while NOA mutants were normally recruited to the abscission site - called midbody-, all ZF ubiquitin binding mutants were not, suggesting that ZF also serves as a new midbody localization domain. Indeed, we showed that GFP-ZF is specifically recruited to the midbody. In addition, this midbody targeting activity cannot be replaced by other ZF-based UBDs such as the UBZs of RAD18, WRNP1, NEMO, NDP52 and the NZFs of TAB2 and A20 (NZF7), indicating that CEP55 ZF is specifically involved in CEP55 midbody localization. In line with this, we also showed that CEP55 endogenous partners

such as MKLP-1, Alix and TSG101 specifically interact with CEP55 in a ZF dependent manner. Our work sheds ligth on an unexpected role of CEP55 in non-degradative ubiquitin signaling during cytokinetic abscission. These results will be also related to two new lethal human diseases in order to provide a molecular basis as to how CEP55 mutations can cause these pathologies.

P.1.3-021
Using yeast cells as a model to study the biogenesis of bacterial secretins

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Beta-barrel proteins are found in the outer membrane (OM) of Gram-negative bacteria, chloroplasts and mitochondria. The assembly of these proteins into the corresponding OM is conserved and in each case facilitated by a dedicated protein complex. Secretins form large, multimeric pore in the OM of Gram-negative bacteria. These pores are part of type II and III secretion systems and are thus crucial for pathogenicity. Recent structural studies indicate that secretins form a structure rich in β-strands. However, little is known about the exact mechanism by which the secretins complexes assemble into the OM.

Based on the conservation of biogenesis of β-barrel proteins, we use yeast as a model system to dissect the stages in the assembly process of secretins. To that end we analyze the biogenesis of PulD (T2SS), SsaC (T3SS) and InvG (T3SS) in wild type cells or in cells mutated for known mitochondrial import and assembly factors. Our initial results suggest that secretins can be expressed in yeast cells, where they are enriched in the mitochondrial fraction. Interestingly, deletion of mitochondrial import factor affect to a various extent the assembly of secretins into the mitochondrial OM. The contribution of these findings to our understanding of the membrane integration process of secretins will be discussed.

P.1.3-022
Molecular assembly of archaeal peroxiredoxin

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Peroxiredoxins (Prxs) are thiol peroxidases that reduce hydrogen peroxide and alkyl peroxides to water and to the corresponding alcohols, respectively; they are found in organisms belonging to all biological kingdoms. Prx has a variety of quaternary structures, involving monomers, dimers, decamers, and dodecamers. Prx from *Pyrococcus horikoshii* (PhPrx) is a decameric protein formed by ring-type assembly of five dimers. To engineer the quaternary structure of PhPrx, we created a mutant PhPrx (PhPrx6m) by introducing six point mutations designed to dissociate PhPrx into dimers. Although PhPrx6m was a dimer in solution, the six dimers assembled into a dodecamer following crystallization. In the crystal structure, PhPrx6m was overoxidized, and the peroxidatic cysteine was in sulfonic acid form and two cysteines in the C-terminal region were linked by an intramolecular disulfide bond. Thus, we characterized the wild-type PhPrx overoxidized by hydrogen peroxide (PhPrxPer). Analytical ultracentrifugation showed that PhPrxPer had a higher molecular mass in solution than PhPrx. This was confirmed by analysis of the crystal structure of PhPrxPer, which was found to form a ring-type dodecamer composed of six dimers. The monomeric structures of PhPrx6m and PhPrxPer differed from that of PhPrx in the relative orientation of two domains, reflecting the number of dimers in the ring-type assembly. Unlike PhPrx, homologous peroxiredoxin from *Aeropyrum pernix* (ApPrx) did not undergo hexameric association. This property can be explained by the stronger connection between the two domains in ApPrx due to its C-terminal extension relative to PhPrx.

P.1.3-023
Aberrant cytoplasmic localization of wild-type nucleophosmin due to association with its leukemia-related mutants is not prevented by C21F mutation targeting the oligomerization domain

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Mutation-induced aberrant cytoplasmic localization of nucleolar phosphoprotein nucleophosmin (NPM) likely contributes to NPM immunogenicity in acute myeloid leukemia (AML) cancer cells. NPM localization is highly affected by the mutation subtype, in particular by the presence/absence of tryptophan W288, which is crucial for NPM nucleolar localization, and by the type of acquired NES sequence at the C-terminus of the resulting mutated protein. We have constructed two groups of AML-related NPM mutants (NPMmut): mut A, B and Nm represent the abundant mutation types lacking the W288 and acquiring weak NES motif L-xxx-V-xx-V-x-L whereas rare mutations E and H retain the W288 and carry a strong NES motif L-xxx-L/F-xx-V-x-L. Plasmids with genes for fluorescent protein fused to NPMmut variants were transfected into HEK-293T cells and localization of fluorescing NPM variants was analyzed by confocal microscopy. Accordingly to the two categories, mutation types A, B and Nm showed mostly cytoplasmic localization whereas types E and H localized in both the cytoplasm and the nucleoli. However, all the mutation subtypes drove wild-type NPM (NPMwt) into the cytoplasm due to heterooligomer formation. Co-immunoprecipitation confirmed the interaction between the endogenous NPMwt and the fluorescent protein-labeled NPMmut, irrespective of the mutation subtype. Surprisingly, targeting the oligomerization by C21F substitution, formerly reported to abolish the NPM oligomerization, did not lower the cytoplasmic fraction of the NPMwt. Moreover, the presence of endogenous NPM in GFP-precipitate of cells transfected with GFP-labeled C21F-NPM mutant proved retained interaction potential of C21F-NPM. In conclusion, all the tested NPM mutation subtypes were able to mislocalize the NPMwt and the interaction between NPMwt and NPMmut persisted after C21F mutation introduction.

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P.1.3-024
Designer inhibitors with single MMP specificity

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In nature, families of enzymes are frequently regulated by broad inhibitors that can bind to all family members with similar affinities. However, for biomedical applications, it is important to design inhibitors that target only one family member among many. Therefore, utilizing a broad natural inhibitor as a scaffold for design of an inhibitor with single specificity is a challenging task due to the high structural and sequence similarity among targets. In principle, single-target specific inhibitors could be generated either through computational design or through directed evolution. We explore both approaches to convert a broad matrix metalloproteinase (MMP) inhibitor, the N-terminal domain of tissue inhibitor of metalloproteinases 2 (N-TIMP2) into a specific inhibitor of either MMP-9 or MMP-14. In one approach, we used computational methods to design a small N-TIMP2 library with mutations focused to the binding interface, while in another approach, we introduced random mutations throughout the N-TIMP2 gene. In two parallel experiments using yeast surface display, we selected N-TIMP2 mutants with higher binding specificity towards MMP-9 or MMP-14. After three rounds of selections, we expressed six N-TIMP2 variants and measured their target and off-target affinities. Our results show that both random mutagenesis and computationally designed libraries could produce N-TIMP2 mutants with enhanced specificity towards MMP-14 relative to MMP-9 and vice versa. Yet, computational design is slightly better at improving the overall binding specificity of N-TIMP2 when tested against a panel of MMPs. Our results provide new insights regarding evolution of promiscuous proteins and define the best strategies for the design of inhibitors with single-target specificities.

P.1.3-025
Structural determinants of dimerization specificity of Zinc-finger-associated domain

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Zinc-finger-associated domain (ZAD) is dimeric zinc-coordinating domain widely present within Arthropodan zinc-finger transcription factors. Besides zinc-ion-coordinating cysteines these domains possess very low sequence similarity. To study the possibility of formation of heterodimers we used multiple sequence alignment and selected a few clusters of ZAD-proteins with consensus homology over 45% which obviously recently evolved as a result of gene duplication. Cross-dimerization within clusters was studied using yeast two-hybrid assay. Heterodimerization was observed in 6 out of 9 clusters. Crystal structure is known for one of heterodimerizing ZAD-domain – Grauzone, and another structure of CG2712 ZAD-domain, which is unable to form heterodimers with its homologue Zeste-white 5, was recently solved by our group. Close examination of conserved residues within each pair lead us to conclusion that most specificity restraints resulted from the interaction between zinc-coordinating module and C-terminal part of alpha-2 helix. This was further confirmed by swapping experiments where this helix was completely or partially substituted by the same helix from homologous protein. In the case of heterodimerizing proteins (Grauzone and CG15073) most amino-acid substitutions between them along dimerization interface obviously do not result in a loss of existing or acquiring of new molecular contacts. On the opposite within homodimerizing pair there are several substitutions resulting in loss of some contacts and formation of new hydrophobic bonds. Introduced single amino acid mutations did not result in change of binding specificity. Therefore it seems likely that heterodimerization is still possible in this case but thermodynamically is much less favorable than homodimerization.

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P.1.3-026
Identification of core cellulolytic enzymes from Talaromyces cellulolyticus strain S6-25

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The cellulose-degrading fungus strain *Talaromyces cellulolyticus* (formerly *Acremonium cellulolyticus*) Y-94 and derivatives of Y-94 with higher cellulase productivity, designated strains TN and C-1, represent the best-characterized cellulase-producing members of the genus *Talaromyces*. Six cellulolytic enzymes critical for the hydrolysis of lignocellulosic biomass from strain CF-2612, derivative of C-1, were recently identified: two endoglucanases (Cel5A and Cel7B), two cellobiohydrolases CBHII (Cel6A) and CBHI (Cel7A) that attack the nonreducing or reducing end of a cellulose chain, β-glucosidase (Bgl3A), and xylanase (Xyl10A). In this work, the core cellulolytic enzymes composition of strain S6-25, TN derivative, was studied. It was shown that cellulose system of S6-25 had the same cellobiohydrolases, β-glucosidase, xylanase and one endoglucanase (Cel5A) as those for strain CF-2612, but instead of Cel7B, S6-25 cells expressed another endoglucanase (Cel5C). Two cellobiohydrolases, Cel7A and Cel6A, represent about 60% (w/w) of secreted proteins and are the main enzymes of cellulase complex. The RT-qPCR analysis of chromosomal *cel6A, cel7A, cel3A, cel5A and cel5C* genes copy number in Y94 and S6-25 strains showed that increasing of activity of cellulase complex in S6-25 strain at least partially may be explain by appearance of *cel7A* additional copy in cellular genome. The carboxymethyl cellulose (CMC) degrading activities of Cel5A and Cel5C were analyzed using CMC with average MW 700,000 and MW 90,000 (Sigma-Aldrich, USA). The reduced sugars released were analyzed using the DNS assay. The specific activity of Cel5C in comparison with Cel5A was about the same on CMC (MW 90,000), but about three-fold lower on CMC (MW 700,000) as a substrate. So, the high cellulase yield (FPU/g carbohydrate) and specific cellulase activity of strain S6-25 may be connected with increased Cel7A expression as well as variation in substrate specificity of expressed endoglucanases.

P.1.3-027
Fungal bioluminescence system: luciferin, luciferase and luciferin biosynthesis

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Many living organisms emit light, a phenomenon named bioluminescence. There are estimated to exist ~40 different chemical mechanisms underlying the generation of "cold light". The energy required for light production is generated by the oxidation of a small organic molecule, luciferin, catalyzed by a specific enzyme, luciferase.

More than 100 species of bioluminescent higher fungi are known. The international research group led by the speaker reported elucidation of fungal luciferin in 2015. In 2016 the same team identified and cloned fungal luciferase and the enzymes of luciferin biosynthesis. Discussed will be structure elucidation of fungal luciferin, cloning of fungal bioluminescnce enzymes, light emission mechanism and perspectives of practical applications of fungal bioluminescence.

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P.1.3-028
The photosystem II subunit S dynamics under stress

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The increased spectral range absorption of light exerted by pigments within Light Harvesting Complexes (LHCs) proves an important advantage under low light conditions, in higher plants. However, in the exposure to excess light, oxidative damages and ultimately cell death can occur. It proved, thus, utmost important for the photosynthetic organisms to develop a down-regulatory mechanism called Non-Photochemical Quenching (NPQ). Quantifying this mechanism at the atomic level is still very uncertain. There are several components of the photosynthetic apparatus that are actively involved in NPQ. Apart from the LHCs, and the xanthophyll cycle, the Photosystem II Subunit S (PsbS) is a 22-kDa integral membrane protein that is essential for the response of the photosynthetic apparatus to high-light and it is activated by the protonation of key lumen-exposed glutamate residues. Atomistic details on its involvement in NPQ remain still a mystery. However, It is widely accepted that NPQ (qE) is co-regulated by low lumen pH and ion fluxes (K^+, Ca^{2+}, Mg^{2+}, Cl^-) in Lumen-Stroma areas. It has also been proposed that the activated PsbS may strongly interact with some LHCs enabling quenching by providing an alternative environment for some pigments within these LHCs, or by changing the membrane organization and dynamics. In this study, PsbS (pdb code 4ri2) is embedded in a lipid bilayer model membrane (400–500 POPC lipids, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). NPQ conditions are simulated by perturbations in the thylakoid lumen ionic load. Zeaxanthin (Zea) of the xanthophyll cycle that is produced under NPQ is also embedded in the membrane. We employ large-scale Molecular Dynamics simulations to probe the PsbS conformational changes, membrane dynamics, or Zea binding that activate PsbS. We identify two distinct PsbS forms (active-inactive), in response to A) the lumen acidification or ion fluxes, and B) the Zea binding, revealing a PsbS-NPQ relation at the atomic scale.

P.1.3-029
Single-molecule spectroscopy of protein-DNA interactions

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Competence in *Bacillus subtilis* is the ability to uptake foreign DNA from the extracellular space. The initiation of this process is a prime example for stochastically driven phenotype switching caused by gene expression noise. The interaction between the transcription factor ComK and its promoter is key for this switch, but the origin of the stochasticity remained elusive. Here, we use single-molecule spectroscopy to study the interaction between the transcription factor ComK and its promoter DNA. Our results show that the structural changes in the promoter during ComK-binding include local rearrangements that are more complex than a simplistic bending model. In contrast to earlier reports, we observe nanomolar affinities (~20 nM) with a cooperativity of binding that varies drastically along the promoter sequence. Our results suggest that at least 10 ComK-molecules bind to its promoter sequence. Together with the estimated *in vivo* copy number of ComK (~100 proteins/cell) this suggests the formation of only a few ComK-DNA complexes per cell. Such a low number of transcription factor-DNA complexes is common for gene expression noise, which may provide an explanation for the stochastic entry into competence.

P.1.3-030
Characterization and effects of binding of food-derived bioactive phycocyanobilin to bovine serum albumin

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Microalga *Arthrospira platensis* (Spirulina) is the next big superfood thanks to composition and the numerous health-related benefits. Phycocyanobilin (PCB) is a blue tetrapyrrole chromophore of C-phycocyanin, the main protein of the microalga Spirulina, with numerous proven benefits for human health. Bovine serum albumin (BSA) is blood and food protein capable to bind various bioactive ligands. In this study, we examined and characterized the binding of PCB to BSA and how it affects protein and ligand stability or activity under physiological conditions. Protein fluorescence quenching and microscale thermophoresis results have shown high-affinity binding ($K_a = 2 \times 10^6$/M). Spectroscopic titration experiment with molecular docking analysis revealed two binding sites on BSA for PCB at the inter-domain cleft and at the subdomain IB, while CD spectroscopy indicated the stereo-selective binding of *P* conformer of pigment to protein. In contrast, previous studies have found binding of *M* conformer of PCB to Human Serum Albumin (HSA) at subdomains IB and IIA. Although HSA and BSA have high sequence similarity, subtle differences between the tertiary structures of the two albumins is the most likely explanation for the partial divergence in the binding location of the tetrapyrrole ligand. BSA-PCB complex has increased thermal stability than free protein. Gel electrophoresis data from pepsin digestion study suggest that saturated PCB binding slightly increases digestive stability of protein. Although complex formation partly masked the antioxidant properties of PCB and BSA, a mutually

protective effect against free radical-induced oxidation was found. These results point to subtle differences between PCB binding for bovine *vs.* human serum albumin and that Spirulina health supplements consumption with BSA-containing foods could change bioavailability and bioactivities of participating molecules.

P.1.3-031
Kinetics of aggregation of phosphorylase kinase from rabbit skeletal muscle

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Phosphorylase kinase (PhK; EC 2.7.1.38) plays a key role in the regulation of glycogen metabolism in skeletal muscle. PhK with molecular mass of 1320 kDa has a complex molecular organization. The PhK molecule consists of four subunits that form a hexadecamer $(\alpha\beta\gamma\delta)_4$, where the γ-subunit possesses the catalytic activity and other subunits regulate its activity. Ca^{2+} and Mg^{2+} ions stimulate PhK activity by inducing changes in the tertiary and quaternary structure of the molecule and also stimulate association/aggregation of PhK hexadecamer molecules. Ca^{2+}-free PhK and PhK molecules in the presence of Ca^{2+} and Mg2+ ions have different conformations and physicochemical properties. The kinetics of association/aggregation of PhK from rabbit skeletal muscle was studied at 40°C, close to the average physiological temperature of the rabbit, using dynamic light scattering (40 mM Hepes, pH 6.8; 0.1 mM Ca^{2+}, 10 mM Mg^{2+}, 0.1 M NaCl). The initial rate of aggregation was calculated from the kinetic curves describing an increase in the light scattering intensity with time. Based on the analysis of the initial rate of aggregation, depending on the initial concentration of the protein, it has been concluded that the order of aggregation with respect to protein is equal to unity. Thus, the rate-limiting stage of heat-induced aggregation of PhK under used conditions is the stage of unfolding of the protein molecule. Construction of a plot showing the relationship between the light scattering intensity and hydrodynamic radius (R_h) of protein aggregates indicates that the initial stage of PhK aggregation is the stage of formation of the start aggregates. It was shown that the hydrodynamic radius of start aggregates formed at PhK concentration of 0.1 mg/mL is 29 nm and increased to 56 nm at [PhK] = 0.8 mg/mL.

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P.1.3-032
Negative charge and membrane tethered viral 3B cooperate to recruit viral RNA dependent RNA polymerase 3Dpol

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RNA dependent RNA polymerases (RdRp) are key enzymes for +RNA viruses. These enzymes are not present in human genome which makes viral RdRp an ideal target for drug design. Therefore, RdRps are highly studied enzymes, however, their regulation is not fully understood. Most +RNA viruses replicate at replication organelles (ROs); membranous structures derived from the host intracellular membranes (Golgi or ER most often). These ROs provide microenvironment needed for efficient viral replication and also provide shelter from innate intracellular immunity while serving as a platform for viral replication. The lipid hallmark of the RO membrane is phosphatidylinositol 4-phosphate (PI4P). However, +RNA viruses do not possess any phosphatidylinositol 4-kinase (PI4K), instead they hijack the human enzyme PI4K. Many picornaviruses use the Golgi resident acyl-CoA-binding domain-containing protein-3 (ACBD3) to hijack the lipid kinase PI4KB. Using pure recombinant proteins and biomimetic model membranes we show that the nonstructural viral 3A protein is sufficient to induce membrane hyperphosphorylation given the proper intracellular cofactors PI4KB and ACBD3. However, our bio-mimetic *in vitro* reconstitution revealed that not PI4P but rather the negative charge is responsible for the recruitment of RdRp enzyme to the viral replication sites. Additionally, we show that membrane tethered 3B protein cooperates with the negative charge to increase the efficiency of RdRp membrane recruitment.

P.1.3-033
Interaction of the N-terminal extension of myosin essential light chain-1 with F-actin studied by fluorescence resonance energy transfer

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Rabbit fast skeletal muscle myosin has two isoforms of the essential light chain (ELC), called LC1 and LC3. The LC1 differs from LC3 by the presence of N-terminal extension of 41 residues containing seven pairs of Ala-Pro repeats, which form an elongated structure, and two pairs of Lys residues near the N-terminus. When isolated myosin head (myosin subfragment 1, S1) binds to F-actin, these Lys residues may interact with the C-terminus of actin thus forming an additional actin-binding site on S1. Here we applied fluorescence resonance energy transfer to measure for the first time the distances between Cys374 on actin and different sites on the N-terminal extension of LC1 associated with S1. Cys374 of actin was labeled with 1,5-IAEDANS as a donor, and S1 was reconstituted with various recombinant LC1 mutants which were fluorescently labeled with 5-IAF (acceptor) at different positions in their N-terminal extension and then introduced into the S1 regulatory domain. At physiological ionic strength (120–150 mM NaCl) and S1:actin molar ration equal to 1:3 (i.e. under conditions when the LC1 N-terminal extension interacts with actin), the following distances were calculated between Cys374 on actin and different sites on the N-terminal extension of LC1: >6 nm to Cys41, 4–5 nm to Cys15 located among Ala-Pro repeats, and 3–4 nm to Cys residues located near Lys residues at the N-terminus. At higher ionic strength (above 300 mM NaCl) and S1:actin molar ration equal to 1:1 (i.e. under conditions preventing interaction of the LC1 N-terminus with actin) all these distances significantly increased. These results are consistent with the previously proposed concepts and also reveal new interesting details of the molecular mechanism of the interaction of LC1 N-terminal extension with actin, which may play an important role in actin-myosin interaction during muscle contraction.

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P.1.3-034
Regulation of cell migration by Arpin binding to the Arp2/3 complex and Tankyrase

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Actin nucleation plays a key role in cell migration. The Arp2/3 complex nucleates the branching of actin at the leading edge of the moving cell to form lamellipodia. Recently, Arpin was identified as an inhibitory protein of the Arp2/3 complex. Arpin's C-terminal acidic domain is homologous to the A-domain of nucleation promoting factors (NPF), thereby it is thought that Arpin inhibits the Arp2/3 complex by competing with the NPF. Recently, by immunoprecipitation and mass-spectrometry, new Arpin and Arp2/3 complex binding partners, Tankyrases 1 and 2 (TNKS1/TNKS2) were identified. TNKS binding sites for Arpin and Arp2/3 complex partially overlap, suggesting a possible competition between these biochemical entities. TNKS are therapeutic targets in a variety of cancers, but there is currently no structural model of these proteins, mainly due to their flexibility. Here we generated the stable cell line expressing the mutant form of Arpin that does not bind TNKS, but binds the Arp2/3 complex. We have analyzed and compared the directional persistence of cells, expressing wild type and mutant Arpin. Interestingly, cells expressing mutant Arpin have longer cell trajectories, compared to the cells, expressing wild type Arpin. Additionally, by co-immunoprecipitation we demonstrated that binding of Arpin to the Arp2/3 complex *in vivo* requires TNKS binding. To understand the mechanism of Arpin-TNKS interactions we used single particle electron microscopy. We have purified TNKS1 using affinity chromatography. Purified proteins were applied to a carbon-coated grid, contrasted using 1% aquatic solution of UA, and investigated in a JEOL 2100 electron microscope. We derived the reconstructions of full-length TNKS1 and TNKS1 bound to Arpin. Nanogold labeling was used to identify the N-terminal part of TNKS. The obtained data suggests that TNKS may work as an intermediate carrier helping Arpin to bind and inhibit the Arp2/3 complex.

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P.1.3-035
Structural and biophysical characterization of the PI4KB:14-3-3 protein complex

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Phosphatidylinositol 4-kinase IIIβ (PI4KB) produces the Golgi pool of phosphatidylinositol 4-phosphate (PI4P). PI4P serves as a signaling molecule, and, additionally, as a precursor for higher phosphoinositides. PI4KB is a soluble cytoplasmic enzyme with no membrane binding properties and is recruited to the membranes by the Golgi resident ACBD3 protein. However, the regulation of PI4KB activity is not understood in detail. One of PI4KB interaction partners are 14-3-3 proteins. 14-3-3 proteins bind PI4KB upon its phosphorylation by protein kinase D, however, the structural basis of PI4KB regulation by 14-3-3 proteins is unknown. Here, we characterized the PI4KB:14-3-3 protein complex biophysically and structurally. We discovered that the PI4KB:14-3-3 protein complex is tight and is formed with 2:2 stoichiometry. Our structural analysis revealed that the PI4KB:14-3-3 protein complex is flexible but mostly within the disordered regions connecting the 14-3-3 binding site of the PI4KB with the rest of the PI4KB enzyme. We observed, that the *in vitro* measured enzymatic activity of PI4KB is not directly modulated by 14-3-3 proteins. However, 14-3-3 proteins protect PI4KB from degradation. The biological role of binding of 14-3-3 proteins to PI4KB is not clear, but we hypothesize that 14-3-3 proteins regulate PI4P production via stabilization of PI4KB in the intracellular environment.

P.1.3-036
Structural basis for vinyl sulfone inhibition of the SmCB1 drug target from the human blood fluke

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Schistosomiasis caused by parasitic blood flukes of the genus *Schistosoma* afflicts over 200 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a gut-associated peptidase that digests host blood proteins as a source of nutrients. In our recent work we demonstrated that SmCB1 is a drug target for vinyl sulfone peptidomimetic inhibitors. Now we performed a detailed analysis with a unique set of 30 vinyl sulfone derivatives with diverse substituents. The inhibitors were screened *in vitro* against recombinant SmCB1 and ex vivo against *S. mansoni*. Two most effective inhibitors in terms of IC_{50} values and parasite suppression were complexed with SmCB1, and high resolution crystal structures were determined. Analysis of 3D structures and inhibition profiling identify key binding interactions and provide insight into SmCB1 inhibition specificity. Our work provides a footing for the rational design of anti-schistosomal chemotherapeutics.

P.1.3-037
Mitochondrial protein import: a mutagenesis approach to studying the structural and functional properties of Tim50

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The mitochondrion is an essential organelle functioning in various pathways in the eukaryotic cell from energy production, metabolism of amino acids and lipids, assembly of iron sulphur clusters, to the regulation of apoptosis. Throughout evolution mitochondrial genes were shifted to the nuclear genome. Therefore, despite the fact that the mitochondrion contains its own circular DNA and ribosomes, most (99%) mitochondrial proteins are encoded by the nucleus. These proteins are translated on cytosolic ribosomes and are sorted via large complexes serving as translocases and insertases to one of the four mitochondrial compartments: outer membrane, inner membrane, intermembrane space and the matrix. Our research focuses on the TIM23

complex that mediates import of proteins targeted to the matrix and some of the inner membrane and intermembrane space proteins. Tim50 is an essential subunit of this complex which serves as a receptor that recognizes the precursor and transfers it to Tim23 which forms the import channel. Both proteins expose conserved domains into the intermembrane space that interact with each other. This Tim23-Tim50 interaction is essential for proper protein import across the mitochondrial inner membrane. The objective of this work is to elucidate the molecular mechanism of function of the yeast TIM23 complex with emphasis on the role of Tim50. To this end, we generated a random Tim50 mutant library that enabled us to screen for yeast exhibiting temperature-sensitive growth, due to the fact that defective mitochondrial protein import is reflected usually in a temperature-sensitive phenotype. By using this approach, we identified specific residues that are essential for the function of Tim50. Our results show that mutating these residues results in temperature-sensitive growth and accumulation of mitochondrial precursor proteins *in vivo*. The identity of the components that interact with the identified residues are under intense investigation.

P.1.3-038
Recombinant expression of natural killer cell activating immunocomplex NKp80:AICL and its structural characterisation

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Natural killer cells (NK cells) play one of the key roles in our immune system. They have ability to eliminate infected, stressed of malignantly transformed cells. Recognition of the target cell is promoted by surface NK cell receptors. NKp80 and its myeloid-specific activating ligand AICL are members of C-type lectin-like structural family with C-type lectin-like domain (CTLD). Immunocomplex NKp80:AICL is involved in lysis of malignant myeloid cells as well as in crosstalk between NK cells and monocytes and takes part in stimulation of immune response during early stage of inflammation.

This immunocomplex is an interesting target for therapeutic use of NK cell cytotoxicity. Activation of NK cells through AICL:HER2-scFv fusion construct recognizing HER2 tumor antigen, while displaying AICL, as NK cell activating ligand, on tumor surface, was described recently. All these applications are limited with the lack of structural data, because structure of this immunocomplex is still unknown. Further structural description of NKp80:AICL interaction interface might be helpful for design of novel immunotherapeutics.

Recombinant expression of NKp80 and AICL ectodomain was optimized. We used mammalian expression system of modified human embryonic kidney cell line 293 (HEK293) to produce glycosylated proteins in inducible way. Sequence of AICL ectodomain was modified, respecting CTLD disulfide motive, which means that odd cysteine was mutated to serine. Yield and stability of prepared protein is greatly enhancer compared to wild-type construct. Immunocomplex was analysed using analytical ultracentrifuge and mass spectrometry. Amount of protein was sufficient for initial crystallization trials.

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P.1.3-039
The effects of membrane composition on the dimerization of transmembrane domains of receptor tyrosine kinase

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Structural studies of membrane proteins are of great importance and interest, with solution and solid-state NMR spectroscopy being very promising tools for that task. One of the most widely used and prospective type of membrane mimetics for this purpose is isotropic lipid-detergent bicelles. While large anisotropic bicelles are well-studied, it is generally unclear, whether the small particles could adequately mimic the properties of lipid bilayer. Additionally, in order to be sure that NMR structures of transmembrane proteins have any biological relevance, it is necessary to understand, how these structures are affected by the changed properties of membrane mimetics. In the present work, we addressed several issues regarding the validity of bicelles analyzing the influence of bicelle size and detergent type on the energy and manner of dimerization for ErbB4 transmembrane domain. Our data supports the membrane mimetic propensity of small isotropic bicelles and their general correspondence to the lipid bilayer. Next, we aimed to investigate the possible signaling impact of the lipid composition around the transmembrane domains of various cell receptors. There is a number of hypotheses implying the potential active role of cell membrane in the signal transduction process by receptor tyrosine kinases, and here we investigated the effects of membrane thickness and saturation of the lipid fatty acid tails in bilayer on the free energy and spatial structure of ErbB4 transmembrane domain dimer. We show that membrane properties affect the stability of certain dimeric conformations, which indicates the potential ability of membrane to cause the switching between active and inactive states of the receptor.

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P.1.3-040
'Divide and conquer' approach for structural NMR studies of voltage-gated sodium channels. Mapping of binding interface with gating-modifier toxin

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Voltage-gated Na^+ channels are essential for the functioning of cardiovascular, muscular, and nervous systems. The α-subunit of eukaryotic Na+ channel consists of ~2000 amino acid residues and encloses 24 transmembrane (TM) helices, which form five membrane domains: four voltage-sensing (VSD) and one pore domain. This complexity significantly impedes structural studies of full-sized Na^+ channels. The modular organization gave an idea for studying of the isolated VSDs of human skeletal muscle Nav1.4 channel. The isolated VSD-I and VSD-II (~150 a.a., four TM helices, S1-S4) were produced by cell-free expression. The secondary structure and dynamics of VSD-II was studied by NMR in LPPG micelles. The secondary structure of VSD-II showed similarity with the bacterial Na^+ channels. The fragment

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of S4 helix between the first and second conserved Arg residues probably adopts 3/10-helical conformation. ^{15}N-relaxation data revealed characteristic pattern of μs-ms time scale motions in the VSD-II regions sharing expected interhelical contacts. VSD-II demonstrated enhanced mobility at ps-ns time scale as compared to isolated VSDs of K+ channels.

The binding interfaces in the complex of VSD-I with spider gating-modifier toxin Hm-3 were mapped by NMR data in the micellar environment. The β-hairpin of Hm-3 peripherally associates with the S3 helix and S3-S4 extracellular loop of VSD-I. The complex is stabilized by electrostatic interactions of toxin Lys residues with negatively charged groups of VSD-I and by hydrophobic interactions. Two different hydrophobic interfaces on the Hm-3 surface are responsible for the interactions with the membrane and VSD-I. The proposed 'divide and conquer' approach could significantly facilitate structure-function studies of Na+ channels.

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P.1.3-041
Induction of fibrillogenesis of a number of biologically active peptides in the presence of triazavirin

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At present, triazavirin (sodium salt of 2-methylthio-6-nitro-1,2,4-triazolo [5,1-c] -1,2,4-triazin-7 (4H) -one, (TZV)), a synthetic analogue of guanine, is actively being investigated as a new antiviral drug. While searching for possible TZV targets in humans, a feature was observed, namely the ability to induce fibrillogenesis of a specific peptide (beta-amyloid N-terminal fragment analogue, or Aβ (1-20)). These structures (fibrils) caused Alzheimer's disease. The present work focused on the search for any potential adverse drug events, and on elucidating the mechanisms behind the self-organization of these peptides into ordered structures. Additionally, melittin (Mel) peptide was chosen for study, due to its ability to self-associate. For studying the binding of TZV with Mel and Aβ (1-20), mass spectrometry (Matrix Assisted Laser Desorption/Ionization, MALDI) with the inclusion of ^{18}O-containing standards was used. Molecular modeling (with application of the docking procedure) was performed (Molsoft ICM-Pro version 3.8-3), and transmission electron microscopy, utilizing negative contrast, was also carried out. The MS-MALDI studies demonstrated that, in the presence of TZV, Mel and Aβ (1-20) form associates, which lead to peptide precipitation. Subsequent analysis of the sedimented components and their ratios, carried out with MS-MALDI, showed the formation of TZV- Mel and TZV-Aβ (1-20) complexes. Modeling of the complexation (TZV-Mel and TZV-Aβ (1-20)) made it possible to establish two key interactions: terminal phenylalanines form the stacking interaction with the aromatic component of TZV; and adjacent (positively charged) Arg (in the case of Mel) and Lys (in the case of Aβ (1-20)) form strong hydrogen bonds with TZV's nitro group. These mechanisms (stacking and hydrogen bonds) play the main role in the ligand's ability to bind with its target. Electron microscopy showed that peptide associates have fibrillar structures.

P.1.3-042
In vitro evaluation of cytotoxic and anti-inflammatory activities of Romanian Balta-Alba mud extracts

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The use of mud extract contributes to a long term stability of therapeutic effects, thus avoiding common inconveniences of conventional drugs, like installation of therapeutic resistance and adverse effects. Cytotoxicity testing was performed *in vitro* using ATCC-CRL-9855 cell cultures and MTS (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega). The anti-inflammatory action was evaluated by cytokine measurements using Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel kit and analyzed using Luminex 200 system (Luminex Corp., TX, USA). We focused to establish if these 13 mud extracts have cytotoxic effects (MTS assay) and to what extent. The extracts were provided by Pell-Amar Cosmetics as spray-dried powders. For this purpose, we used different concentrations - ranging 3 to 75 mM, considering an "average" MW of 90 for extracts, at different cell densities (5000/10,000 cells/well) and incubation times (48/72 h). For 10,000 cells incubated for 72 h – IC50 were 247 mM for sample 1, 386 mM for sample 3, 410 mM for sample 5 and 373 mM for sample 7. For 5000 cells at 72 h – IC50 were 440 mM for both samples 3 and 5. IC50 could not be calculated for 48 h exposure, although a dose-effect relation could be observed. Our results indicated the relatively low-cytotoxic effects of the mud extract analyzed. The mud extracts were demonstrated to modulate cytokine release, generating profiles that are characteristic to anti-inflammatory activities (incresed level of IL-10; $P < 0.05$) and decrease of pro-inflammatory cytokines release (IL-1beta, IL-4, IL-6 and IL-8; $P < 0.01$) with statistical significance. Using a combination of *in vitro* assays, mud extracts could be classified and ranked for their cytotoxiciy and specific activity, providing an effective screening system for the discovery of potential therapeutic compounds.

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P.1.3-043
GroEL chaperonin interaction with denatured protein chains: the role of electrostatic forces and GroEL ligands

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An interaction with polypeptides lacking rigid spatial structure is a well-known property of GroEL (Hsp60) chaperonin. Here, we demonstrate that the interaction of GroEL with such polypeptides is provided by both hydrophobic and electrostatic forces, and it depends on chaperonin ligands (ATP, ADP, and GroES). A number of fluorescently labeled native and denatured protein

chains were tested for interaction with GroEL by polarized fluorescence, size-exclusion chromatography, native electrophoresis, and affinity chromatography on the basis of denatured proteins. The results allow the following conclusions. Firstly, interaction of GroEL with negatively charged polypeptides requires the presence of either bivalent cations (Mg^{2+} or Ca^{2+}) or medium ionic strength (~200 mM), while positively charged polypeptides interact with GroEL at a low ionic strength even in the native state. Secondly, GroEL ligands (ADP and ATP) reduce chaperonin affinity for denatured polypeptides, while the co-chaperonin GroES practically expels denatured proteins from their complexes with GroEL. Thirdly, the lifetime of the complex (GroEL-denatured protein) in the presence of GroES is evaluated to be less than 6 s, which is much less than the time of ATP-mediated GroEL/ES working cycle. Furthermore, it has been shown that GroES competes with the denatured protein (malate dehydrogenase) for binding to GroEL.

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P.1.3-044
The fluorescence spectroscopy study of the interaction between proCaspase-2 and the 14-3-3 protein

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Apoptosis is a process of programmed cell death that maintain number of cells in tissues. Caspases were identified to play a crucial role in apoptotic pathways and they are named due to their specific cysteine protease activity. Our research is focused on human procaspase-2 and its interaction with the 14-3-3 protein which was described in *Xenopus laevis*. The sufficient NADPH level induces phosphorylation of caspase-2 and 14-3-3 protein binding prevents procaspase-2 maturation and as a result inhibiting the apoptosis. The nutrient depletion promotes the 14-3-3 protein release and caspase-2 activation. Procaspase-2 as an inactive proenzyme consists of 3 domains: CARD, p18 and p12. CARD and p18 are connected with linker containing phosphorylated Ser^{139} and Ser^{164}. Our recent research confirmed the phosphorylation dependent interaction of procaspase-2 with the 14-3-3 protein. In current study we tested conformational behavior of procaspase-2 and its changes upon complex formation with 14-3-3 using time resolved fluorescence measurements. Four procaspase-2 mutants and WT containing single tryptophan residue were prepared at positions 151, 188, 218 and 426, to sample various regions of procaspase-2. Values of mean fluorescence lifetimes clearly show the different vicinity in individual mutants after the 14-3-3 binding with exception of Trp^{188}. Data obtained from fluorescence anisotropy determined Trp^{151} and Trp^{426} with flexible vicinity with significant restriction after 14-3-3 binding. On the other hand area around Trp^{188} and Trp^{218} has been shown rigid.

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P.1.3-045
Comparative modeling and molecular dynamics studies of the oligopeptidase B from *S. proteamaculans*. Insights into the enzymatic activation mechanism

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The Oligopeptidase B (OpdB; EC 3.4.21.83) is a trypsin-like peptidase from prolyl oligopeptidase family (clan SC, family S9). A characteristic feature of the family is a seven-bladed β-propeller N-terminal domain that impedes penetration of large globular proteins into the active center located in a cavity at the interface with C-terminal catalytic domain. The known OpdB enzymes from both parasitic protozoa and infectious bacteria are important virulence factors of the corresponding infections and can be promising targets for therapeutic treatment upon development of their selective inhibitors by structure-based rational design. Only two protozoan OpdBs' crystal structures have been obtained, namely from *L. major* and *T. brucei*. These 2 structures demonstrate the importance of the interface between the catalytic and propeller domains for enzymatic activity and stability of the OpdBs. The interface is stabilized by hydrogen bonds as well as salt bridges and one of the latter plays a key role in transition of the enzyme from an open (inactive) to closed (active) form. This important element in the molecular architecture of protozoan OpdBs is not conserved in proteobacterial OpdBs including the peptidase from *S. proteamaculans* (PSP), which is the object of our study. To elucidate the mechanism underlying PSP activation we have created 3D models of both forms of PSP. The molecular dynamics simulation of the models allowed identifying of charged amino acid residues participating in the formation of stable interdomain contacts important either for the enzyme activation or stabilization, which was further confirmed by site-directed mutagenesis. Proposed mechanism of PSP activation may be useful for both modulation of enzymatic features of bacterial OpdBs and development of their small-molecule inhibitors.

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P.1.3-046
Structural basis underlying different stability and DNA-binding properties of two mycoplasmal HU-proteins

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The histone-like DNA-binding (HU) protein is one of the major nucleoid-associated proteins involved in DNA supercoiling and compaction into bacterial nucleoid as well as in all DNA-dependent transactions including replication, recombination, repair and transcription. This small positively charged dimeric proteins bind DNA in a non-sequence specific manner promoting DNA superstructure. HU proteins are absent from eukaryotic cells and are therefore promising pharmacological targets for the development of antibacterial drugs. In the present study two isotope-labeled recombinant HU proteins from mycoplasmas *S. melliferum* (HUSpm) and *M. gallisepticum* (HUMgal) were produced, and their structures in free and DNA-bound forms studied by nuclear magnetic resonance spectroscopy (NMR). Using comparative modeling and molecular dynamic simulation with distance and dihedral constraints derived from triple-resonance NMR data of the $^{13}C/^{15}N$-labeled recombinant proteins we obtained all-atom models of HUMgal and HUSpm and described their backbone flexibility. We have found that while both proteins fold into dimeric conformation typical for HU proteins, the structure of HUMgal is very labile, especially in the absence of DNA, whereas HUSpm structure is more rigid. This finding may explain differences in the thermal denaturation patterns we observed in differential scanning calorimetry experiments performed for both proteins. Moreover, the differences in backbone flexibility of the two studied proteins affect their ability to interact with various DNA structures, which we suppose to reflect the complex and pleiotropic role of HU proteins in bacterial cells. Obtained molecular dynamics models of HU proteins may be applied in studies of DNA-protein interaction as well as in designing of small-molecule HU inhibitors.

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P.1.3-047
A change in the aggregation pathway of bovine serum albumin in the presence of chemical chaperones

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Chemical chaperones are widely used by biochemists working on the design of agents, which are able to efficiently suppress protein aggregation. To elucidate the mechanism of suppression of protein aggregation by polyamines (putrescine, spermidine), arginine and its derivatives (arginine amide and arginine ethyl ester), we used test systems differing in the kinetic regime of the aggregation process, namely the test systems based on dithiothreitol (DTT)-induced aggregation of bovine serum albumin (BSA) at 45 °C (0.1 M Na-phosphate buffer, pH 7.0; [DTT] = 2 mM) and thermal aggregation of BSA at 70 °C (0.1 M Na-phosphate buffer, pH 7.0). In the case of DTT-induced aggregation of BSA the rate-limiting stage of the overall aggregation process is that of unfolding of the protein molecule (the order of aggregation with respect to protein is equal to unity). In the case of heat-induced aggregation of BSA the rate-limiting stage is that of aggregation of unfolded protein molecules (the order of aggregation with respect to protein is equal to 2). The kinetics of BSA aggregation was monitored by dynamic light scattering and asymmetric flow field-flow fractionation. To characterize the effect of chemical chaperones under study on thermostability of BSA, differential scanning calorimetry was used. On the basis of the obtained data the mechanism of DTT-induced and heat-induced aggregation of BSA in the presence of polyamines, arginine and its derivatives has been proposed. It is assumed that the chemical chaperones under study stabilize the native form of protein, however stimulate the sticking of existing aggregates. To prove this mechanism, the plots of the hydrodynamic radius of protein aggregation versus the portion of aggregated protein have been constructed.

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P.1.3-048
Interplay between protein kinetic stability and thermal flexibility

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Kinetic stability is a key parameter to comprehend protein behavior and it plays a central role to understand how evolution has reached the balance between function and stability in cell-relevant timescales. Protein flexibility is a widely used concept to understand many aspects of protein behavior. However, what should be understood by the statement that a protein is more or less flexible than another?. In this work, we propose a new concept regarding protein flexibility, namely thermal flexibility, which refers to the change of residue displacements due to a temperature gradient. As such, thermal flexibility quantitatively measures the increment of the size of the conformational space available to the protein when energy in provided. Using an approach that includes simulations, protein engineering, and calorimetry, we show that there is a clear correlation between kinetic stability determined by differential scanning calorimetry and protein thermal flexibility obtained from a novel method based on temperature-induced unfolding molecular dynamics simulations. Thermal flexibility quantitatively measures the increment of the conformational space available to the protein when energy in provided. The $(\beta/\alpha)8$ barrel fold of two closely related by evolution triosephosphate isomerases from two trypanosomes are used as model systems. The kinetic stability-thermal flexibility correlation has predictive power for the studied proteins, suggesting that the strategy and methodology discussed here might be applied to other proteins in biotechnological developments, evolutionary studies, and the design of protein based therapeutics.

P.1.3-049

Withdrawn

P.1.3-050

The role of intrinsically disordered fragments of human transcription factors YY1 and YY2 in recognizing specific DNA sequences

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Yin Yang 1 (YY1) is an ubiquitously expressed and conserved transcription factor, named after variety of functions it performs. The protein consists of a regulatory domain localized to the N-terminus and a C-terminal DNA binding domain (DBD), which is composed of four C2H2-type zinc fingers. Unusually, YY1 can act either as repressor, activator or initiator of transcription, depending on DNA sequence or cellular context. YY1 regulates various promoters including viral and protooncogenic genes.

The N-terminal fragment of human transcription factor YY1 was recently confirmed to be intrinsically disordered [1] and although it does not interact directly with DNA, it influences the affinity of DBD to recognized sequences [2].

The aim of our ongoing research is to compare the properties of YY1 and its retoposon YY2. Little is still known about both structure and function of the latter [3]. Partial similarity between YY1 and YY2 provides opportunity to investigate details of their structure and mechanism of interaction with DNA. Here we present various spectroscopic studies of YY1 and YY2 structures and their interaction with specific DNA sequences.

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P.1.3-051

Peculiarities of the RNA interactions of the human heterodimeric Ku protein

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Heterodimer protein Ku consists of two subunits Ku70 and Ku80. It is a DNA-binding protein showing a high affinity to DNA ends, which plays a key role in the DNA double-strand break repair by non-homologous end-joining mechanism (NHEJ). In addition, its role was shown in other cellular processes, i.e. transcription, telomere maintenance, V(D)J-recombination and some others. Ku was identified as a participant of HIV-1 replication at the stages of integration and/or transcription. There are only some shreds of evidence of the human Ku protein interaction with RNA: Ku binds to telomerase RNA, HIV-1 TAR RNA, a hairpin at the 5′-end of p53 mRNA. We developed a simple method for Ku heterodimer expression in the prokaryotic system and performed a systematic *in vitro* analysis of Ku/RNA binding using gel-shift assay with this recombinant human Ku protein and synthetic RNAs. We identified RNA structural determinants important for interactions with the Ku protein. Ku was not found to interact with a perfect linear double-stranded RNA and weakly interacts with a duplex RNA containing a bulge. The highest Ku affinity was detected toward a hairpin RNA structure containing an extensive single-stranded region or a bulk bulge just near the loop. A double-stranded DNA displaces RNA ligands from the Ku/RNA complexes, and this fact indicates a common binding site for the double-stranded DNA and hairpin RNA binding. Interestingly, Ku tightly binds to the second loop of nuclear non-coding 7SK RNA (7SK-L2), which is an essential cellular transcription regulator participating in particular in HIV-1 transcription. The 7SK-L2 structure resembles the structure of HIV-1 TAR RNA, and Ku interacts with both RNAs with a comparable affinity. These interactions might be important for understanding the mechanism of Ku influence on transcription of cellular and HIV genes.

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P.1.3-052

Detection of pair-wise intermolecular interactions in large protein complexes using NMR tr-NOE and specific methyl labeling of HIV-1 gp120 in complex with a CCR5 peptide

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The interaction between HIV-1 envelope glycoprotein gp120 and the CCR5 chemokine receptor is crucial for viral entry thus serving as an ideal target for entry inhibitors. The molecular details of gp120 interactions with the N-terminal segment of CCR5 (Nt-CCR5) are still unknown mostly due to the lack of experimentally derived data on pair-wise interactions. NMR is in a unique position to provide such information even from such weakly bound complexes. We have reported the use of transferred NOE (Tr-NOE) experiment for the detection of pair-wise interactions between a mostly dimeric gp120 complexed with a CD4 mimic peptide (~110 kDa) with a 27-residue peptide representing the N-terminal segment of CCR5 (Nt-CCR5) exhibiting fast-exchange. Intermolecular interactions were identified using asymmetric deuteration. Here we report another strategy which uses Tr-NOE and ^{13}C edited NOSEY. The high molecular weight and the fact that gp120 is glycosylated and can only be expressed in mammalian or insect cells possess significant challenges. Specific methyl labeling has revolutionized NMR of large proteins by applying specific ^{13}C protonated methyl on a fully deuterated background. Here we incorporate into gp120 deuterated methionine residues in which the methyl moiety is ^{13}C labeled and protonated. The other amino acids are unlabeled. Using methyl-TROSY HMQC we have recorded an ^{1}H-^{13}C correlation spectrum showing all expected methionine residues with good signal to noise ratio and linewidth. This is attributed to the high SNR for methyls, the TROSY effect and the partial deuteration of Met residues. Specific changes in chemical shifts can be detected upon the addition of Nt-CCR5 to gp120 complexed with a CD4-mimic peptide. Future studies will incorporate a third NOSEY dimension that will allow the detection of intermolecular interactions with Nt-CCR5.

P.1.3-053
Characterizing the protein-protein interactions between SARS corona virus and its cellular receptor and its neutralizing antibody

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In early 2000s', a previously unknown corona virus was isolated from patients with severe pneumonia, and was recognized as the infectious agent. The newly emerged disease has been named Severe Acute Respiratory Syndrome (SARS). Subsequently, the new virus has been named SARS coronavirus (SARS-CoV). Similarly to many viruses of the Coronaviridae family, the infection mechanism of SARS-CoV is mediated by a Spike (S) protein – a transmembranal glycoprotein on the surface of the viral envelope. This protein has two functional domains: S1 and S2. S1 mediates viral interaction with a cellular receptor; it was demonstrated that angiotensin-converting enzyme 2 (ACE2) of a target human cell efficiently binds the S1 subunit of the SARS-CoV S protein, allowing viral infection. 80R Antibody is a high affinity recombinant human monoclonal neutralizing antibody (nAb) that was isolated from convalescent individuals. This nAb is directed against the S protein of SARS Co-V. It inhibits viral infection by competing with ACE2 for association with S1 domain of the S protein, and binding S1 with higher affinity. Both ACE2 and 80R bind the same motif within the Receptor Binding Domain (RBD) of S1 as can be concluded from the crystal structures of the two complexes. In spite their overlapping contact area with RBD, there are several RBD mutants reported to lose binding to one of the proteins whilst maintaining contact with the other. In order to understand what stabilizes these interactions, we employed Molecular Dynamics (MD) simulations. The simulations used crystal structures of both RBD-ACE2 and RBD-80R complexes, as well as complexes with an in-silico generated RBD D480A mutant. Each complex was simulated in all-atom resolution with three repeats of 200 ns. In the present study we will show the main interacting residues at the interface and the evaluation of the stability of the complexes as was computed by a "Strength of Interaction" scoring function developed in our lab.

P.1.3-054
The basis for NleD selectivity on its MAP kinase substrates

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Mitogen-Activated Protein kinases are a Ser/Thr family, divided mainly into three groups: p38, JNK and ERK. They are conserved in all eukaryotes and activated by dual phosphorylation on the TXY motif located on their activation loop. Type III secretion system (T3SS) is a molecular syringe used by pathogenic bacteria to deliver "effector" proteins directly into the host cytoplasm. One of these effectors is NleD: a Zn^{+2}-dependent protease which cleaves non-phosphorylated forms of p38 and JNK at their TXY motif, but fails to cleave ERK. In this study, we examined molecular characteristics that dictate NleD selectivity. Swapping the activation loops between ERK2, JNK2 and p38α resulted in cleavage of all variants indicating that there are factors other than the loop composition that permit NleD activity. Consequently, we have examined the role of structural determinants in the three-dimensional vicinity of the cleavage site, considering regions that are similar in JNK2 and p38α and differ in ERK2 producing several ERK2 variants in these specific regions, yet none of them were cleaved by NleD. Structural studies of p38α and ERK2 variants showed an intriguing trend. In this context, the swapped activation loops exhibited high flexibility which can be correlated to the cleavability by NleD. Conversely, one of the ERK2 mutants at the G-helix region exhibited a defined conformation of the activation loop and was not cleaved. One can thus conclude that the NleD activity is dependent on the flexibility of the cleavage site and required the substrate induced fit to obtain the active conformation. In cases where the activation loop has a rigid conformation (ERK2 and activated forms of MAP kinase) NleD is unable to promote the enzymatic reaction. Our results demonstrate a case in which a pathogen utilizes the distinction between similar proteins by exploiting cell response for its purposes.

P.1.3-055
Proteins evolve on the edge of supramolecular self-assembly

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In cells, over one third of proteins self-associate with multiple copies of themselves to form symmetric homomers. These protein complexes entail unique geometric and functional properties, underlining the virtue of symmetry in proteins. Yet, symmetry can also pose a risk. In sickle cell disease, the symmetry of hemoglobin exacerbates the effect of a mutation, resulting in harmful fibril formation. Here we assessed the universality of this Achilles heel by determining how readily mutations can induce homomers to further self-assemble. We predicted that mutations solely increasing surface hydrophobicity could frequently induce *de novo* intermolecular interactions driving polymerization. We investigated twelve distinct homomers and, remarkably, we observed their polymerization in all cases, with seven forming micrometer-long fibrils *in vivo*. Biophysical measurements and electron microscopy indicated that mutants self-assembled in their folded states. Though surface mutations are often regarded as benign due to their minimal impact on protein stability, we exposed their dramatic potential to trigger *de novo* interactions and polymerization when compounded by symmetry. Accordingly, an analysis of all symmetric proteins of known structure revealed strategically placed charged residues at sensitive surfaces patches, suggesting a mechanism for protection against misassembly in these regions. The potential of symmetric proteins to polymerize upon mutation is thus a general mechanism by which protein fibrils can form *in vivo*, is a target of negative selection, and can be exploited in protein design and nanotechnology.

P.1.3-056
Chimeric human mitochondrial PheRS exhibits editing activity to discriminate nonprotein amino acids

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Mitochondria are considered as the primary source of reactive oxygen species (ROS) in nearly all eukaryotic cells during respiration. The harmful effects of these compounds range from direct neurotoxicity to incorporation into proteins producing aberrant molecules with multiple physiological problems. Phenylalanine exposure to ROS produces multiple oxidized isomers: tyrosine, Levodopa, ortho-Tyr, meta-Tyr (m-Tyr), and so on. Cytosolic phenylalanyl-tRNA synthetase (PheRS) exerts control over the translation accuracy, hydrolyzing misacylated products, while monomeric mitochondrial PheRS lacks the editing activity. Recently we showed that "teamwork" of cytosolic and mitochondrial PheRSs cannot prevent incorporation of m-Tyr and L-Dopa into proteins. Here, we present human mitochondrial chimeric PheRS with implanted editing module taken from EcPheRS. The monomeric mitochondrial chimera possesses editing activity, while in bacterial and cytosolic PheRSs this type of activity was detected for the (ab)2 architecture only. The fusion protein catalyzes aminoacylation of tRNAPhe with cognate phenylalanine and effectively hydrolyzes the noncognate aminoacyl-tRNAs: Tyr-tRNAPhe and m-Tyr-tRNAPhe.

P.1.3-057
Effects of various mutations in α- or β-chains of tropomyosin on structural and functional properties of its αβ-heterodimers

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Tropomyosin (Tpm) is an actin-binding coiled-coil protein that plays a key role in the regulation of contraction of skeletal and cardiac muscles. Fast skeletal muscles express two Tpm isoforms, α and β, which are the products of different genes. As a result, dimeric Tpm molecules are usually represented by αα-homodimers and αβ-heterodimers (ββ-homodimers are unstable and therefore occur very rarely). The study of Tpm αβ-heterodimers is particularly important because various pathologies of skeletal and cardiac muscles are accompanied by an increase in the expression of Tpm β-chains, which causes an elevation in the content of αβ-heterodimers. It has been proposed that Tpm αβ-heterodimers can essentially differ from αα- and ββ-homodimers in their structure and functional properties. However, by now all works involving recombinant Tpm preparations (in particular, all studies of the effects of various mutations in the Tpm molecule on its structure and properties) have been performed exclusively with αα- and ββ-homodimers because preparation of recombinant Tpm αβ-heterodimers is a very difficult task. We applied different methods (CD, DSC, co-sedimentation with actin, *in vitro* motility assay, etc.) to investigate the effects of various mutations in α- or β-chains of Tpm (namely, stabilizing substitutions G126R, D137L and G126R/D137L in the α-chain or myopathic mutations R133W and N202K in the β-chain) on the structure and properties of Tpm αβ-heterodimers and to compare them with the effects of these mutations on the properties of αα- and ββ-homodimers. The results showed that the properties of Tpm αβ-heterodimers with substitutions in α- or β-chain can substantially differ from those of Tpm homodimers with the same substitutions in both chains. Thus, our results clearly indicate that the effects of myopathic mutations in the Tpm β-chain should be studied only on the Tpm αβ-heterodimers.

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P.1.3-058
A new non-canonical pathway of Gαq protein regulating mitochondrial trafficking in hippocampal neurons

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A novel localization of heterotrimeric G proteins at the mitochondria and their implications on the physiology of the organelle has been recently reported. In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane dynamics. Together with Gβ (that binds to Mfn1), Gαq stabilizes elongated mitochondria and cristae structure. Gq is also necessary for the maintenance mitochondrial membrane potential and the activity of the respiratory chain and mitochondrial ATP synthesis. Surprisingly, Gαq is necessary for the supercomplex assembly at the inner membrane. The molecular mechanism of action of heterotrimeric G proteins at the mitochondria is still unknown. A recent MS-proteomic analysis has helped us to decipher the Gq-interactome ("Gq-mitoproteome"). We have utilized mitochondrial enriched fractions from four different cell lines, among them the Gq/11-MEF knockout, the recover Gαq-MEF-knockout, MEFs wild type and NIH3T3 cells, as well as, two different anti-Gq antibodies. The new candidate binding proteins are being analyzed by their capacity to interact to Gαq. Among the candidates we found Armadillo-like-domain containing protein Armcx3, also known as Alex3. This mitochondrial outer-membrane protein is involved in the regulation of mitochondrial aggregation and trafficking, among other processes. IP studies showed that the constutitive-active mutant of Gq, GqR183C, interacts specifically with Alex3 as well as the mitochondrial Rho-GTPase Miro1, one of the main regulators of mitochondrial transport in neurons. In summary, our group postulates a new non-canonical mitochondria-function of heterotrimeric G proteins that involves their translocation to the mitochondria and the interaction with several mitochondrial partners.

P.1.3-059
Novel method of quantification of protein interactions in living cells

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Nowadays, life-science researchers utilize plenty of methods aiming in quantification of protein-protein interactions. These methods range from simple biochemical experiments, through molecular biology methods, towards advanced proteomic analysis. In this way, myriads of valuable data for biology, medicine and pharmacology were provided. However, majority of experiments was performed on fixed cells or extracted proteins. Therefore, detailed information about *in vivo* dynamics of protein-protein interactions is still missing, but substantially needed. We present a fluorescence correlation spectroscopy (FCS) method of protein oligomerization analysis. As a protein of interest, we chose dynamin-related protein 1 (Drp1) which is involved in mitochondrial fission process. Our method base on precise determination of length-scale dependent hydrodynamic drag of

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cytoplasm. It was proved, that cytoplasmic hydrodynamic drag (d_h, also interpreted as viscosity) depends on a probe's size. Therefore, first step of our research was determination of diffusion coefficients (D_{diff}) of probes of known sizes (GFP, Calcein-AM, dextrans) in cytoplasm of HeLa cells. These results were utilized for evaluation of a scaling equation, and, subsequently, for determination of D_{diff} expected for certain oligomers of Drp1. Next, D_{diff} of GFP-fused Drp1 was measured by FCS in HeLa. Different Drp1 mutants were investigated (monomer, dimer, wild type). Results indicate that there is an equilibrium between dimeric and tetrameric form of wild type Drp1 in cytoplasm. Length-scale dependence of d_h enabled separation of D_{diff} of these two forms (D_{diif} of dimer was 1.5 fold bigger than D_{diff} of tetramer, in contrast to constant viscosity conditions). Thus, quantity of dimer and tetramer forms could have been determined. Moreover, equilibrium constant of tetramer formation could have been calculated and it was 5×10^{-4}/M for wild type Drp1.

P.1.3-060
Live monitoring of bacterial peptide uptake using lab-on-a-chip technology

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The majority of experiments with cells are performed after removal from their natural environment in a time dependent manner. Under these conditions cells are exposed to synthetic environments, which lack biochemical and mechanical interactions with the extracellular matrix, and direct cell–cell contacts with the neighboring cells that vary in time and space. Unfortunately most experimental conditions are poor in mimicing natural environments; thus microfluidic systems may provide an alternative approach to monitor live cells in their natural environments. Microfluidic systems are small platforms with a typical length scale of one hundred nanometers to several hundreds of micrometers, consisting of channel systems connected to liquid reservoirs by liquid connectors. Even though the initial goal of microfluidics was to reduce sample consumption, lab-on-a-chip technology has successfully provided portable, fast, controllable and reproducible operations for biological processes such as drug screening, cell counting, and enzymatic assays. Previously, we have proposed novel beta-lactamase inhibitory chimeric peptides with the potential to translocate across the bacterial cell wall and inhibit periplasmic beta-lactamase. Motivated by the findings on structure-activity relationship studies with the cell-penetrating peptide, pVEC, our approach involved the addition of LLIIL residues to the beta-lactamase inhibitory peptides. These peptides were examined for their antibacterial activities and effects on bacterial membrane permeability and surface structure. In the current work, our aim was to construct a live cell imaging system for the bacterial uptake of pVEC and a chimeric beta-lactamase inhibitory peptide using the lab-on-a-chip technology. Using this system, we were not only able to successfully monitor uptake of both peptides, but also observed the cell growth inhibition due to their antibacterial features.

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P.1.3-061
Cooperation of PtdInsP2 and PtdSer in synaptotagmin-1 membrane binding

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Synaptic transmission in the nervous system is mediated by the regulated release of neurotransmitters (stored in synaptic vesicles) from presynaptic nerve terminals. Upon arrival of an action potential, voltage-gated Ca^{2+}-channels open, leading to a rise in cytoplasmic Ca^{2+} concentration and the fusion of synaptic vesicles with the presynaptic plasma membrane. The primary Ca^{2+} sensor for neuronal exocytosis is synaptotagmin-1 (syt-1), a synaptic vesicle protein that consists of a short luminal domain, a transmembrane α-helix and two cytoplasmic C2 domains, referred to as C2A and C2B. Synaptotagmin-1 binds phosphatidylserine (PtdSer) and phosphoinositides (PtdIns) via its C2 domains, but the molecular details of this process are not fully understood. Using quantitative thermodynamic, kinetic and structural methods, we show that synaptotagmin-1 binds to PtdIns(4,5)P_2 via a polybasic lysine patch in the C2B domain, which may promote the priming or docking of synaptic vesicles. Furthermore, Ca^{2+} neutralizes the negative charges of the Ca^{2+}-binding sites, resulting in the penetration of synaptotagmin-1 into the membrane, via binding of PtdSer, and an increase in the affinity of the polybasic lysine patch to phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2).Our results demonstrate that PtdSer and PtdIns(4,5)P_2 act in a synergistic manner to enhance the penetration depth of synaptotagmin-1 and to reduce the dissociation rate from the membrane while the association rate remains unchanged. Additionally,our data provide strong evidence that PtdIns binds to the polybasic lysine patch on the C2B domain and does not compete with PtdSer for sites in the Ca^{2+}-binding sites. Finally,we conclude that both membrane penetration and the increased residence time of synaptotagmin-1 at the plasma membrane are crucial for triggering exocytotic membrane fusion.

P.1.3-062
Structure-function relationship and chemical stability of yeast D-amino acid oxidase

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D-amino acid oxidase (DAAO) plays important role in living systems. The enzyme from yeast *Trigonospsis variabilis* (TvDAAO) is also of high practical importance. It is used in biocatalytic two-enzyme process of preparation of 7-amino cephalosporanic acid from cephalosporin C. The main drawback of TvDAAO is insufficient thermal and chemical stability.

Using high speed sedimentation analysis and light scattering technique it was shown that oligomeric state of TvDAAO depends on concentration. At concentrations 5–30 μg/mL the enzyme is a homodimer. At concentration higher than 50 μg/mL oligomeric state became tetrameric and higher. At concentrations lower than 0.5 μg/mL dimer TvDAAO dissociates to subunits. Stability of dimer can be improved by addition of FAD. Analysis

of TvDAAO activity at different concentrations showed that only dimer is active.

Hydrogen peroxide is the product of TvDAAO reaction. It can inactivate the enzyme through oxidation of Met and Cys residues. Site-directed mutagenesis of four non-conservative Met residues showed that these residues do not control the TvDAAO chemical stability but substitution Met156Leu increased thermal stability. Cys108 is placed in D-amino acid binding domain. Site directed mutagenesis of Cys108 for Ala and Ser residues improved the chemical but not thermal stability. Substitution Cys108Phe increased both chemical and thermal stability. Amino acid changes Cys108Phe and Cys108Ala resulted in increase of catalytic efficiency with cephalosporin C by 3 and 4-fold, respectively. The second Cys residue in position 298 is placed in oxygen channel of active site. It was changed with four residues. Amino acid changes Cys108Phe and Met156Leu were combined with substitution Phe54Ser to produce active with cephalosporin C stable mutant enzyme with increased thermal and chemical stability.

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P.1.3-063
Homology modeling and characterization studies on gamma subunits of heterotrimeric G proteins in *Oryza sativa* and *A. thaliana*

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Heterotrimeric G proteins, composed of alpha, beta, and gamma subunits, act as molecular switches to turn on intracellular signaling upon arrival of external stimuli at membrane receptors. In plants they participate in multiple developmental processes from seed germination, early seedling development to organ shape determination, as well as and in defense mechanisms, hormone perception and ion-channel regulation.

Recently, it was demonstrated that the components of G proteins and intrinsic signaling and network mechanisms in plants are significantly different from those in the animal counterparts. One of these differences is the complex family of plant gamma subunits which may be directly involved in signaling and is likely to play a role in the specificity of signaling. The present study involves computational analyses for modeling the structures RGG1 and AGG1, the gamma subunits from rice and Arabidopsis respectively, and the biochemical and biophysical characterization of the two purified proteins. Homology modeling yielded an extended helical structure for both RGG1 and AGG1 proteins. In experimental characterization studies, RGG1, RGG2 and additionally AGG1 were expressed in *E. coli.* and purified from bacteria for dynamic light scattering (DLS) and circular dichroism spectroscopy (CD). Stability of structures was investigated in thermal denaturation experiments. Comparative results will be presented and discussed in the context of plant gamma subunit function(s).

P.1.3-064
Analysis of the *B. pumilus* endopeptidase gene regulatory region

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Minor secreted *B. pumilus* metalloproteinase is a unique protein. This is the only isolated enzyme that combines the classification characteristics of the astacins and adamalizines families of the metzincin clan. In addition, MprBp metalloendopeptidase is the first and the only adamalysin-like enzyme of prokaryotes to date. Eukarotic adamalysin-like enzymes play an important role in human life and health. They are involved in cellular migration, in the development of muscle tissue and the processes of fertilization. Their role in the occurrence and development of such serious diseases as inflammatory processes, rheumatoid arthritis, oncological diseases, aging, allergy and asthma is great. The purpose of this work was to study the functions of MprBp metalloproteinase in bacilli cells. To understand the functional role of the enzyme in a cell, it is important to establish ways of regulating the expression of its gene. To assess the effect of different regulatory systems on the expression of mprBp, we analyzed the promoter region of the gene for the presence of specific binding sites with regulatory proteins. In the promoter of the mprBp gene were found four binding sites with the phosphorylated form of the DegU protein (DegS-DegU signal transduction system), four binding sites with the Spo0A regulatory protein (sporulation control system), six binding sites with the regulatory protein TnrA (nitrogen exchange system) and 2 binding sites with the regulatory protein CcpA (carbon cell exchange system). The presence of binding sites with these regulatory proteins indicates the likely involvement of MprBp in trophic processes in the cell, as well as in the process of sporulation.

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P.1.3-065
Nanobodies as tools for studying dynamics of multidomain proteins

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Nanobodies, or single-domain antibodies, are recombinant derivatives of the unusual heavy-chain only antibodies produced by camels, llamas and sharks. The small size of the nanobodies (molecular weight of 12–15 KDa) in combination with high binding affinity and specificity makes them uniquely suitable as high precision tools for protein NMR. We have introduced nanobodies as probes of protein dynamics, and used them to analyze interactions between the metal binding domains of the human copper transporter ATP7B (Wilson disease protein) involved in the regulation of the activity and trafficking of ATP7B in the cell. The six cytosolic copper binding domains of ATP7B are connected by flexible linkers for a total length of 630 amino acids (MBD1-6). The individual domains in MBD1-6 do not form a compact structure and are highly mobile. In the cell, the MBDs interact with each other in a copper dependent manner, and these interactions are believed to trigger relocalization of ATP7B from the *trans*-Golgi network to the cytosolic membrane vesicles and plasma membrane, and activate copper removal from the cell. The molecular mechanism of this regulation is unknown. Using the complete MBD1-6 as an antigen, we have produced and characterized a panel of nanobodies against various metal binding domains of ATP7B. Binding sites of the selected nanobodies were mapped by chemical shift perturbation analysis, and their binding affinity characterized using isothermal titration calorimetry. Analysis of changes in the dynamics of individual MBDs caused by the nanobody binding to the neighboring domains

revealed transient interdomain interactions, and outlined two distinct dynamically correlated domain groups within MBD1-6. Taken together with our studies of ATP7B interaction with the copper chaperone protein Atox1, the nanobody-assisted analysis of conformation and dynamics of MBD1-6 led us to the structure-based mechanism of ATP7B regulation by copper in the cell.

P.1.3-066
SimRNP: a new method for fully flexible modeling of protein-RNA complexes and for simulations of RNA-protein binding

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Macromolecular complexes composed of proteins and nucleic acids play fundamental roles in many biological processes, such as the regulation of gene expression, RNA splicing and protein synthesis. Structures of some of these complexes have been experimentally determined, providing insight into mechanisms of their biological activities. However, for a great majority of protein-nucleic acid complexes, high-resolution structures are only available for some isolated components, often accompanied with low-resolution information about the overall shape (e.g. from cryo-EM or SAXS) or about the proximities and interactions of these components (e.g. from chemical cross-linking experiments). Given the scarcity of experimentally determined structures, computational techniques can be used to integrate heterogeneous pieces of information, guide structure elucidation and subsequently determine the mechanisms of action and interactions between the components. Our group has developed computational tools for protein and RNA 3D structure prediction, which covered approaches for template-based and template-free modeling. These tools, including the GeneSilico metaserver and Frankenstein Monster approach for proteins, and ModeRNA and SimRNA for RNA have been validated in CASP and RNA Puzzles experiments, respectively. Recently, we combined our approaches for protein and RNA modeling, and developed a method for modeling of proteins, RNAs, and protein-RNA complexes. SimRNP uses a coarse-grained representation of protein and RNA molecules, utilizes the Monte Carlo method to sample the conformational space, and relies on a statistical potential to describe the interactions in the folding process. It allows for modeling of complex formation for assemblies comprising two or multiple protein and RNA chains. To our knowledge, this is the first method that allows for fully flexible modeling of protein-RNA binding, e.g. with components of unknown structure or which are disodrered in isolation.

P.1.3-067
APC/C regulation via CDH1-MAD2L2 interaction

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Correct progression through mitosis is regulated by the Anaphase-Promoting Complex/Cyclosome (APC/C). The APC/C is an essential mitotic E3 ligase, controlling the accuracy of mitosis and G1 phase. It has two activators - CDC20 to CDH1 both are also APC/C substrates. MAD2L2, also known as REV7, was recently shown to participate in APC/C regulation. MAD2L2 binds CDH1 and prevents CDH1 from premature binding and activation of the APC/C. Canonicaly, at the onset of anaphase MAD2L2 is rapidly degraded by APC/C^{CDC20}, releasing CDH1 to bind and activate the APC/C. Loss of MAD2L2 leads to premature association of CDH1 with the APC/C and unscheduled destruction of APC/C^{CDH1} substrates. APC/C premature activation leads to accelerated mitosis and frequent mitotic aberrations. Thus, MAD2L2 helps to safeguard the switch between APC/C^{CDC20} and APC/C^{CDH1} during the metaphase-to-anaphase transition, thereby contributing to mitotic fidelity. Our goal is deepen the understanding on the MAD2L2-CDH1 complex, its regulation and influence on the APC/C activity. We present here *in vitro* mapping of MAD2L2 and CDH1 interaction, suggesting that the conserved C-BOX on CDH1 is responsible for their binding. Understanding the mechanism of action of MAD2L2-CDH1 complex and the effect on APC/C activity is important for dissecting the different roles of MAD2L2 and developing therapeutic agents, designed and targeted to manipulate either APC/C activation or DNA damage response.

P.1.3-068
A single point mutation increases the catalytic activity of *B. subtilis* aminopeptidase (BSAP) by two orders of magnitude

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Aminopeptidases catalyze the cleavage of single amino acid from the amino terminus of peptides and proteins. These enzymes are involved in a wide range of biological processes, such as protein digestion, protein degradation and cell-cycle control. The goal of the current study was to explore the structure-function relationship of the bacterial metallo-aminopeptidase from Bacillus subtilis (BSAP). The detailed three-dimensional structure of BSAP has recently been determined by X-ray crystallography, demonstrating that the enzyme consists of two main domains, a TIM barrel catalytic domain and a protease-associated (PA) domain. Based on this structure, the C-terminal tail appears to interact with the PA-domain near the active site and potentially blocking it. The main interactions are made by residues Arg153, Asp155 and Tyr158 in the PA- domain. In addition, Glu452 appears to coordinate tightly with one of the two Zn ions in the active site, thereby potentially interfering with substrate binding. To study the exact roles of the PA domain and the carboxy-tail of BSAP, we prepared a series of mutated forms of the enzyme, including deletions and key amino acid replacements. Native BSAP and its mutants were over-expressed in *E. coli* and purified using cation exchange chromatography. Preliminary results indicate that the Asp155Ala and Tyr158Phe mutations do not affect significantly the activity towards p-nitroanilide derived Arg and Lys at 30°C. In contrast, however, the Glu452Asn replacement resulted in a 100-fold improvement in the specificity constant kcat/KM ($9.1*10^3$ $mM^{-1}*s^{-1}$), confirming that Glu452 plays a key role in the mode of action of BSAP. Moreover, the melting temperature of the Glu452Asn mutant increased by ~5 °C, suggesting that the improvement in enzyme catalysis can not be attributed to higher enzyme flexibility. Nevertheless, the detailed catalytic mechanism of this novel double-zinc metallo-aminopeptidase is yet to be revealed.

P.1.3-069
Kinetic and structural studies of Xyn52B2 glycosynthase suggest enzyme product release as the rate-limiting step of the reaction

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Glycosynthases are catalytic mutants of retaining glycoside hydrolases that catalyze the synthesis of oligosaccharides from their corresponding glycosyl-fluoride donors and suitable acceptors. In these enzymes, the nucleophile is replaced by a smaller non-nucleophilic residue. Enzymatic synthesis of oligosaccharides provides an attractive alternative to the classical synthetic chemical methods, since it enables a complete control over newly generated anomeric centers, and the reaction can be performed in aqueous solution under mild conditions.

The β-xylosidase Xyn52B2 nucleophilic mutant (E335G) from *Geobacillus stearothermophilus* has already proved to be useful for glycosynthesis applications. The enzyme can catalyze the self-condensation reaction of α-D-xylopyranosyl fluoride, providing mainly α-D-xylobiosyl fluoride.

By using two cycles of directed evolution, an improved variant of Xyn52B2-E335G was isolated containing in total ten random mutations. Recently, the crystal structures of Xyn52B2-E335G and its improved variant, with the glycosyl-fluoride donor and the product bound to the active site, were obtained. These 3D structures, together with rigorous kinetic analysis of selected mutations (out of the ten random ones) allowed to pinpoint the crucial amino acid substitutions contributing to glycosynthesis and suggest that product release is the rate-limiting step of the glycosynthase reaction.

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P.1.3-070
Structural studies of macrophage migration inhibitory factor interaction with ceruloplasmin

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Ceruloplasmin (Cp) is natural inhibitor of macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine which is a pharmacological target for treatment cancer, septic shock etc. We present structural studies of MIF-Cp complex formation as combination of small-angle scattering, X-ray and molecular docking. Biochemical data shows inhibitions of all three active sites of MIF trimer by Cp. Conformational changes of active site induced by inhibitor phenyl isothiocyanate (PITC) which mimics substrate is necessary for MIF interaction with Cp. PITC modified MIF was crystallized in microgravity by counter diffusion method, data were collected using synchrotron radiation. Atomic resolution structure was used for MIF-Cp modeling by molecular docking. SAXS measurements of MIF-Cp in concentration 10 mg/mL were perform on AMUR-K diffractometer. Unstability of MIF-Cp complex leads to capture one Cp molecule bound to MIF trimer. Docking model was corrected after fit with SAXS curve. Cp sites (near labile copper binding sites) 585–595 and 924–933 interact with MIF, site 585–595 contacts with entrance of MIF active site. Our model of MIF-Cp interaction was confirmed by 1.4 Å X-ray structure of MIF modified with bigger size inhibitor fluorescein isothiocyanate (FITC). According our biochemical data MIF modification by FITC prevent association with Cp. Superposition of FITC-MIF crystal structure with MIF-Cp model reveals steric clash of FITC with 585–596 site of Cp. Our low resolution MIF-Cp model is a basis for understanding of mechanism of MIF inhibition by Cp and design of new MIF inhibitors.

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P.1.3-071
Characterization of iron ions associated with the *E. coli* Dps protein and their influence on the protein oligomeric form

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The Dps protein has a dodecameric form and performs a number of functions in the bacterial cell. Being the main architectural factor of the bacterial nucleoid at the stationary phase; it affects gene expression during rapid growth and oxidizes Fe^{2+}, accumulating them inside the protein cavity. We found, that during the process of Dps purification, a reversible dissociation of its dodecamers occurs, which is accompanied by a loss of iron ions. Using the 1,10-phenotroline method it was found that the purified protein contains only two iron ions per monomer, which corresponds to the Dps apo-form. Both electrophoretic fractionation and gel filtration showed that titration with Mohr's salt ($(NH4)_2SO_4 \times FeSO_4 \times 6H_2O$) led to accumulation of iron ions within the protein cavity, and also stabilized the dodecameric form of Dps. The composition of iron ions associated with Dps was analyzed using Mössbauer spectroscopy at 77°K. The obtained components of the spectrum are associated with two non-equivalent positions of iron atoms in an octahedral surroundings, one being more distorted than the other. Thus, it can be assumed that the inorganic core of the Dps protein consists of β-FeOOH clusters, at least one of which has ability to stabilize dodecameric form of Dps.

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P.1.3-072
Synchrotron study of *Escherichia coli* ferritin Dps inorganic core

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The Dps protein of *Escherichia coli* (*E. coli*) is the prototype of a particular family of bacterial ferritines with a DNA-binding ability. It is a nanoscale particle consisting of 12 identical subunits with a known crystal structure of about 9 nm in diameter and an inorganic core of about 5 nm in diameter. The physico-chemical structure of the core have been less studied and it is usually assumed that its composition is mainly represented by Fe^{2+} oxidation products in the ferroxidase centers of the protein. However, the physical properties of iron ions, characterized under anaerobic conditions *in vitro*, indicate a more complex core composition in the native Dps protein. We used ultrahigh vacuum synchrotron experiments with X-ray absorption near edge structure (XANES) spectroscopy of the iron absorption edge in the soft X-ray region to get the direct experimental information about *charge state of iron ions*. Supporting experiments were performed with the use of transmission electron microscopy and dynamic light scattering that made it possible to estimate the size of the inorganic core (about 6 nm) and the absence of a general atomic ordering of iron ions in it. High sensitivity of synchrotron XANES spectroscopy allowed also detecting the presence of both trivalent and divalent iron ions in the octahedral and tetrahedral environment of oxygen atoms in the prepared samples. The composition of the iron ions in the core of the native Dps protein, which was isolated from bacteria grown under aerobic conditions, is thus more complex than is commonly believed.

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P.1.3-073
The identification of a protein involved in formation of cytoskeleton-like structures in Mycoplasma gallisepticum cells

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Mycoplasmas are very successful pathogens of humans, mammals, birds, insects and plants. They lack cell wall and resistant to many conventional antibiotics. *Mycoplasma gallisepticum* causes chronic respiratory diseases in poultry that leads to economic losses in poultry farming. Now an actively search for new potential targets for the control of mycoplasmal infections take place. The proteins of cell division and motility are potential targets for the new antibacterial therapies. Long enough in our laboratory unique cytoskeleton-like structures resembling microtubules of higher eukaryotes were found in the cells of *M. gallisepticum*. It was assumed that these structures can directly participate in the motility of the mycoplasma cells. However, the protein that forms these structures has never been identified. It was only revealed that it has a molecular weight approximately to 40 kDa and interacts with anti-tubulin antibodies. In this study, using immunoprecipitation with antibodies against the conserved porcine tubulin epitopes (TU-01, Abcam) and mass spectrometric analysis, we identified this protein in the cell extract of *M. gallisepticum*: GapA (37.96 kDa), glyceraldehyde-3-phosphate dehydrogenase. It is known that GapA protein homolog of eukaryotes, GAPDH, can interact with tubulin and regulate the formation of microtubules. In addition, GapA *M. gallisepticum* participates in the adhesion and motility of the mycoplasma cells. We also analyzed proteins in a precipitate formed after treatment of *M. gallisepticum* cells with Triton X-100. Among insoluble proteins GapA was also detected in noticeable amounts. Maybe GapA *M. gallisepticum* is an important part of cytoskeleton or it participates in its regulation. If so, then it can be considered as a convenient target for new antibacterial drugs. Soon we plan to identify proteins that interact with GapA in *M. gallisepticum*.

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P.1.3-074
Crystallographic structure of destabilase from Hirudo medicinalis – a small bifunctional enzyme with isopeptidase and lysozyme activities

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Destabilase is an enzyme secreted by the salivary gland cells of medicinal leeches, which was shown to possess lysozyme and isopeptidase activities. This enzyme attracts interest because it expresses thrombolytic activity through isopeptidolysis of the ε-(γ-Glu)-Lys bonds that cross-link polypeptide chains in stabilised fibrin and can dissolve old clots. Destabilase is a member of i-type lysozymes (from "invertebrate"), some of which were assumed to have similar bifunctional activity. Several crystallographic structures are available for the representatives of this family, which gives structural basis for assessing their functional activity. However, destabilase structure from *Hirudo medicinalis* - the most pharmacologically relevant i-type lysozymes - remains uncovered. Here, we showed the first high resolution X-ray structure of the protein. The recombinant destabilase-lysozyme isoform 2 was produced in the human cell line. Transfected cells secreted mature recombinant protein into the cultural liquid. The destabilase was purified in two stages using metall-chelating chromatography and cation-exchange chromatography on carboxymethyl-sepharose. The protein was crystallized using sitting-drop approach and the crystallographic data were collected at the European Synchrotron Radiation Facility up to 2.3 Å resolution. Our new crystallographic model reveals the arrangement of

the protein functionally important sites and allow us to critically reconsider the existing data for its structure-function relationship.

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P.1.3-075
Non-conserved interactions maintain a common quaternary octameric structure of a new family of esterases

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Protein oligomerization contributes to the stability and function of proteins. The acetyl-xylooligosaccharide esterase, Axe2, from the thermophilic bacterium *Geobacillus stearothermophilus* represents a new family of carbohydrate esterases belonging to the SGNH superfamily of hydrolytic enzymes and has a unique doughnut-like homo-octameric configuration composed of four homo-dimers. The dimers of Axe2 are held together mainly by clusters of hydrogen bonds involving Tyr184 and Arg192, as was demonstrated by site directed mutagenesis. The dimeric mutants had lower catalytic activity towards 2-naphthyl acetate compared to the wild type, indicating the necessity of the octameric structure for catalysis. The uncharacterized homologous protein from *Alicyclobacillus acidocaldarius* (PDB no. 3RJT) has the same octameric structure as Axe2 that is maintained by different set of amino acids, involving Asn183. As in Axe2, the dimeric mutant was inactive. These findings led us to investigate five more proteins from this group, which potentially have non-conserved interactions to create the same octamer as Axe2 and 3RJT. Indeed, we revealed for the first time a group of proteins with diverse interactions responsible for a common octameric configuration. We suggest that proteins in this new esterases family evolved from a common octameric ancestor, and maintain this favorable structure using different interactions to increase stability under certain environmental conditions and/or by changing substrate specificity.

P.1.3-076
Conserved cysteines mutagenesis of Mus m 1 allergen: when protein engineering meets the needs of allergen specific immunotherapy

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The lipocalin Mouse Urinary Proteins (MUPs) is an ensemble of ligand binding proteins and represent the major mouse allergen Mus m 1 for humans. Once an individual is sensitized, specific allergen immunotherapy, through gradual allergen administration, induces tolerance and reduces reactivity to the allergen, preventing the progression to severe allergy manifestations. The use of recombinant allergens for the formulation of allergy vaccines can overcome the pitfalls associated with the difficult standardization and management of native allergen sources. We investigated the role of the disulfide bridge (C64-C157) and of the free C138 residue focusing on allergen structural stability, folding reversibility and immunogenicity. Four recombinant MUP mutants, C138S, C138A, C157A or C138-157A, were studied by light spectroscopy techniques and patient IgE-mediated degranulation test. Preliminary structural characterization confirmed that they retain a native-like fold. The C138 mutants performed similarly to the wt protein in a ligand binding assay with the fluorescent probe N-phenyl-naphthylamine, while those bearing the substitution of C157 revealed a weaker binding of the ligand. Thermal unfolding experiments attributed a significant loss of thermal stability only to C157A and C138-157A. Degranulation similar to wt and complete reversibility of unfolding were obtained only with C138A that, after gradual cooling, recovered the native conformation. We conclude that only the mutation C138A preserves protein functionality, stability and immunoreactivity; more than that it suppresses the free and reactive thiol group responsible for incorrect disulphide bond formation and irreversible unfolding. We will exploit this MUP mutant to formulate allergen diagnostics and vaccines, which, ultimately, might improve the management of allergic diseases.

P.1.3-077
Macrophage migration inhibitory factor dynamics and biological functions

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Macrophage migration inhibitory factor (MIF) is best known for its pro-inflammatory activity. It is expressed in every human cell, is present in the cytosol with small amounts in the nucleus, and is exported to extracellular milieu when cells are stressed. It has distintct functions in all of the cellular compartments it is found. It was recently reported to have nuclease activities, it functions as a signaling molecule in the cytosol interacting with various proteins, and activates CD74, CXCR2, and CXCR4 extracellularly. Based on its three-dimensional structure, MIF has other functions that have not yet been described. For example, the three-dimensional structure of MIF shows it is a homotrimer with topology similar to two microbial enzymes, 4-oxalocrotanate tautomerase (4-OT) and 5-carboxymethyl-2-hydroxymuconate isomerase (5-CHMI), with nothing in common among the three sequences other than a catalytic base in a cavity between subunits. The natural substrate for MIF has been elusive but two "subrate mimics" have been identified. In addition, MIF and 5-CHMI, but not 4-OT, also share a solvent channel along its 3-fold axis which was presumed to be a consequence of homotrimerization. Given so many functions of MIF, we used molecular dynamics and residues with high centrality to study some of the functions of MIF. We verified a residue that is involved in protein-protein interactions and the enzymatic site. We also identified a gating residue at the solvent channel with high centrality. By disrupting pathways for coupling to the catalytic site and receptor binding using mutagenesis, we identified allosteric pathway from the gating residue to both of these sites that regulates its activity. This study reveals that MIF has multiple surface sites that play a role for its multiple biological activities.

Molecular Machines in Action

P.3.1.A-001
Targeting Type IV secretion traffic ATPases to stop dissemination of antibiotic resistance genes

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Antibiotic resistance is becoming a pressing public health concern. The main mechanism for the dissemination of antibiotic resistance genes between gram-negative bacteria is the horizontal transfer of genetic material in a process known as conjugation. Therefore, the search for specific conjugation inhibitors (COINs) is crucial for winning the battle against antibiotic resistance bugs. Unsaturated fatty acids (uFAs) and 2-alkynoic fatty acids (2-aFAs) are the only effective and specific inhibitors of conjugation found so far. We have found that the Type IV secretion system ATPase TrwD, the VirB11 homolog in the conjugative plasmid R388, is the molecular target of these inhibitors.

VirB11 proteins belong to the super-family of secretion AAA+ traffic ATPases. These proteins form hexameric rings in which each monomer is caracterized by a catalytic region (C-terminal domain, CTD) and a region that interacts with the cytoplasmic site of the membrane (N-terminal domain, NTD) connected both of them by a flexible linker of a variable length. We have identified the mode of action: COINs bind to a pocket comprised by NTD and linker, preventing the pivoting movement of NTD over CTD. Interestingly, even though saturated fatty acids are not COINs, palmitic acid (major component of bacterial membrane phospholipids) interacts directly with TrwD and binds to it. Characterization of kinetic parameters and docking predictions indicate that uFAs and saturate palmitic acid compete for the same binding site of TrwD. Moreover, uFAs incorporate to the bacterial membrane when added to the growth media, affecting the natural phospholipid composition. All this suggests that the mode of action is by affecting the interaction of TrwD with the bacterial membrane. In short, our results do not only contribute to a better understanding of VirB11 proteins but also lie the foundations for the rational design of more potent and effective drugs to control dissemination of antibiotic resistance genes.

P.3.1.A-002
Hidden phage Vp16 PDF features are essential for deformylase activity

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Prokaryotic proteins must be deformylated before the removal of their first methionine. Peptide deformylase (PDF) is indispensable and guarantees this mechanism. Recent genome sequencing studies highlighted over $2x10^4$ putative peptide deformylase sequences. Furthermore, unpredicted modified bacterial PDF genes have been retrieved from many viruses. Sequence comparisons with other known PDFs reveal that viral PDFs are devoid of the key ribosome-interacting C-terminal region. Little is known regarding these viral PDFs, including the capacity of the corresponding encoded proteins to ensure deformylase activity. We provide here the first evidence that viral PDFs, including the shortest PDF identified to date, *Vp*16 PDF, display deformylase activity *in vivo*. Large scale N-terminomics characterization reveals that *Vp*16 PDF has substrate specificity similar to that of other bacterial PDFs. However, our integrated biophysical and biochemical approaches also reveal hidden and unique functions of the unusual C-terminus. The high-resolution crystal structures of *Vp*16 PDF, free or bound to the potent inhibitor actinonin, reveal a classical PDF fold and also an unexpected crucial role for the ultimate residue tethering the active site. Our study underscores the structural and molecular characteristics of the unusual C-terminal Ile residue that sustains deformylase activity in the absence of the otherwise indispensable C-terminal domain.

P.3.1.A-003
Metal selectivity studies of the cation diffusion facilitator protein family

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Cation diffusion facilitator (CDF) is a ubiquitous family of divalent transition metal cations (DTMCs) transporters. Each member of this family transports a specific DTMC from the cytoplasm to the extracellular environment or into intracellular compartments. Severe diseases – from skin lesions in infants to type-II diabetes and Alzheimer's disease – that are caused by dysfunctional human CDF proteins, emphasize the importance of these proteins for normal cell function. Most CDF proteins contain two domains, the regulatory cytoplasmic domain (CTD) that during metal binding changes its conformation, and the transmembrane domain (TMD), where the DTMCs are transported through a specific tetrahedral binding site. Although CDF proteins have been extensively studied over the past two decades, the exact factors governing their metal specificity remained elusive. Aiming to understand these factors, we combine general computational analysis of the TMD and specific biochemical and structural analysis of the CTD. We scanned the protein data bank (PDB) and performed a structural-based comprehensive analysis to characterize DTMCs binding propensities exclusively in proteins. We then utilized this general analysis to study the correlation between the metal selectivity and phylogenetic classification of CDF proteins. We found that although DTMCs generally show different binding preferences, CDF proteins phylogenetic classification and the related TM binding site composition cannot solely determine the metal selectivity of these proteins. These results led us to investigate whether the CTD relates with metal selectivity as well, using as a model protein the CDF protein MamM from magnetotactic bacteria. Here we present crystal structures of MamM CTD bound to different metals and biochemical analysis of its metal binding, both emphasize the importance of CDFs' CTD as a regulatory element and suggest a further role for the CTD in DTMCs' selection.

P.3.1.A-004
CglI cleaves DNA using a mechanism distinct to other ATP-dependent restriction endonucleases

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The restriction endonuclease CglI from *Corynebacterium glutamicum* recognizes an asymmetric 5′–GCCGC–3′ site and cleaves the DNA 7 and 6/7 nucleotides downstream on the top and

bottom DNA strands, respectively, in an NTP–hydrolysis dependent reaction. CglI is composed of two different proteins: an endonuclease (R.CglI) and a DEAD–family helicase–like ATPase (H.CglI). These subunits form a heterotetrameric complex with R_2H_2 stoichiometry. However, the R_2H_2.CglI complex has only one complete nuclease active site sufficient to cut one DNA strand suggesting that two complexes are required to introduce a double strand break. Here we report studies to evaluate the DNA cleavage mechanism of CglI. Using one– and two–site circular DNA substrates we show that CglI does not require two sites on the same DNA for optimal catalytic activity. However, one–site linear DNA is a poor substrate, supporting a mechanism where CglI complexes must communicate along the 1–dimensional DNA contour before cleavage is activated. Based on triplex displacement assays and cleavage assays on two–site linear DNA, we propose that ATP hydrolysis by CglI produces translocation on DNA preferentially in a downstream direction from the target, although upstream translocation is also possible. Our results are consistent with a mechanism of CglI action that is distinct from that of other ATP–dependent Restriction–Modification enzymes.

P.3.1.A-005
Interplay between sigma region 3.2 and secondary channel factors during promoter escape by bacterial RNA polymerase

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Initiation of gene transcription depends on the recognition of specific promoter elements by RNA polymerase (RNAP) and associated factors. During promoter escape, RNAP must break the established protein-DNA interactions to allow efficient RNA elongation. In bacterial RNAP, the main role in promoter recognition is played by the s subunit of RNAP. Conserved region 3.2 of the sigma subunit (sigma finger) was proposed to contribute to promoter escape by interacting with the 5′-end of nascent RNA, thus facilitating s dissociation. RNAP activity during transcription initiation can also be modulated by protein factors that bind within the secondary channel and reach the enzyme active site. Gre factors facilitate the release of aberrant abortive products by stimulating their cleavage by RNAP. DksA modulates the stability of promoter complexes but its effects on promoter escape have not been studied. To monitor the kinetics of promoter clearance in real time, we used a molecular beacon assay in which fluorescence of site-specifically labeled s subunit is changed depending on its specific interactions with promoter DNA. We showed that noncanonical initiating substrates, including short primers lacking the 5′-triphosphate moiety or NAD^+/NADH, have only minor effect on the rate of promoter escape by *Escherichia coli* RNAP. At the same time, mutations in region 3.2 the sigma subunit decrease the rate of promoter clearance on various promoters. Depending on the promoter, Gre factors and DksA differently modulate promoter escape by RNAP; in particular, GreB can either stimulate or inhibit the transition to productive RNA elongation. Exonuclease footprinting revealed that stimulation of promoter escape by Gre factors correlates with accumulation of stressed transcription complexes with scrunched DNA. In summary, our data reveal complex interplay between protein factors and growing RNA during the initiation-to-elongation transition.

P.3.1.A-006
Translesion RNA synthesis by RNA polymerase from the radioresistant bacterium *Deinococcus radiodurans*

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DNA lesions can significantly affect the process of transcription by interfering with the activity of RNA polymerase (RNAP). Consequently, RNAP serves as one of the main sensors of DNA damage in the cell. In bacteria, transcription complexes stalled at DNA lesions can be directly recognized by either Mfd translocase or UvrD helicase, that bring other components of the nucleotide excision pathway (NER) to the sites of damage. *Deinococcus radiodurans* is a highly stress-resistant bacterium, that encodes universal as well as lineage-specific regulators of transcription and DNA repair. We investigated the ability of RNAP from *D. radiiodurans* to transcribe damaged DNA templates *in vitro* and demonstrated that various lesions (8-oxoguanine, O-6-methylguanine, thymine glycol, thymine dimers, AP-sites, 1-N6-ethenoadenine) significantly affect both the efficiency and fidelity of RNA synthesis. Furthermore, *Deinococcus*-specific Gfh factors stimulate RNAP stalling at damaged DNA sites, likely by interacting with the enzyme active site and stabilizing its inactive conformation. As a result, Gfh factors increase sensitivity of the stalled complexes to the action of either Mfd or UvrD, which can translocate RNAP in opposite dirrections to expose the DNA lesion. The results suggest that *Deinococcus*-specific factors might have evolved to increase the efficiency of DNA damage recognition by the transcription machinery.

P.3.1.A-007
Trimeric photosystem I is involved in higher order functional supercomplex formation in thylakoid membranes of Synechocystis sp. PCC6803

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Cyanobacterial photosystem I (PSI) forms trimers in thylakoids of *Synechocystis sp*. PCC6803 at optimal growth conditions. Measurements of physical properties of isolated monomeric and trimeric PSI failed to reveal any substantial differences between them. We used wild type *Synechocystis* (WT) and *psaL*$^-$ mutant deficient in the PsaL protein responsible for PSI trimerization to measure response to different light intensities and kinetics of P700 oxidation and reduction in whole cells. This enabled us to gain information not only about the electron transport activity of PSI but also about performance of its functional partners. Response to light, measured as the ratio of the $P700^+$ centers pool at given light intensity to the total number of P700 centers in the sample, is impaired in mutant compared to WT. The formation of $P700^+$ is facilitated and this state is stabilized in trimeric PSI as shown by oxidation/reduction kinetics. Methyl viologen and diuron (MV+DCMU) treatment renders reduction rates identical and light intensity-dependent in both strains. MV+dibromothymoquinone (DBMIB) treatment slows down the reduction of $P700^+$ in WT cells but not in the mutant. The ratio of quantum yield of photochemical process in PSI, Y(I), quantum yield of non-photochemical energy dissipation due to donor

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side limitation, Y(ND), and due to acceptor side limitation, Y (NA), is similar in WT and mutant cells in control conditions. Treatment with MV+DCMU induces significant Y(NA) in mutant but not in WT cells. MV+DBMIB induces strong Y(ND) in WT cells. Mutant cells do not show any response to MV+DBMIB. Observed differences in electron transport activity and performance between PSI trimer-containing *Synechocystis* cells and *psaL*$^-$ mutant cells can be explained by the involvement of PSI trimers in formation of higher order complexes with other subunits of photosynthetic electron transport chain that results in facilitated formation and stabilization of P700$^+$ state in WT cells.

P.3.1.A-008
The effects of abiotic factors on telomere length

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Telomeres are evolutionarily conserved essential protein-DNA structures necessary for genome stability. The length of telomeric DNA appears to be genetically determined but varies highly between human individuals at birth. We have previously shown that natural populations (ecotypes) of the model plant *Arabidopsis thaliana* display significant variation in telomere length set point. While telomere length is known to be under strong genetic control in this plant species, it is currently unclear whether different environmental factors can also affect this phenotype. To address this question we studied the effects of varying environmental conditions on telomere length in several *Arabidopsis* ecotypes and mutant plants deficient in telomere maintenance genes. First, we examined the influence of nutrient limitation on telomere length. We showed that both wild type and mutant plants grown on different phosphorus sources showed no changes in telomere length. Next, we studied whether drought can influence telomere length. Plants were grown in wet and dry conditions for 25 days and their relative leaf water content was measured to gauge the extent of drought. Despite having a substantial difference in leaf water content, wild type and mutant plants displayed no statistically significant changes in telomere length between drought-treated and control plants. Finally, we analyzed telomere length in wild type plants grown in different laboratories with varying growth regimes, which included variations in growth temperature and light conditions. Interestingly, telomeres were shorter by 200–300 bp in plants grown at higher temperature. We conclude that environmental factors may indeed affect *Arabidopsis* telomere length.

P.3.1.A-009
Chemical biosupercapacitors for biomedical application

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The development of miniature autonomous bioelectronic devices that function in the human or animal internal environments is one of the most popular areas of bioelectronics. In recent works, a concept was developed for the creation of charge-storing fuel cells, or in other words self-charging supercapacitors based on (bio)electrodes with a dual function of generation and accumulation of electric charge and operating in both continuous and pulse modes.

The main purpose of this work is to create a potentially implantable biodevice with a dual function of generation and accumulation of electrical charge on the basis of a membraneless nanobiocomposite biocathode with CNT/PANI/MvBOx composite material and a bioanode with GOx/AuNPs composite material, as well as investigation of their stability and efficiency in solutions close to the human blood.

Nanobiocomposite materials are widely used as components of electronic devices for biomedical applications (biosensors, biofuel cells, biobataries, etc.) Modern bioelectronic devices based on nanocomposite materials can be used to influence organs and tissues, as well as for point delivery of drugs.

Electrically conductive polymers are usually synthesized by chemical methods in an acid medium by oxidative polymerization of the monomer. This approach has a number of disadvantages, in particular, contamination of the final product with residual monomers and oxidant degradation products. Therefore, in this paper, electrochemical and enzymatic methods for the synthesis of electrically conducting polymers have been tested, which may be an alternative to chemical polymerization.

P.3.1.A-010
A subunit of transcription factor TFIIIC is controlled by PKA signaling pathway and thereby mediates regulation of RNA polymerase III transcription by carbon source

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Regulation of RNA polymerase III (Pol III) in yeast *Saccharomyces cerevisiae* in response to carbon source is mediated by general Pol III - repressor, Maf1. Upon a shift of yeast cells from glucose rich media to a medium with a non-fermentable carbon source the Pol III occupancy on tRNA genes was markedly decreased. Surprisingly, that shift also decreased the Pol III occupancy on tRNA genes in a strain lacking Maf1 protein indicating Maf1-independent regulation of Pol III association with tDNA in response to glucose availability. This is consistent with Maf1-independent effect of PKA kinase on regulation of Pol III by carbon source shown previously. Now we demonstrate that Tfc3 protein is involved in Pol III regulation by carbon source and is directly controlled by PKA. Tfc3, a part of TFIIIC transcription factor, is predicted to be phosphorylated by PKA at three serine residues. We showed, that the level of primary tRNA transcripts was decreased in *tfc3* mutant with inactivated PKA-phosphorylation sites when cells were transferred from medium with non-fermentable carbon source to glucose medium. Moreover, phosphosite mutations in Tfc3 affect interactions within TFIIIC complex and Bdp1 (subunit of TFIIIB) with Tfc4 (subunit of TFIIIC) in carbon source dependent manner. Our analysis indicated that *tfc3* mutant has defect in formation of the Pol III initiation complex and down regulates tRNA transcription. This study is supported by the National Science Center (UMO-2012/04/A/NZ1/00052).

P.3.1.A-011
mNeonGreen versus GFPs: a benchmark for the detection of single molecules

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Fluorescent proteins (FPs) are indispensable tools for studying protein functions in model systems and live cells. Here we characterise mNeonGreen (mNG), a further developed monomeric FP from the primitive chordate *Branchiostoma lanceolatum*, in comparison to the widely used variants EGFP and AcGFP from *Aequorea* species. Imaging of single immobilized FPs showed that mNG provides indeed up to three-fold higher signal-to-noise ratio than GFPs, whereby the total photon yield was largely outbalanced by the equally reduced photostability. Performing a statistical analysis on individual bleach-steps of domains contained in multimeric mNG fusion constructs revealed that 24% remained non-fluorescent after purification. Performing fluorescence correlation spectroscopy (FCS) in solution, where proteins experience shorter exposure times, the counts per particle (CPP) of mNG were consistently increased with respect to EGFP (1.4-fold) and AcGFP (3-fold) within a reasonable excitation power regime (<2 kW/cm²). As previously reported, all FPs were quenched in acidic buffers, albeit with a different response and dynamic signature: while GFPs occupy progressively protonation-dependent dark states (flickering, EGFP 50–300 μs, AcGFP 300–500 μs), flickering of mNG (50–200 μs) never exceeded 25% of the amplitude and, in stark contrast to the GFPs, showed a reversed pH-dependence. The molecular brightness of fluorescent mNG species remained stable across a broad range of pH values, although spectral deconvolution indicates transitions between three pH-dependent chromophore states. In cells, the CPP of multimeric mNG in both cytoplasm and nucleus increased predictably with a signal increment of 78% per domain that might be suitable to calibrate stoichiometric binding reactions. Thus, mNG appears as a superior fluorescent label for single molecule approaches and in particular for FCS, where the labelled proteins are observed in their diffusive state.

P.3.1.A-012
Discovery of geroprotective capacity of human placental preparation on *Caenorhabditis elegabs* model

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The process of aging is a serious and unsolved problem until now. Important direction in studying of aging process is the reassessment of drugs capacity that increase life expectancy and act against age-related diseases. The aim of our study was to evaluate the geroprotective properties of placental preparation LAENNEK, which technology of production was developed by Bioproducts Co. Ltd. In Japan. Today, the therapeutic effect of placental therapy was identified in hematology, gynecology, dermatology and aesthetic medicine. We used *Caenorhabditis elegans* model as well as eukaryotic cells, human fibroblasts specifically, to study geroprotective capacity of LAENNEK. This preparation was tested based on aging markers such as life extension, resistance to thermal and oxidative stresses, and the accumulation of β-galactosidase (SA-β-gal) in senescent human cells.

Studies have shown that the *C. elegans* lifespan increased by 92% in the presence of LAENNEK, compared to the control animals. Moreover, the greatest effect was achieved at the homeopathic doses of the preparation - 179 ng/ml. After 8 hours of incubation at 35 °C in the presence of LAENNEK nematodes survival was by 20% - 27% higher, compared to the population of control nematodes. After 24 hour of incubation, all tested nematodes were died, while 59% of nematode populations in the presence of LAENNEK remained alive. In the presence of 100 mM paraquat the nematode's life expectancy was increased by 67% in the presence of LAENNEK during 120 hours of incubation. The resistance of nematodes to 100 μM H_2O_2 was increased by 100% during the 140 hours of incubation in the presence of hydrogen peroxide. LAENNEK greatest effect of 18.7% reduction of the β–galactosidase (SA-β-gal) level in senescent human fibroblast cells was observed at the minimal concentrations of LAENNEK. The essential declining of senescent fibroblast cell's population by7.8% was observed in the presence of LAENNEK during 45 days of incubation.

Our experimental results were coincide with the anti-aging and regenerative effects as well as enhancing immunity and human body's defenses in general, which were observed during LAENNEK use by aesthetic medical clinic RHANA in its practical applications. Thus, these findings in both, pre-clinical and clinical studies, let consider LAENNEK as a promising candidate for future development as a new drug with geroprotective potential.

P.3.1.A-013
The ribosomal protein uL22 modulates the shape of the nascent protein exit tunnel

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Nascent proteins progress through an elongated tunnel until they exit from the ribosome. Biochemical, genetic and structural studies have shown that the tunnel is not just a passive path, but also has regulatory properties. Erythromycin is a clinically useful antibiotic that binds to an rRNA pocket in the entrance of the ribosomal exit tunnel and interferes with the progression of nascent chains. Commonly, resistance to erythromycin is acquired by alterations of rRNA nucleotides that interact with the drug. Mutations in the β-hairpin of ribosomal protein uL22, which is rather distal to the erythromycin binding site, also generate resistance to the antibiotic. Interestingly, most of these mutations do not reduce the affinity of erythromycin to the bacterial ribosome. We have determined the crystal structures of the large ribosomal subunit of *Deinococcus radiodurans* with a three residue insertion mutation in uL22 that renders resistance to erythromycin in its apo and erythromycin bound states. These structures reveal that the β-hairpin of L22, which is naturally positioned on the tunnel walls is shifted toward the center of the exit tunnel, triggering a cascade of structural changes among rRNA nucleotides that propagates to erythromycin binding pocket and increases its flexibility. Based on our results, we suggest a feasble mechanism that explains how nanscent proteins can be translated when erythromycin is bound to the ribosome. Furthermore, our findings support recent studies showing that the interactions between uL22 and specific sequences within nascent chains trigger conformational rearrangements in the exit tunnel that are essential for the translation of specific genes.

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P.3.1.A-014
The kinesin-5 mitotic motor Cin8 localization and activity are being differentially regulated by three Cdk1 phosphorylation sites in its catalytic domain

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The bipolar kinesin-5 biological nano-motors perform essential functions in mitotic spindle dynamics by ATP-driven motility along microtubules (MTs) as well as crosslinking and sliding apart antiparallel MTs of the spindle. It was previously demonstrated in our lab, that the *S. cerevisiae* kinesin-5 Cin8 is differentially phosphorylated during anaphase in at least one of the three cyclin-dependent kinase 1 (Cdk1) sites located in its catalytic domain: S277, T285 and S493. It was also demonstrated that this phosphorylation affects Cin8 localization to the mitotic spindle, and mitotic spindle elongation. Moreover, phosphomimic variant in which the three sites were replaced with aspartic acid exhibited lesser to non attachment to MTs *in vitro*, indicating that phosphorylation of at least one of these sites is required for Cin8 detachment from the mitotic spindle.

Here we studied the effect of each phosphorylation site in the catalytic domain on the regulation of Cin8 activity during mitosis. For this purpose, we quantitatively characterized the effect of multi-site phosphorylation of Cin8 at the three Cdk1 sites using *in vitro* phosphorylation assay, *in vivo* fluorescence intensity distribution analysis parallel and perpendicular to mitotic spindle, yeast viability test and immunostaining. We found that although the three Cdk1 sites undergo similar phosphorylation *in vitro*, *in vivo* they differ in the timing of their phosphorylation, and the strength of their effect. Thus they exhibit differential regulation of Cin8 activity during anaphase in cells, with the S277 site being most important in regulating Cin8 localization to the mitotic spindle, while the S493 site affecting the rate of spindle elongation.

P.3.1.A-015
Influence of cellular factors on oxygen consumption and transmembrane proton transport in cytochrome c oxidase

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Cytochrome *c* oxidase (CcO) consumes the major part of oxygen, and the changes in oxygen pressure influence on energy supply of the whole cell. Nitric oxide (NO) is one of the well-known inhibitors of CcO activity. The kinetics of the reaction (CcO with NO) are quite different with various CcO intermediates. Depending on the intracellular oxygen concentration and the resultant dominant redox state of the enzyme, the interaction between CcO and NO has a range of signaling consequences for cells in the perception of changes in oxygen concentration and the initiation of adaptive responses. In the present work the respiratory rate dependence on oxygen concentration is modeled under different medium parameters such as matrix pH and nitric oxide concentration. CcO activity was estimated via computer algorithm based on stochastic approach where the catalytic cycle of the enzyme is reported as a set of consecutive transitions between distinct states with various number of metabolites. The modeling showed that the part of fully reduced enzymes has decreased by 19% with increase of oxygen concentration from 1 μM to 15 μM, and has decreased in addition by 3% with increase of oxygen concentration to 70 μM. CcO efficiency (H^+/e^-) decreased from 0.98 to 0.9 with the increase of pH in physiological range according to lowering of proton transport rate through the D-channel, while oxygen flow through a unitary protein was not affected by the changes in pH. Addition of 10 nM nitric oxide lowered oxygen flow to $128 \pm 1\ s^{-1}$ at oxygen saturation. The modeling of pumping efficiency dependence on oxygen concentration using three schemes which differed in a binding order of oxygen indicated no significant distinction in the results for the schemes. Thus, we can assume that oxygen is able to bind to fully reduced binuclear center of CcO only. It was also remarkable that virtual simulations denoted nitric oxide could recover H^+/e^- efficiency of cytochrome *c* oxidase modified by external pH.

P.3.1.A-016
Tetrameric assembly of hGBP1 is crucial for both stimulated GMP formation and antiviral activity

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Interferon-γ inducible human guanylate binding protein-1 (hGBP1) shows a unique characteristic that hydrolyses GTP to a mixture of GDP and GMP through successive cleavages, with GMP being the major product. Like other large GTPases, hGBP1 undergoes oligomerization upon substrate hydrolysis, which is essential for the stimulation of activity. It also exhibits antiviral activity against many viruses including hepatitis C. However, which oligomeric form is responsible for the stimulated activity leading to enhanced GMP formation and its influence on antiviral activity, are not properly understood. Using mutant and truncated proteins; our data indicate that transition-state induced tetramerization is associated with higher rate of GMP formation. This is supported by chimaeras that are defective in both tetramerization and enhanced GMP formation. Unlike wild-type protein, chimaeras did not show allosteric interactions, indicating that tetramerization and enhanced GMP formation are allosterically coupled. Hence, we propose that after the cleavage of the first phosphoanhydride bond GDP·Pi-bound protein dimmers transiently associate to form a tetramer that acts as an allosteric switch for higher rate of GMP formation. Biochemical and biophysical studies reveal that sequential conformational changes and interdomain communications regulate tetramer formation via dimer. Our studies also show that overexpression of the mutants, defective in tetramer formation in Rep2a cells do not inhibit proliferation of hepatitis C virus, indicating critical role of a tetramer in the antiviral activity. Thus, the present study not only highlights the importance of hGBP1 tetramer in stimulated GMP formation, but also demonstrates its role in the antiviral activity against hepatitis C virus.

P.3.1.A-017
Simulation of the proton transport through half-channels of FoF1-ATP synthase

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F_oF_1-ATP synthase is unique energy transformer in a cell that acts as a molecular motor driven by the energy of the electrochemical gradient of hydrogen ions. In this paper, proton transport through the F_o factor ATP synthase is investigated, which is one of the most important processes necessary for the synthesis of ATP. Despite the long-term biochemical and structural studies, mechanism and characteristic time of proton transfer remain unknown. It is assumed that protons can move along the chain of amino acid residues and water molecules contained in the half-channels of the membrane part of the enzyme. In order to determine the direction of a proton transfer between centers, it is necessary to calculate the transition probability, which is inversely proportional to the time of elementary transfer. The times of proton transfer from one charged center to another are given by the quantum-mechanical model of one-dimensional motion. The Monte Carlo simulation of the possible pathways of a single proton transfer through the inlet half-channel with their statistical weights is carried out. The average time of proton transfer was $1.66 \cdot 10^{-10}$ s. Two amino acid residues (Asp119 and His245) were found to be essential for the proton transport. Exclusion of them from the pathway led to an increase in proton transport time, which is in agreement with known experimental data on site-specific mutagenesis. The effect of the membrane potential was observed on proton conductivity of the half-channel. Taking it into account the proton transport time has been decreased. New data obtained using this approach could be applied to both simulation of the proton transport process in protein channels and to develop a deeper understanding of the energy transformation mechanisms.

P.3.1.A-018
Transcription of damaged templates by Escherichia coli RNA polymerase and its mutant variants

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The activity of DNA-dependent molecular machines, such as nucleic acid polymerases, can be greatly affected by changes in the DNA structure. During transcription, DNA lesions can either induce nucleotide misincorporation by RNA polymerase (RNAP) or induce RNAP stalling, which can have pronounced effects on DNA repair and replication. However, molecular mechanisms underlying the diverse effects of various types of DNA lesions on transcription remain only partially understood. We investigated the influence of common DNA lesions on the activity of *Escherichia coli* RNAP and the fidelity of transcription *in vitro*. Most analyzed lesions significantly inhibited transcription and increased nucleotide misincorporation. To reveal RNAP regions important for transcription of damaged DNA templates, we obtained a series of mutant RNAP variants with amino acid substitutions in regions involved in contacts with the template DNA nucleotide and the incoming NTP. The strongest effect on transcription was observed for substitutions in the bridge helix and the trigger loop in the RNAP active site. In particular, substitutions of nonconserved residues I937T and G1136M in the trigger loop increased RNAP activity at the sites of DNA lesions likely by stimulating NTP incorporation. Thus, translesion RNA synthesis by bacterial RNAPs can be modulated by mutations of nonconserved residues in the RNAP active site. This work was supported by the grant 17-14-01393 of the Russian Science Foundation.

P.3.1.A-019
Functions of the bacterial RNA polymerase sigma subunit region 3.2 in transcription initiation on ribosomal RNA promoters

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Bacterial RNA polymerases (RNAPs) rely on a specialized transcription initiation factor, the sigma subunit, for initiation of RNA synthesis. The sigma subunit of RNAP has been implicated in all steps of transcription initiation, including promoter recognition and melting, RNA priming, abortive initiation, promoter escape and formation of promoter-proximal pauses. Previously, we showed that sigma subunit region 3.2 is involved in these processes by positioning the DNA template in the active site, stimulating binding of initiating NTPs (iNTP), and facilitating dissociation of the sigma subunit at later stages of initiation through direct interactions with the nascent RNA chain. In this work, we demonstrate that region 3.2 plays a crucial role in transcription initiation at a ribosomal RNA promoter (rrnBP1). RrnBP1 forms highly unstable complexes with RNAP and the efficiency of transcription initiation from this promoter was shown to depend on the concentration of the first initiating nucleotide (ATP). In addition, the activity of rrnBP1 during the stationary phase is suppressed by the stringent response factors DksA and ppGpp. We show that amino acid substitutions and deletions in sigma region 3.2 significantly increase the stability of RNAP complexes with rrnBP1 without having major effects on the values of apparent Michaelis constant for iNTPs. On the other side, mutations in region 3.2 make promoter complexes less sensitive to transcription suppression by DksA and ppGpp both *in vitro* and *in vivo*. This suggests that sigma region 3.2 plays a key role in the formation of unstable complexes of RNAP with rRNA promoters and in the regulation of rRNA synthesis at various stages of cell growth. This work was supported by a grant from the President of the Russian Federation for young scientists (MK-9567.2016.4).

P.3.1.A-020
Effects of suppressor mutations in *Escherichia coli* RNA polymerase on various steps of transcription

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Bacterial RNA polymerase (RNAP) plays a central role in coordination of transcription with other genetic processes, including DNA repair, replication, and RNA translation. Previously, it was shown that *rpo** mutations in *E. coli* RNAP suppress mutations in various DNA repair and DNA processing factors. This suggested that *rpo** mutations affect the interplay between these processes, probably by changing the properties of transcription complexes and directly affecting translesion transcription. However, the effects of *rpo** mutations on RNAP activity *in vitro* and

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in vivo remain largely unknown. We therefore obtained nine *rpo** substitutions located at several sites around the main RNAP channel in *E. coli* RNAP and analyzed their properties *in vitro*. It was shown that some, but not all, *rpo** mutations significantly decreased stability of promoter complexes. Most *rpo** mutations did not affect the average rate of RNA elongation but stimulated hairpin-dependent transcriptional pausing. At the same time, none of the *rpo** mutations affected transcription termination. Furthermore, the mutations did not change the efficiency of translesion RNA synthesis. Thus, previously reported *in vivo* phenotypes of the *rpo** mutations can be likely explained by changed interactions of RNAP with other regulatory factors that couple transcription with DNA repair and replication. This work was supported by the grant MK-8983.2016.4 of the President of Russian Federation for Young Scientists and grant 17-14-01393 of the Russian Science Foundation.

Protein Folding and Misfolding

P.3.1.B-001
The study of amyloidogenic properties of plant proteomes

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Amyloids represent protein fibrils with highly ordered structure possessing unique physical and chemical properties. Historically, amyloids were mainly perceived as the lethal pathogens causing dozens of incurable diseases in humans and animals. Nevertheless, in the last fifteen years it has become clear that amyloid is a biologically important form of proteins participating in the numerous biological processes. Functional amyloids were found in animals, fungi, bacteria, and archaea, but practically nothing is known about amyloids in plants. The goal of this study was to analyze amyloidogenic properties of the plant proteomes. We screened proteomes of more than 20 plant species using WALTZ and SARP algorithms. WALTZ algorithm is based on the position-specific matrices and predicts hydrophobic regions that tend to be amyloidogenic, while SARP algorithm detects low complexity regions enriched with Q or N, which is a particular feature of amyloids. We found that proteomes of plants are highly enriched with amyloidogenic regions. The number of QN-rich amyloidogenic proteins in the plant proteomes is 2–5 times higher than in *Saccharomyces cerevisiae*, which is a traditional object for studying QN-rich amyloids. The proteomes of plants are also rich in amyloidogenic regions predicted by WALTZ. The proteome of *Pisum sativum* is extremely enriched in proteins containing such regions, whose fraction reaches 13.5%. We performed functional analysis and demonstrated that the fraction of amyloidogenic proteins is significantly higher in several groups of proteins, the most important of which is seed storage proteins constituting an important part of human diet. The storage proteins of different plants form fibrous gels in specific conditions, but their amyloid properties were never tested before. Taking together, our data suggest that seed storage proteins may adopt amyloid state. This work is supported by the Russian Science Foundation grant No 17-16-01100.

P.3.1.B-002
Identification of novel amyloids in bacterial proteomes

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Amyloids are protein fibrils with characteristic spatial structure called cross-beta. They play important pathogenic and functional roles and have been found in different taxonomic groups, from prokaryotes to animals. Prokaryotes are the group, in which all known amyloids are implicated in physiological functions. At least nine groups of prokaryotic proteins have been shown to form amyloid fibrils that are involved in the biofilm formation, toxin storage, overcoming the surface tension as well as formation of the cell sheaths. We performed an analysis of the amyloidogenic properties within proteomes of socially important species, *Escherichia coli* and *Rhizobium leguminosarum*. The proteomes of these bacteria are characterized by relatively low content of QN-rich proteins predicted by SARP algorithm (1.06 and 1.66%, respectively), but have very high content of hydrophobic amyloidogenic regions predicted by WALTZ (about 13% of proteins in each proteome), which makes these species promising objects for proteomic screenings for novel amyloid-forming proteins. YghJ is a metalloprotease involved in the intestinal colonization by enterotoxic *Escherichia coli*. Using previously developed method for proteomic screening of amyloid proteins, we demonstrated that YghJ forms detergent-resistant aggregates at the endogenous level of production. Moreover, metalloprotease domain of YghJ forms unbranched fibrils which bind Thioflavine-T and exhibit apple-green birefringence upon Congo Red staining. We may conclude that bioinformatic screening represents a powerful tool for identification of novel amyloids in the proteomes of different species. The work of AAN, KSA, AOK, and MVB on *Rhizobium* is supported by Russian Science Foundation grant No 17-16-01100 and performed in ARRIAM, the work on *Escherichia* is supported by the grant of the President of Russia (MK-3240.2017.4) in SPbSU.

P.3.1.B-003
Physicochemical properties of Sup35NMp aggregates and their infectivity

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Prion and other neurodegenerative diseases are associated with misfolded protein assemblies called amyloids. The Sup35 protein (Sup35p) is a translation termination factor of *Saccharomyces cerevisiae* yeast. Its transition into prion isoform leads to formation of [PSI^+] factor. Only N-terminal part of Sup35p (Sup35NMp) is essential for [PSI^+] propagation. The prion formation is accompanied by Sup35p aggregation. Previously obtained in our laboratory alleles of $sup35^{KK}$ (designated M1–M5) contain double substitutions of polar amino acids for charged ones (two lysines). Such changes in the protein, according to theoretical assumptions and obtained experimental data, affect the prion structure and the properties of corresponding aggregates. Using TEM (transmission electron microscopy) we showed that these proteins with substitutions spontaneously form sodium dodecyl sulfate-resistant fibrillar aggregates whose width is increased compared to wild type (WT) protein. Addition of the sonicated fibrils to monomeric protein seeds its aggregation. Then

we used fibrils of Sup35NM-MXp to induce aggregation of native Sup35NMp. Further TEM analysis revealed that in all cases, except Sup35NM-M2p, width of obtained fibrils was increased compared to WT. We also showed that substitutions M1, M2, M4 lead to a slight increase in thermal stability in comparison with aggregates of the WT. We found out that all aggregates of proteins with substitutions are infectious to the same extent upon protein transformation. Overall, we observed no statistically significant differences in the thermal stability of investigated fibrils and their infectivity. The authors acknowledge St. Petersburg State University for research grants (1.37.291.2015, 15.61.2218.2013). This work also was supported by the grants of the President of the Russian Federation (MK-512.2017.4) and "RRCMCT" SPbSU.

P.3.1.B-004
Kinetic mechanism of aggregation of UV-irradiated glycogen phosphorylase b

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Test systems of different kinds are used to characterize the anti-aggregation efficiency of molecular chaperones of proteinous nature and low-molecular-weight chemical chaperones. Test systems based on aggregation of UV-irradiated proteins are of special interest because such test systems allow studying the protective action of different agents at physiological temperatures. To characterize the changes in the structure of the molecule of glycogen phosphorylase *b* (Ph*b*) from rabbit skeletal muscle induced by UV radiation, measurements on the enzyme activity, circular dichroism and differential scanning calorimetry were used. The kinetics of UV-irradiated Ph*b* (UV-Ph*b*) was studied at 37 °C (0.03 M Hepes buffer, pH 6.8, containing 0.1 M NaCl) using dynamic light scattering in the wide range of the protein concentrations. It has been shown that the order of aggregation with respect to protein is equal to unity. The conclusion has been made that the rate-limiting stage of the overall process of aggregation is the heat-induced structural reorganization of UV-Ph*b* containing concealed damages. The formation of the protein states containing UV-induced concealed damages should be taken into account in studies of the action of ultra violet on biological systems. The study was funded by the Russian Science Foundation (grant number 16-14-10055).

P.3.1.B-005
Characterizing the size of protein aggregate particles using a combination of NMR diffusion measurements and dynamic light scattering: case study of RRM2 domain from protein TDP-43

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The second RNA recognition motif (RRM2) from neuropathological protein TDP-43 is a small globular domain. Pulsed-field-gradient stimulated-echo (PFGSTE) NMR diffusion experiments and dynamic light scattering (DLS) measurements performed on a sample of RRM2 yield diffusion coefficients $1.38 \pm 0.01 \cdot 10^{-10}$ and $1.40 \pm 0.08 \cdot 10^{-10}$ m^2/s respectively, in excellent agreement with theoretical predictions. *In vitro* oxidation of RRM2 leads to formation of disulfide-bonded dimers followed by domain unfolding and assembly of the protein into soluble aggregate particles (APs). These particles prove to be unstructured and highly inhomogeneous, preventing their direct observation by HSQC spectroscopy – except for RRM2 C-terminal tail which remains flexible and solvated. In particular, the C-terminal residue N76 produces a sharp signal with the same intensity as in the control (unoxidized) sample. This signal has been used for PFGSTE NMR measurements on the oxidized sample, yielding the diffusion coefficient $0.55 \pm 0.02 \cdot 10^{-10}$ m^2/s. This is significantly different from the value determined by the DLS method, $0.40 \pm 0.01 \cdot 10^{-10}$ m^2/s. The difference can be understood once we recognize that DLS and PFGSTE NMR experiments provide different sampling of the polydisperse AP ensemble. Specifically, DLS is more biased toward bigger particles (weighted according to r^6, where r is the AP's radius) compared to PFGSTE NMR (weighted according to r^3 in the specific case when the signal originates from the fully flexible tail). Thus, the diffusion data from DLS and PFGSTE NMR measurements can be combined to characterize the size distribution function of the aggregate particles. We have shown that these data are consistent with the exponentially distributed r values and predict amply hydrated aggregate particles, ca. 3 grams of hydration water per gram of protein. The work has been supported by the RSF grant #15-14-20038.

P.3.1.B-006
Non-native disulfide bridges in protein-peptide complex and in protein homodimers trigger unfolding and aggregation

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We have investigated the complex between SH2 domain from proto-oncogene tyrosine kinase Fyn and low-affinity pTyr-containing peptide imitating the C-terminal sequence of the kinase. Guided by the structure of the complex PDB ID 2MRK, we sought to engineer a disulfide bridge between the peptide and the protein. Toward this goal we introduced a cysteine-for-glycine substitution yielding the peptide FynC with the sequence EPQ-pYQPCENL. Unexpectedly, formation of the intermolecular disulfide bond between the peptide residue C7 and the proximal protein residue C239 has led to protein unfolding accompanied by aggregation.

We hypothesized that formation of an adventitious intermolecular disulfide bridge (either engineered or engendered by oxidative stress) is likely to trigger protein unfolding. The key to this behavior is the reduced ability of disulfide-bonded complex to refold and its increased propensity to misfold, which makes it vulnerable to large thermal fluctuations. Indeed, we have shown that disulfide conjugate FynSH2:FynC cannot be renatured from solution of urea unless treated by DTT. Similar behavior has also been observed for other proteins, including RRM2 domain from neuropathological protein TDP-43 and ubiquitin-like protein SUMO2, which undergo disulfide-mediated dimerization under oxidizing conditions.

The emerging picture suggests that covalent peptide inhibitors, e.g. engineered cysteine-containing peptides, can strongly perturb their protein targets. Along the same lines, formation of non-native disulfide bridges under oxidative stress leads to protein unfolding followed by aggregation. This effect contributes to build-up of proteinaceous bodies in a number of neurodegenerative disorders. The work was supported by the RSF grant 15-14-20038.

P.3.1.B-007
Role of crystallins aggregation in cataract development: a potential target for non-surgical treatment of cataract

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Clarity of the lens is indeed an essential requirement for vision. Transparency of the lens mainly depends on the well-ordered arrangement of crystallin proteins. Constant age-related changes cause modification and disruption of crystallin protein arrangement which in turn results in aggregation and precipitation of crystallins. Recent reports support a notion that proteolytic cleavage of α-crystallin releases short peptides of high amyloidogenic propensity. Subsequently, these amyloids can interact with the native crystallins and make the milieu turbid which then leads to cataract formation. Surgical removal of cataract lens is the only available remedy till date. However, various socio-economic and geopolitical circumstances restrict the masses to avail the offered treatment. Moreover, several post-operative complications associated with surgical measures pose an added obstacle. Therefore, it is the need of the hour to design an alternate non-invasive and cost-effective strategy, which can benefit the large population. In the present study, we were able to isolate the amyloids from cataract lens. The isolated amyloids showed Congo red binding, Thioflavin-T binding, and fibrillar morphology when analyzed under transmission electron microscope. Our study suggests that the lens amyloids possess an association with native crystallins that renders the lens turbid. Furthermore, we have also observed that the relative concentration of amyloids in cataract lens is age-dependent. This study suggests that the peptide aggregation leads to recruitment of native crystallins and other proteins, thereby, provide a seeding effect in the age-dependent cataract. Further, characterization of lens amyloids will prove to be helpful in revealing the underlying molecular mechanism of cataract formation. This study also paves a way in the identification of potential drugable targets and thus will impart an alternate treatment strategy in cases of age-dependent cataract.

P.3.1.B-008
Interaction of a new derivative of fluorescent probe thioflavin T with insulin amyloid fibrils

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Thioflavin T (ThT) fluorescence is a proven method to detect amyloid fibrils that formation frequently accompany the development of many serious human diseases, including Alzheimer's, Parkinson's, prion, and many other diseases. Despite the obvious advantages of ThT for amyloid fibrils testing *in vitro*, the spectral properties of this dye restrict its applicability for testing of amyloid plaques in the cells and tissues. We investigated the properties and interaction to amyloid fibrils of a new benzothiazol dye trans-2-[4-(dimethylamino) styryl]-3-ethyl-1,3-benzothiazolium perchlorate (DMASEBT), which has a similar molecular structure with ThT, but differs from it by the length of the chain connecting the benzthiazol and aminobenzene rings, by the absence of a methyl group at the C2 atom and the replacement of the methyl group at the N7 atom of the benzothiazol ring by the ethyl group. Quantum-chemical calculations and spectral studies found that DMASEBT (like ThT) has properties of a molecular rotor. Interaction of DMASEBT with insulin amyloid fibrils is characterized by a high binding constant and a significant increase in fluorescence quantum yield of the dye, which are important properties of the fluorescent probe. In addition, the absorption and fluorescence spectra of DMASEBT bound to fibrils are significantly shifted to longer wavelengths (to the "transparency window" of biological tissues) in comparison to the spectra of ThT. The obtained results indicate that the new synthesized dye is a useful probe for amyloid fibrils detection and study of their formation. The present work is a step towards to the creation of fluorescent dyes that can be used for immediate testing of amyloid aggregates *in vivo*. This work was supported in part by grants 16-04-01614 and 16-54-00230_Bel from the Russian Foundation of Basic Research, the Program "Molecular and Cell Biology" of the Russian Academy of Sciences and RF President Fellowship SP-1982.2015.4.

P.3.1.B-009
Role of residues in conserved sequence region in formate dehydrogenases from different sources

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Formate dehydrogenase (FDH, EC 1.2.1.2.) catalyses a reaction of formate ion oxidation coupled with reduction of NAD^+ to NADH. The enzyme is a widely spread in nature.

Analysis of FDH sequences showed several common features in primary structure of the enzymes. For example, catalytically important Gln residue (Tishkov, V. et al. FEBS Lett. 1996) is placed in conserved motive – XPQP, where X in most bacterial enzymes is Phe (>90%), while in plants X is presented by Asn, Asp, Phe, and Tyr. In this work we studied role of residues in position X in three formate dehydrogenases from moss *Physcomitrella patens* (PpaFDH, X - Asn), bacterium *Staphylococcus aureus* (SauFDH, X - Tyr) and yeast *Ogataea parapolymorpha* (OpaFDH, X - Tyr). The next replacements were introduced in the FDHs - Y/A, Y/F and Y/Q in SauFDH, Y/D, Y/E, Y/A, Y/S in OpaFDH and N/D, N/E in PpaFDH. Data obtained showed that the residue X in XPQP motive plays a very important role in catalysis and protein stability in all studied FDHs. The best results for enzyme stability showed the substitutions of X to negatively charged residues Asp (PpaFDH and OpaFDH) and Glu (OpaFDH). In the case of SauFDH the replacements Y/A and Y/F practically did not changed the enzyme properties, but substitution Y/Q resulted in high increase of Km for both substrates - NAD^+ and formate, and decrease of enzyme stability.

The first Pro residue in motive XPQP in some very rare cases is substituted by another residue For exapmle, PpaFDH and FDH from yeast *Saccharomyces cerevisiae* (SceFDH) instead of Pro in the this position have Ala and Lys residues, respectively. The changes Ala/Pro in PpaFDH and Lys/Pro in SceFDH resulted in high increase of stability (about 4–10 times compared to wild type enzyme), but at the same time Km for NAD^+ and formate (PpaFDH) or only Km for formate (SceFDH) also increased.

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P.3.1.B-010
Modulation of prion protein folding and aggregation state by naturally occurring small molecule compounds

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Misfolding of proteins susceptible to amyloid transformation and aggregation leads to the development of severe neuropathologies, such as prion diseases, Alzheimer's and Parkinson diseases. One of the viable approaches towards slowing down these diseases is based on the compounds capable of binding to the prion protein (or its aggregates), thus inhibiting its conversion into the pathologic form and hindering further aggregation. Using molecular docking approach, we have recently suggested a binding site in native prion protein (PrP) for cinnamic acid derivatives. We have studied the activity of 8 cinnamic acid derivatives bearing different substituents in o-, m-, and p- positions of cinnamic acid in amyloid fibril formation, "seeding" and spontaneous agregation assays. We should also note that ferulic acid (L8) is a naturally occuring component of some foods and herbs including coffee beans, red wine, and *Scutellaria baicalensis*. The tested compounds were found to be active in amyloid fibril formation and "seeding" assays, monitored by ThT fluorescence, and the most pronounced effect was found for compounds L3, L6, L7, and L8. Based on the obtained results, a brief SAR analysis was performed on the cinnamic acid derivatives as PrP aggregation inhibitors. Substituents in 3- and 4- (m- and p-) positions were found to have the most pronounced effect. Compounds L6 and L7 bearing an acetamidoxy group in position 4 have shown the most promising effect in PrP aggregation assays, so we suppose that a polar substituent in this position is crucial for ligands with increased potency. Not only the tested compounds were shown to have no cytotoxity, but also some extent of cytoprotective effect. Therefore, we conclude that cinnamic acid derivatives are perspective candidates as prion protein aggregation inhibitors, while of special interest is ferulic acid which is naturally abundant in coffee beans.

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P.3.1.B-011
Soluble expression and easy purification of human bone morphogenetic protein-2

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Bone morphogenetic proteins (BMPs) are growth factors that are part of the transforming growth factor-β super family. The clinical application of BMP2 has helped an increasing number of patients achieve bone regeneration in difficult cases of bone healing. Overexpression of rhBMP-2 in *Escherichia coli* was previously reported, however, production of rhBMP-2 in *E. coli* is hampered by formation of inclusion bodies. Our goal was to develop an effective method to improve production of the soluble form of active BMP-2 omitting any refolding steps. The construct was based on modified pET-23 expression vector. In order to obtain a soluble form of the protein the construct containing ORF encoding hBMP-2 with His-tag was fused with SlyD that is a folding helper with both chaperone and peptidyl-prolyl cis-trans isomerase activities. The TEV protease cleavage site was inserted between SlyD and the ORF. Codons in the ORF encoding hBMP-2 were optimized in order to reach high level expression in *Escherichia coli*. The soluble precursor protein has been expressed in *E. coli* BL21 (DE3). After purification by Ni-chromatography the precursor protein has been cleaved by TEV protease at standard conditions, and in order to get rid of His-SlyD part and uncleaved precursor protein, as well as His-tagged TEV protease, Ni- chromatography has been applied for the second time. Pure hBMP-2 has been obtained in protein fraction unbound to Ni-resin. The biological activity of rhBMP-2 was confirmed by detection of increasing alkaline phosphatase level in MG63 cells and in mesenchymal stem cells, as well as by detection of mineralization on surface of fibroin microcarriers used for cell cultivation. The new approach makes it easy to obtain soluble, biologically active rhBMP-2 for laboratory and clinical use.

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P.3.1.B-012
An impact of inter-monomer and inter-domain interactions on the properties of near-infrared fluorescent proteins engineered from bacterial phytochromes

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Near-infrared fluorescent proteins (NIR FPs) engineered from bacterial phytochromes (BphPs) with the absorbance and fluorescence in the "NIR transparency window" of biological tissues meet the requirements for probes for deep-tissue in vivo imaging. Spectral properties of NIR FPs, composed of two PAS and GAF domains are affected by a position of the covalent attachment of their natural biliverdin chromophore (BV), with the BV-Cys^{PAS} adduct being more red-shifted relative to the BV-Cys^{GAF} adduct. The covalent binding of BV and its linkage position influences the stability of NIR FP holoprotein. Earlier, covalent binding of the chromophore in dimeric NIR FPs was proposed to depend on inter-monomer and inter-domain interactions. The fraction of the non-covalently bound chromophore in dimeric NIR FPs resulted in decreased quantum yield and brightness of the proteins. Here, we studied the biochemical, structural and spectral properties of a monomeric NIR FP, termed BphP1-FP, and its mutants in buffer solutions and in the presence of denaturant. BphP1-FP has two reactive Cys residues (Cys^{PAS} and Cys^{GAF}) while its mutants bears either Cys^{PAS} or Cys^{GAF}, or none of them. All BphP1-FP variants were monomeric. The elimination of inter-monomer interactions led to the absence of the non-covalently bound BV in the BphP1-FP variants. It also confirmed the inter-monomeric allosteric influence on the BV interaction with the monomers in dimeric NIR FPs. The unfolding of monomeric BphP1-FP variants in apoform was irreversible that was in contrast to dimeric NIR FPs, thus indicating that the inter-molecular contacts contribute to folding of NIR FPs. The increased stability of BphP1-FP is likely connected with the inter-domain cross-linking of the PAS and GAF domains to BV. We suggest

that similar structural changes in dimeric NIR FPs remove inhibition of the BV covalent binding and increase their stability.

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P.3.1.B-013
Detection of flexible portion of protein chain in Sup35NM amyloid fibrils by means of diffusion-filtered NMR experiment

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The *Saccharomyces cerevisiae* protein Sup35p is a part of the translation termination complex. A prion form of this protein has been intensely studied as a model for disease in higher organisms. In particular, the amyloidogenic portion of this protein, Sup35NM, attracted significant attention. One of the questions that remain unresolved is the status of M domain within Sup35NM: is it an integral part of the fairly rigid fibril architecture, or does it remain partially disordered? Historically, two types of experiments have been used to target flexible elements of protein fibrils: (*i*) solid-state HSQC experiments under MAS conditions and (*ii*) solution-state HSQC experiments on static samples. In both cases it is usually assumed that all protein material in the sample is sequestered in the fibrils. Such assumption is, generally speaking, incorrect. Protein fibrils always exist in a state of dynamic equilibrium with monomeric (oligomeric) species. Our diffusion experiments on a solid-state sample of Sup35NM demonstrated that ca. 20% of the signal originates from monomers, characterized by high translational mobility, whereas the remaining 80% belongs to the flexible fibril tails. Furthermore, we have prepared a dilute solution sample of Sup35NM, where ca. 85% of the signal was associated with monomers and the remaining 15% represented the flexible fibril tails. A comprehensive series of diffusion and relaxation measurements on this sample produced a self-consistent picture of dynamic equilibrium involving monomers and fibrils in slow exchange with each other. The addition of the diffusion filter to the standard HSQC sequence has allowed us to isolate the signals from the flexible parts of the fibril. Finally, the use of Sup35NM sample selectively labeled with ^{15}N in valine positions made it possible to delineate the boundary between the flexible and the rigid portions of the fibrils. This work was supported by the RSF grant 15-14-20038.

P.3.1.B-014
Is the structure and function of fusion proteins dependent on order of their domains?

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Nowadays, the study of proteins moves from the mapping of their functions to the studies of communications between their basic protein units - domains. The information on domain structures and interactions in their synthetic compositions can be used to decrypt inter- and / or intra- communication and allosteric modulation leading to specific domain functions. We proposed a new synthetic proteins composed of a well structurally characterized PDZ3 domain (part of ZO-1) which acts as a cytoskeleton and membrane protein, and the artificial protein molecule TrpCage. The designed proteins are linked by a flexible glycine linker in two different orders: PDZ3-TrpCage and its reverse form TrpCage-PDZ3. To decipher changes in the PDZ3 domain as part of the fusion two-domain proteins we used biochemical, biophysical methods and structural studies. To find out the changes in the binding affinity / specificity of PDZ3 in chimeras we used natural interacting peptides with the domain. Our data suggest a different behaviour of the both observed chimeras. The PDZ3-TrpCage protein consists of one domain, unlike the two-domain TrpCage-PDZ3 protein has a lower stability and tendency to aggregation. We assume that the order of domains in the protein context is an essential factor of allosteric modulation domain and determines their specific function.

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P.3.1.B-015
Evaluation of structural features of recombinant Mus a 5 allergen from Musa acuminata

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Reliable diagnostic reagents are prerequisites for successful therapeutic strategies in food allergy. Molecular basis for allergy to banana fruit is based on six IgE reactive proteins. Mus a 5 allergen is glucanase whose abundance in banana depends on various endogenous and exogenous factors. Replacement of food allergen extracts with well-defined individual allergens in a component resolved allergy diagnosis is promising concept for improved allergy diagnosis.

Aim of this work was to produce recombinant Mus a 5 for component resolved allergy diagnosis.

The allergen of interest is produced as 6His tag at the C terminus of the protein in *E. coli*. The protein is purified under native conditions by ion metal affinity chromatography and ion exchange chromatography (patent P-2015/0783). Recombinant protein is characterized by 2D PAGE, CD spectroscopy and gel chromatography. Immunological reactivity was tested in dot blot and ELISA by employing rabbit polyclonal antisera produced against banana proteins.

Recombinant Mus a 5 cDNA encodes a protein of 313 amino acid residues with predicted molecular mass 34436.70 and calculated p*I* 6.14. The yield of recombinant Mus a 5 was 20 mg per L of cell culture. By using experimentally obtained CD dataset and K2D3 method for prediction of protein secondary structure 77.2% of α helix and 2.8% of b strand were estimated in the recombinant Mus a 5. The spectrum shows two negative bands with minima at approx. 210 and 222 nm, characteristic of a protein with an appreciable α-helix content. The overall shape of the spectrum is similar to that of many α/b proteins, including the barley 1,3-b-glucanase. Recombinant Mus a 5 showed IgG reactivity in dot blot and ELISA with anti-banana polyclonal antibodies.

Recombinant Mus a 5 was produced as 6His tag protein and resembles the structure of beta-barrel folding motif. Mus a 5 preserved IgG binding reactivity and should be tested as a reagent for in vitro allergy diagnosis.

P.3.1.B-016
Optimization of production of the functionally active water-soluble recombinant membrane proteins and cold adapted enzymes

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Recombinant DNA technologies are an integral tool for fundamental and applied research. Possibilities of overexpression of target genes in host cells allow us to obtain the target recombinant protein in large-scale amounts that are several orders of magnitude higher than the amounts of their native analogs in the natural source. Thus, we have succeeded to obtain the recombinant functional cold-adapted enzymes in *E. coli*: α-N-acetylgalactosaminidase and α-galactosidase from the marine bacteria Arenibacter latericius KMM 426T and Pseudoalteromonas sp. KMM 701, which are interesting in their ability to convert A and B human erythrocytes, respectively. As a result of optimizing the expression conditions, the contents both of the recombinant proteins in the crude cell extracts were increased up to 200 mg per 1 liter of the transgenic bacterial culture. One of the most interesting possibility of genetic engineering is obtaining functional water-soluble proteins, including water-soluble membrane proteins. This makes it possible to study proteins in a folded native-like form without using detergents and denaturants which as known prevent some important studies of recombinant proteins with a living cells. Using the protein-chaperone DsbC, optimized expression conditions and composition of the expression medium made possible to obtain the functionally active water-soluble OmpF porin from Yersinia pseudotuberculosis, which is used in ELISA as effective antigen for the differential diagnostics of pseudotuberculosis. It has been shown that the purified water-soluble porin possesses similar immunogenic properties and antigenic characteristics with the previously obtained insoluble recombinant analogue. Similarity of spatial structure both of these proteins was proved by circular dichroism and intrinsic protein fluorescence.

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P.3.1.B-017
In silico identification and in vitro validation of Hsp60 inhibitor compounds

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Type I chaperonins are essential for cell viability. These proteins are found in chloroplasts, mitochondria and bacteria. In these compartments, chaperonins mediate the folding of newly translated, imported and stress denatured proteins. The 60 KDa chaperonin (mitochondrial Hsp60), is a Type I chaperonin found in the mitochondria. Together with the co-chaperonin, Hsp10, it uses ATP hydrolysis to drive conformational changes that facilitate folding of bound proteins. In addition to its protein folding activity, Hsp60 is also involved in inflammation, apoptosis regulation and carcinogenesis. We hypothesize that an inhibitor of Hsp60 that is targeted to mitochondria might enhance apoptosis in cancer cells and might have anti-cancer potential. In order to identify potential inhibitors, we used virtual screening methods, aimed at discovering small molecules that dock well to the nucleotide binding pocket of Hsp60, based on the recently published crystal structure. These compounds were designed to covalently bind to a cysteine residue located near the ATP binding site of Hsp60. The identified compounds were examined for their ability to inhibit the protein folding function of Hsp60 in vitro. Our results show that some of these designed compounds significantly inhibit protein folding by Hsp60, using two different in vitro substrates: Malate dehydrogenase and citrate synthase. However, the inhibitory effect was observed at a hundred-micromolar concentration range. We will attempt to characterize the binding site of the small molecules to Hsp60 using Mass Spectrometry and their specificity using mutagenesis. An understanding of how these small molecules interact with the chaperonin will provide the basis for developing more potent inhibitors of Hsp60.

P.3.1.B-018
Biophysical characterization of insulin amyloid – eukaryotic model membrane interaction: mechanism of insulin fibrillation intermediates induced membrane disruption

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Injection amyloidosis or "Insulin Ball" is associated with therapeutic use of insulin. The formation of amyloid ball at the site of injection reduces the insulin bioavailability and results in increase of therapeutic cost. There is great interest in understanding the interactions between insulin and eukaryotic model cell membrane that play an important role in amyloidosis of insulin. The heterogeneous environment, which results from the aggregation process, makes characterization of discrete steps difficult. By coupling high and low resolution spectroscopic techniques with microscopic analysis, we have been able to probe early interactions, which promote the binding of the insulin to b-pancreatic model cell membrane lipid surfaces. More specifically, the solid state NMR data in conjugation with single molecule fluorescence correlation spectroscopy (FCS) results show that the insulin amyloid intermediates interact with the lipid membrane while the dye leakage assay confirms the mechanism of membrane disruption similar to that of Aβ40 and hIAPP, occurring via a two-step mechanism. This work will help to differentiate the mode of interaction of insulin amyloid – eukaryotic cell membrane / β-pancreatic cell membrane models at an atomic resolution.

P.3.1.B-019
Flavivirus methyltransferase as target for virus treatment

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Many members of the genus *Flavivirus* in the family *Flaviviridae* are arthropod-borne and cause human disease. Dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), tick-borne encephalitis virus (TBEV) and Zika virus (ZIKV) are all emerging or reemerging pathogens. The flavivirus methyltransferase (MTase) is responsible for N-7 and 2′-O methylation of the viral RNA cap. The N-7 methylation of the cap structure is essential for RNA stability and stimulates their translation into viral protein. The 2′-O-methylation of the cap structure was demonstrated to protect viral RNA from being recognized by host cell sensors. These facts suggest that viral RNA capping

enzyme, the methyltransferase, is an attractive target for development of antivirals. In our study we cloned ZIKV and TBEV – strain HYPR MTase domain sequence into a pET SUMO expression system with an N-terminal His_6 tag. The MTase domains were produced in *Escherichia coli* Rosetta bacterial strain, purified and e*nzyme* assays were performed. Thereafter the MTases were crystallized. Crystal structures of almost all available flavivirus methyltransferases contain *S*-adenosyl-*L*-methionine (SAM), the methyl donor molecule that co-purifies with the enzymes. For the purpose of removal SAM we tried denaturation using urea, but the refolding process of MTase was accompanied by protein precipitation. However, we observed that high phosphate concentrations during protein purification lead to SAM dissociation. Our next aim is the crystallization of ZIKAV MTase with small molecule fragments to obtain starting hits for inhibitor design. We assume that our findings could lead to new treatment strategy of *Flaviviruses*.

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P.3.1.B-020
Amyloids of antimicrobial peptides: friend or foe

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Antimicrobial peptides (AMPs) are small peptides synthesized by all the organisms starting from bacteria to mammals and constitute an important part of innate immunity. They are generally classified into α-helical, β-sheet, mixed α/β and random coil based on their natural propensity to form secondary structure. Our recent study suggests that most of the β-sheet forming AMPs share considerable sequence similarities with many pathological amyloidogenic peptides such as Aβ, IAPP etc. In contrast, it has been reported that in addition to cellular toxicity, some of the naturally occurring amyloid structure possess antimicrobial activity *in-vivo*. These observations indicate the relationship between two opposite but highly relevant classes of peptides (antimicrobial and amyloidogenic peptides). Initially, we retrieved all the β-sheet forming AMPs from the AMP database (http://aps.unmc.edu/AP/main.php) and examined their aggregation propensity using computational algorithms (TANGO, AGGRESCAN etc). Surprisingly, around 50% of the total β-sheet forming AMPs were predicted to be amyloidogenic, further confirmed by using the synthetic analogs of the peptides *in-vitro* where they could form visible aggregates under physiological pH and temperature conditions. These aggregates display bathochromic shift in the Congo red absorbance spectra, strong fluorescence increase in Thioflavin T binding and fibrillar morphology under transmission electron microscopy which are the defining hallmarks of typical amyloids. The striking aggregation behaviour of AMPs renders them to acquire different structures and provides a unique sense of conformation specific functional attributes. As these AMPs are shown to form amyloid structures under physiological conditions, their existence and relevance under *in-vivo* system become an interesting aspect for further exploration of these AMPs.

P.3.1.B-021
A structural approach for understanding the mechanism of function of the mitochondrial Hsp60

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Chaperonins are large oligomeric proteins that assist in the folding of newly translated, stress denatured and translocated polypeptides. Also known as Hsp60, these folding machines consist of two heptameric rings that sequester partially folded proteins in the inner cavity of their barrel-like structure and assist their refolding in an ATP-dependent manner. Type I chaperonins exist in eubacteria, mitochondria and chloroplast and their activity requires the assistance of a co-chaperonin, Hsp10. Despite the high homology among Type I chaperonins, recent studies point to unique structural and functional properties of the mammalian mitochondrial chaperonin system implying a distinctive mode of action. Worth mentioning here is the fact that ADP acts as potent inhibitor of ATP-dependent protein folding by the bacterial chaperonin, GroEL, but not by Hsp60.

In order to gain insight into the unique effect of nucleotides on Hsp60 function, we set out to analyze its structure and function in the presence of transition state analogues of various nucleotides. To this end, we used X-ray crystallography in an effort to obtain high-resolution structures of different intermediates of the complex. We have recently succeeded in obtaining crystals of Hsp60 in complex with Hsp10 that diffract at low resolution, in the presence of a non-hydrolyzable ATP analogue. In the present work, we report on our progress in improving the quality of the crystals and their diffraction.

P.3.1.B-022
mRNA sequences evolved to control protein folding via ribosome pausing

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The genetic code is redundant, and many organisms show a bias in their use of synonymous codons. Codon bias is thought to influence various levels of gene expression control, perhaps the most surprising one being protein folding. Synonymous codons are translated at different rates by the ribosome, and since many proteins fold co-translationally, the elongation rate was suggested to influence nascent peptide chain folding. A proposed underlying mechanism suggests that pausing after each independently-folding unit that emerges from the ribosome allows correct folding before the rest of the chain emerges. The influence of synonymous mutations on protein folding and function was shown only for a few proteins, and a clear and general understanding of the phenomenon is still missing. In this work we aim to identify the structural elements in proteins that require ribosome pausing for correct folding. To this end, we analyzed 1,115 orthologue protein groups from *E. coli* and *B. subtilis* to identify occurrences of significant translation attenuation, compared to a randomized control model. The result set was screened to find enriched motifs with suspected roles in protein folding. Preliminary results suggest a new determinant that can explain selection for ribosome pausing: homo-dimerization may be assisted by ribosomal pausing after the dimerization surface emerges, in order to allow the consequent ribosome to translate the complemental monomer.

This hypothesis will be tested experimentally for a set of proteins, for which synonymous mutations will be created and their effect on protein function will be assessed.

P.3.1.B-023
Can we read genes?

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In principle we should be able to "read genes", i.e. to tell the stable structures based on the gene sequence alone, but, after 60 years of active research this is still not commonly achieved. Why? In the first place since the reductionist approach is not applicable, due to the weak coupling of many interactions in each molecule. It is a multibody problem. A solution to the problem depends, among other factors, on our ability to systematically determine what sequence elements direct formation of key intramolecular contacts along the polypeptide backbone, *in situ,* in the context of the whole molecule. Our long range goal is to identify sequence elements that form the initial contacts and thus arrest the molecules into a directed pathway of fast folding. While most research efforts focus at the rate of passage through the transition state ensemble we focus our attention at the earliest formed contacts. We use various modes of FRET and fluorescence measurements, microfluidic mixing methods and perturbation mutagenesis. We hypothesize that non-local contacts along the chain are key factor in avoiding aggregation and accelerating the folding pathway. This is the "loop hypothesis" and so far we have strong evidence in support of it. Knowledge of just few non-local contacts formed early in the folding transition is key to extending the simulation of the folding to the average size proteins. New methods and results will be presented.

P.3.1.B-024
Oxidative folding of bovine β-lactoglobulin by using a selenium reagent

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β-Lactoglobulin (βLG), an important component of mammalian milk, has attracted an interest in relation to the folding and unfolding processes. For bovine βLG, there are two major genetic variants (βLG-A and βLG-B), both of which contain five Cys residues forming two SS linkages between Cys66–Cys160 and Cys106–Cys119 with the other one (Cys121) being left in a reduced thiol form. Although the structural properties have been elaborately studied in the presence of a denaturant, the folding behaviors of βLG are not well understood. In this study, the oxidative folding pathways of bovine βLG-A have been studied by using a strong and selective oxidant, DHS^{ox}, developed in our laboratory. When fully reduced βLG-A (R) was reacted with 1 eq DHS^{ox} at pH 8.0 and 5 °C, SS formation was completed in one minute, generating several SS intermediates (I-1 to I-6) along with native βLG-A (N) and unreacted R as observed in RP-HPLC. With incubation, initially formed SS intermediates were gradually converged to I-6 and N probably through rapid SS reshuffling and slow air oxidation. The yield of N was about 60% after 24 h. The numbers of SS linkages present in the SS intermediates were determined by ESI-MS after the modification with AEMTS. The results suggested that among the 1SS intermediates SS reshuffling occurred fast under the employed conditions and there is at least one metastable 1SS species. On the other hand, N and I-6 are significantly stable among the 2SS intermediates. I-6 would be a dead-end, which cannot be refolded to N. When the similar oxidative folding was carried out at 35 °C, N and I-6 were regenerated more rapidly. In the meantime, I-6 was not observed in the presence of 1.25 M GdnCl at 5 °C, suggesting that I-6 can be converted to N if it is unfolded. Based in these observations, it was found that the control of reaction temperature as well as the denaturation of misfolded I-6 was important for effective oxidative folding of bovine βLG-A.

P.3.1.B-025
Chaperones expression levels are critical to form and maintain proper organization of myosin heavy chain proteins in *Caenorhabditis elegans*' muscle tissue

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Molecular chaperones regulate the folding and unfolding of proteins, and are able to direct unwanted proteins to degradation. In a previous study, we demonstrated that during the differentiation process of *Caenorhabditis elegans'* muscle cells, chaperone genes are expressed differentially. Some of these genes are tissue-specific while others are widely expressed throughout the organism, but with different expression levels. Thus, we asked whether this differential expression pattern is required in order to maintain proper protein-fold homeostasis of the proteome of each unique tissue. We began by mapping the chaperones and co-chaperones that are required for proper myosin heavy chain protein organization in the Sarcomere. Two chaperones are known to be involved in this process, the muscle-specific UNC-45 and Hsp90. Utilizing sensitive background mutants, we discovered three additional Hsp90 co-chaperones, *CeSti1*, *CeAha1* and *CeP23*. The knockout of these 3 co-chaperones result in severe motility decline of the animals, and disorganization of the myosin filament in the Sarcomere. Moreover, over expression of each of the three co-chaperones, or Hsp90, also resulted in motility decay and myosin filament disorganization. This suggest that the tissue specific proteome requires a well-balanced chaperon folding environment.

Chromatin Structure and Epigenetic Modifications

P.3.2-001
Epigenetic control of microglia polarization in brain pathologies

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Microglia are the innate immune cells of the central nervous system. After injury, infection or tumor development pathological factors induce microglia polarization, which results in production of factors shaping brain microenvironment. Microglial can switch between the inflammatory (M1) and cytoprotective, immunosuppressive (M2) activation. Transcriptomic analysis of microglial cultures exposed to lipopolysaccharide (LPS) or glioma-conditioned medium (GCM) revealed activation of distinct signaling pathways and specific patterns of gene expression. Mechanisms how external signals generate the complex and sustained patterns of gene expression are poorly understood. We hypothesized that epigenetic modifications may lie beneath microglia polarization. We found that the promoters of genes crucial for either phenotype are unmethylated and in open chromatin state. Using chromatin immunoprecipitation we detected reduced histone acetylation of

those genes in tumor-polarized microglia associated with the increased histone deacetylase (HDAC) expression and activity. Changes in repressive histone modifications after GCM or LPS were delayed and correlated with transcription down-regulation of stimulus-specific genes. HDAC inhibitors blocked functional changes associated with GCM or LPS treatment and stimulus-induced transcription up-regulation. Pretreatment with GCM established repressive marks at inflammation-related genes and reduced cells responsiveness to LPS. HDAC inhibitors restored the expression of LPS-inducible genes, which points to the role of epigenetic mechanisms underlying "the innate immune memory". Our results demonstrate that inflammation-related genes are epigenetically prone to be induced, while erasure of histone acetylation marks followedM2 genes is critical for glioma-induced microglia polarization. This opens gateways to therapeutic strategy based on modulation of innate immune cells withHDAC inhibitors.

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P.3.2-002
Insights into epigenetic environmental stress response and adaptation by chromatin modifications in barley (Hordeum vulgare)

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Plant epigenetics has gained vast interest, not only as basic research but also as a possible new source of beneficial traits. The mechanisms of epigenetic regulation could be exploited to broaden plant phenotypic and genetic variation, which could improve long-term plant adaptation to environmental challenges. Adverse environmental conditions, e.g. drought, determine plants to prematurely senescence with precarious recycling and major losses in yield. The plastid-nucleus located protein Whiry1 acts as an upstream regulator of leaf senescence binding to the promoter of senescence associated genes (SAGs) like senescence marker gene HvS40.

Our work was conducted on barley (Hordeum vulgare), wild-type and transgenic lines with a knockdown of Whirly1 (Hvwhy1kd) exhibiting a delayed senescence. Barley plants were grown under untreated and drought stress conditions. The study aimed to reveal the epigenetic indexing at senescence and stress responsive gene HvS40. Stress-responsive loading with histone modifications at 6 gene regions of HvS40 (2 regions in the promoter, one region around translation start site and 3 regions located in the gene body) was analysed by ChIP and quantified by RTq-PCR. Drought is causing in wildtype a significant increase in the levels of euchromatic mark H3K9ac all over the analyzed gene regions which correlates with a massive induction of HvS40, while no substantial increase of H3K9ac in why1kd plants was observed.

The results suggest that drought induced expression of HvS40 is under epigenetic control but the heritability of these histone modifications loading has to be studied especially because it is known that heritability of epigenetic modifications offers an attractive possible mechanism of adaptive processes.

P.3.2-003
Adult and larval subloci of the zebrafish major alpha/beta-globin gene cluster possess different epigenetic state and spatial chromatin structure

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In warm-blooded vertebrates the α- and β-globin genes are organized into domains of different types and are regulated in different fashion. In cold-blooded animals, in particular in tropical fish Danio rerio, α- and β-globin genes are gathered in common gene clusters. The major cluster of Danio rerio globin genes is located on the 3rd chromosome and includes α- and β-globin genes of embryonic-larval and adult types. Upstream to the cluster the gene c16orf35 is located, which in warm-blooded vertebrates harbors the main regulatory element of the α-globin gene domain. In the study, using the technique of transient transfection of erythroid cells with genetic constructions, we have demonstrated that a functional analog of MRE of warm-blooded animals is located in the 5th intron of Danio rerio c16orf35 gene. This enhancer stimulates activity of promoters of both adult and embryonic-larval α- and β- globin genes. Also we have studied the spatial organization of the major cluster. Using chromosome conformation capture (3C and 5C techniques), we have compared the spatial configuration of the α- and β-globin domain in red blood cells (RBCs) of Danio rerio, expressing embryonic-larvae or adult globin genes. Moreover, we have discovered genetic element, located between subdomains of embryonic-larval and adult globin genes, which demonstrates enhancer-blocking activity.

P.3.2-004
Interaction between CLAMP and MSL2 is required for specific recruiting of MSL complex to X chromosome

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The male-specific lethal dosage compensation complex (MSL-DCC) selectively assembles on the X chromosome in *Drosophila* males and activates gene transcription twice through histone acetylation. A MSL recognition element (MRE) sequence motif nucleates the initial MSL association, but mechanism of its recognition remains unknown. These sequences are marked by two proteins: male specific MSL2 and zinc finger protein CLAMP which is expressed through both sexes. We identified that Msl2 directly interacts with Clamp and mapped the domains required for interaction between these proteins. Deletion of the CLAMP-interacting domain in MSL2 resulted in loss of binding of MSL complex to the X chromosome. These results suggest a key role of interaction between MSL2 and CLAMP in recruiting of MSL complex to X chromosome. The work was supported by the Russian Scientific Foundation (grant No. 14-24-00166).

P.3.2-005
Heterogeneity of telomeric piRNA clusters in *Drosophila melanogaster* germline

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Telomeres of *Drosophila melanogaster* are maintained by transpositions of three non-LTR retrotransposons *HeT-A, TART* and *TAHRE* to chromosome ends. To maintain proper chromosome length and genome integrity, their transposition rates should be tightly regulated, especially in germline. In *Drosophila* germline, telomere length is controlled by telomeric piRNA produced from telomeric element transcripts. Previous analysis of ovarian small RNA deep sequencing data revealed abundant telomere-specific piRNA from both genomic strands suggesting that telomere regions represent dual-strand piRNA clusters, but it is still unclear whether all telomeric sequences are equally efficient in the piRNA production. We took advantage of transgenic constructs *P{EPgy2}* located at different telomeric regions and use them as a unique marks that allowed us to examine piRNA production and chromatin structure of repetitive telomeric sequences. Ovarian small RNA libraries sequencing and subsequent bioinformatic analysis revealed that transgene inserted at retrotransposon *TAHRE* of 2nd chromosome produces abundant piRNA, whereas transgenes inserted within retrotransposon *TART* of 4th and 3rd chromosomes generate low amount of small RNAs. Chromatin immunoprecipitation analysis (ChIP) demonstrated accumulation of trimethylated histone H3 lysine 9 (H3K9me3) and heterochromatin protein 1 (HP1) at all studied telomeric transgenes. H3K9me3 chromatin mark is recognized by HP1 and its homologues. The germline-specific HP1 homologue Rhino binds specifically to the dual-strand piRNA clusters providing piRNA processing of precursor transcripts. ChIP results showed that Rhino is associated only with telomeric transgenes generating abundant piRNAs and located within *TAHRE* and in telomere associated sequences. Collectively, our findings reveal heterogeneity of telomeric piRNA clusters in their chromatin structure and piRNA-production ability. This work was supported by the Russian Science Foundation (16-14-10167).

P.3.2-006
Self-association of G-rich dsDNA into G4/IM-synaptic complex

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G-quadruplexes (G4s) are noncanonical nucleic acid structures consisting of planar guanine tetrad arrangements. They play important role in regulation of key cellular processes, such as DNA recombination, replication, transcription and others. Wherever there is a G-quadruplex forming sequence in one strand the complimentary strand contains a C-rich sequence capable of forming an I-motif (IM). Although structures of G4s and IMs themselves are well-characterized, little is known about their behavior in dsDNA. Here we report AFM studies of DNA duplexes ($\approx$ 200 bp) containing $(G_3T)_nG_3$ sequences ($n = 1 \div 5$) in the middle regions. In the case of $n \geq 3$, the middle regions could fold into G4/IM; while in the case of n = 1, 2, no intrastrand noncanonical structures are possible. Surprisingly enough, AFM scanning of the duplexes revealed intermolecular G4/IM-synaptic complex in all cases (the images contained cruciform and higher order structures). No signs of such complex were visible in AFM images of the control duplex that lacks the $(G_3T)_nG_3$ middle region (the double helixes do not interact with each other). It should be noted that low-ionic strength solutions (10 mM KCl, 10 mM Tris*HCl, pH 5.6) were used in our AFM experiments, while in vivo KCl concentrations are substantially higher, which makes G4/IM-synaptic complex more likely. Molecular modeling was used to elucidate possible structures of the G4/IM-synaptic complexes, FRET-experiments were also performed to confirm two possible structures of the complexes in the case of n = 1, 2. Collectively, our findings suggest that formation of G4/IM-synaptic complexes may be an important factor of chromatin remodeling, recombination, replication, and transcription processes.
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P.3.2-007
Studying RNA-DNA interactome of the cell

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It is becoming increasingly evident that eukaryotic genomes encode thousands of non-coding RNAs, many of which are now implicated in diverse biological processes. In particular, non-coding RNAs have been demonstrated to regulate all aspects of gene expression, including transcription, processing and post-transcriptional control pathways. To study the functions of non-coding RNAs in chromatin, we developed a new genome-wide approach, designated Red-C (RNA ends on DNA capture), to simultaneously map the sites of DNA binding for all RNA molecules present in the nucleus. The method is based on adapter-mediated ligation of RNA and DNA in situ followed by high-throughput sequencing of the chimeric RNA-DNA molecules. By using various cell models, we study RNA-DNA interactome of the cell in differentiation, disease, aging, and other physiological processes. Our results give promise for greatly improving the in vivo functional characterization of non-coding RNAs. This work was supported by the Russian Science Foundation (grant 14-24-00022).

P.3.2-008
Pancreatic beta-cells plasticity – the role of stearoyl-CoA desaturase 1

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Type 2 diabetes (T2D) is characterized by two major pathological abnormalities: insulin resistance in peripheral tissues and progressive loss or dysfunction of insulin-producing pancreatic β-cells. The current understanding that loss of β-cell mass in T2D is primarily induced by β-cell death has been recently challenged. Over the past decade genetic and epigenetic studies of endocrine cells within the pancreas revealed a certain degree of β-cells plasticity under metabolic stress, supports the view that active regulation of gene expression is essential for the maintenance of functional β-cells identity. In the present study we tested the hypothesis that stearoyl-CoA desaturase 1 (SCD1), which plays a significant role in fatty acids metabolism, affects expression patterns of transcription factors involved in maintenance pancreatic β-cells identity via epigenetic mechanisms. The experiments were carried out *in vivo*

on SCD1 knock-out (SCD1 KO) mice and *in vitro* on INS-1E β-pancreatic cell line, where lipotoxicity was induced by palmitic acid treatment. On both *in vivo* and *in vitro* models we observed lower expression of transcription factors which are crucial to maintaining β-cells identity (Pdx1, FoxO1, Isl1) and reduced methyltransferase 1 (Dnmt1) protein level in comparison to appropriate controls. We showed that pancreatic islets of SCD1 KO mice are smaller, secrete less insulin after glucose stimulation and are characterized by increased glucagon level comparing with wild type mice. Furthermore, we noticed that inhibition of SCD1 by specific inhibitor (A939572) as well as silencing of SCD1 gene expression by siRNA, result in DNA hypomethylation in pancreatic β-cells and leads to significant changes in methylation status within *Pdx1* promoter region. Obtained results suggest that SCD1 activity may influence the identity of pancreatic β-cells through DNA methylation pattern alterations.

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P.3.2-009
Lysine methylation signaling at chromatin

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Greater than fifty methyltransferases (PKMTs) are predicted to be present in the human proteome; however, the catalytic activity and substrate specificity for the majority of these enzymes is unknown.

Using the protoarray system, we have identified PAK4 as a new substrate for methylation by the PKMT SETD6. Our data demonstrate that SETD6 methylates PAK4 both in vitro and at chromatin in cells. Interestingly, depletion of SETD6 in various cellular systems significantly hinders the activation of the Wnt/beta-catenin target genes. PAK4 was recently shown to regulate beta-catenin signaling, and we show that SETD6 is a key mediator of this pathway. In the presence of SETD6, the physical interaction between PAK4 and beta-catenin is dramatically increased, leading to a significant increase in the transcription of beta-catenin target genes.

In a second project, we have utilized the protoarray system to define SETD3 interactome and identified 172 new interacting proteins. We uncovered and validated a novel interaction between SETD3 and the transcription factor FoxM1 which has been shown before to regulate the expression of VEGF. We provide evidences for a new methylation signaling at chromatin which describe a functional interplay between SETD3 and FoxM1 in the activation of VEGF expression under normoxic and hypoxic conditions.

Taken together, these findings provide new insight into PKMT biology and demonstrate that the methylation of non-histone substrates serves as negative or positive regulators of transcriptional signaling at chromatin.

P.3.2-010
Breaking good: +1 nucleosome destabilization by nicking

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The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei of human peripheral blood mononuclear cells to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Elution profiles reflecting relative destabilization were measured in the case of the TSS-proximal H3K4me3 and H3K27ac marks, while the nucleosomes carrying any of 14 different other PTMs were significantly more resistant. Nicking, including treatment of live cells with bleomycin, did not affect the stability of nucleosomes carrying the above active marks, while those of the second group were all destabilized. H3K4me3 by itself doesn't destabilize in view of the elution profiles recorded on H3K4-trimethylated LacO-arrays. NFRs generated by salt elution outside TSSs did not destabilize nucleosomes either. We suggest that the H3K4me3 and H3K27ac active marks specify dynamic nucleosomes accomodating already relaxed DNA sequences, facilitating the eviction of the +1 nucleosome during pause release, while most other nucleosomes hold the DNA in constrained superhelices. This conclusion is supported by the results of mapping of DNA breaks carrying free 3'OHs in DNA immunoprecipitation – NGS experiments. PN and IL are shared first authors. Support: OTKA 72762, 101337, Fulbright fellowship (G.Sz), TÁMOP 4.2.2.A-11/1/KONV-2012-0023, TÁMOP 4.2.4. A/2-11-1-2012-0001, GINOP-2.3.2-15-2016-00044.

P.3.2-011
Distinct pattern of epigenetic DNA modifications in leukocytes of colorectal carcinoma patients and in patients with precancerous conditions - benign adenoma and inflammatory bowel diseases

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The most plausible mechanism responsible for active DNA demethylation involves ten-eleven translocation (TET) proteins which catalyze oxidizing 5-methylcytosine to 5-hydroxymethylcytosine, and then to 5-formylcytosine and this further to 5-carboxylcytosine. Also 5-hydroxymethyluracil may be generated by TET enzymes and has epigenetic functions. 5-hydroxymethylcytosine is profoundly reduced in many types malignant cells including colorectal cancer. Vitamin C and vitamin A act as modulators of TET activity and enhance 5-hydroxymethylcytosine production. In this study we investigated 4 groups: healthy control, patients with inflammatory bowel disease, individuals with benign polyps (colon adenoma) and patients with colorectal cancer. In all participants we measured: i/ the level of epigenetic DNA modifications and marker of oxidatively modified DNA namely 8-oxo-7,8-dihydro-2'-deoxyguanosine in leukocytes using isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry; ii/ the expression of TETs mRNA using RT-qPCR and iii/ the plasma concentrations of vitamins A and C using chromatographic assays. We observed that all patients groups had significantly lower levels of 5-methylcytosine and 5-hydroxymethylcytosine compared to control group. Similar trend was found in the level of 5-hydroxymethyluracil. Patients with inflammatory bowel disease were characterized by the highest levels of 5-formylcytosine and 8-oxo-7,8-dihydro-2'-deoxyguanosine. Individuals with colorectal cancer had the lowest concentration of vitamin C and A. Inflammatory bowel disease group were

characterized by the highest expression of TET1 and TET2. In conclusion, for the first time, we found that every studied setting: healthy status - precancerous conditions - colorectal cancer has characteristic pattern of epigenetic modifications in leukocytes' DNA.

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P.3.2-012
Alcohol applied during preimplantation in vivo induces changes in the expression and CpG methylation of placental Igf2/H19 locus

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The present study examines a potential link between ethanol-induced placental growth retardation and changes in the normal epigenetic profile of the *Igf2*/*H19* imprinted locus. Preimplantation is a very sensitive stage to the action of teratogens like ethanol during mouse and human embryogenesis. Two doses of ethanol – 2.9 and 5.8 g/kg were administered at 3.5 day post coitus (dpc) to pregnant C57BL/6 mice. Placentas harvested at 11.5 dpc were used for analysis of growth restriction, gene expression and CpG methylation. Expression of the *Igf2* and *H19* imprinted loci were examined together with the *de novo* and maintenance methyl-transferases Dnmt1, Dnmt3a, Dnmt3b. CpG methylation was assessed in all differentially methylated regions (DMRs) of the *Igf2*/*H19* locus, i.e. DMR0, DMR1, DMR2 and DMRH19. Ethanol-exposed placentas showed decreased growth in comparison to control group. Real-time PCR analysis demonstrated a dose-dependent reduction of *Igf2* expression and increased levels of *H19* after alcohol treatment. The higher dose of 5.8 g/kg ethanol decreased the DNMT1 and DNMT3a expression levels as well as the methylation of DMRH19 and DMR0 in placentas. Spearman's rank correlation determined a significant correlation between placental weight and methylation of DMR0, but no significance with DMRH19. Mediation analysis revealed that there was a statistically significant indirect effect of 5.8 g/kg dose of ethanol on placental weight, mediated through the change of methylation levels of DMR0. Reduced weight of the placentas subsequently appears as the mediator in the indirect effect the high 5.8 g/kg ethanol dose has on embryonal weight. Thus, our results provide the first in-depth analysis of the preimplantation epigenetic mechanisms implicated in the intrauterine growth restriction (IUGR) - one of the key features of fetal alcohol syndrome (FAS).

P.3.2-013
Long non-coding RNAs expression pattern in cervical oncogenesis

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One of the most common type of cancer that affects women worldwide is cervical cancer. An important role in cervical oncogenesis is played by the two viral oncogene (E6 and E7) of high-risk human papillomaviruses (hrHPV). Recently, there is evidence that illustrates an association between long non-coding RNAs (lncRNAs) expression pattern and oncogenesis. This study aims to investigate lncRNAs expression profile in cervical cancer in relationship with hrHPV infection. Specific siRNA was used in HeLa cell line in order to silence E7HPV18 oncogene. From treated/untreated cells total RNA was isolated and using LncProfiler qPCR Array Kit (System Biosciences) lncRNAs expression levels were determined. Next, using qRT-PCR expression levels for selected lncRNAs were evaluated in patient samples (n = 29) and controls (n = 20). We found that from a total of 90 lncRNAs differential expressed in E7-siRNA treated/untreated cells, 24 were up-regulated whereas 66 showed a down-regulated expression profile. Moreover, it was noted that 15 lncRNAs exhibited a significant difference between treatments (fold change > 2; $P < 0.05$) from which Hoxa11as (fold change:74.8), ANRIL (fold change:27.93), Gomafu (fold change:3.27), NEAT1 (fold change:2.87), UCA1 (fold change:2.7), E2F4 antisense (fold change:2.65) presented the highest differences. Hoxa11as and ANRIL were selected for validation in patient's samples and both show significantly reduced levels of expression in cervical cancer samples as compared with controls ($P = 0.024$; respectively $P = 0.0193$). The expression levels correlate with tumour grade, size and lymph node metastasis. Notably, an association between ANRIL expression levels and hrHPV infection was observed ($P < 0.05$). The obtained results showed a different lncRNAs pattern of expression in HPV induced-cervical oncogenesis and suggested a potential role for these epigenetic factors in this pathology. This study was supported by PNII RU-TE-2014-4-2502.

P.3.2-014
The effect of hrHPV infection on DNA methylation process durin cervical oncogenesis

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Human papilloma virus (HPV) is considered the aetiologic agent of cervical neoplasia and infection with high risk types HPVs was associated with cervical dysplasia and carcinogenesis. DNA methylation and histone modifications are the main mechanisms of epigenetic regulation. MBD proteins exhibit methyl-CpG-binding domains that mediate the interaction with methylated DNA. The aim of this study was to establish the influence of hrHPVs infection on MBDs, DNMTs expression pattern and 5-mC percentage in cervical oncogenesis. In this purpose DNA and RNA were isolated (TriZol) from cervical samples (159 patients and 40 control samples). Gene expression levels (MBD1,3,4 and DNMT1,3a) were investigated using qRT-PCR. $2^{-\Delta\Delta Ct}$ method was used to establish n-fold gene expression. 5-mC percentage was estimated with 5-mC DNA ELISA (Zymo Research). Significant differences were found in DNMT1 gene expression especially between CIN2 + and SCC samples ($P = 0.0013$), whereas for DNMT3B gene the expression level was increased in tumor samples (mean = 2.406 ± 1.088) as compared to the precursor lesions ($P = 0.0027$). A significant increase was observed for MBD1 gene between CIN2 + and SCC ($P = 0.046$) samples, while MBD3 gene found a significant difference in expression levels between CIN1 and CIN2 + ($P = 0.0229$). MBD4 gene showed a significantly high level of expression in tumors (mean = 2.824 ± 0.9320) ($P = 0.004$). The correlation between the MBDs level of expression, and the percentage of 5-mC, showed a good connection with MBD1 levels (r^2=0.3551, $P = 0.0056$). These results illustrate the involvement of epigenetic alterations in cervical oncogenesis and could serve as a starting point for diagnosis and prognosis. PNIIRU-TE-2014-4-2502.

P.3.2-015
Genetic determinant of epigenetics

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Genomic regulation is an integrated mechanism which incorporates genetic and epigenetic components. Although epigenetic profiles vary dramatically between cells with the same genetic information in multicellular organism, recently it has been shown that genetic features (DNA sequence) to some extend determine epigenetic profiles of a cell. For the majority of epigenetic profiles, in particular for histone modifications, genetic determinants are largely unknown. The question remain unanswered: how the enzymes performing most of histone modifications are targeted to specific regions of the genome. To address this question we first determine the regions of variable histone modifications (present only in some of the studies cells types). Among several histone modifications, acetylation (H3K9ac, H3K27ac) was one of the most variable. Than we performed a correlation analysis between the presence of the histone modification and the expression of DNA binding proteins (transcriptional factors, TFs) and long non-coding RNA with the aim to determine potential candidates that might target epigenetic complexes to genomic regions. As a last step, for sub-selected lncRNAs and transcriptional factors we computationally predicted DNA binding motifs and regions where lncRNAs could form triplexes with double stranded DNA. We determined several promising lncRNAs and TFs that could contribute to establishing the profile of H3K27ac. The work is supported by RSF grant 15-14-30002 to YAM.

P.3.2-016
A universal model underlying topologically associating domains

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Hi-C is a genomic technique that measures pairs of DNA loci that are in spatial contact. Hi-C has led to the key discovery of contact patterns known as Topologically Associating Domains (TADs), which are thought to constitute a novel higher-order chromatin organization. TAD-like patterns have been observed in a wide spectrum of species ranging from bacteria to mammals. In mammals, TADs have been suggested to specify secluded regulatory microenvironments for enhancers and promoters. Thus, it is of major importance to understand what TADs are, how these 3D structures are encoded in the 1D genome and how they may affect gene regulation. Here we show, using a probabilistic model, that the observed TAD-like patterns, over the entire range of species, can be explained as the joint action of independent insulation events, averaged over a population of cells. Importantly, this suggests that TADs can be encoded by local genomic events (such as the binding of a protein to DNA) that occur independently of one another, without the requirement of any locus-specific loops. As a consequence, our model allows to robustly predict the effects of genomic perturbations on 3D genome organization. We use this to demonstrate that our model can predict the effects of genomic rearrangements in a conserved developmental locus and provide a quantitative explanation of how these rearrangements cause regulatory rewiring - ultimately leading to limb malformation. Finally, we demonstrate how our model allows us to identify species-specific mechanisms underlying insulation with high accuracy.

P.3.2-017
Urinary levels of DNA damage markers and active demethylation products of 5-metylcytosine – a non-invasive assessment of the whole-body epigenetic status

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The aim of this work was to urinary measurement of epigenetic DNA modifications. Products of active DNA demethylation are released into the bloodstream and eventually excreted with urine. Therefore, whole-body epigenetic status can be assessed non-invasively on the basis of the urinary excretion of epigenetic modifications: 5-hydroxymethylcytosine (5-hmCyt), 5-formylcytosine (5fCyt), 5-carboxylcytosine (5caCyt) and 5-hydroxymethyluracil (5-hmUra). We applied isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) for direct measurement of 5-hmCyt, 5fCyt, 5caCyt and their deoxynucleosides in the same urine sample. The study was performed in four groups: control group of healthy volunteers, the adenoma patient group, the inflammation patient group and the carcinoma patient group. Human urine contained all the modifications except from 5-formyl-2'-deoxycytidine (5-fdC) and 5-carboxyl-2'-deoxycytidine (5-cadC). A significant difference in the urinary excretion of 5-hmdC was found between healthy subjects and carcinoma patient group, as well as strong correlations between the majority of analyzed compounds.

P.3.2-018
β-actin dependent global chromatin organization and gene expression programs control cellular identity

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During differentiation and development, cell fate and identity are established by waves of genetic reprogramming. Although the mechanisms are largely unknown, during these events dynamic chromatin reorganization is likely to ensure that multiple genes involved in the same cellular functions are co-regulated depending on the nuclear environment. Here, we report novel evidence that nuclear actin significantly contributes to these mechanisms. Using a high content screening platform for phenotypic profiling of β-actin knockout mouse embryonic fibroblasts (MEFs) we discovered that heterochromatin is repositioned from the perinuclear region towards the nuclear interior while both repressive (H3K9me3) and active (H3k4me3) epigenetic marks are significantly altered. Transcriptional profiling of the β-actin knockout MEFs by RNA-Seq showed these global chromatin alterations accompany changes in gene expression patterns, influencing cellular identity. Specifically, gene programs involved in angiogenesis, cytoskeletal organization and myofibroblast features were upregulated in β-actin knockouts. Using in vitro angiogenesis assays and gel contraction assays we show that β-actin knockout MEFs indeed acquired both angiogenic and contractile features, otherwise absent in wild type cells. These novel features are β-actin-dosage dependent as heterozygous MEFs with a single β-actin allele display intermediate phenotypes. Furthermore these features are due to a unique function of β-actin in the nucleus since the majority of alterations observed in the β-actin knockout MEFs at both chromatin and transcriptional level are rescued by

reintroducing β-actin in the cell nucleus. We propose that nuclear actin consolidates the chromatin environment by controlling the ATPase activity of the SWI/SNF (BAF) remodeler at both nuclear envelope and nuclear interior, contributing to segregate heterochromatin at the perinuclear region while maintaining euchromatin levels at multiple genomic sites.

P.3.2-019
Dictyostelium discoideum chromosomes are partitioned into unstable globular domains

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Recent advances enabled by the Hi-C technique had unraveled many principles of chromosomal folding that have been subsequently linked to disease and gene regulation. In particular, Hi-C revealed that chromosomes of mammals and fruit flies are organized into Topologically Associating Domains (TADs), evolutionarily conserved compact chromatin domains that influence gene expression. However, we still know remarkably little about chromatin architecture in other organisms. To explore the principles of chromosomal folding in a popular model organism, soil-living amoeba *Dictyostelium discoideum*, we performed Hi-C in two biological replicates and constructed high-resolution interaction maps that revealed the presence of small (20–80Kb) loose globular domains. Though biological replicates demonstrated high correlation (>95%), only 52% of globular domains were located at the same position in both replicates, suggesting general instability of globules. For comparison, in fruit flies 42–67% of TADs were reported to retain positions in different cell lines. However, similar to previous observations for TADs of fruit flies and mammals, boundaries of globular domains of *D. discoideum* were enriched with actively transcribed genes and housekeeping genes, while differentially expressed genes were preferentially found within globules. Moreover, chromatin compactness decreased with the increase of the gene transcription level. Additionally, we found that binding sites of transcription factors were enriched at boundaries of globules, though this result could be partially explained by the fact that transcription factor binding sites were located near gene promoters. Taken together, these observations suggest that despite general instability of globular domains of *D. discoideum*, they resemble TADs of multicellular eukaryotes in their association with prominent functional features of the genome.

P.3.2-020
Evaluation of in vivo biotinylation by next generation sequencing

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We used earlier developed Proximity Utilizing Biotinylation with native chromatin immunoprecipitation (PUB-NChIP) approach to study chromatin in proximity to a nuclear protein of interest. The preferential labelling of chromatin proximal to nuclear envelope protein (NE) was achieved by co-expressing in HeLa cell line a biotin ligase (BirA) targeted to emerin, together with a biotin acceptor peptide (BAP) fused to histone macroH2A. Upon biotin pulse, BirA specifically biotintylates BAP domain of BAP-macroH2A histone-fusions in proximity to emerin. Specificity of PUB-NChIP *in vivo* biotinylation was further evaluated by sequencing of DNA associated with the subpopulation of BAP-macroH2A histones proximate to BirA-emerin. For library construction, DNA fragments were converted to blunt ends, purified and then ligated to barcode adapters. The resulting DNA fragments with a size of 200–220 bp (150-base-read libraries) were selected using 2% agarose gel. Library sequencing was performed on Ion Torrent PGM system using chip 318. The sequencing was also conducted for input DNA (HeLa cell line total digested chromatin from the same experiment) as a control. Analysis of the data was carried out using sequences from the database of lamin-associated domains (LAD), against which the obtained DNA sequences were compared. The experimental sample showed a much higher degree of overlapping with the LAD database in DNA associated with the subpopulation of BAP-macroH2A histones proximate to BirA-emerin compared to the control, non-biotinylated sample, indicating the specificity of the *in vivo* biotinylation approach. Evaluation of PUB-NChIP *in vivo* biotinylation by next generation sequencing, developed in our work, may shed a new light on chromatin organization, and have a general relevance for study of nuclear proteins. This study was supported by Ministry of Education and Science of the Republic of Kazakhstan (grant no. 0390/GF4).

P.3.2-021
Function of phosphoinositides in the cell nucleus

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Phosphoinositides are glycerol-based phospholipids. They are composed of hydrophobic fatty acid tail and hydrophilic inositol head. Phosphoinositides are important cytoplasmic signalling molecules. They participate in various processes such as modulation of ion channels and transporters, membrane dynamics and cellular movement. Despite the absence of membranes inside of the nucleus, phosphoinositides are implicated in essential nuclear processes. Through the interaction with their binding partners, they regulate DNA damage response, DNA transcription and RNA processing. Phosphatidylinositol 4-phosphate (PI4P) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) are the most abundant phosphoinositides, however, their nuclear functions are still poorly understood. Our preliminary data show that nuclear PI4P and PI(4,5)P2 interact with many RNA-binding proteins including heterogeneous nuclear ribonucleoproteins (hnRNPs). hnRNPs are associated with RNA polymerase II transcripts and have important roles in mRNA processing. We focus our research on hnRNP U. One portion of hnRNP U is found in the soluble hnRNP particles and another portion is tightly associated with chromatin. hnRNP U also plays a role in the initiation of RNA polymerase II transcription. We would like to investigate the impact of phosphoinositide binding on hnRNP U function in the cell nucleus. Altogether, our study will bring new insights into nuclear functions of phosphoinositides.

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P.3.2-022
Characterizing transcriptional enhancers of epilepsy-associated genes in human inhibitory interneurons

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Epilepsy is one of the most common neurological disorders. It is a complex and heterogeneous disorder which makes it difficult to precisely diagnose and provide effective treatment. A major and underexplored cause of epilepsy could be mutations in gene regulatory elements, such as enhancers. Disruption of enhancer function, and subsequently the regulatory networks that are involved in differentiation and migration of interneurons, can lead to early onset of epilepsy, such as infantile spasms (IS). However, the enhancers that regulate epilepsy-associated genes are unknown. Here, we characterized active enhancers in human inhibitory-like interneurons that regulate the expression of IS-associated genes. Using ChIP-seq and RNA-seq, we generated a genome-wide regulatory map of human embryonic stem cells (hESC) differentiated into inhibitory GABAergic-like interneurons. Then, we analyzed the activity of several enhancer candidates and found that they can drive enhancer activity in the developing brain in a zebrafish model. We focused on the regulation of transcription factors, such as *FOXG1* that known to be involved in neurodevelopmental disorders including epilepsy. The identified enhancers showed activity patterns that mimic the targeted gene expression. Furthermore, *FOXG1* enhancers showed redundancy effect that suggests the important of enhancer activity in executing expression patterns. The results of this study provide a novel dataset of the regulatory landscape that controls the spatiotemporal gene expression in inhibitory interneurons and highlights genomic regions in the human genome where mutations could lead to IS.

P.3.2-023
Hormonal regulation of the tet enzymes determines pituitary gonadotrope Development

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Tet enzymes catalyze DNA hydroxymethylation and demethylation, and thus have a central role in regulation of gene expression, especially during development. We have shown that Tet1 and Tet2 are expressed in proliferating gonadotrope precursor cells which go on to regulate reproductive function through production of gonadotropin hormones, LH and FSH. The Tet enzymes have distinct effects on the expression of *Lhb*, with Tet1 repressing its expression in partially-differentiated gonadotropes, and Tet2 having a stimulatory effect. Tet1 is expressed in the gonadotropes of young 6–10 d old mice while this cell population expands, possibly due to relief from the negative feedback of maternal steroids. However Tet1 is then dramatically down-regulated and is not expressed in fully-differentiated gonadotropes, leading us to ask how the Tet enzymes are regulated in these cells. We found that several hormones along the reproductive axis repress Tet1 expression, including the hypothalamic GnRH, estrogens and androgens. Chromatin immunoprecipitation revealed that the receptors for estrogen (ESR1) and androgen (AR) bind upstream of the Tet1 promoter suggesting that the effects are direct. To determine the role of gonadal steroids *in vivo*, we examined the effects of ovariectomy and castration on Tet levels in the gonadotropes of adult mice. In both sexes, gonadectomy led to an increase in gonadotrope cell numbers accompanied by elevated *Tet1* mRNA levels with low levels of *Lhb*, while treatment with estradiol or DHT lowered the levels of *Tet1* mRNA to the same levels as in intact adults. These results suggest a role for Tet1 in proliferation of the gonadotrope precursor cells which is repressed by the reproductive hormones to allow completion of gonadotrope differentiation. Our findings thus reveal novel epigenetic pathways though which mammalian reproductive function is controlled, as well as shedding further light on the role and regulation of Tet enzymes in differentiated cells.

P.3.2-024
MOZ/MORF complex components expression levels in HPV-cervical cancer

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Human papilloma virus (HPV) represents the etiologic factor of malign genital neoplasia. The mechanism by which HPV induce tumorigenesis is played by the actions of E6 and E7 oncogenes and their interaction with different host regulatory molecules. Lately it has been shown that histone acetyltransferases (HATs), important chromatin remodelling modulators, are involved in various types of cancer. KAT6A and KAT6B are two components of MOZ/MORF complex known to have a histone H3 acetyltransferase activity. We aim to study the expression levels of this two components in HPV-cervical neoplasia. For this we used HeLa cell line in order to silence E7 oncogene. Also, were investigated 90 cervical specimens from women with hrHPV-induced dysplastic cervical lesions (CIN1; CIN2/3) and squamous cervical carcinoma (SCC), and 25 women (with negative cytological smears and HPV DNA negative) as controls. From all the samples RNA was isolated (TriZol) and HATs expression levels were evaluated by qRT-PCR. The obtained data showed increased levels of expression for KAT6A (n-fold: 2.5) and KAT6B (n-fold: 3.8) in E7-siRNA treated cells/control. Moreover, investigating KATs levels in cervical patients we noted that both KAT6A and KAT6B presented significantly decreased levels in cervical samples vs. control. For KAT6A the lowest levels were observed in SCC patient (mean n-fold: 0.218; $P = 0.0065$) and precancerous levels (mean n-fold: 0.853; $P = 0.03$) compared with the control group. KAT6B displayed as well the most reduced levels in cervical cancer samples (mean n-fold: 0.151; $P = 0.003$) and the levels were significantly correlated with clinical parameters such as FIGO stage and lymph node metastasis ($P < 0.05$). Our results indicate that KAT6A and KAT6B could be new important epigenetic factors involved in cervical oncogenesis and underlines the importance of studying their involvement in this pathology. Study supported by PNII RU-TE-2014-4-2502.

P.3.2-025
Glutarate derivatives as modulators of TET proteins activity. The level of L-2-hydroxyglutarate is significantly increased among colorectal cancer patients compared to controls

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Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide. One of the fundamental processes driving initiation and progression of CRC is the accumulation of genetic and epigenetic changes in colon epithelial cells. Methylation of cytosine residues in DNA is key epigenetic event, and the central epigenetic mark is 5-methylcytosine (5-mCyt). An active DNA demethylation process is responsible for DNA hypomethylation and activation of previously silenced genes, and is controlled by 2-ketoglutarate(2-KG)-dependent enzymes – TETs. It has been demonstrated that TETs oxidized DNA 5-mCyt residue to 5-hydroxymethylcytosine, which level is depleted in human cancers. Some metabolites may play a role as co-substrates or inhibitors of TETs. A hallmark example are point mutations in genes encoding isocitrate dehydrogenase (IDH) enzymes. IDH proteins catalyze decarboxylation of isocitrate to 2-KG. Mutant IDH enzymes preferentially reduce 2-KG into 2-hydroxyglutarate (2-HG), precisely D-2HG, since there are two possible enantiomers (L- and D-2HG). Physiological cellular level of 2-HG is maintained by 2-hydroxyglutaric dehydrogenase, however, the action of this enzyme is not sufficient to counteract its overproduction in IDH-mutated cancer cells. Consequently, 2-HG accumulates to micromolar concentrations in plasma/urine. The first reported mutation in IDH was found in CRC. Literature data suggest, that increased level of 2-HG does not always accompany the IDH genes mutations. We measured plasma concentration of L/D-2HG (0.77 μM/0.23 μM) among CRC patients compared to controls (0.20 μM/0.17 μM) using UPLC-MS/MS method. We observed ~2.5-fold increased urinary level of L-2HG in CRC patients compared to controls. Molecular changes in the DNA methylation pattern, similar to those caused by functional mutations in TET proteins, might have been the result of metabolic alterations in cells.This work was supported by NSC grant No. 2013/09/B/NZ5/00767.

P.3.2-026
Targeted epigenetic editing in the brain to control neuronal refinement and memory in aging and Alzheimer's disease

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Epigenetic changes have emerged as fundamental mechanisms to define transcriptional regulation of plasticity genes and hence regulate critical brain functions. However, the mechanisms are not well understood. Here we examined epigenetic modifications at the PSD95 gene promoter in rat hippocampus from embryonic to adult stages. We find that the PSD95 gene promoter lacks DNA methylation and classical repressive histone marks at any given developmental stage. By contrast, Increased PSD95 gene expression during development is accompanied by enrichment of active histone marks at its promoter. We analyzed PSD95 epigenetic parameters in hippocampal tissue and designed a PSD95-specific epigenetic editing strategy: A PSD95 Zinc finger DNA-binding domain was engineered and fused to effector domains to either repress (G9a, Suvdel76, SKD) or activate (VP64) transcription, respectively generating zinc finger proteins (ZFPs) or artificial transcription factors (ATFs). We show that the PSD95-6ZF-fusions can re-write several of the critical epigenetic marks and bi-directionally modulate endogenous PSD95 gene expression in cultures. Expression of these constructs in hippocampi impacted several hippocampal neuron plasticity processes, including spine maturation and synaptic plasticity. Finally we tested the potential applications of the designed epigenetic tools for gene therapies in disorders where PSD95 expression is aberrant, including in aging and Alzheimer's disease. Intriguingly, PSD95-ATF transduction rescued memory deficits in aged mice and in an Alzheimer mouse model. Conclusively, this works validates PSD95 as a key player in memory and establishes epigenome editing as a potential therapy to treat human neurological disorders. This work was supported by several funding agencies, including Fondecyt 1140301 (BvZ) and FONDAP 15090007 (MM).

P.3.2-027
Coordinated epigenetic mechanisms control the expression of the bone master Sp7 gene during mesenchymal lineage commitment

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Sp7 is a critical regulator of osteoblast differentiation. The mechanism underlying its restricted bone-lineage expression and the regulatory events orchestrating its activation in osteoblasts remain to be elucidated. Here we simultaneously assess histone modifications, chromatin remodeling and DNA methylation processes that coordinately control the Sp7 expression during mesenchymal lineage commitment. We find that Sp7 gene repression is mediated by DNA methylases DNMT1 and DNMT3a, histone deacetylases HDAC1, HDAC2 and HDAC4, and complexes containing the histone methylases Suv39h1 (H3K9) and Ezh1/2 (H3K27). In contrast, Sp7 gene activation involves critical changes in histone modifications, accompanied by decreased nucleosome enrichment and DNA demethylation mediated by the chromatin remodeling complex SWI/SNF and the DNA methyl hydroxylases Tet1/Tet2, respectively. Inhibition of DNA methylation triggers changes in the histone modification profile and chromatin remodeling events leading to Sp7 gene expression. Tet1/2 silencing prevents Sp7 expression during osteoblast differentiation as it impairs DNA demethylation and alters the recruitment of histone methylase (COMPASS)-, histone demethylase (Jmjd2a/Jmjd3)-, and SWI/SNF-containing complexes, to the Sp7 promoter. The dissection of these interconnected epigenetic mechanisms that govern Sp7 gene activation reveals a hierarchical process where regulatory components mediating DNA demethylation play a leading role.FONDECYT 1170878 and FONDAP 15090007.

P.3.2-028
Non-coding transcripts of tandemly repeated sequences LL2R can form splicing factors depot in *Gallus gallus*

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Non-coding tandem LL2R repeat ('lumpy loop 2 repeat') of 440 bp monomer in length is localized on the long arm of chicken (*Gallus gallus domesticus*) chromosome 2. The RT-PCR screening of different chicken tissues allowed us to reveal that LL2 R tandemly repeated cluster is transcribed in all analyzed cell types of different stages of chicken development – from growing oocytes and embryos to different adult somatic tissues and cultured malignized cells. LL2R transcription was verified via northern-blotting technique. We have also shown that nascent LL2R transcripts enrich the RNP-matrix of unusual transcription units called 'lumpy loops' on lampbrush chromosome 2. Immunostaining of the lampbrush chromosome 2 has demonstrated that LL2R repeat cluster is associated with the elongating form of RNA-polymerase II. LL2R transcripts are enriched with the marker protein of nuclear speckles SC35 and spliceosomal snRNPs forming the nuclear domains in 'lumpy loops' region of chicken growing oocytes. RNA fluorescent *in situ* hybridization of the lampbrush chromosome 2 pretreated by one of the RNaseH, RNAseIII or RNaseR has revealed that enriching 'lumpy loops' LL2R transcripts are predominantly single-stranded and may include circular RNAs. Moreover, bioinformaticaly we have found the presumed binding sites for two micro RNAs. The goal of this study was to advance in LL2R transcripts function understanding in two directions. Firstly, we analyzed the LL2R RNP complex composition by performing RNA-immunoprecipitations with different antibodies against several splicing factors followed by western blotting and RT-PCR. Secondly, we have tried to reveal circular RNA LL2R transcripts using RNaseR pretreatment of total RNA isolated from chicken cells followed by RT-PCR with specific primers to the presumed circular LL2R structures. Together our data suggest that the non-coding tandem repeat LL2R transcripts may function as splicing factors depot forming the specific nuclear domains. This work was supported by a grant of the President of the RF (#MK-1630.2017.4).

P.3.2-029
Modulation of cellular CpG DNA methylation by Kaposi's sarcoma associated herpesvirus

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Kaposi's sarcoma associated herpesvirus (KSHV, HHV-8) is the etiological agent of Kaposi's sarcoma (KS), and is tightly associated with primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). DNA methylation at CpG dinucleotides is an epigenetic mark dysregulated in many cancer types. Several previous studies have analyzed in details the CpG methylation of the KSHV episomal genomes, but little is known on the impact of KSHV on the human genome. Our knowledge on cellular CpG methylation in the context of KSHV infection is currently limited to four hyper-methylated human genes; p16INK4a (CDKN2A), the TGF-beta type II receptor (TbetaRII), PDZ-LIM domain-containing protein 2 (PDLIM2) and H-cadherin (CDH13). Therefore, we decided to perform a comprehensive CpG methylation analysis to reveal the human methylome in KSHV-associated malignancies. We have performed the Infinium HumanMethylation 450K and EPIC BeadChip arrays and identified a panel of hyper-methylated cellular promoters in KSHV infected cells. Surprisingly, on top of these newly methylated sites, our analysis revealed a major differential genome wide hypo-methylation of up to 27% of the genome in PEL cells. We have intersected our genome wide methylation analysis with RNA-seq to add functional outcomes for virally induced methylation changes. We could correlate many downregulated genes to promoter hyper-methylation, and upregulated genes to Hypo-methylation. In addition, we show that treating the cells with a de-methylating agent leads to re-expression of these downregulated genes, indicating that indeed DNA methylation plays a role in the repression of these human genes by KSHV. Genome wide analysis of CpG methylation in KS samples revealed initial directed hyper-methylation, followed with increase in hypo-methylation as the KS progresses from Plaque to Nodule. This study extends our understanding of the relationship between epigenetic changes induced by KSHV infection and tumorigenesis.

P.3.2-030
G4-binding proteins: the focus on chromatin remodelers

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DNA G-quadruplexes (G4s) are noncanonical architectures that reportedly exist in vivo in oncogene promoters, telomers and other genomic regions. Recent data support G4 interference with replication, transcription, translation, recombination and histone code maintenance, however, relatively little is known about G4 interactome. We profiled labeled G4 oligonucleotides against ca. 9000 human proteins using ThermoFisher protein microaarays, correlated our data with the data in the literature, and verified some of the protoarray results by optical and electrophoretic methods. Our data confirmed the affinities of heterogenous ribonucleoproteins for G4 DNA and revealed new G4-recognizing transcription and splicing factors. Intriguingly, they also suggest G4s recognition by several histone chaperons. Our top candidates for G4 recognition include PAD4 (a chromatin remodeler that catalyzes histone citrullination), SPT16 (a FACT complex subunit that facilitates transcription-dependent nucleosome disassembly and reassembly), and BRD3 (a bromodomain family member that assists transcription from hyperactylated chromatine templates). The conventional view of chromatin rearrangements implies no direct interactions between DNA and SPT16, BRD3 or PAD4. Our findings indicate that these proteins might actually interact with noncanonical DNA, similarly to the known G4-affine chromatin remodeler ATRX. The putative G4-binding sites of BRD3, PAD4 and SPT16 were identified using molecular modeling experiments. To summarize, our preliminary results suggest that the passage of transcription machinery through G-rich regions might be more complex than previously thought, and a number of chromatin remodelers might be involved.This work was supported by Russian Science Foundation [14-25-00013].

P.3.2-031
Structure of complexes of DNA with HMGB1 chromosomal protein in presence of linker histone H1

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The chromatin of the eukaryotic cells comprises of DNA and variety of nuclear proteins. DNA interacts with histones and non-histone proteins and forms nucleoprotein complex. Among such proteins, the most interesting are the linker histone H1 and the protein HMGB1. H1 histone is a conservative and tissue-specific protein. H1 interacts with linker DNA and plays an essential role in the post nucleosomal level of the structural organization of the chromatin. Non-histone chromosomal protein HMGB1 is the member of a large family of High Mobility Group proteins. Despite the fact that HMGB1 presents in the cells of all investigated organisms its functions remain unclear. The interest in HMGB proteins is also explained by the fact that structural motifs similar to its DNA-binding domains (HMGB domains) were found in many regulatory proteins. Similar to histone H1, HMGB-proteins bind to linker DNA regions. Sometimes HMGB1 is regarded as "architectural" factor of transcription. It participates in the assembly of transcriptionally active multi-protein complexes on DNA. The presence of histone H1 may inhibit the formation of such complexes. However, it is still not clear what kind of interplay between these two proteins takes place in the chromatin. We investigated tertiary complex DNA-H1-HMGB1 on the different stages of formation of complexes using UV Circular Dichroism (CD) and IR/UV absorption spectroscopy, atomic force microscopy. The combined influence of HMGB1 and H1 on the structure of formed complexes with DNA is not a result of competitive interactions between proteins. Structural organization of such complexes depends not only on DNA–protein interactions but also on the interaction between the protein molecules bound to DNA. The observed interaction between the HMGB1 and the H1 stimulates DNA condensation forming large DNA–protein complexes. The work was supported by Russian Foundation for Basic Research (15-08-06876) and Russian Science Foundation (17-14-01407).

Single molecule conformational dynamics of the ABC transporter BtuCD

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In all kingdoms of life ATP Binding Cassette (ABC) transporters translocate a diversity of molecules across cell membranes. They are involved in most physiological processes and are tightly linked to human disease and to multidrug resistance (MDR) of tumor and bacterial cells. Structural studies of ABC transporters have provided an invaluable context for understanding their mechanism of action. However, since crystal structures provide static pictures of highly dynamic machines, almost nothing is known of the conformational dynamics of ABC transporters. Herein, we studied the dynamics of an ABC transporter using single molecule FRET (smFRET). We observed that in each of its hydrolysis intermediate states, the vitamin B_{12} ABC transporter BtuCD, adopts a single dominating conformation with very little population heterogeneity. We also saw that a single molecule is conformationally stable, and does not spontaneously fluctuate between different conformations. These observations are very different from those made with secondary transporters, and describe for the first time an ATP-driven system that shows such conformational plasticity.

Redox Regulation of Biological Activities

P.3.3.A-001
Oxalomalate reduces expression and secretion of VEGF in the retinal pigment epithelium and inhibits angiogenesis

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Clinical and experimental observations indicate a critical role for vascular endothelial growth factor (VEGF), secreted by the retinal pigment epithelium (RPE), in pathological angiogenesis and the development of choroidal neovascularization (CNV) in age-related macular degeneration (AMD). RPE-mediated VEGF expression, leading to angiogenesis, is a major signaling mechanism underlying ocular neovascular disease. Inhibiting this signaling pathway with a therapeutic molecule is a promising anti-angiogenic strategy to treat this disease with potentially fewer side effects. Oxalomalate (OMA) is a competitive inhibitor of $NADP^+$-dependent isocitrate dehydrogenase (IDH), which plays an important role in cellular signaling pathways regulated by reactive oxygen species (ROS). Here, we have investigated the inhibitory effect of OMA on the expression of VEGF, and the associated underlying mechanism of action, using *in vitro* and *in vivo* RPE cell models of AMD. We found that OMA reduced the expression and secretion of VEGF in RPE cells, and consequently inhibited CNV formation. This function of OMA was linked to its capacity to activate the pVHL-mediated HIF-1a degradation in these cells, partly via a ROS-dependent ATM signaling axis, through inhibition of IDH enzymes. These findings reveal a novel role for OMA in inhibiting RPE-derived VEGF expression and angiogenesis, and suggest unique therapeutic strategies for treating pathological angiogenesis and AMD development.

P.3.3.A-002
Sigma-1 receptors are involved in modulation of Ca2 + responses induced by glutoxim and molixan in macrophages

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Sigma-1 receptors, ligand-regulated molecular chaperones, are located in endoplasmic reticulum membranes at the interface with mitochondria. Their ligands are endogenous steroids, antidepressants, antipsychotics, anticonvulsants, and analgesics. Sigma-1 receptors interact with target proteins, including ion channels and receptors, and modulate many cellular processes. It was found that interacting with inositol 1,4,5-trisphosphate receptors, sigma-1 receptors modulate Ca^{2+} signaling processes in cells.

Earlier, we have shown that disulfide-containing immunomodulators glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA VAM, Saint-Petersburg)

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and molixan® (complex of glutoxim with nucleoside inosine) cause biphasic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages.

To elucidate the possible involvement of sigma-1 receptors in the effect of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages we used sigma-1 receptor antagonist neuroleptic haloperidol, widely used for treatment of schizophrenia.

Using Fura-2AM microfluorimetry we have found that macrophage preincubation with 30 μg/ml haloperidol for 6 min before 100 μg/ml molixan addition leads to a significant suppression of both Ca^{2+} mobilization (on average, by 49.3 ± 8.1%) and subsequent Ca^{2+} entry (on average, by 47.6 ± 9.7%), induced by molixan. Similar results were obtained in experiments with glutoxim.

Thus, we have demonstrated for the first time that sigma-1 receptor antagonist haloperidol inhibits both phases of the Ca^{2+} response induced by glutoxim or molixan, which indicates the possible involvement of sigma-1 receptors in signaling cascade triggered by these immunomodulators in macrophages. Our results also indicate that it is inadvisable to use glutoxim or molixan in combination with antipsychotic haloperidol in clinical practice.

P.3.3.A-003
Stizolophus balsamita Lam. isolated culture extracts as the antioxidant agents

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Plant cell redox homeostasis is formed as a result of the balance between the accumulation of reactive oxygen species and functioning of the antioxidant enzymes or non-enzymatic antioxidants. The production level of the last ones by plant tissue cultures is of importance also taking into account their value for the pharmaceutical and food industry. So, the aim of this investigation was to study the antioxidant activity (AOA) of *Stizolophus balsamita* Lam. medicinal plant tissue culture extracts (ethanol, methanol, water, hexane, acetone, chloroform). *S. balsamita* isolated culture was obtained, using Murashige and Skoog (MS) mineral-based nutrient media: MS1 – supplemented with indole-3-acetic acid (IAA) (2.0 mg/l) and kinetine (0.2 mg/l); MS2 – 6-benzylaminopurine (BAP) (2.0 mg/l) and IAA (0.5 mg/l).

According to the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay results the highest radical scavenging activity possessed methanol and acetone extracts of *S. balsamita* tissue culture, growing on MS2 medium (both of cultures were tested at the end of their exponential growth phase). Their IC_{50} values were reached 250–300 μg/ml. Further analyses were carried out with the most active fractions. TBARS (thiobarbituric acid reactive substances) assay data showed that malondialdehyde-equivalent quantity was approximately 4-fold lower against control under the influence of 1 mg/ml extracts. AOA of *S. balsamita* extracts was investigated also by microbe test systems, using *Escherichia coli* GC 4468 wt; *E. coli* GC4468 sodA49 (sodA-lacZ) and *E. coli* BN 407 (iucC-lacZ) mutant strains. It was tested the extracts capability to support aerobic growth of *E. coli* strains in the presence of 4 mM H_2O_2. The obtained data showed that pretreatment of the bacteria with the extracts (50 mg/ml in DMSO) 20 min before adding H_2O_2 increased the resistance of bacteria against oxidative stressor.

The results show the great potential of *S. balsamita* isolated culture extracts as antioxidant agents.

P.3.3.A-004
Decrease of external oxygen concentration as an early response to cell wall injury of Chara corallina

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Molecular oxygen plays a crucial role in plant metabolism. O_2 is a source of reactive oxygen species (ROS) and oxidative stress. The excessively generated ROS plays both signaling and protective function in mechanically stressed plants. Generation of ROS in microwounded cells is presumably mediated by the plasma membrane NADPH-oxidase that transfers electrons from cytoplasmic NADPH to extracellular oxygen with a concomitant production of ROS, H_2O_2 in particular. Microscopic injuries associated with ROS generation might be accompanied by oxygen concentration changes in the apoplast. Appropriate methods for oxygen measurements on the cellular and subcellular levels are necessary to reveal O_2 alterations. In the last decades significant progress has been made in the development of miniature sensors, including oxygen- and ROS-sensitive sensors. Recent invention of nanoscale electrochemical probes provides the opportunity to gain a deeper understanding of oxygen and ROS metabolism upon mechanical stress in plant cells. Our data obtained by applying carbon-filled quartz micropipettes with platinum-coated tips (oxygen nanosensors) showed a considerable drop in oxygen concentration at *Chara corallina* cell surface in response to microperforation of the cell wall (CW). The oxygen gulp activated by mechanical stress is dependent on stretching of plasma membrane, calcium fluxes across plasmalemma and dynamical rearrangements of micritubules. We tested possible involvement of the suppression of photosynthesis, the enhancement of respiration, and the activation of the plasma membrane NADPH oxidase as an origin of oxygen decline upon CW microwounding. The results provide evidence for major role of plasmalemmal NADPH-oxidase in the discovered local drop of O_2 content.

P.3.3.A-005
Development of sensitive SERS-based approaches to study redox state of cytochrome C in living mitochondria

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The redox state and the conformation of cytochrome C (cyt C) depend on interaction with complexes of the electron transport chain (ETC), the electron transfer efficiency and properties of mitochondria intermembrane space (IMS). Impairment of electron transfer leads to overproduction of reactive oxygen species and cytochrome C-related apoptosis. This implicates mitochondria in several human diseases, including hypertension and cardiac dysfunction. Therefore, methods for direct investigation of cyt C redox state and its conformational dynamics in living mitochondria are essential for understanding of mechanisms

underlying pathologies and development of new diagnostic tools. We applied silver nanostructures of different morphologies to obtain a great selective enhancement of Raman scattering from cyt C in living mitochondria using surface-enhanced Raman spectroscopy (SERS). We demonstrated that SERS spectra of mitochondria placed on nanostructures and excited with 532 nm laser provide information about redox state and conformation of cyt C in intact functional mitochondria. Our results indicated that: (1) induction of respiration by pyruvate, malate, succinate and ADP increases reduced cyt C peak intensity, whereas peaks of oxidized cyt C prevail during basal respiration in the absence of substrates; (2) the decrease in the IMS in mitochondria caused by valinomycin or hypotonic medium increases overall SERS intensity and results in appearance of cyt B peaks; (3) application of protonophore FCCP or oligomycin affects relative input of reduced and oxidized cyt C peaks into the overall SERS spectrum. The developed technique was applied to investigate mitochondria of normotensive Wistar Kyoto rats and spontaneously hypertensive rats. We showed that SERS-based approach to study cyt C is sensitive to rate of respiration and ATP-synthesis, IMS properties and cardiac pathologies. NAB acknowledges financial support from RFBR and Moscow Government (grant 15-34-70028-mol_a_mos).

P.3.3.A-006
Sugar binding and peroxide bond cleavage – a concerted action of fungal Hybrid B heme peroxidases

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Fungal Hybrid B heme peroxidases are unique multidomain proteins consisting of an evolutionary conserved N-terminal heme b peroxidase domain fused C-terminally with various sugar binding domains. Among phytopathogenic ascomycetes these domains are represented mainly by carbohydrate binding motifs CBM34 or CBM21. Here we present a detailed molecular phylogeny of these peculiar oxidoreductases, their inducible expression during oxidative stress as well as their first biochemical functional data. We have focused our research on two paralogs of Hybrid B heme peroxidases MagHyBpox1 and MagHyBpox2 from the hemibiotrophic ascomycete *Magnaporthe oryzae* that causes rice and wheat blast diseases worldwide. As detected by means of RT-qPCR methodology the expression of *maghyBpox1*-mRNA and *maghyBpox2*-mRNA increased 7-fold and 4-fold, respectively, upon addition of 5 mM H_2O_2 in the cultivation medium for 20–30 min at 28°C. Adding 5 mM peroxyacetic acid to growing fungus for the same time period and the same temperature led up to 31-fold and 6-fold increase of the respective hyBpox transcripts. The shorter codon-optimised MagHyBpox1 paralog was expressed heterologously in the methylotrophic yeast *Pichia pastoris* and the secreted recombinant heme protein was purified with a combination of affinity and anion exchange chromatography. We report the typical UV/vis and EPR spectral features of ferric and ferrous proteins and kinetic parameters of the peroxidase activity probing up to twelve potential electron donors. Moreover, we show tight binding of recombinant Hybrid B peroxidase 1 to soluble starch followed spectroscopically with the KI/I_2 method. Obtained kinetic and spectral results of highly purified MagHyBpox1 are discussed and compared with other previously investigated members of the peroxidase-catalase superfamily. Our research was supported by Austrian Science Fund (project P27474-B22), by the Slovak Grant Agencies VEGA (grant 2/0021/14) and APVV (grant APVV-14-0375).

P.3.3.A-007
Structural basis for redox sensitivity in Corynebacterium glutamicum diaminopimelate epimerase: an enzyme involved in L-lysine biosynthesis

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Diaminopimelate epimerase (DapF) is one of the crucial enzymes involved in l-lysine biosynthesis, where it converts l,l-diaminopimelate (l,l-DAP) into d,l-DAP. DapF is also considered as an attractive target for the development of antibacterial drugs. Here, we report the crystal structure of DapF from *Corynebacterium glutamicum* (*Cg*DapF). Structures of *Cg*DapF obtained under both oxidized and reduced conditions reveal that the function of *Cg*DapF is regulated by redox-switch modulation *via* reversible disulfide bond formation between two catalytic cysteine residues. Under oxidized condition, two catalytic cysteine residues form a disulfide bond; these same cysteine residues exist in reduced form under reduced condition. Disulfide bond formation also induces a subsequent structural change in the dynamic catalytic loop at the active site, which results in open/closed conformational change at the active site. We also determined the crystal structure of *Cg*DapF in complex with its product d,l-DAP, and elucidated how the enzyme recognizes its substrate l,l-DAP as a substrate. Moreover, the structure in complex with the d,l-DAP product reveals that *Cg*DapF undergoes a large open/closed domain movement upon substrate binding, resulting in a completely buried active site with the substrate bound.

P.3.3.A-008
Hypericin: a potent inhibitor of glutathione reductase purified from baker's yeast

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Glutathione is a non-protein thiol peptide and widely distributed among all living organisms. Antioxidant defense mechanisms and xenobiotic detoxification mediate the formation of oxidized glutathione (GSSG) which is then converted to reduced glutathione (GSH) by the action of glutathione reductase (GR), in expense of NADPH. Hypericin is one of the active constituents of the St. John's Wort (*Hypericum perforatum L.*) and has antidepressant and antiviral actions. It is also used in photodynamic therapy of several oncological diseases. Here, we aimed to clarify the interaction of hypericin with GR (E.C. 1.6.4.2) purified from baker's yeast (*S. cerevisiae*). Activity of the GR was measured at 340 nm by using different hypericin concentrations at either fixed 1 mM [GSSG]-variable [NADPH] or fixed 0.1 mM [NADPH]-variable [GSSG]. Hypericin inhibited GR in a dose dependent manner with an IC_{50} value of 19.7 μM (calculated from Hill plot). When the variable substrate was GSSG, inhibition type was competitive, K_m and K_i were found as 190 ± 40 μM and 2.92 ± 0.73 μM, respectively. The V_m was calculated as 242 ± 15 U/mg protein. On the other hand, when the variable substrate was NADPH, inhibition type was linear-mixed type competitive and the K_s, K_i and α values were 15.8 ± 1.6 μM, 2.63 ± 0.50 μM and 3.48 ± 1.31, respectively. The V_m was calculated as 232 ± 8 U/mg protein. Competitive inhibition with GSSG shows that hypericin binds to the GSSG binding site. On the other hand, linear-mixed type competitive inhibition with

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NADPH indicates that although hypericin binds to the GSSG site it is a huge molecule and it also affects the binding of NADPH because GSSG and NADPH sites are close to each other.

P.3.3.A-009
Antidepressant fluoxetine inhibits baker's yeast glutathione reductase

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Glutathione reductase (E.C. 1.6.4.2) has a central role in detoxification due to the fact that its ability to regenerate the reduced glutathione (GSH) which is a central antioxidant molecule from oxidized glutathione (GSSG) by using NADPH. GSH scavenges and eliminates superoxide and hydroxyl radicals non enzymatically or functions as an electron donor to several enzymes. Fluoxetine is an antidepressant drug and is known to carry out its action as a selective serotonin re-uptake inhibitor (SSRI) by blocking serotonin transporter. In this study, we investigated the interaction of fluoxetine with glutathione reductase (GR) purified from baker's yeast. The optimum temperature, optimum pH and energy of activation were determined to characterize the enzyme. The purity of the enzyme was also confirmed by electrophoresis. In the presence of fluoxetine, GR activity was followed at fixed 1 mM [GSSG]-variable [NADPH] and fixed 0.1 mM [NADPH]-variable [GSSG]. Single protein and activity bands were obtained on native PAGE. GR also gave single band on SDS-PAGE with a Mr of 49 kDa. Optimum pH, optimum temperature, activation energy and Q_{10} were found as 7.65, 57°C, 3,544 calories and 1.26, respectively. GR was inhibited by fluoxetine in a dose dependent manner and from Hill plot IC_{50} was calculated as 0.88 mM. When the variable substrate was GSSG, linear-mixed type competitive inhibition was observed with fluoxetine. K_s, K_i and α values were calculated as 111 ± 5 μM, 279 ± 32 μM and 5.48 ± 1.29, respectively. On the other hand, at variable NADPH, the inhibition type was noncompetitive, K_m and K_i values were 13.4 ± 0.8 μM and 879 ± 82 μM, respectively. Linear-mixed type competitive and noncompetitive inhibitions suggest that fluoxetine binds to a site between GSSG and NADPH binding sites but much closer to the GSSG site. Thus, it competes with GSSG binding but then noncompetitive inhibition with variable NADPH can be explained by the conformational change of the enzyme.

P.3.3.A-010
The effect of biologically active substances from *Inula britannica* and *Limonium gmelinii* on the antioxidant status in liver tissue of laboratory mice

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Most environmental pollutants have toxic and mutagenic effects on organisms as a result of activation of free radical formation and inhibition of DNA repair system. The increased formation of free radicals leads to an increase in lipid peroxidation (LPO). In this regard, search for protectors from the effects of xenobiotics is becoming urgent challenge. Many biologically active substances (BAS) of natural origin are potential antioxidants and can increase resistance to toxic and mutagenic effects of a wide range of pollutants. The aim of the research was to study the antioxidant potential of BAS from medicinal plants *Inula britannica* (Compositae) and *Limonium gmelinii* (Plumbaginaceae). The oxidant and antioxidant potential of plant extracts were determined by lipid hydroperoxides (LHP) and malondialdehyde (MDA) content in mice liver by the extraction-spectrophotometric method. Unsymmetrical dimethylhydrazine (UDMH) was used as an oxidant, known for its toxic and genotoxic effects. It was found that UDMH statistically significantly enhanced LPO in intoxicated animals compared to intact animals. The LHP and MDA content with single intraperitoneal exposure to UDMH at dose of 6.6 mg/kg increased by 3.55 ($P < 0.01$) and 1.84 ($P < 0.05$) times, respectively. The LHP and MDA content with long-term (10-day) exposure were higher of 4.02 ($P < 0.01$) and 2.07 times ($P < 0.01$), respectively. In mice taking BAS from *I. britannica* and *L. gmelinii* at doses of 50.0; 100.0 and 150.0 mg/kg, the content of LPO products were at the control level. It indicates that the BAS at the used doses had no oxidant activity. The combined effect of BAS and UDMH on mice showed statistically significant decrease of the LPO level, induced by UDMH, to the control level. Thus, the extracts from *I. britannica* and *L. gmelinii* have antioxidant activity in labotatory mice. Currently, the plant extracts are being tested for antigenotoxic activity.

The work was done within the framework of the MES project (No.GR 0115RK00378).

P.3.3.A-011
The use of redox active natural substances in the treatment of deoxynivalenol poisoning

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It is evident from our recent in vivo study (10-week experiment on the toxicity of lead acetate (1/30 LD50), that humic acids at a dose 0.5 and 1% are either efficient in lowering oxidative stress load as seen in decrease in superoxide dismutase activity, or in the mechanisms of reversing the adverse peroxidation effect of lead and effort to eliminate this metal by chelation in cooperation with reduced glutathione. The occurance of mycotoxins produced by spp. Fusarium (mostly deoxynivalenol, DON) in food and feed ranges from 43–74% in EU countries. The aim was to investigate the possibility of preventing and treating poisoning with DON by applying these natural substances permitted by the EMEA, and monitoring the selected oxidative stress markers in plasma, liver, heart, kidney tissue. The experiment (Ro-2559/16-221) included a total of 72 Sprague Dawley male rats, and lasted for 4 weeks. The animals were divided into 6 groups thus allowing to monitor the effects of the administered compounds. DON was fed in the feed mixture at doses exceeding 100% and 200% limiting values, and humic acid at 1%. DON intoxication led to a significant decrease in the levels of reduced glutathione, more in the heart than liver but also in kidney, and almost as well for both dosages. The activity of glutathione peroxidase also declined markedly. We found increased glutathione reductase activity in the heart and kidneys at a 100%, but low at 200% DON overdose. In plasma, the values of the above parameters were not significantly altered except for superoxide dismutase. The activities of glutathione-transferase, associated with resistance to apoptosis and carcinogenesis, were increased most significantly. Histochemical detection of Hsp 70 is currently under way. Concomitant administration of humic acids led to the approximation of enzyme activities to those in the control group. The correction was less noticeable in 200% DON overdose. The study was supported by VEGA 1/0782/15.

P.3.3.A-012
Ex vivo identification of plastid thioredoxin targets in Nicotiana benthamiana plants

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Post-translational redox modifications provide an important mechanism for the control of major cellular processes. Thioredoxins (Trxs), key actors in this regulatory mechanism, are ubiquitous proteins that catalyze thiol-disulfide exchange reactions in several cellular compartments. In chloroplasts, Trxs f and m act as transmitters of the redox signal by transferring electrons to downstream target enzymes, reducing them and thereby regulating many essential plastid processes. The number of characterized Trx target enzymes has greatly increased in the last years. Two procedures, one based on thiol specific probes and the other on mutant Trx affinity trapping, facilitated the labeling or isolation of potential Trx targets that were later identified with proteomic approaches. However, all of them were *in vitro* procedures lacking Trx isoform specificity. With this background, we have developed a new *ex vivo* approach based on the overexpression of his-tagged single-cysteine mutants of Trx f or m into *Nicotiana benthamiana* plants. The over-expressed mutated Trxs, capable of forming a stable mixed disulfide bond with target proteins *in planta*, were immobilized on affinity columns packed with Ni-NTA agarose and the covalently linked target proteins were eluted with DTT. A total of 1478 proteins were identified by mass spectrometry-based proteomics and 645 proteins were also quantified. The statistical analysis show 63 and 62 potential protein targets for Trx f and Trx m respectively, with a 56% overlapping between both isoforms. Already known partners were identified, validating the relevance of the approach. Additional functional analysis showed that the captured targets are involved in several processes such cell redox homeostasis, translation and carbon metabolism.

P.3.3.A-013
Impact of Di(2-ethylhexyl) phthalate administration on various trace elements in rat serum

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Di(2-ethylhexyl) phthalate (DEHP), is used in the production of polyvinyl chloride and belongs to phthalatic-acid esters which is a widely-used plasticizer for synthetic polymers. DEHP may have effects such as potential endocrine disruptive, reproductive toxicity, inducing carcinogenesis that lead to fetal death and malformations in the laboratory animals. The aim of this study was to investigate the effects of DEHP administration on trace elements concentrations in serums samples of rats fed with various concentrations of DEHP.

6 weeks old, 24 pubertal male Wistar albino rats (200–220 g) were used. All treatments were administered daily by oral gavage and indicated procedure took four weeks. The dosing solution was prepared by mixing the compound with corn oil and diluting in series with corn oil to the desired concentration of 100, 200 and 400 mg/kg DEHP. The male rats were divided into four groups. In the negative control group, the animals were only given corn oil oral gavage. Other groups were dosed with 100, 200 and 400 mg/kg/day of DEHP. At the end of 28th day, we take blood samples were taken from heart after fasting 12 h. Serum was separated and frozen until analysis. Serum samples were diluted to 1:600 with 65% nitric acid (Merc Millipore) for (ICP-MS) determination. Trace elements concentration in the serum samples were measured with Agilent 7700x ICP-MS (Agilent Technologies Inc., Tokyo, Japan) equipped with octupole reaction system with Helium collision cell for spectral interference removal.

Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Ti, U, V, Zn were investigated in the serum samples of rat fed with DEHP. Our data have indicated that concentration levels of Na, Ca, Rb, K and Cs significantly decreased in serum samples of rats feed with increased concentrations (100, 200, 400 mg/kg/day) of DEHP. Fe and Se concentrations in the indicated serum samples have significantly increased in each concentrations.

P.3.3.A-014
In vitro toxicity of silica nanoparticles in human lung fibroblasts

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Silica nanoparticles (SiO_2 NPs) are some of the most widely used nanomaterials in various biomedical applications. Therefore, toxicity of nanoparticles depends on physicochemical parameters such as particle size, shape, surface charge, composition and stability. The goal of the current study was to investigate the *in vitro* effects of amorphous silica nanoparticles (7 nm) on human lung fibroblasts (MRC-5 cells) after 24, 48 and 72 h. MRC-5 cells were seeded and grown in standard culture conditions for different periods of time. Cells unexposed to NPs were used as control. A series of parameters including F-actin organization, lysosomes formation, intracellular reduced glutathione (GSH) levels, superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels were analyzed after treatment with SiO_2 NPs. The exposure of MRC-5 cells to 62.5 μg/ml of SiO_2 NPs showed no changes of actin filaments, though the level of lysosomes significantly increased after 48 and 72 h as evidence of NPs internalization. Initiation of oxidative stress in human lung fibroblasts was demonstrated by the increase of SOD enzymatic activity starting with 48 h in comparison with control cells. Moreover, a decrease of the intracellular GSH content was significant correlated with the increase of the MDA levels for the same time points of 48 and 72 h. In conclusion, this study reveals that nanotoxicity of SiO_2 NPs on MRC-5 cells is associated with lysosome production, modulation of antioxidant activity and oxidative stress.

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P.3.3.A-015
Determining short-term effects on the activity of antioxidant enzymes in the rat uterus: the example of ibogaine

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The anti-addiction agent ibogaine interacts with different types of neural transmitter receptors, but also decrease cellular ATP level, followed by increased cellular respiration and production of reactive oxygen species, which change redox homeostasis. We wanted to investigate the effects of single dose of 1 or 20 mg/kg body weight of ibogaine (dose range commonly used for therapeutic purposes) on activity of antioxidant enzymes (SOD1 and SOD2, catalase - CAT, glutathione peroxidase - GSH-Px, glutathione reductase - GR and glutathione S transferase - GST) in rat uterus, 6 and 24 h after treatment. Three month-old virgin female rats were treated *per os* while in estrus phase of estrous cycle (determined by examination of vaginal smear). A problem in this experimental design was that activity of antioxidant enzymes changes during the estrous cycle (SOD2 activity is lower, GSH-Px and GR activities are higher in estrus compared to metestrus). Since estrus phase lasts 15–24 h, the female rats treated in estrus, after 6 h will still be in estrus, in most cases, but after 24 h they will be in metestrus. Therefore we used two approaches for 6 h treatment. First, females were treated immediately upon completion of vaginal smear, and sacrificed 6 h later (in the time of treatment they were in estrus phase). The others were treated with a delay of 18 h, and sacrificed 6 h later, so that in the time of sacrifice, they were in the same phase as the 24 h group (metestrus). The dose of 20 mg/kg has lowered CAT activity, but, in general, canonical discriminant analysis shows that the phase of the estrous cycle in the time of sacrifice has a greater impact than a dose of ibogaine. Thus, selection of the phase of the cycle, appropriate control groups and statistical model are of crucial importance in order to distinguish the effects of treatment from the effects of estrous cycle phase changes.

P.3.3.A-016
Organophosphate insecticide induced hematologic and hepatotoxic changes in rats: the protective effect of selenium

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Chlorpyrifos (CPF) is a broad-spectrum organophosphorus insecticide widely used for medicine and agriculture. Among antioxidants, selenium (Se) used as nutritional supplements, is an essential element for humans, which improves the activity of the seleno-enzyme. The aim of the study was to investigate the ameliorative properties of selenium (Se) against CPF induced hepatotoxicity by erythrocyte osmotic fragility, lipid peroxidation and antioxidant defence system on rats.

Thirty male Sprague Dawley rats were randomly divided into five groups and distilled water, corn oil, CPF (13.5 mg/kg bw), sodium selenite (3 mg/kg bw) and a combination of chlorpyrifos and sodium selenite were given by gavage for 5 times a week during 6 weeks. At the end of the study, erythrocyte osmotic fragility *was measured in blood; Malondialdehyde* (MDA) and *Glutathione peroxidase* (GPx) were analysed in kidney tissues.

While erythrocyte osmotic fragility *and* MDA activity significantly increased; GPx significantly reduced in the CPF treated group compared to all other groups. There were not any significantly changes between the distilled water, corn oil and selenium treated groups for all parameters. The study concluded that the exposure of CPF caused to increased erythrocyte osmotic fragility due to elevated lipid peroxidation. Also, the use of selenium as a antioxidant appeared to be beneficial to rats, and a great extent in restoring the oxidative damage induced by CPF exposure.

P.3.3.A-017
Multi component antioxidative and antimicrobial activity of the secondary metabolites

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In recent years, microbial carotenoids, fatty acids and phenolic acids gain a considerable interest in nutrition due to their performance for preventing or delaying degenerative diseases by enhancing activity on immune responses. The development of novel drugs against drug resistant pathogens is the main need for the threat of a wide range of serious infections.

In this study, the combined anti-microbial and anti-oxidative activity of the fatty acid methyl esters, carotenoids and phenolic acids were investigated. As a representative components, β-carotene, β-cryptoxhantin, rutin, catechol, and food industry fame mix were selected. The anti-microbial activity of the samples that contain individual and multi-component model oxidative agents were interpreted by micro-broth dilution and disc diffusion methods for the gram-positive and gram negative strains as *Staphylococcus aeureus ATCC 6538 and Escherichia coli ATCC 25922*. The result was obtained by measuring the optical density at 600 nm and the microbial free zone area diameter respectively. Three replicates were performed for each bacterium the mean values are presented. Anti-oxidative activity of the samples were determined spectrophotometrically by means of Trolox equivalent antioxidative activity and gallic acid equivalent total phenolic content.

As a result, phenolic acids showed a lower antimicrobial activity compared with the fame mix and carotenoids. The individual anti-microbial activity of the components are in an increasing order of catechol, rutin, fame mix, β-carotene, and β-cryptoxhantin respectively. Individual anti-oxidative activity of the fame mix was lower than the phenolic acids. In the case of combined anti-oxidative and anti-microbial activity effect investigation, synergistic effect was observed. *E. coli* strain was a much resistant strain than *S. aeureus* with respect to micro-broth and disc diffusion method results.

P.3.3.A-018
Time line redox profiling of yeast cell subpopulations using fluorescence based cell sorting methodology

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Aging has always been a mysterious part of life, and we often ask ourselves: Why do we age and how does our body cope with aging? Here we present a new methodology, which enables us to monitor redox changes of chronologically aged cells, and to sort

out cells based on their oxidation levels. This methodology is based on the *in vivo* redox sensor, Grx1-roGFP2, expressed in distinct organelles and allowing quantification and sorting of cells according to their redox status in real time. By using this approach we dissected the heterogeneity of yeast sub-populations according to their oxidation status during chronological aging and defined physiological and proteomic characterization of these subpopulations. We ascertained that at early age redox status defines growth and division rate, while in older yeast it varies as a combination of both redox status and age. Furthermore, we have determined that the proteome of early aged yeast cells depends mostly on the redox status and not necessarily their chronological age. Finally, we have found that yeast cells can maintain their redox status until they hit a redox threshold of maximum oxidation, which depends on division and most probably density. This research opens a window into a better understanding of redox mechanisms within the cells and presents us with a novel technique to further inquire into chronological aging.

Systems Biology

P.3.3.B-001
Cell functional enviromics of Pichia pastoris cells

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Microorganisms constantly monitor their surroundings for the availability of nutrients and other chemicals, using both external and internal sensors to respond dynamically to environmental changes. Integration of the external environment with metabolism occurs through the intake of compounds from the environment and results, for example, in a transcriptional response or an allosteric interaction with an enzyme. As a whole it displays a very complex network of interactions. In this project, we set forth a reverse environment-to-function reconstruction technique, i.e. Cell functional enviromics (CFE), to investigate the global physiological response of cells to complex environmental dynamics. CFE is a technique for cellular function reconstruction through the collection and analysis of dynamic envirome data. The key principle is measuring multiple environmental variables over time and then to infer causal biological mechanisms using appropriate systems analysis tools. In this study, the CFE technique was applied to a recombinant *Pichia pastoris X33* strain constitutively expressing a single-chain variable fragment (scFv) antibody. Two 50 liter cultivations were performed in fed-batch mode with and without amino acids supplementation. Exometabolite profiling was performed by 1H-NMR, which allowed, together with other standard analytic techniques, to acquire data of 21 environmental variables over time. The resulting data set was subject to systems analysis to identify the most likely causal metabolic functions and respective environmental regulation patterns. We have identified 5 predominant metabolic functions along with their environmental regulation patterns. The resulting *Pichia pastoris* functional enviromics map displays unprecedented detail on environmental regulatory functions over cellular function with many practical applications, namely in clone selection, culture media composition optimization, and bioreactor optimization.

P.3.3.B-002
A network integrative approach to unravel new links between NMD and mRNA processing pathways

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Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that recognizes and selectively degrades mRNAs carrying premature translation termination codons (PTCs). This process has been associated with many genetic diseases and some forms of cancer caused by nonsense or frameshift mutations that introduce PTCs. Moreover, recent studies have shown that NMD is also involved in the regulation of a large number of transcripts, suggesting a major role in the control of gene expression. To further investigate the biological relevance of NMD and how this process can be modulated, we used a network analysis approach that integrates 1) protein-protein, 2) kinase-target, 3) phosphatase-target, 4) miRNA-target, 5) transcription factors-target, 6) gene co-expression, 7) ubiquitination and 8) signaling interactions. The generated network was used to find novel NMD-associated proteins, prioritizing candidates with simultaneous interactions with different mRNA processing pathways (mRNA splicing, mRNA transport, mRNA translation and mRNA decay). Taking in account all information sources integrated in our network, we have created a scoring algorithm to identify new potentially important players in NMD, which can be essential to further understand the interplay between mRNA translation, PTC definition and NMD. Due to the diversity of regulatory links integrated in this workflow, we propose it can be applied to find molecular bridges between related biological processes and generate novel hypotheses about the molecular mechanisms co-regulating these phenomena.

P.3.3.B-003
Characterizing the effect of interferons on the monocyte compartment

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Monocytes are innate immune cells which belong to the mononuclear phagocyte system along with macrophages and dendritic cells. Formed in the bone marrow (BM) from hematopoietic stem cells, Ly6C+ monocytes exit the BM and enter the blood stream, where they circulate and can either retain their Ly6C expression or down-regulate it. Ly6C+ blood monocytes express a large arsenal of chemokine receptors and therefore can be rapidly recruited to sites of tissue damage, inflammation or injury, while the Ly6C- cells patrol the blood vessels and apparently maintain endothelial integrity. Ly6C+ monocytes also have a major role in steady state, as they constantly replenish the pool of monocyte-derived macrophages which reside in various tissues such as the intestine, skin, heart and peritoneum.

Previous studies have shown that secretion of cytokines from the interferon family following pathogen infection induce activation of blood monocytes and their recruitment to the sites of invasion. However, the characterization of the full monocytes compartment – BM, blood and tissue – following activation by these cytokines was not yet performed. Therefore, the effect of interferons on monocyte development and activation remains poorly understood.

Here we performed a comprehensive study to examine and characterize interferon-activated monocytes by using FACS, CyTOF and RNASeq of sorted monocytes from challenged mice vs. controls. This study aims to shed new light on the effect of

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interferons on monocytes in the BM, blood and tissue, in an attempt to better understand the mechanisms behind host defense against pathogen invasion.

P.3.3.B-004
Principles of cellular resource allocation revealed by condition-dependent proteome profiling

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Growing cells must coordinate protein translation with metabolic rates. Central to this coordination is ribosome production. Ribosomes drive cell growth, but translation of ribosomal proteins competes with production of other proteins. Theory shows that cell growth is maximized when all expressed ribosomes are constantly translating. To examine whether budding yeast function at this limit of full ribosomal usage, we profiled the proteomes of cells growing in different environments. We find that cells produce an excess of ribosomal proteins, amounting to a constant ≈8% of the proteome. Accordingly, ≈25% of ribosomal proteins expressed in rapidly growing cells does not contribute to translation and this fraction increases with decreasing growth rate. These excess ribosomal proteins are employed during nutrient upshift or when forcing unneeded expression. We suggest that steadily growing cells prepare for conditions demanding increased translation by producing excess ribosomes, at the expense of lower steady-state growth.

P.3.3.B-005
Systems biology approach to time-course investigation of ATRA-induced HL-60 cell line differentiation

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Promyelocytic cell line HL-60 is a conventional model for study on granulocytic differentiation under ATRA treatment. The ultimate molecular mechanism, which underlies promyelocytic cell maturation, remains unclear. We applied systems biology approach that includes transcriptomic and proteomic profiling followed by bioinformatics modeling to determine molecules involved in ATRA-induced differentiation of HL-60 cell line. Transcriptomic and proteomics profiling were preformed within time range from 0.5 to 96 h after ATRA treatment. Using geneXplain platform (www.genexplain.com) we have determined transcription factors (TFs) that can regulate differentially expressed proteins (DEPs) most probably and that were expressed in HL-60 cells at mRNA level according to our transcriptomic data. Among them VDR, RXRα, HIC1, GATA2, AML3, and IRF-7A were 2-fold differentially expressed (P-value < 0.05). TFs STAT1, YY1 and HIC1 were identified at proteome level. Next, we have performed upstream analysis in geneXplain platform and found key molecules (retinoic acid receptor α (RARα) and poly (ADP-ribose) polymerase 1 (PARP1)) regulating above mentioned TFs set. The upstream analysis results were visualized as a modeling scheme. CASP9, UBC9, IKBA, DNA-PKcs, and PARP1, the molecules of modeling scheme, were differentially expressed at transcriptomic level. At the same time, PARP1, DNA-PKcs, UBC9, CASP3, and CSBP1 were detected as proteins. Hypermethylated in cancer 1 protein (HIC1), which is known to cooperate with p53 tumor suppressor, was up-regulated at transcriptomic level (up to 9-fold) and expressed at proteome level starting 24 h after ATRA treatment according to our data. Since p53 gene is deleted in HL-60 cell line, we suggest that HIC1, being tumor suppressor itself, could act instead of non-expressed p53, and our modeling scheme represents the way to bypass the affects of p53 mutation in HL-60 cell line under ATRA treatment. This work is supported by RSF grant No.16-44-03007.

P.3.3.B-006
A community-based Naïve Bayes method for classifying oncogenes and tumor suppressors using fusion protein-protein interaction networks

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Since the first cancer genome sequencing in 2008, various large-scale studies have been conducted to catalog tumor-specific mutation and identifying genes that act either as oncogenes or tumor suppressors. The general approach for characterization of cancer genes as onco-genes or tumor suppressers are entirely unavailable. Our approach is based on the fusion breakpoints from our previously developed ChiTaRS-3.1 database, and the protein-protein interaction networks of our publicly available ChiPPI server for the onco-genes and tumor suppressors' classification, using a Naïve Bayes approach. In this study, we consider as a training set of the protein-protein interaction networks of 150 fusions and 300 their parental proteins in leukemia, lymphomas, sarcomas and solid tumors. We performed the modularization of the protein-protein interactions to identify communities using a 'community attachment score', composed of degree, network diameter, clustering coefficient, and interaction score of the community. We hypothesize that proteins that appear in the same community are likely to have their similar molecular functions. We found that the number of communities are over-represented in leukemia, lymphomas (70%), in sarcomas (30%) and solid tumors (10%), due to the presence of more connected clusters. Further, in sarcomas, due to less compact communities, the number of open links are over-represented, resulting in more interacting proteins that tend to attach to these communities. The situation in solid tumors is more interesting: the number of communities are significantly reduced, giving rise to ubiquitous proteins. For example, known oncogenes including TRAF7 and ALK that are missed by previously published tools at the p-value 0.05 cutoff, are easily detected by our method. Our method may be further useful for the classification of proteins with unknown functions, not only for oncogenes and tumor suppressors, but also for mutations in the specific cancer sub-types.

P.3.3.B-007
Gene architectures that minimize cost of gene expression

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Gene expression burdens cells by consuming resources and energy. While numerous studies have investigated regulation of expression level, little is known about gene design elements that govern expression costs. Here we ask how cells minimize production costs while maintaining a given protein expression level, and if there are gene architectures that optimize this process. We measured fitness of ~14,000 *E. coli* strains, each expressing a reporter gene with a unique 5′ architecture. By comparing cost-effective and ineffective architectures we found that cost per protein molecule could be minimized by lowering transcription

levels, regulating translation speeds, and utilizing amino acids that are cheap-to-synthesize and that are less hydrophobic. We then examined natural *E. coli* genes, and found that highly expressed genes have evolved more forcefully to minimize costs associated with their expression. Our study thus elucidates gene design elements that improve the economy of protein expression in natural and heterologous systems.

P.3.3.B-008
Linking stem cell function and growth pattern of intestinal organoids

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Intestinal stem cells (ISCs) require well-defined signals from their environment in order to carry out their specific function. Most of these signals are provided by neighboring cells that form a stem cell niche, whose shape and cellular composition self-organize. Major features of these processes can be studied in ISC-derived organoid culture. In this system, manipulation of essential pathways of stem cell maintenance and differentiation results in well-described growth phenotypes.

We here provide an individual cell-based model of intestinal organoids that enables a mechanistic explanation of observed growth phenotypes. In simulation studies of the 3D structure of expanding organoids, we study interdependences between Wnt- and Notch- signaling which control the shape of the stem cell niche and thus the growth pattern of the organoids. In these simulations, similar to culture experiments, changes of pathway activities change the cell composition of the organoids and, thereby, affect their shape. Based on the simulation results, we identify spatial constrains for ISC maintenance. Moreover, switches between bistable Wnt-states can explain spontaneous transitions between cyst-like and branched growth pattern, known to occur during long term expansion. These results suggest ongoing stem cell adaptation to in vitro conditions by stabilizing Wnt activity.

Our study exemplifies the potential of individual cell-based modeling in unraveling links between molecular stem cell regulation and 3D growth of tissues. This kind of modeling merges experimental results in the fields of stem cell biology and cell biomechanics constituting a prerequisite of a better understanding of tissue regeneration as well as of developmental processes.

P.3.3.B-009
Characterizing the interaction and dynamics between duplicated transcription factors

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Gene duplication plays a critical role in evolution. Duplicated genes serve as a template for the emergence of novel proteins through the acquisition of mutations. Understanding the process by which duplicated genes gain new functions is therefore of major interest. Duplication of transcription factors (TFs) is of a particular interest, as it promotes rewiring of regulatory networks: Mutations can change the TFs DNA binding domains or their regulatory domains thereby tuning the effected genes or the regulating environmental inputs. In my research I am characterizing the functional divergence of NRG1 and NRG2, duplicated repressors of transcription of *Saccharomyces cerevisiae* - that originated by a whole genome duplication 100 million years ago. In addition I am also characterizing the interaction between NRG1/2 to Msn2/4, a pair of duplicated transcriptional activation genes that emerged from the same ancestral gene through prior duplication. The four TFs share the same DNA binding motif and have overlapping target genes. By examining the distinct function, dynamics, and regulation of each duplicate, as well as the interaction between them, my study will try to define a new paradigm for the divergence of TF-duplicates and its contribution to the evolution of gene regulatory networks.

P.3.3.B-010
Exploring horizontal gene transfer through in-lab evolution

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The ability of an organism to generate phenotypic variation is a key driving force in evolution. Genetic variation can be achieved by local events such as point mutations and indels or via more massive changes such as gross chromosomal rearrangements, changes in chromosome number and even changes in the entire cell ploidy. However, while all of these within-genome change can improve existing genes, rarely do they enable the cell to discover a completely new and innovative solution. Another mean of generating variation is horizontal gene transfer (HGT) - the acquisition of new genetic material from outside the cell. Indeed HGT has been shown to play a major role in evolution, mainly in the microbial word. However, currently most our knowledge regarding the role of HGT in evolution originates from bioinformatic analysis of existing genomes. While powerful, these methodologies do have limitations. In order to experimentally explore the effect of HGT-based solutions on cells adaptation to stress and follow the dynamics of HGT-based solutions compared to within-genome solutions, we established an in-lab evolution pipeline to follow HGT under controlled settings. Using this pipeline, we evolved the competent microbe *Bacillus subtilis* for 500 generations in stress conditions in the presence of various sources of foreign DNA in the growth media and followed the evolutionary dynamics. We detected the transfer of dozens of DNA fragments of various sizes into different locations in the genome of the recipient cells. Further, we found that all integration events occurred via homologues recombination, thus resulting in a replacement of an existing fragments rather than duplication, and were highly dependent on the phylogenetic distance between the recipient and donor DNA. Interestingly, our results suggest that HGT in *B. subtilis* seems to occur in bursts in which many fragments are simultaneously integrated into a single cell thus providing a mean for massive genomic variation.

P.3.3.B-011
Optimizing the production of bulk chemicals from carbon monoxide using a genome-scale model of *Clostridium autoethanogenum*

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Recent international directives promoting the reduced consumption of fossil fuels have warranted methods for effective carbon recycling. Subsequently, *Clostridium autoethanogenum* has attracted academic and industrial interest through its ability to convert syngas components (CO, CO_2 & H_2) into valuable platform chemicals, including ethanol and 2,3-butanediol - a jet fuel

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additive. Developing the metabolic conversions catalysed by *C. autoethanogenum* into an efficient bioprocess requires the accurate prediction of optimal metabolic steady states, which in turn necessitates the construction of a genome-scale model (GSM). We have successfully constructed a predictive model, suitable for the integration of omics data sets and prediction of gene knock-out targets, consisting of 795 reactions and 786 metabolites. Our model-simulated growth yields agree well with experimentally observed specific growth rates, while elementary modes analysis (EMA) confirms the availability of metabolic routes for acetate, ethanol, lactate and butanediol production. Elevated ethanol production is predicted to result from a reduction in pH levels. Similarly, we found that the switch from acetate to ethanol production occurs with increasing CO uptake rates under non-carbon limited conditions, finally leading to lactate production as a consequence of electron stress. Our results are consistent with trends observed in continuous cultures. Our interdisciplinary approach for the construction, analysis and application of a genome-scale model provides insight into biological and biochemical principles which govern experimentally observed metabolic behaviour. Our results offer a rationale to aid the optimization of commodity chemical production from waste gases on an industrial scale.

P.3.3.B-012
Expression homeostasis during DNA replication

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Genome replication introduces a stepwise increase in the DNA template available for transcription. Genes replicated early in S phase experience this increase before late-replicating genes, raising the question of how expression levels are affected by DNA replication. We show that in budding yeast, messenger RNA (mRNA) synthesis rate is buffered against changes in gene dosage during S phase. This expression homeostasis depends on acetylation of H3 on its internal K56 site by Rtt109/Asf1. Deleting these factors, mutating H3K56 or up-regulating its deacetylation, increases gene expression in S phase in proportion to gene replication timing. Therefore, H3K56 acetylation on newly deposited histones reduces transcription efficiency from replicated DNA, complementing its role in guarding genome stability. Our study provides molecular insight into the mechanism maintaining expression homeostasis during DNA replication.

P.3.3.B-013
Duplication of transcription factors enables a low-noise transcription system

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Gene duplication is a major force of innovation in evolution. Duplications create the opportunity for new specialized functions or increased levels of proteins. Duplicated Transcription factors (TF) usually keep their pre-duplication target motifs and in many assays are shown to be redundant, discarding the specialization hypothesis. Furthermore, TFs are by and large lowly expressed genes, negating also the need for increased dosage. To study the evolutionary forces preserving duplicated TFs during evolution, we focused on MSN2 and MSN4 which drives the general stress response in yeast. These factors regulate the same genes and have similar nuclear localization dynamics. We showed that each factor can replace the other in activating target gene expression, raising the question of why both factors were conserved in evolution. The role of each factor in generating the stress response was condition dependent, due to different upstream regulation of their levels. This difference was explained by high divergence in the TFs promoters architecture, which was shown to be functional by mutating specific regulatory regions. Changing the mean expression or the noise level of their expression had a strong cost on growth in unperturbed conditions. This supports the importance of accurate regulation of these factors expression. We showed that the current regulatory configuration defines a low-noise expression system, infeasible with only one factor. Moreover, this configuration allows a quick response to perturbations without a toll on growth. Our results suggest that duplication events can result not only in redundancy or specialization, but also in a noise buffering system that provides a selective advantage.

P.3.3.B-014
Cell cycle dependent expression variation between yeast species

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Regulation of gene expression is an important source of phenotypic diversity between close species, yet the genetic basis of most differences remains unknown. Within a species, gene expression profiles can vary greatly along the cell cycle, as up to 20% of the genes show cell-cycle dependent expression pattern. Therefore quantifying how expression divergence between species changes along the cell cycle is of major interest and a key step in understanding the genetic and molecular mechanisms underlying phenotypic variations. We performed a deep resolution RNA-seq on synchronized cultures of two closely related yeast species: *S. cerevisiae, S. paradoxus* and their hybrid. These two species differ both in cell cycle regulation and growth pattern. Interestingly, the hybrid displays different and faster cell cycle progression from both parents, indicating possible regulation rewiring. By measuring genome wide allele-specific mRNA expression, we are able to find genes that show expression differences between the species, and distinguish whether it resulted from changes in regulatory sequences of the gene itself (*cis*) or from changes in upstream regulatory factors (*trans*). We quantify the relative contribution of cis and trans effects during the different phases of the cell cycle, and highlight possible cell cycle regulators leading to the hybrid faster growth.

P.3.3.B-015
DNA methylation in *Mycoplasma gallisepticum*

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Mycoplasma gallisepticum belongs to the class Mollicutes and characterized by reduced genome, lack of cell wall and many metabolic pathways, and also easy to culture and non-pathogenic to humans. Due to this, it is convenient model object for system research of minimal cells. It is interesting to investigate the DNA methylation sites for whole-genome level in *M. gallisepticum* and estimate their influence for functioning of the cell.

For the identification of DNA modifications, we sequenced by SMRT (single-molecule real-time) method the wild-type *M. gallisepticum* S6 and its knock-out mutant (Tn_02360) in specificity subunit of system restriction-modification (SRM).

We determined that from annotated DNA modification systems the only one SRM type I is active. Genes of

methyltransferase GCW_02355, specificity subunit GCW_02360 and restrictase GCW_02365 code it. This SRM methylates DNA by N6-nitrogen of adenine in ANCNNNNCCT motif (methylation occurs at both adenines of a double-stranded motif in the direct and complementary strands).

The maximum density of the methylation sites found in the genome of *M. gallisepticum* in the genes of some adhesins and proteins for evading the immune response, while transposases and mobile elements are usually located in demethylated regions. Methylation sites are rarely found in the promoters of genes.

Almost all sites of modifications are methylated nearly completely. There are only 5 hypo-methylated sites where methylated fraction is about 0.75 in both strands. 3% of sites are hemy-methylated and methylation is absent in the strand AGGNNNNGNT.

Comparision of protein composition between wild-type *M. gallisepticum* S6 and its knock-out mutant Tn_02360 was carried out by two-dimensional electrophoresis with differential staining.

This work was supported by Russian Science Foundation 14–24–00159 «Systems research of minimal cell on a *Mycoplasma gallisepticum* model.

P.3.3.B-016
Decoding the evolution of transcriptional networks

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Divergence of gene expression plays a key role in phenotypic variation and speciation. Still, whether divergence relies on *cis*-regulatory elements in the DNA or regulatory protein factors, is unclear. Here, we utilize the closely related yeast species *S. cerevisiae* and *S. paradoxus*, to study these driving forces, and their contribution to large scale rewiring in gene expression. We apply a perturbation-based approach, to a deletion library in which 46 transcription factors playing a central role in different cellular processes were deleted on the genetic backgrounds of the two species and their hybrid. Using transcription profiling, we compared the contribution of each transcription factor to the gene expression program in the two species. We further analyzed the regulatory network of each species, and defined the mechanisms by which these networks were diverged, by assessing their preservation in the hybrid. This study sheds light on the interplay between *cis* and *trans* effects and their implications for the large scale divergence of the transcription regulatory network.

P.3.3.B-017
Reconstructing functional interactome of the candidate tumor suppressor protein CTCF: from protein-protein interactions to predicted functional modules

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The CCCTC-binding factor (CTCF) is a multifunctional zinc-finger transcription factor, known as "the master weaver of the genome" for its unique role in the 3D genome organization. This highly conserved protein acts as a transcription factor and occupies a myriad of target sites across the human genome. CTCF was shown to occupy its target sites in normal and cancer cells in a cell-type specific manner depending on the cellular context. This differential occupancy is coordinated by CTCF protein interaction partners and results in cell-type specific regulation of target genes. Current evidence only loosely links CTCF to cancer as a candidate tumor suppressor gene and there is a lack of experimental studies in this field. Vast experimental omics data, which is related with various biological questions and stored in diverse repositories, offers unprecedented possibilities to fill the gap. In this study, multiple datasets are retrieved from the selected omics databases and analyzed in the context of CTCF interactions and cancer. The employed integrative bioinformatics approach links CTCF-associated interactomics, transcriptomics and functional annotation patterns by reconstructing the putative CTCF protein-protein interaction network. The data analysis pipeline, which is mainly based on R, Python and Cytoscape software, utilizes diverse data mining and vizualizaton algorithms. The resulting putative CTCF interaction map links CTCF with distinct molecular processes and co-expression pattern of CTCF interaction partners provides indications about the underlying cellular mechanism. The highlighted interactions and differential expression profiles of the underlying proteins offer clues about potential functional modules and cell-type specific functions of CTCF. This work complements experimental CTCF research and the preliminary data provides novel insights into implication of this candidate tumor suppressor protein in cancer.

P.3.3.B-018
Analysis of immunoglobulin repertoires with unique molecular identifiers

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Unique Molecular Identifiers (UMI) used to assign sequencing reads to individually labeled cDNA or gDNA molecules is a powerful tool to achieve high-throughput error-free analysis of immunoglobulin (IG) repertoires. UMI allow to count molecules that enter the library preparation step thus making the sampling bottlenecks visible. Grouping of sequencing reads that carry the same UMI and thus cover the same starting molecule dramatically improves quality of long range sequencing, allowing for up to 750-nt lengths to be sequenced in an almost error-free manner. Thus UMI-based analysis provides accurate sequencing covering the whole variable region, and efficiently eliminates PCR and sequencing errors without using frequency-based error correction which would discard true hypermutating IG subvariants. This approach allowed us to perform a comprehensive analysis of full-length IG sequences from plasma B-cells emerged in young and old donors during the course of vaccination and uncover age-related differences in both somatic hypermutation profiles and class-switch recombination process. This work was supported by the Russian Science Foundation grant 14-14-00533.

P.3.3.B-019
Understanding the detailed functional 3D chromatin quasi-fibre multi-loop aggregate/ rosette architecture and dynamic organization of the genomes

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With a systems genomics combination of a novel superior selective high-throughput high-resolution chromosomal interaction capture (T2C), a novel FCS microscopy technique, polymer simulations, analytical mathematical modelling, and architectural/ DNA sequence scaling analysis we determined and cross-proved the final architecture of genomes with unprecedented molecular resolution and dynamic range from some base pairs to entire chromosomes: for several genetic loci of different species, cell type, cell cycle, and functional states a chromatin quasi-fibre exists with 5 ± 1 nucleosome per 11 nm, which folds into stable 40–100 kbp loops forming stable aggregates/rosettes connected by a ~50 kbp linker. This architecture shows minor though characteristic functional differences which are only variations of a theme and is a general property from bacteria to mammalian genomes. Both architecture and DNA sequence are tightly evolutionarily entangled based on the co-evolutionary connection to its function – the storage, translation, and finally replication of genetic information, i.e. the stability, accessibility, and reproduction ability of genomes are balanced. In principle genome architecture, dynamics, and function show a strong interdependence of genotype and phenotype, forming an inseparable system described by a multi-listic genome mechanics from the single base pair to the entire genome/cell nucleus. Hence, we not only determined the three-dimensional organization and dynamics but also show that this is a general phenomenon, explaining why genomes are organized in such a consistent systems manner. Thus, our approach not only opens the door to "architectural and dynamic sequencing" of genomes, but after ~170 years of intense research on this topic we also finally get a broad understanding of genome architecture and function, which has fundamental consequences for further basic research as well as diagnosis, treatment, and genome manipulation/engineering in the future.

P.3.3.B-020
The protein-protein interaction networks of cancer fusions and their alterations are cancer phenotype specific

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Fusion proteins in cancers are usually produced by chromosomal translocations. Fusions often function as onco-proteins or cancer drivers. One of the best-known examples is the BCR/ABL oncogenic fusion protein, considered to be the primary oncogenic driver of Chronic Myelogenous Leukemia. The identification of this specific fusion event led to the development of a drug, Imatinib that is highly specific for inhibiting the fusion kinase, resulting in a breakthrough treatment for a poorly responsive disease. More recently, gene fusions in prostate cancer, in particular, have been shown to drive carcinogenic processes such as invasiveness (the TMPRSS2/ERG fusion). Gene fusions are being recognized as important diagnostic biomarkers in malignant hematological disorders, and childhood sarcomas.

To demonstrate the systematic identification of protein-protein interactions of fusions, uncovering their influence on networks and on cancer phenotypes, we present here Chimeric Protein-Protein-Interaction (ChiPPI) method. It uses a "domain-domain co-occurrence" (DDCOS) score to calculate protein-protein interaction likelihood. The DDCOS score is based on the previous observations about the preference of domain-domain interactions and the systematic co-occurrence.

We applied ChiPPI to the analysis of 11,528 fusion from our ChiTaRS-3.1 database. Thus, using ChiPPI, we found a loss of tumor suppressors from fusion protein networks and the novel inclusion of onco-proteins. We tested the power of ChiPPI to resolve differences in cellular metabolism in different cancer types, i.e. leukemia/lymphoma, sarcoma and solid tumors. While certain pathways are enriched in all three cancer types (Wnt, Notch, TGF beta), there are distinct patterns for leukemia (p53 pathway, EGFR signaling, DNA replication, and CCKR signaling), for sarcoma (p53 pathway, and CCKR signaling), and solid tumors (FGFR and EGFR signaling). Thus, ChiPPI is the systems-biology method for analyzing protein networks in cancer.

P.3.3.B-021
Characterization of ZEB2 enhancers during brain development

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Zinc finger E-box binding homeobox (*ZEB2*) is a transcription repressor that is essential regulator of the nervous system during development. De-novo heterozygous mutations in the *ZEB2* are associated with multiple neurological defects, including Mowat–Wilson syndrome, due to haploinsufficiency of *ZEB2* expression during development. Therefore, mutations in ZEB2 enhancers that regulate its neuronal expression might lead to a similar phenotype. However, *ZEB2* enhancers are yet to be characterized. Using enhancer associated ChIP-seq data, we identified 13 sequences in the Zeb2 locus that might function as neuronal enhancers during development. Using zebrafish enhancer assay, we characterized nine neuronal enhancers, when four function as notochord enhancers and two function as specific neuron enhancers. Interestingly, the four notochord enhancers which drove similar GFP expression pattern do not have homology in their sequence, which led us to further determine the transcription factors that activate these enhancers. We found the TF repertoire that is likely to regulate the activity of these enhancers that required for the spatiotemporal expression of ZEB2 during neuronal development. The characterized enhancers shed light on the mechanism of action of *ZEB2* regulation and can serve as potential sequences that should be screened for mutations in patients whom no mutation at *ZEB2* coding region were found.

P.3.3.B-022
Landscapes of t cell receptor repertoires

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Individual repertoires of T cell receptors (TCR) are tightly shaped first by positive and negative selection in thymus, and then on the periphery. The resulting TCR landscape depends on HLA context, differs for the functional T cell subsets, and changes during the life. I will describe how the methods of high-

throughput immune repertoires profiling and comparative bioinformatic post-analysis allow us to study the functional features of TCR repertoires for the T cells subsets in mice and human. This work was supported by the Russian Science Foundation grant 16-15-00149.

P.3.3.B-023
Mesenchymal stem cells and graphene-based substrates as novel grafts in cardiac tissue regeneration

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Regenerative medicine focus on utilization of innovative achievements of stem cells (SCs) and biomaterial investigations to develop novel strategies for tissue repair. Human umbilical cord Wharton's jelly -derived mesenchymal SCs (hUC-MSCs) as one of the promising type of SCs that may be used in biomedical applications. Moreover, utilization of novel biocompatible scaffolds as artificial niches modulating SCs fate are of wide interest. Thus, the aim of the study was to evaluate the potential of graphene-based scaffolds as a culture surfaces for culture and differentiation of hUC-MSCs.

Graphene oxide (GO) and reduced graphene oxide (rGO) were prepared from graphite according to the Marcano method. We tested different size and thickness of graphene flakes to determine the most effective graphene substrate for hUC-MSCs culture. Next, we investigated the effect of GO and rGO on the biological and functional properties as well as effectiveness of cardiomyogenic differentiation of hUC-MSCs cultured on different types of graphene-based substrates.

Obtained results revealed that graphene-based substrates constitute non-toxic surfaces for hUC-MSCs, but their effect depends on the thickness of graphene layer and the level of graphene reduction. Highly reduced rGO flakes inhibit cell proliferation and survival of hUC-MSCs. Moreover, analysis of cell trajectories demonstrated the influence of type of GO solvent on speed of cell migration. Importantly, results shown that graphene may enhance hUC-MSCs differentiation toward cardiomyocytes *in vitro*.

Our study provides evidence that graphene-based substrates, constitute a suitable surfaces for hUC-MSCs propagation and may enhance their selected biological and therapeutical properties. However, further studies are required to analyze the impact of several graphene-based materials and their potentially applicability in cardiac regeneration.

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P.3.3.B-024
Whole genome mapping of heterochromatin in migrating cells and its effects on transcription

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Epigenetic mechanisms such as histone modifications are essential for the regulation of chromatin structure and gene expression. Previously we found that migration of melanoma cells is associated with and dependent on global heterochromatin formation. This heterochromatinization includes an increase level of various histone modifications including H3K9me3 and H3K27me3, which was also found in positive correlation with tumor progression. More recently, this phenomenon has been reported in additional cell types, suggesting it is a general signature of migrating cells. Previously, the H3K9me3 and H3K27me3 levels in migrating cells were determined by immunostaining, a technique that is not able to localize the histone modifications to specific genomic loci. Here, we analyze for the first time the genomic distribution of H3K9me3 and H3K27me3 in migrating cells in high resolution using chromatin immunoprecipitation following by sequencing (ChIP-seq). We show that the relative distributions of both histone modifications between the various genomic elements (i.e. promoters, enhancers, gene bodies and repetitive elements) are maintained similar upon induction of migration, while there is an increase in the genomic coverage by these modifications in migrating cells. Interestingly, instead of signal accumulation in peaks, the signal becomes more diffused in migrating cells while generating lower number of defined peaks. Apparently, only H3K9me3 peaks are wider in migrating cells in comparison to control cells. Associations between these histone modifications and cellular transcriptional profile as measured by RNA-seq in migrating cells are presented. In the long run our research may help to identify new targets for treatment against metastatic tumors.

P.3.3.B-025
Glutamine synthetase from *Esherichia coli*: a modeling of isotope exchange reactions using a stochastic approach

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One of the most critical and ubiquitous biochemical object is glutamine synthetase – a key component of nitrogen metabolism by catalyzing the synthesis of glutamine from glutamate and ammonia and ensure the regulation of their levels in almost all organisms. It is important to note that the active site of GS is rather conservative among all species. Therefore, one needs to investigate the catalytic mechanism of GS, because the mechanisms of the enzyme regulation under hyperammonemia and other pathological conditions remain unclear. One of the commonly used experimental procedure in the study of reaction mechanisms is the isotope exchange at chemical equilibrium. Our model focused on stochastic simulation based on the description of single bacterial GS as a series of state transitions, which described using the thermodynamic constants (e.g. Gibbs energy). All the possible states and transitions between them was set in accordance with relevant «Random Ter-Ter» mechanism. The basis of imitation algorithm is a step-by-step calculation of state probabilities with the subsequent estimation of both rate constant and exchange rates for each «substrate-product» pair. As a result, the dependence of exchange rate between the substrates and the products $[^{14}C]Glu \leftrightarrow Gln$, $[^{14}C]ADP \leftrightarrow ATP$ and $[^{32}P]P_i \leftrightarrow ATP$ on the concentrations of various metabolites was obtained. Retrieved values of $K_{0.5}$ are in a good agreement with the *in vitro* experimental data. The major conclusion is that there is a tendency for presence of dominant pathways as a part of a fully random mechanism. Such a preffered order binding of the substrates always relevant for the case of no reduction of the exchange rate. Apart from that, our results eliminate the possibility of the strictly ordered sequential metabolites binding, confirming appropriate experimental findings described in the literature. Further research can be focused on applying the obtained computational model of the enzyme to study a Gln-Glu cycle *in silico*.

Molecular Neuroscience

P.3.4-001

Recategorized

P.3.4-002
Chronic toluene exposure, hippocampus-dependent spacial memory and hippocampal structure. Experimental study

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Toluene and toluene-containing volatile substances are the most commonly abused solvents with a demonstrative addictive potential in humans. The central nervous system is one of the main targets: chronic toluene exposure is associated with long-lasting neurological, behavioral, neurochemical, and structural impairments. In the present study, we evaluate immediate and persisting effect of chronic toluene exposure on spatial memory and hippocampal structure in adult male Wistar rats. Each animal separately was exposed to toluene vapor (2000 ppm) or clean air for 3–5 min/day, during 20 d. Immediate effect of toluene chronic exposure was evaluated immediately after the end ofchronic inhalation, while persisting effect – 90 days after the end of toluene exposure. Impairment in spatial long-term memory was assessed using escape latency and Morris water maze test 24 h after training. Behavior in Water Maze comprise the acquisition and spatial localization of relevant visual cues that are subsequently processed, consolidated, retained and finally retrieved in order to successfully navigate and thereby to escape water. The hippocampus is a key structure for place learning, and the water maze procedures have been considered as a hippocampus-dependent. Therefore in parallel with behavioral studies, quantitaitve analysis of cell loss was performed in all layers of hippocampal CA1 and CA3 areas. The results revealed that in toluene-treated animals the long-term spacial memory in rats is worsened. Moreover, in both hippocampal areas significant loss of cells was observed in pyramidal cell layers. Such data are one more evidence of the role of hippocampus in toluene addiction.

P.3.4-003
Nerve growth factor and its receptor encoding genes in ischemic stroke

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Despite the important role of the nerve growth factor (NGF) and its receptor (NGFR) in the survival and maintenance of neurons in ischemic stroke (IS), data regarding the relationships between variations in the encoding genes and stroke are lacking. In the present study, we evaluated the association of the functional polymorphisms in *NGF* (rs6330 and rs4839435) and *NGFR* (rs2072446, rs11466155 and rs734194) genes with IS in an Armenian population. In total, 170 patients with IS (males/females: 88/82; mean age ±SD: 56 ± 9.7 years) and 200 healthy subjects (males/females: 112/88; mean age ±SD: 49 ± 9.2 years) were enrolled in this study. DNA samples of patients and healthy controls were genotyped using polymerase chain reaction with sequence-specific primers. The genotyping analysis showed that genotype distribution of the studied single nucleotide polymorphisms (SNPs) in study groups were concordant with HWE ($P > 0.05$). The results obtained indicate that the minor allele of rs6330 (P_{corr} = 2.4E-10) and rs2072446 (P_{corr} = 0.02) are significantly overrepresented in stroke group, while the minor allele of rs734194 (P_{corr} = 8.5E-10) was underrepresented in diseased subjects. In IS patients group, Mann-Whitney U test revealed statistically significant difference of the volumes of ischemic area between *NGF* gene rs6330*T minor allele carriers and non-carriers ($P = 0.023$). Furthermore, 27% of the patients experienced second or third IS and also there were significantly higher number of recurrent IS in T minor allele carriers group compared to non-carriers (P = 1.0E-6). Finally, no association with IS were observed for rs4839435 SNP of the *NGF* gene and rs11466155 SNP of the *NGFR* gene. In conclusion, our findings suggest that the *NGF* rs6330*T and *NGFR* rs2072446*T minor alleles might be nominated as a risk factor for developing IS and *NGFR* rs734194*G minor allele as a protective against this disease at least in Armenian population.

P.3.4-004
Dissecting sporadic ALS in a geographical cluster of patients: a multidisciplinary study

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Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder that results in paralysis and leading to death in 3–5 years. Genetic and environmental factors are involved in the pathogenesis of this disease and metals metabolism has been linked to ALS. Proteomic studies are currently being performed to search for possible biomarkers. Here we present a study aimed at investigating different aspects of the disease, based on a multidisciplinary approach. The cohort of ALS patients that we analyzed includes seven patients, all originating from a common, restricted, geographical area and five matched controls. Environmental exposure is the same for all these subjects.

SOD1, FUS, TDP43, C9ORF72 and APOE genotypes were evaluated. For metal quantitation, samples of serum and whole blood were analyzed by ICP-MS. For proteomic analyses, immobilized pH gradient covered the 4–10 and 3–7 pH range both in reducing and non-reducing conditions. Levels of DNA oxidation were evaluated by a comet assay. Statistical analyses were carried out with Student's t-test and Artificial Neural Networks.

As concentration resulted significantly lower in patients than in controls (Auto-CM analysis linked closely high concentrations of Al and Se to the ALS group. Levels of metals in whole blood have been correlated with levels in serum.

Proteomics data show that some proteins related to Acute Phase Response (APR) and lipid homeostasis are decreased in patients. Apoε4 allele is more represented in the patient's group than in controls'.

Impaired metal homeostasis, attributable to environmental exposure, could lead to mineral overload. Metals can compete for the binding sites of metal-containing proteins. The different expression of the APR proteins reported could be a reflection of the disease status of the subjects analyzed. Enrichment in Apoε4 allele frequency in patients may provide a link between neurodegeneration and lipid metabolism disturbances.

P.3.4-005
Novel centrally active antidote for intoxication by sarin and VX nerve agents

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Sarin and VX are organophosphorus nerve agents (OPNA), which show their toxic effect through the irreversible inhibition of the acetylcholinesterase (AChE, E.C. 3.1.1.7.) and butyryl-cholinesterase (BChE, E.C. 3.1.1.8) that normally degrade acetyl-choline (ACh). This inhibition compromises normal cholinergic nerve signal transduction in the peripheral and central nervous system synapses, leading to a cholinergic crisis with minor to medium symptoms, as well as to life-threatening impairments (*e.g.* respiratory failure and seizures). Survivors of attacks with these nerve agents can also experience long-term neurological impairments. The antidote treatment comprises an antimuscarinic drug, an oxime reactivator of the OPNA-inhibited enzyme and an anticonvulsive drug. Standard reactivators (*e.g.* 2-PAM, HI-6, obidoxime) are not efficient for every OPNA, and since they have positively charged quaternary ammonium in their structures, they are ill-equipped to cross the brain-blood barrier (BBB). Novel uncharged and therefore possibly centrally active reactivators were synthesised and evaluated by *in vitro* and *in silico* methods. The *in vitro* reactivation assay showed that they are promising reactivators of hAChE/hBChE inhibited by VX, as well as good reactivators of hAChE inhibited by sarin. *In silico*-determined physicochemical parameters enabled the prediction of BBB permeability. The cell line cytotoxicity profile showed that, at the most common therapeutic dose, they do not induce significant cell death. Induction of reactive oxygen species (ROS) production in cells was also tested to evaluate if they would have a synergistic or antagonistic effect toward one of the secondary mechanisms of neurotoxicity induced by OPNA (*i.e.* oxidative stress induction). Further *in vivo* BBB permeability potential and therapeutic efficacy is to be determined to conclude on the overall effectiveness of uncharged reactivators.

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P.3.4-006
Toluidine blue O, but not thionine, alters APP processing in 3xTg-AD mice

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Alzheimer's disease (AD) is a neurodegenerative disease marked by accumulation of amyloid-β (Aβ) peptides formed by sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases and neurofibrillary tangles comprised of hyperphosphorylated tau. Additionally, abnormalities in the cholinergic activity are also linked to cognitive impairment and memory loss in AD. Although majority of current on-market drugs target the cholinergic system, the aim of the ongoing research about AD therapy is to design drugs that ameliorate many aspects of the neuropathological hallmarks of AD. According to our earlier studies, two phenothiazine-derived compounds, toluidine blue O (TBO) and thionine (TH), seem to be potential therapeutic candidates for AD because they were found to be potent inhibitors of acetyl- and/or butyryl-cholinesterase at nM-μM levels and they were found to modify the amyloid metabolism by reducing extracellular Aβ40, Aβ42 and sAPPα levels in a dose-dependent manner in PS70 cells. The aim of this study was to investigate whether TBO and TH may moderate amyloid pathology in an *in vivo* model of AD. 3xTg-AD mice, which mimic the AD progression in humans, at 7.5 or 13 months of age, were injected intraperitoneally with 4 mg/kg TBO, TH or PBS containing 5% ethanol as control daily for 30 days. After treatment, the levels of Aβ40 and Aβ42, as well as APP and β-secretase were analyzed in the hippocampal homogenates by ELISA or Western blot. Our results showed that TBO, but not TH, reduced the insoluble hippocampal Aβ40 and Aβ42 levels in both young and aged mice significantly compared with control. On the other hand, TBO treatment caused no apparent change in the expression of APP and β-secretase in 3xTg-AD mice at late pathological stage. Overall, our *in vivo* data support the idea that TBO may be a promising drug candidate in the treatment of AD.

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P.3.4-007
Early spontaneous activity as a regulator of interneuron migration and integration in the mouse cerebral cortex

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At early stages of embryonic development, the brain demonstrates spontaneous activity in the form of intracellular calcium fluctuations. The cerebral cortex, the largest part of the mammalian brain, is responsible for motor, association and sensor functions among others. Two major classes of neurons regulate network activity in the cerebral cortex: excitatory (pyramidal) and inhibitory (interneurons) neurons. Although interneurons represent only the minority of all the neurons, they are crucial for the proper network function. Intriguingly, interneurons enter each layer of the developing cortex only when pyramidal neurons had already populated these sites and demonstrate correlated calcium activity. As interneurons reach their positions in target layers of the cortex, their intrinsic spontaneous intracellular calcium activity changes and migration ceases. This process may be connected to the increasing of chloride uptake provided by the expression of the K-Cl cotransporter KCC2 (Bortone D, Polleux F.,2009). However, this effect has not been studied *in vivo*. A novel and robust method for controlling neuronal activity *in vivo* is a chemogenetic approach for modulation of G protein-coupled receptors signalling. Designed Receptors Exclusively Activated by Designer Drugs (DREADDs) are receptors that can be selectively expressed in specific cell populations and activated by inert drugs, which gives a stable but temporally restricted effect *in vivo*. The main goal of our study is to address *in vivo* the mechanisms regulating migration and functional network integration of interneurons in relation to spontaneous activity of developing cortical circuits and chloride homeostasis. For this purpose, we made use of a DREADD system on early postnatal stages in a mouse model. Collected data indicates that silencing of pyramidal neurons significantly disturbs the normal distribution of interneurons in the mouse cerebral cortex.

P.3.4-008
α-Cobratoxin conjugated with fluorescent protein is a novel molecular tool for nicotinic acetylcholine receptor research

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The research aims at creation and structural-functional characterization of a new class of biomolecular tools for ion channel visualization. In the course of the work, three chimeric molecules were designed and obtained, then their physiological activity was evaluated. Nicotinic acetylcholine receptors (nAChR) belonging to the Cys-loop ligand-gated ion channels are an important group of integral membrane proteins. This subtype of cholinergic receptors provides the transmission of nerve impulses in neuronal and neuromuscular synapses. Function of diverse nAChR isoforms and their interaction with different ligands are in the focus of modern biochemistry and neurobiology. Moreover, certain diseases are associated with the dysfunction of these ionotropic receptors, and selective remedies are highly anticipated. In this communication we present three new hybrid ligands of nAChR constructed from fluorescent proteins and α-cobratoxin (CbTx) that bind selectively to nAChR. CbTx was purified from *Naja kaouthia* cobra venom and belongs to the family of "three-finger" toxins. Three fluorescent proteins (FP) were used for construction of the chimeras: TagGFP2, TagBFP, and mKate2. The resulting hybrid nAChR ligands comprise four parts: FP, CbTx, a flexible linker between FP and CbTx modules, and a His-tag utilized for protein purification. Chimeric proteins TagGFP2-CbTx, TagBFP-CbTx, and mKate2-CbTx were produced using a bacterial expression system. Biological activity of the fluorescent chimeras was assessed using the radioligand binding assay. The new tools will find application as selective fluorescent nAChR labels in current neurobiology.

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P.3.4-009
Unconventional myosin VI is important for DOCK7 localization and activity in the brain

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Myosin VI (MVI) is a unique unconventional motor that moves toward the minus end of actin filaments. It is involved in endocytosis, cellular trafficking, cell migration and adhesion. The spontaneous mutation of a mouse Myo6 gene resulted in a characteristic circling phenotype (termed as Snell's waltzer, SV) with sensorineural deafness and neurological symptoms accompanied by abnormalities in other organs. Our pursuit of MVI function in neurosecretory PC12 cells resulted in identification of DOCK7, a guanidine nucleotide exchange factor (GEF) for Rac1 and Cdc42 GTP that is crucial for axon formation, as a novel MVI binding partner [Majewski et al. (2012) Biochem Cell Biol 90:565-574]. We also characterized the MVI-DOCK7 interaction sites and showed that in PC12 cells the interaction was important for DOCK7 activity and NGF-stimulated protrusion formation [Sobczak et al. (2016) Biochim Biophys Acta 1863:1589–1600].

Herein we aimed at elucidation of the role of MVI-DOCK7 interaction in the brains of the wild type (WT) and SV mice. In hippocampus of WT brains, DOCK7 colocalized with MVI mainly in the perinuclear region. In the SV brains, DOCK7 distribution was more diffusive, not resembling the puncti-like defined structures visible in the WT samples. Also, the absence of MVI affected DOCK7 activity as revealed by estimation of the levels phosphorylated (active) forms of DOCK7 and its downstream effector SAPK/JNK kinase. Moreover, a significant increase of the levels of GFAP (glial fibrillary acidic protein) and caspase-3 were observed in the in hippocampus and cerebral cortex of SV brains. Thereby, our data indicate that in the brain MVI is important both for DOCK7 distribution and activity, and that this interaction could play important role(s) in neuronal functions.

P.3.4-010
Mechanisms of inhibition glioma cells proliferative activity by 2-hexadecenal

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2-Hexadecenal (Hex) forms in the body from sphingosine-1-phosphate in a reaction catalyzed by sphingosine-1-phosphate lyase. Previously, we established a non-enzymatic pathway of Hex formation under the action of HOCl on sphingosine, sphingosine-1-phosphocholine, sphingosine-1-phosphate and also on rat glioma C6 cells. It was shown that Hex regulates redox processes in these cells and reduces their proliferative activity by cytoskeleton reorganization. The purpose of this work is to establish the mechanisms of Hex effect on the proliferation of C6 cells.

It has been shown that cells cultivation with Hex (0.35–350 μmol/l) causes a decrease in the proliferation index from 10 ± 3.2 to 90 ± 2.9%. Using propidium iodide, it has been found that cultivation cells with Hex in a concentration range of 0.35–100 μmol/l for 24 h does not affect cells viability. Only in the presence of 350 μmol/l Hex a decrease in the survival rate by 34 ± 1.6% was observed, indicating the necrosis of cells under these conditions. Using flow cytometry method and the Annexin V-FITC apoptosis marker, it was found that the cells cultivation with 35 μmol/l Hex for 4 hours leads to apoptosis of 11.7 ± 2.3% of cells. Addition a 350 μmol/l of this aldehyde causes an increase in the number of apoptotic cells up to 28 ± 3.1%. Cultivation of cells with Hex (0.1–100 μmol/l) leads to a significant decrease in mitotic activity due to the redistribution of cell number in different fission phases. Using specific inhibitors of signal transduction pathway components participating in apoptosis initiation, it has been determined that PI3K, ERK ", JNK and p38 MAP kinases are involved in the regulation of proliferative activity of glioma cells by Hex.

Thus, Hex causes a decrease in the proliferative activity of rat glioma C6 cells by reducing fission rate and triggering apoptosis.

P.3.4-011
PKCβII translocation to mitochondria as a component of endogenous neuroprotection

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Protein kinases C (PKC) constitute a family of enzymes which regulate an action of other proteins by phosphorylation of their Ser/Thr residues. PKCs are reported to be involved in mitochondrial metabolism after ischemia-reperfusion injury (I/R). Because

of the mitochondrial engagement in cell death and survival, PKC activity in mitochondria, triggered by I/R, may be responsible for further fate of the cell. We previously demonstrated that the β isoform of PKC, due to ischemia injury, translocates to mitochondria, mainly in ischemia-resistant part of gerbil hippocampus. We hypothesize that the translocation is a factor responsible for endogenous neuroprotection. To reveal the role of PKCβs in neuronal survival we used widely acknowledged model of brain ischemia: in vivo- 5′ transient brain ischemia in gerbils. First, we proved that PKCβII translocation to mitochondria is tightly connected with post-ischemic neuron survival in I/R resistant hippocampal area (CA2-4, DG). Next, to understand the mechanism of this process, we used proteomic approach to select potential mitochondrial interactors of PKCβII. In post-ischemic mitochondria the analysis recognised proteins involved in energetic metabolism (e.g. the Krebs cycle or the electron transport chain), amino acids metabolism and scaffold proteins. Till now, we have confirmed the direct interactions between PKCβII and α and β subunits of ATP synthase and pyruvate dehydrogenase. The impact of PKCβII on these proteins activity is under investigation. In summary, we suggest that mitochondrial proteins might be regulated by PKCβII phosphorylation and validation of this process should contribute to elucidate a mechanism of PKCβII-mediated endogenous neuroprotection. The study is supported by National Science Centre grants: 2014/15/D/NZ3/02784 and 2012/05/B/NZ3/00415.

P.3.4-012
The transglutaminase-2 "transamidome" in Aβ-exposed primary rat hippocampal neurons

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Type 2 transglutaminase (TG2) is a calcium-dependent protein cross-linking enzyme activated in mis-folding diseases (e.g. Alzheimer's disease). In vitro TG2 has been implicated in the generation of toxic Aβ oligomers, and Aβ and tau are substrates of TG2-mediated transamidation in brain. Although TG2 may be directly involved in the progression of neurodegeneration, the underlying mechanism remains unclear. Here we have characterised TG2 in primary rat embryonic hippocampal neurons exposed to Aβ, to mimic a neurodegenerative environment. Dual immunofluorescence staining of TG2, and either presynaptic marker vGlut1 or postsynaptic marker Shank2 revealed that TG2 is mainly located at neuronal pre-synapses. Stimulus with Aβ induced a 6-fold increase and spread of TG2 activity in situ, when visualised by incorporation of fluorescent primary amine substrate FITC-cadaverine into endogenous γ-glutamyl-substrates. To identify potential substrates of TG2-transamidation ("TG2 transamidome") we employed a global quantitative proteomic approach involving incubation of hippocampal neurons with FITC-cadaverine either in the presence of Aβ, or of Aβ with a TG2 inhibitor. Proteins cross-linked to FITC-cadaverine were immunoprecipitated (IP) from total cell lysates with an anti-FITC antibody, proteolytically digested and analysed via SWATH™-MS/MS proteomics (n = 3) using a spectral library produced by classic shotgun-MS. Differences between Aβ and Aβ-TG2 inhibitor (representing the background) were identified at a confidence ≥70%. Furthermore, the proteome of hippocampal neurons with and without Aβ treatment was also resolved, with a coverage of 1000 + , and interrogated with the "TG2 transamidome". Six proteins were found to be specific substrates of TG2-transamidation upon Aβ. An ionic channel protein was a highly specific substrate of TG2 and its involvement in the modulation of calcium homeostasis at neuronal synapses, and thus further TG2 activity, is currently being investigated.

P.3.4-013
Kinetic behaviour of horse serum butyrylcholinesterase with fluoxetine

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Butyrylcholinesterase (3.1.1.8.; BChE) is a serine esterase which is found in vertebrates. BChE plays a major role in the detoxification of natural as well as synthetic ester bond-containing compounds. It is also responsible for the elimination of acetylcholine when acetylcholinesterase is inhibited. Alterations in BChE activity is associated with the diseases. Particulary, cholinergic system abnormalities are correlated with the formation of senile plaques in Alzheimer's disease (AD). Current therapeutic approaches use cholinesterase inhibitors in the treatment of AD. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and easily passes through the blood-brain barrier.

Purity and molecular weight of the horse serum BChE enzyme were confirmed with native and SDS-PAGE. Optimum pH and optimum temperature were determined for the characterization of the enzyme. Five different fluoxetine concentrations (5–80 μM) and six different BTC concentrations (0.05–2 mM) were used in inhibition kinetic studies.

The molecular weight of the tetrameric, dimeric and monomeric forms of BChE was calculated as 380, 190 and 95 kDa, respectively. Optimum pH of the enzyme was 8.1. Optimum temperature, energy of activation (E_a) and temperature coefficient (Q_{10}) were calculated as 36.4°C, 1526 cal/mol and 1.19, respectively. In kinetic studies, V_m was found to be 20.59 ± 0.36 U/mg protein and K_m was calculated as 194 ± 14 μM. Fluoxetine inhibited BChE competitively. Half maximal inhibitory concentration, IC_{50}, and K_i were found to be 104 μM and 36.3 ± 4.7 μM, respectively.

Low K_i value suggests that fluoxetine is a potent inhibitor of BChE even at therapeutic doses.

Further studies on the molecular mechanism of the BChE inhibition by fluoxetine and its clinical use should be performed since BChE is responsible for the appearance of the pathologic hallmarks of the some diseases such as plaque formation in AD.

P.3.4-014
Non-steroidal anti-inflammatory agent, mefenamic acid potentiates and inhibits GABAA receptors acting via distinct binding sites

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Nonsteroidal anti-inflammatory drugs besides of suppression of prostaglandins synthesis may modulates a variety of ion channels including type A receptors of γ-aminobutyric acid ($GABA_AR$). We combined electrophysiological and modeling approaches to study the mechanisms of mefenanic acid (MFA) interaction with $GABA_AR$. Patch-clamp recordings showed that MFA strongly potentiates GABA-induced currents in acutely isolated rat Purkinje cells. At concentration of 3–100 μM MFA increased the amplitude of currents with EC50 ≈ 15 μM and maximal potentiation up to 700%. The further growth of MFA concentration caused an inhibitory effect on $GABA_AR$ which was dependent on membrane voltage. We found that potentiating effects of

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MFA and general anesthetic etomidate (ETM) were non-additive, suggesting that MFA acts through the binding site targeted by etomidate. We built the model of open $\alpha_1\beta_2\gamma_2$ $GABA_AR$ based on the GluCl X-ray structure and used Monte-Carlo energy minimization to predict the MFA binding site(s). There are experimental data indicating that etomidate efficacy strongly depends on mutations of α_1 M236 and β_2 M286 residues. We imposed distant constraints to find the ETM and MFA binding modes in the transmembrane $\beta(+)/\alpha(-)$ interface. We showed that both ETM and MFA form H-bonds with β_2 M2 R269 (19′) and N265 (15′) residues, van-der-Waals contacts with hydrophobic residues β_2 M3 Met286, Phe289 and α_1 M1 Leu232, Pro233, Met236. In order to find an inhibitory site we pulled (and simultaneously rotated) MFA through the pore. Our calculations predict the existence of two binding sites located at 6 and 31 Å of the pore depth. In the upper site MFA mainly interacts with charged and polar residues 20′, 21′. In the lower site hydrophobic residues from −2′, 2′ and 5′ rings and polar residues 2′, 6′ are strongly contributed in MFA stabilization. The complete pore occlusion in the upper site is achieved by binding of two MFA molecules simultaneously. Supported by RFBR grant 15-04-02010.

P.3.4-015
Neuroprotective effects of α2-adrenoblocker mesedin on astroglia and neurons upon exposure to hypoxia

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It has been previously reported that mesedin (2-(2-methyIamino-4-thiazolyl)-1,4-benzodioxan hydrochloride), α2-adrenoblocker, possess also anti-hypoxic properties and reveal the protective features in the rat model of stroke. However, the mechanisms of cytoprotective function of mesedin under hypoxia induced brain damage remains unexplored. The goal of this study is to investigate the protective features of mesedin on the mouse neurons, neural progenitors and astroglial cells in in-vitro models of hypoxia. Quantification of lactate dehydrogenase (LDH) release from cells in a mouse brain primary culture at day 7 in-vitro after 48 h incubation with the different (10 μM, 100 μM and 1 mM) concentrations of mesedin was assessed as a marker of cell survival upon hypoxic injury. Administration of mesedin led to a remarkable decrease of LDH in the cell culture supernatant, while the highest concentration appeared to be the most effective. In regard to deleterious effects of hypoxia on the synaptic plasticity, cholinergic activity and on the inflammatory response of the brain tissue in various neurologic disorders we assessed the mRNA expression of respective markers in the brain primary culture upon hypoxia/normoxia after mesedin administration. Mesedin increased the expression of PSD95 and IL 10, mRNA only upon hypoxia, while a decrease in IL-6 and enhanced expression of ChAT failed to reach statistical significance. Evaluation of mesedins' influence of on the survival, differentiation of neurons and generation of neural precursor cells under normoxic control and hypoxia revealed that mesedin led to an increase in the number of β-III tubulin-positive neurons and nestin/β-III tubulin-positive precursors upon both, normoxic and hypoxic culture conditions. It can be concluded that mesedin protects neurons from hypoxia-induced death, possibly via IL-10 production, increases postsynaptic density marker and shifts the population of nestin-positive cells towards neuronal lineage.

P.3.4-016
The homeobox gene Anf/Hesx1 underlies emergence of the vertebrate telencephalon: important proof from lampreys

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Accumulated evidences indicate that the core genetic mechanisms regulating early patterning of the brain rudiment in vertebrates are very similar to those operating during development of the anterior region of invertebrate embryos. However, mechanisms underlying obvious morphological differences between elaborated vertebrate brain and its simpler invertebrate counterpart still remain unknown. Recently, we have hypothesized that the emergence of the most anterior unit of the vertebrate brain, the telencephalon, could be the result of appearance in their ancestors of a unique homeobox gene, *Anf/Hesx1*, which is absent in all invertebrates and regulates the most early steps of the telencephalon development in vertebrates. However, the fact that *Anf* failed to be detected during recent genome sequencing of one of the most basal extant vertebrate species, the lamprey, seriously compromises this hypothesis. Here we report cloning of *Anf* in three lamprey species and demonstrate that this gene is indeed expressed in embryos in the same pattern as in other vertebrates and executes the same functions by inhibiting expression of the anterior general regulator *Otx2* in favor of the telencephalic regulator, *FoxG1*. Our finding confirms a unique role of *Anf* class homeobox in the telencephalon emergence during vertebrates evolution and the embryonic development. This work was supported by RFBR grant No.15-04-04343.

P.3.4-017
Inflammation-dependent cerebrospinal fluid hypersecretion from the choroid plexus in post-hemorrhagic hydrocephalus

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Intraventricular hemorrhage (IVH) frequently causes post-hemorrhagic hydrocephalus (PHH) as a result of impaired cerebrospinal fluid (CSF) homeostasis. The most common treatment for PHH is surgical CSF shunting, a procedure fraught with complications. PHH has been classically attributed to impaired intra- or extraventricular CSF reabsorption, but little experimental evidence supports this theory. We now demonstrate that IVH causes a toll-like receptor-4 (TLR4) and NF-κB-mediated inflammatory response of the choroid plexus epithelium that is associated with a ~3-fold increase in bumetanide-sensitive CSF secretion. The increased CSF secretion is secondary to inflammation-dependent increase in the functional expression of the Ste20-type stress kinase STK39 (SPAK), which interacts with, phosphorylates, and stimulates the Cl^- cotransporter NKCC1 at the choroid plexus apical membrane. Genetic depletion of TLR4 or SPAK, or intracerebroventricular delivery of drugs that antagonize TLR4-NF-κB signaling or the SPAK-NKCC1 complex, normalize hyperactive CSF secretion rates and reduce post-IVH ventriculomegaly. These data uncover a previously unrecognized contribution of CSF

hypersecretion in the development of PHH, demonstrate a novel role for innate immunity in the regulation of the internal brain milieu, and identify a kinase-mediated regulatory pathway of CSF secretion that can be targeted with repurposed, FDA-approved drugs for the non-surgical treatment of PHH.

P.3.4-018
TCPTP and conexins mediate the effect of leptin on hypothalamic astrocyte morphology

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Leptin plays a key role in the hypothalamus to regulate energy homeostasis and has emerged as a modulator of neurodevelopment of the hypothalamic circuitries involved in the feeding behavior. Morphological changes of astroglial cells are known to occur during brain development. Thus, in this study we investigated the role of leptin in the morphology of hypothalamic astrocytes using primary cell culture obtained from one-day newborn rats. We also investigated the role of T-cell protein tyrosine phosphatase (TCPTP), a counter-regulatory molecule of leptin signaling and conexin 43 (CX43) and CX30 that participate in the function and maintenance of astrocyte morphology. Procedures were approved by Ethics Committee for Animal Use. At 80% of confluence, the astrocytes were treated with leptin (0, 100, 1000, 5000 ng/ml) for 24 h. Treatment with LPS (500 ng/ml) was used as a positive control. The stimulus with LPS increased ($P < 0.01$) the *Ptpn2* and *Gja1* mRNA expression and it also increased TCPTP and CX43 immunoreactivity and the soma area of astrocytes. Leptin treatment (5000 ng/ml) showed similar results, but not *Gja1* expression. To assess the role of TCPTP in leptin effects, knockdown of *Ptpn2* induced by siRNA was carried on before the treatment of the cells with leptin or LPS. The silencing of *Ptpn2* (siRNA *Ptpn2*) reversed not only the gene expression induced by LPS and leptin, but also it prevented the morphology changes induced by these stimuli. Remarkably, silencing of *Ptpn2* increased *Gja6* mRNA expression ($P < 0.01$) and CX30 immunoreactivity. The stimulation with LPS increased the mRNA expression of the *Il6*, *Il1b*, *Tnf* and *Ptpn1*. The *Ptpn2* siRNA further increased these responses, demonstrating that *Ptpn2* has a counter regulatory action on the expression of these cytokines and *Ptpn1*. These data indicate that leptin affects the morphology of astrocytes and this effect is likely to be mediated by TCPTP, which in turn modulates the expression of gap junction protein CX30.

P.3.4-019
A role for salt inducible kinase 2 (SIK2) in Müller Glia reprogramming

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Müller cells are the main glial cells of vertebrate retina and function to maintain retinal homeostasis and integrity. In fish, Müller cells proliferate, gain stem cell characteristics and generate new neurons upon injury for retina regeneration. Proliferation is the critical step for retina regeneration. Injury dependent changes in mammalian Müller cells are similar to that observed in fish, however regeneration of neurons is not detected in mammals. Very limited retina regeneration is observed in mammals upon stimulation of Müller cell proliferation by exogenous growth factors, such as FGF2. The strict control of Müller cell proliferation has been proposed to limit retinal regeneration in mammals. In this context, we aimed to provide clues for the potential contribution of SIK2, as a negative regulator of FGF2-dependent Müller cell proliferation, to Müller cell reprogramming. To test this, we used MIO-M1 cells, which are known to transdifferentiate into neurons upon FGF2 stimulation, as a model system. In our experiments, MIO-M1 cells were treated with FGF2 for 0–7 days. In agreement with literature, transdifferentiation potential of MIO-M1 cells were confirmed with formation of nestin-positive neurospheres with self-renewal capacity. Increase in Pax6 and Chx10 transcript levels showed that MIO-M1 cells dedifferentiate into progenitors. Decrease in vimentin expression with increase in calretinin and Prox1 expression verified that MIO-M1 cells lose their glia characteristics and differentiate into neurons, particularly horizontal cells. Our results show that ERK activity is required for increase in Müller cell population. Significant decrease in SIK2 protein level in early phase of the process permits Müller glia to re-enter cell cycle. Overexpression of SIK2 blocked ERK activation, cell cycle entry and further transdifferentiation process. Based on these, we suggest that SIK2 negatively regulates Müller glia reprogramming through downregulation of ERK activity.

P.3.4-020
Human neuromodulator Lynx1: action on nicotinic acetylcholine receptors in the brain and its role in cognitive processes

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Lynx1 is the endogenous GPI-anchored neuromodulator of nicotinic acetylcholine receptors (nAChRs) in the mammalian brain. Presently, Lynx1 is considered as one of the factors which regulate the neuronal plasticity. In rodents the Lynx1 is involved in cognitive processes such as memory and learning. The mechanisms underlying neuromodulatory activity of Lynx1 remain insufficiently studied.

For the first time we demonstrated that recombinant analogue of Lynx1 activates α7-nAChR in the rodent brain. Moreover, we demonstrated that Lynx1 competes with soluble oligomeric β-amyloid peptide Aβ(1-42) for binding with different nAChR subunits extracted from the brain. It should be noted that Aβ(1-42) is considered as the toxic form of β-amyloid peptides associated with cognitive deficits in Alzheimer's disease (AD). Aβ(1-42) interacts with and regulates the function of several nAChR subtypes. We found that Lynx1 abolishes the Aβ(1-42)-induced cytotoxity on mouse cortical neurons. Effect of Lynx1 on long-term potentiation (LTP) in the hippocampus, which underlies learning and memory, was studied. Pre-incubation of hippocampal slices with 10 μM Lynx1 during one hour showed significant increase in LTP amplitude (~ 70%) in CA1 region. Contrary, 200 nM Aβ (1-42) inhibited LTP. Co-application of Aβ(1-42) with Lynx1 fully abolished the LTP blockade, caused by Aβ(1-42). Moreover, we showed that administration of mouse cortical neurons with Aβ(1-42) leads to decrease of Lynx1 gene expression. This is in line with our observation that cortical Lynx1 level is decreased in transgenic 3xTg-AD mice.

Data obtained point on Lynx1 as important regulator of the cognitive processes, which could activate α7-nAChRs in the brain and prevent the blockade of long-term potentiation, caused by β-

amyloid peptide. This might have functional and pathophysiological implications in relation to Alzheimer's disease.

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P.3.4-021
The role of polyamines in etiopathogenesis of schizophrenia

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This work is dedicated to the study of polyamine's role in etiopathogenesis of schizophrenia. Polyaminemia and polyaminuria have been shown in patients with schizophrenia. It was established that levels of polyamines in blood serum and in urine in patients with schizophrenia were dependent on type of course, leading syndrome and duration of disease. Endotoxicosis in schizophrenia is due to significantly higher concentrations of polyamines in average molecular mass fraction of blood serum in patients with schizophrenia then in normal persons. Correlation between concentration of polyamines in blood serum and in urine in patients with schizophrenia and the stage of disease (exacerbation, remission) has been shown. There is a correlation between polyamine levels in blood serum in patients with schizophrenia and such clinical manifestations of disease as "anxiety", "the disorganization of thinking", "hostility" as well as sum of points on Brief Psychiatric Rating Scale (BPRS). In the process of treatment the degree of decrease in polyamine levels is correlating with reduction of such clinical disorders as "the disorganization of thinking", "depression", "suspiciousness", "somatic concern", "motor retardation" as well as with decrease of sum of points on brief psychiatric estimative scale BPRS. The direct action of neuroleptic drugs (structural analogs of polyamines) activated catabolism, inhibited synthesis and normalized polyamine levels in model cell-free test systems, obtained from tissues with increased cellular proliferation. This is considered as a possible mechanism of therapuetical action of these psychotropic medicines. The polyamines participation in etiopathogenesis of schizophrenia is proposed.

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P.3.4-022
HnRNP Q suppresses polyglutamine aggregation by reducing Vaccinia-related kinase 2 mRNA stability

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Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the huntingtin (HTT) gene, which encodes a polyglutamine tract in the HTT protein. Misfolding of proteins containing abnormal expansions of polyglutamine (polyQ) repeats is associated with cytotoxicity in HD. We have previously shown that vaccinia-related kinase 2 (VRK2) downregulates chaperonin TRiC protein levels through the ubiquitin-proteasome system by recruiting the E3 ligase COP1 and by destabilizing USP25 which is deubiquiting enzyme. Here we found that basal level of VRK2 in neuronal cells is regulated by post-transcriptional regulation. HnRNP Q specifically binds to 3′UTR of VRK2 mRNA in neuronal cells and reduces its stability. We report a dramatic increase polyglutamine aggregation by reducing hnRNP Q level. Thus, our results demonstrate that decreased brain hnRNP Q contributes to HD neurological phenotype and open new novel prognostic marker of HD.

P.3.4-023
From proteomics studies to molecular pathways – proteins involved in methamphetamine dependence

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Methamphetamine is a well-known psychostimulant, which exerts its influence on the central nervous system. To identify the changes caused by this drug at the molecular level, several proteomic studies by different groups were performed. Proteomics allows observing the influence of xenobiotics on the protein levels in various types of samples (tissues, cell cultures, body fluids). Identifying those changes may allow finding what kind of proteins/molecular pathways are engaged in the action of a certain drug. In this study, we summed up the results of available proteomic data on this topic published up to date. Our goal was to reveal proteins, which were indicated several times by different proteomics studies. We assume that changes in proteins' expression identified by different proteomics platforms and using different animal models are validated in such a way, and could comprise the trustful markers of changes exerted by methamphetamine. As a result, a list of 80 proteins was generated. To find more information about identified molecules and to interpret the differentially expressed proteins in a biological context, bioinformatic tool: WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) was used. Webgestalt allows for the analysis of detected proteins in different databases comprising information about the function of proteins, such as GO analysis, KEGG database, Pathways Common, and Wikipathways. After bioinformatic analysis of the functions of identified proteins, it was found out, that many of them are engaged in energy metabolism, especially in glycolysis/gluconeogenesis, TCA cycle, and respiratory electron transport. Such meta-analysis of the results generated by various laboratories might be the first step to broadening our knowledge about molecular pathways involved in methamphetamine addiction and neurotoxicity.

P.3.4-024
Efficacy of triple gene therapy based on adenoviral vector- or cell-mediated intrathecal delivery for spinal cord injury treatment in rat model

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The aim of the study is to discover the behavioral, electrophysiological, morphological changes in post-traumatic spinal cord in response to intrathecal administration of triple gene combinations using adenoviral vectors or umbilical cord blood mononuclear cells (UCBMC) as carriers of the therapeutic genes. We investigated the therapeutic efficacy of genes encoding vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF), angiogenin (ANG) and neuronal cell adhesion

molecule (NCAM) in rat model of spinal cord injury (SCI). Therapeutic genes were used in two combinations: VEGF+GDNF+NCAM and VEGF+ANG+NCAM. Adenoviral vectors based on serotype 5 (Ad5) carrying recombinant genes or UCBMC simultaneously transduced with three Ad5 vectors. The therapeutic efficacy was confirmed by (1) improvement of behavioral tests, (2) enhanced recovery of electric response of hindlimb skeletal muscles to electrical stimulation of the sciatic nerve and magnetic stimulation of the cervical spinal cord, (3) greater sparing of gray and white matter. Immunofluorescent staining using antibodies against the functional markers of motorneurons (Hsp27, synaptophysin, PSD95), astrosytes (GFAP, vimentin), oligodendrocytes (Olig2, NG2, Cx47) and microglial cells (Iba1) suggests that morhpo-functional recovery of posttraumatic spinal cords depends on (1) lowering the stress and enhancing the synaptic plasticity in motorneurons; (2) supporting oligodendrocyte proliferation and myelination. Comparative analysis of the obtained data revealed that UCBMC simultaneously transduced with Ad5-VEGF+Ad5-GDNF+Ad5-NCAM has more powerful effects on spinal cord regeneration. Thus our results suggest that intrathecal injection of gene modified UCBMC overexpressing recombinant neurotrophic factors and neuronal cell adhesion molecule represent a novel potentially successful approach for treatment of SCI. This study was supported by the grant of Russian Science Foundation No.16-15-00010.

P.3.4-025
Electrophysiological changes and mucosal permeability in phenotypes of gastroesophageal reflux disease and functional heartburn

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Three different phenotypes of gastroesophageal reflux disease (GERD) such as erosive reflux (ERD), nonerosive reflux (NERD), esophageal hypersensitivity (EH) and functional heartburn (FH) might have different pathophysiological changes within the esophageal epithelium. We aim to investigate the electrophysiological differences and diffusion characteristics as a reflection of tissue integrity using Ussing chamber system. Distal esophageal mucosal biopsies from 14 healthy controls (40.6 ± 11.2 years) and 62 patients with GERD (40 men, 42.9 ± 12.3 years, n = 34 ERD n = 22 NERD, n = 6 EH) and 11 patients with FH were studied. Upper gastrointestinal endoscopy with esophageal biopsies were performed in all patients. Biopsies were put into the chambers to measure the transepithelial resistance (TEER), potential difference (PD) and tissue permeability via fluorescein diffusion within two hours as well as evaluation of dilated intercellular spaces with light microscopy. Esophageal biopsies of healthy volunteers (163.6 ± 41.1 ohms) had significantly higher TEER when compared to total GERD patients (132.5 ± 38.7 ohms). Although the TEER results of whole GERD subtypes were decreased compared to healthy controls, only ERD groups were significantly lower (123.3 ± 29.8 ohms). There was also no significant difference in any parameters between NERD, FH and EH groups. The mucosal permeability of GERD subtypes was significantly higher than the healthy controls. The PPI-unresponsive subjects (n = 10, 94.8 ± 36.5 pmols) were much more permeable to fluorescein compared to PPI-responsive subjects (n = 52, 56.0 ± 32.4 pmols) within all GERD patients (P = 0.009). The TEER and permeability results imply that ERD and NERD groups showed a barrier disruption. However, epithelial permeability was not different in EH and FH groups. The dilatation of intercellular spaces may contribute to increased mucosal permeability in true-NERD and ERD patients. EH and FH patients might have different pathophysiology than others.

P.3.4-026
Development of a method for studying of the glutamate transport in isolated nerve terminals using an electrochemical biosensor

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Glutamate is a main excitatory neurotransmitter in the central nervous system of vertebrates and is involved in almost all aspects of brain work, including cognitive functions like recognition, memory, and learning. Thus, studying of glutamate transport (i.e., release and uptake) in synapses is important for the understanding of glutamate role in different brain processes. In this work we present a method for the evaluation of the rate of glutamate transport by the isolated nerve terminals (synaptosomes). The method is based on electrochemical glutamate-sensitive biosensor. The biosensor consisted of glutamate oxidase that was immobilized on the surface of platinum disc electrode. Glutamate oxidase produced hydrogen peroxide proportionally to the glutamate concentration; this hydrogen peroxide was oxidized on the electrode and generated the biosensor response. It was shown that the selectivity of the biosensor is greatly improved by incorporating a semi-permeable membrane based on phenylenediamine. This allows effective application of the biosensor for biological samples which contain electroactive species like dopamine and ascorbic acid. We optimized conditions for the glutamate oxidase immobilization and studied influence of the working buffer properties on the biosensor work. The biosensor was characterized by good reproducibility of responses and operational stability. Using the biosensor, a procedure for the determination of glutamate in synaptosomal samples was developed and the velocity of glutamate uptake and release by the synaptosomes was measured. For comparison, control experiments using radioactive-labeled glutamate as well as spectrophotometry were carried out, and the biosensor results correlated well with these traditional methods of glutamate determination. The biosensor-based method has following advantages – ease of biosensor use, portative measuring setup, re-usage of the biosensor for multiple analysis and thus low price of one analysis.

P.3.4-027
Analysis of genomic rearrangements in a Turkish cohort of early onset Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide and the prevalence increases up to ~2% with the ages older than 65. In addition to late onset PD

cases, approximately 10% of the patients have an early-onset PD (EOPD) seen before the age of 40. Three genes *PRKN*, *PINK1* and *DJ-1* have found to be strongly related with EOPD pathology. Genomic rearrangements such as whole exonic insertions/deletions in these genes are frequent mutations in EOPD cases at varying degrees depending on the ethnicity of the population. In the present study, we aimed to investigate phenotypical features and the frequency of genomic rearrangements in a subset of Turkish early-onset PD patients with family history. Thirty-five Turkish EOPD patients were screened for genomic rearrangements by using the multiplex ligation-dependent probe amplification (MLPA) method with SALSA P051 and P052 kits. The kits consist of probes for all exons of α-synuclein, PRKN, and PINK1, and specific exons of DJ-1, LRRK2, UCH-L1, ATP13A2, LPA, TNFRSF9, CAV2, CAV1, and GCH1 genes. We identified exonic rearrangements in 46% of Turkish patients. We have identified 11 patients (31%) with homozygous whole exonic deletions, and 4 patients (11%) with heterozygous whole exonic deletions in PRKN gene. Additionally, one patient was found to have homozygous whole exonic deletions in DJ-1 and TNFRSF9 genes. To the best of our knowledge, this is the first genomic rearrangement in DJ-1 reported from Turkey. No genomic rearrangements were found in PINK-1 gene in this cohort. Our results indicated that exon dosage mutations in PRKN gene might be the most common reason for EOPD. In addition, heterozygous PRKN exon dosage mutations have been shown to related with EOPD pathology. To conclude, exon dosage mutations have important genetic and clinical implications in EOPD cases in Turkey.

P.3.4-028
Stress modulation by intranasal oxytocin and variation of this modulation with body weight in chronic stress exposed rats

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Stress, via activation of stress system modulates various physiological parameters like food intake and energy consumption. Understanding the pathogenesis of obesity and mutual interaction between obesity and stress is of vital importance since obesity has become an important public health problem in today's stressful world. The role of oxytocin for control of these mechanisms because of its anxiolytic, anorexigenic effects and being the common ground concerning the hypothalamus cannot be disregarded. On this background we aimed to investigate the effect of intranasal oxytocin (INO) on stress response in obese and normal rats exposed to chronic stress. Cold-immobilization stress was applied for 5 consecutive days to male Sprague-Dawley rats fed either with standard (n = 20) or high fat diet (HFD) (n = 20). Half of the animals in each group were administered INO. Stress response is evaluated via serum and saliva cortisol levels as well as elevated plus maze scores. Prefrontal cortex and hypothalamus oxytocin receptor (OxtR) protein levels were designated using western blot analysis. Stress response of obese animals was higher when compared with control (non-obese) group. Anxiolytic effect of oxytocin was prominent in control group whereas the effect was found to be diminished for obese animals. While OxtR protein levels in prefrontal cortex was found to be invariable according to the bodyweight and exogenous oxytocin administration, levels for hypothalamus was found to be changing according to feeding type and oxytocin administration. Our results indicated that the peripheral and central effects of oxytocin vary with body weight. However, obesity, probably reinforcing the stress condition via central receptors, masks the foretold anxiolytic effects of oxytocin. In conclusion, enlightening the central effect mechanisms of oxytocin is of vital importance and deserves further studies in order to cope with stress and growing problem of obesity and its modulation.

P.3.4-029
Peptidomic and proteomic analysis of cerebrospinal fluid of patients with Guillain-Barre syndrome

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Guillain-Barre syndrome (GBS) is a rare but severe disorder of the peripheral nervous system. To understand the composition change of peptidome and proteome we compared CSF samples from GBS patients and the control group of patients with non-neurological diseases.

The proteome analysis of CSF samples of both groups revealed 1541 proteins, and 491 of them were specific for GBS. As a result of peptidome analysis, 2562 peptides representing 773 proteins were identified in CSF samples. 1873 peptides derived from 591 proteins were specific for GBS samples. CSF peptidome of GBS patients contains peptide fragments of neurofascin, contactin 2 and neuronal cell adhesion molecule. These proteins may be considered as potential targets for autoimmune attack.

We further measured the serum cytokines levels in 5 AIDP patients to verify that the elevated cytokines did not simply penetrate through the blood brain barrier (BBB), but rather originated from the CNS or locally penetrated into the CNS from inflamed regions and cytokine profile of the CSF obtained from AIDP patients, revealed a heterogeneous panel that generally was not directly associated with adaptive immune cells. Our data demonstrate that the CSF from AIDP patients—in contrast to control patients—is enriched with peptides related to the proteins involved in the arrangement of the axonal domains, whereas the over represented proteins identified in the CSF of AIDP patients are mainly linked with the defensive responses to bacteria. These observations are in line with the upregulation of CSF cytokines associated with innate immunity, which in turn surprisingly suggests that the destruction of axons is not accompanied by any indications of a distinct adaptive autoimmune process.

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P.3.4-030
Electrophysiological reaction of neurons to inorganic polyphosphates

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Inorganic polyphosphate (PolyP) is a biopolymer composed of tens to hundreds orthophosphate residues linked by the phosphoanhydride bonds. PolyP presents in all living organisms and plays numerous physiological functions. Micromolar concentrations of PolyP is known to be in mammalian brain. Essential role of PolyP in transduction of signal in astrocytes is shown, but its physiological functions in neurons and glial cells still poorly investigated. Here we study electrophysiological reactions of neurons to PolyP and PolyP - induced death of neurons and glial cells. Crayfish stretch receptor consisting of only two neurons surrounded by glial cells is used as an object. The most obvious

electrophysiological reactions were observed to long chain PolyP (120 orthophosphates). Application of long chain PolyP (100 μM) increased the frequency of action potentials of neurons, but decreased the time of firing of neurons in comparison with control. Long PolyP also increased the level of necrosis of the surrounding glial cells, but stimulate necrosis only in some neurons. Antagonist of P2X purinergic receptors, PPADS, only partially blocked electrophysiological reactions of neurons to long PolyP, but did not influence the level of PolyP – induced necrosis of glial cells. It demonstrates that electrophysiological reaction of neurons to long PolyP could be partly connected with activation of P2X receptors.

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P.3.4-031
Design of an animal model in vivo for hepatic encephalopathy assay

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Hepatic encephalopathy (HE) accompanied by accumulation of ammonium ions in blood is related to the brain dysfunction resulted in toxicants enrichment usually detoxified by liver. The main features of hepatic pathology induced by toxicants can be well simulated using animal models *in vivo*.

The main study idea was to design a TAA-induced hepatic encephalopathy model using rat males of outbred *Wistar* line, which was subjected to acute toxication by the hepatotrophic poison of TAA. Acute hepatic toxication was induced with TAA intra-abdominal injection of 200–600 mg in 0.9% NaCl solution per 1 kg of animal weight. In three days, all the animals were decapitated and some tissue samples of liver, kidney, brain and blood serum were fast taken. Abdominal cavity organs from experimental animals had evident pathology such as some necrosis and haemorrhage foci, some change in liver color. The changes in brains similar to pathological changes in human due to HE were obtained, namely some increase in nerve tissue tonus, cyanosis of forebrain surface. To assess biological-chemical features of pathology level of markers of energy metabolism and target metabolites of glutamine-methionine bicycle in biological samples was assayed. As a result we got some data: 1) the least pyruvate amount in the blood serum was at TAA concentrations of 200 and 300 mg; 2) the highest quantity of ketoglutarate was at TAA concentration of 300 mg; 3) at TAA concentration of 300 mg the malate level was lower than that in control; 4) at TAA concentrations of 200 and 600 mg there was some lactate decrease compared to that in control.

We could conclude that designed HE model *in vivo* shows some typical pathology of liver resulted in increase of lactate, methionine and glutamine levels whereas the amount of pyruvate declines. The model *in vivo* could be used for HE development in animals.

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P.3.4-032
Human γ-tubulin isotypes: differential expression during neuronal development and under oxidative stress

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γ-Tubulins are highly conserved members of the tubulin superfamily essential for microtubule nucleation. Humans possess two γ-tubulin genes. It is thought that γ-tubulin-1 represents ubiquitous isotype, whereas γ-tubulin-2 is found predominantly in the brain, where it may be endowed with divergent functions beyond microtubule nucleation. The molecular basis of the purported functional differences between γ-tubulins is unknown. Previously we have reported that human γ-tubulin-2 nucleates microtubules and rescues mitotic progression in γ-tubulin-1 depleted cells (PLoS ONE 7: e29919, 2012). Here we report discrimination of human γ-tubulins according to their electrophoretic and immunochemical properties depending on C-terminal regions of the γ-tubulins. Using epitope mapping, we discovered mouse monoclonal antibodies that can discriminate between human γ-tubulin isotypes. Real time quantitative RT-PCR and 2D-PAGE showed that γ-tubulin-1 is the dominant isotype in fetal neurons. Although γ-tubulin-2 accumulates in the adult brain, γ-tubulin-1 remains the major isotype in various brain regions. Localization of γ-tubulin-1 in mature neurons was confirmed by immunohistochemistry and immunofluorescence microscopy on clinical samples and tissue microarrays. Differentiation of SH-SY5Y human neuroblastoma cells by all-trans retinoic acid, or oxidative stress induced by mitochondrial inhibitors, resulted in upregulation of γ-tubulin-2, whereas the expression of γ-tubulin-1 was unchanged. Fractionation experiments and immunoelectron microscopy revealed an association of γ-tubulins with mitochondrial membranes. These data indicate that in the face of predominant γ-tubulin-1 expression, the accumulation of γ-tubulin-2 in mature neurons and neuroblastoma cells during oxidative stress may denote a prosurvival role of γ-tubulin-2 in neurons.

P.3.4-033
Physical interaction and gene expression analysis of perineuronal net elements in neuronal differentiation

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Neural extracellular matrix (ECM) which is called perineuronal nets (PNNs) are specialized substructures that surround cell soma and neurites of neurons. Hyaluronan and proteoglycan link protein1 (HAPLN1), tenascin-R (TNR) and aggrecan (ACAN) are the major components of PNNs. In neurodegenerative diseases, their expressions decrease suggesting that these proteins can be related with neurodegeneration. Also it is known that in the absence of PNNs elements, fibulin-2 (FBLN2), which is another ECM protein, interacts with the C-terminal domain of the ACAN and is unusually deposited in the brain suggesting a compensatory role.

In this study we analyzed the physical interaction between HAPLN1 and survival motor protein 1 (SMN1), the major

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protein reduced in spinal muscular atrophy (SMA), which was used as a neurodegenerative disease model. A detailed HAPLN1 and SMN1 network analysis was performed *in-silico*. Also, mRNA levels of HAPLN1, TNR and FBLN2 were investigated by qRT-PCR in differentiated PC12 cell line.

According to *in-silico* analysis, an indirect path between HAPLN1 and SMN1 through FBLN2 was detected. Also, HAPLN1 mRNA level showed 2.9 fold increase in differentiated PC12 cells, compared to non-differentiated state suggesting that HAPLN1 have an important role in formation of PNNs. On the other hand, while mRNA level of TNR remained unchanged, FBLN2 mRNA level was reduced dramatically suggesting that there is no need for FBLN2 when TNR and HAPLN1 are present.

This preliminary data will be used for further investigation of the potential role of PNNs elements in neurodegeneration using *in-vivo* and *in-vitro* disease models.

P.3.4-034
Structure-functional analyzes of the analgesic sea anemone peptide potentiating TRPA1

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The transient receptor potential ankyrin-repeat 1 (TRPA1) receptors play the significant role in initiation and development of neurogenic inflammatory pain as well as in ischemic neurodegeneration. Novel bioactive peptide named Ueq12-1 was isolated and characterized from the sea anemone Urticina eques. Ueq12-1 showed dual activity, both inhibiting bacterial growth of Corynebacterium glutamicum and Staphylococcus aureus, and potentiating TRPA1 ion channel. The 3D structure of Ueq12-1 determined by NMR represents new disulfide-stabilized fold in part similar to defensin-like fold. Injection of Ue-1 in the hind-paw did not cause pain, paw edema or significant thermal hyperalgesia in 2 h. Pretreatment of mice by intravenous injection of Ueq12-1 (0.2 mg/kg) significantly reduced AITC- induced nocifensive behavior. Moreover, paw edema caused by AITC was significantly reduced in within all period of observation. Ueq12-1 (0.2 mg/kg) reversed thermal hyperalgesia within 30 min after i.v. administration of peptide and reduced paw edema at 27% in 24 h in non-specific inflammation in CFA test. The effect of the peptide in the behavioral test was not the result of sedation or behavior impairment since the administration of Ueq12-1 did not change normal behavior of mice in open field test. Most probably, this peptide is able to potentiate and defunctionalize the TRPA1 receptor on sensory neurons or desensitize neurons. To determine the active site of the peptide we analyzed the effects of the N-terminal domain and defensine-like domain of Ueq12-1. We found that both parts of Ueq12-1 are able to potentiate TRPA1 but significantly weaker then parent peptide. Moreover, both peptides were able to reduce the specific response of mice to AITC (TRPA1 agonist). Therefore, small peptides derived from Ueq12-1 could be considered as drug leads for treatment of inflammatory conditions. This work was supported by Russian Science Foundation Grant 16-15-00167.

P.3.4-035
Antipsychotics materials and computational chemistry could help to reveal specific mind functions into the brain

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One possible window that allows us to understand mind is to use antipsychotic materials (or medications). Psychosis is one of the serious mental disorders in which people loss the contact with reality. Schizophrenia is the most tremendous neuropsychiatric disorder and it is characterized by severe distortions of reality. There are various antipsychotics that decrease the symptoms of schizophrenia in the patient. Antipsychotics modify the neurotransmitter systems and therefore, they can be used to explore the neurotransmitter systems. In this investigation, antipsychotic medications have been analyzed using computational chemistry. The main objective of this research is to use different chemical indicators in order to classify the antipsychotic materials. The classification allows us to understand the possible role that these chemicals could have in the mind. The electron donor-acceptor capacity is one the characteristics that was used before to understand sexual selections in birds. In this report, antipsychotic materials are classified as good electron donors or good electron acceptors. Based on this classification, some hypotheses about the importance of electron transfer in the function of these materials in the neurotransmitter systems are presented.

P.3.4-036
Heterologous expression and purification of rhodopsin from radioresistant Antarctic bacterium

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Using optogenetics tools cell processes can be controlled by treating the cell with light. Some microbial rhodopsins are used by optogenetics, but most of them yet unstudied. For further development it is important to have a variety of lite-gated ion pumps and channels. It's important to find out the exact structure of these basic opsins, and to perform mutations to change their selectivity, conductivity and other properties.

One microbial rhodopsin gene (further mentioned as LR1) was found in the genome of Hymenobacter sp. – gram-negative, radioresistant, low-temperature resistant bacterium isolated from an Antarctic lichen (T.-J. Oha et al., Journal of Biotechnology, 227 (2016) 19–20). Ionizing radiation can directly damage DNA and it indirectly damages proteins through reactive oxygen species. Hymenobacter sp. has the specific system of DNA reparation, and it's proteins might have specific resistivity to the mentioned extreme conditions too. Comparison of the amino acid sequence of the unexplored protein with bacteriorhodopsin (BR), halorhodopsin and other seven transmembrane ion pumps showed that they have mostly the same amino acids at key positions responsible for ion transport. The LR1 has at the position 85 (according to BR amino acid order of numbering) a negatively charged glutamic acid instead of the smaller negatively charged aspartic acid. At the position 96 there is neutrally charged glutamine instead of aspartic acid. Thus the properties of the LR1 are supposed to differ from BR. We optimized the DNA for the overexpression of

His-tagged LR1 in *E.coli* and eukaryotes. The heterologous expression was optimized too to achieve best yield of LR1 in membrane fraction. Than it was solubilised and purified. Protein appeared to have two absorption peaks at 530 nm and 425 nm. Further structural and physiological studies to determine molecular mechanisms of ion transport and light-driven functions of LR1 are essential. This work is supported by 16-15-00242 RSF.

P.3.4-037
Effects of exogenous neurometabolites on AKT- and ERK-signaling apoptotic pathways in the cortical neurons in the physiological and pathological (HER2/neu overexpression) aging

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Epidermal growth factor receptors play big role in apoptosis regulation which mediates by various signaling ways. The investigation of apoptosis pathways in age-dependent neurodegeneration can be basis for creating a new strategy for pharmacological correction of age-related disorders. The aim of work was to study effects of exogenous neurometabolites (succinate-containing drug cytoflavin and nootropic drug piracetam) to AKT and ERK signaling pathways in brain cortex neurons during physiological and pathological (HER2/Neu overexpression) aging. We studied HER2/neu transgenic mice, wild-type FVB/N. We evaluated phychoemotional and locomotional status; neuron apoptosis (TUNEL); AKT, ERK expression and level of phosphorilation of these kinases; FAS, p53, caspase-3, Mcl-1 expression (Western blotting).

AKT and ERK cascades which provide a low level of apoptosis, are activated in young FVB/N mice in cortex neurons. In old FVB/N mice, pERK activity decreases, which leads to onset of age-dependent apoptosis proceeding along extrinsic pathway.

In young transgenic mice, activation of pAKT and pERK causes suppression of p53 and FAS-dependent signaling, resulting in a low level of neuronal death. In old HER-2/Neu mice, high pAKT content contributes to neuronal death inhibition.

Cytoflavin and piracetam treatment protects from age-dependent neurogeneration in old FVB/N mice, by an increase of anti-apoptotic protein Mcl-1 synthesis. Obviously, the revealed effects are basic for the anxiolytic, neuroprotective action of studied drugs in aging.

In HER-2/Neu mice cytoflavin and piracetam decreases AKT phosphorylation level, which leads to p53- and FAS-mediated apoptosis. Thus, these drugs affect the HER-2/Neu receptors activity, which leads to decline of their effect on AKT and ERK pathways. It can be concluded that cytoflavin and piracetam under the conditions of HER-2/Neu overexpression provide a moderate apoptosis-stimulating effect, which can be used in clinical practice.

P.3.4-038
Artemisinin conferred ERK-mediated neuroprotection to PC12 cells and cortical neurons exposed to sodium nitroprusside-induced oxidative insult

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The production of nitric oxide (NO) is one of the primary mediators of ischemic damage, glutamate neurotoxicity and neurodegeneration and therefore inhibition of NO-induced neurotoxicity may be considered a therapeutic target for reducing neuronal cell death (neuroprotection). In this study, artemisinin, a well-known anti-malaria drug was found to suppress sodium nitroprusside (SNP, a nitric oxide donor)-induced cell death in the PC12 cells and brain primary cortical neuronal cultures. Pretreatment of PC12 cells with artemisinin significantly suppressed SNP-induced cell death by decreasing the extent of oxidation, preventing the decline of mitochondrial membrane potential, restoring abnormal changes in nuclear morphology and reducing lactate dehydrogenase release and inhibiting caspase 3/7 activities. Western blotting analysis revealed that artemisinin was able to activate extracellular regulated protein kinases (ERK) pathway. Furthermore, the ERK inhibitor PD 98059 blocked the neuroprotective effect of artemisinin whereas the PI_3K inhibitor LY 294002 had no effect. Cumulatively, these findings support the notion that artemisinin confers neuroprotection from SNP-induced neuronal cell death insult, a phenomenon coincidentally related to activation of ERK phosphorylation. This SNP-induced oxidative insult in PC12 cell culture model may be useful to investigate molecular mechanisms of NO-induced neurotoxicity and drug-induced neuroprotection and to generate novel therapeutic concepts for cerebral ischemia therapy.

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P.3.4-039
Syndecan-4 strengthens the effect of integrin on CD90-induced cellular contraction

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In a pro-inflammatory context, reactive astrocytes undergo changes in surface protein expression, and constitute an impediment to axon regeneration in the brain. αvβ3 integrin, a receptor up regulated in reactive astrocytes, interacts with neuronal CD90, suppressing neurite outgrowth and inducing retraction of existing neurites. However, blocking αvβ3 integrin only partially prevents these effects, suggesting the participation of other molecules in CD90-mediated neurite shortening. We propose that the proteoglycan Syndecan-4, another CD90 receptor up regulated in reactive astrocytes, binds to CD90 and acts as a co-ligand with the integrin to induce the effects in neurites. Using neurons seeded over astrocytes and Syndecan-4 silencing, we tested the effect of Syndecan-4 in astrocyte-dependent suppression of neurite outgrowth. We also tested the effect of Syndecan-4 on neurite retraction induced by αvβ3 integrin, using differentiated neurons incubated with a combination of both αvβ3-Fc and Syndecan-4-Fc. After silencing Syndecan-4, our results indicate that neurite outgrowth suppression, when occurring over astrocytes, was induced when CD90-Syndecan-4 interaction was impaired. In addition, such neurite outgrowth over astrocytes was more effective when β3 integrin was also blocked. Furthermore, αvβ3-promoted neurite shortening was faster when co-incubated with Syndecan-4-Fc. Finally, using molecular force spectroscopy, we evidenced a Syndecan-4 and CD90 direct interaction, obtaining quantitative information characterizing the free-energy landscape of the dissociation process. These findings indicate that the combined interaction of astrocytic αvβ3 integrin and Syndecan-4 with neuronal CD90 suppresses neurite outgrowth more effectively and accelerates the contraction of existing processes. Thus, Syndecan-4 is a cell surface component of astrocytes that contributes to the non-permissive environment for axon regeneration generated in the brain. Supported by CONICYT-CHILE.

P.3.4-040
A CRISPR in vivo screen to uncover the mechanisms underlying neuronal remodeling in Drosophila

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Neuronal remodeling is essential for sculpting the mature nervous systems of both vertebrates and invertebrates. It often includes pruning of exuberant connections, followed by regrowth of new, adult-specific ones. Understanding the molecular basis of neuronal remodeling may offer insight into neural degeneration and regeneration during development and disease. The stereotypical remodeling of the *Drosophila* mushroom body (MB) γ-neurons provides a unique model to study both axon pruning and regrowth. Despite recent progress, the molecular pathways governing MB remodeling remain largely unknown, raising the need to screen for new genes involved in the process. Current screening methods in *Drosophila*, however, hold various limitations. Strategies based on mutation analysis often require the application of labor-intensive mosaic techniques, while RNAi-based screens suffer from off-target effects and incomplete gene knockdown. In my project, I have established a novel, CRISPR-based screening method, which enables the formation of homozygous mutant clones for any target gene, in any specific tissue of choice. This is achieved within a single cross and without the need to generate a germline mutation, making this method ideal for high-throughput *in vivo* screening. Our successful pilot screen demonstrated high efficiency of tissue-specific CRISPR in MB γ-neurons. We are now in the initial stages of the large-scale screen, for which we are generating a library of gRNA-expressing *Drosophila* lines targeting hundreds of different genes, which can be crossed to Cas9 expressed in any tissue of interest, thus providing a valuable resource to the worldwide fly community. Preliminary screening has identified the kinase Happyhour (MAP4K3) as important for MB γ-axon pruning. We are now further investigating this finding while also continuing the screening process, in the overall goal of promoting our understanding of the molecular events that occur during neuronal remodeling.

P.3.4-041
The role of the insular cortex in anxiety; from molecules to behavior

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The insular cortex is believed to be involved in anxiety and anxiety-related disorders, based primarily on human imaging studies. However, little is known about its role in anxiety, its position within the currently accepted anxiety-related brain circuitry, and the signaling pathways that allow it to orchastrate behavior. Using a variety of techniques, including in vivo pharmacology, molecular biology and electrophysiology, we have identified the regions of the insular cortex that participate in anxiety-like behavior, and the signaling pathways by which the stress hormones epinephrine and glucocorticoids affect insular activity to orchestrate anxiety responses in rodents.

P.3.4-042
Influence of sound waves with different rhythms to serotonin concentration in rats hippocampus

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Introduction: Because of more pressure to modern people, psychological paths, such as depression, have become to be severe gradually. Music therapy is a simple way of relaxing mind for individual. However, the effect is not satisfied enough. This situation may due to various factors. So it requires more scientific research processes to support music therapy. In our study, we focused on the infuence of music to serotonin concentration variation in hippocampus. Because serotonin is related emotion directly. Addtionally, music is complex, it should be considered respectively on rhythm and tonal frequency levels at least. Compared rhythm with tonal frequncy, we can feel more obviously if the former changes in same music as usual. So rhythm was another parameter needed. We hope our research could answer what kind of music is able to improve mind situation of different people.
Method: (1) Heart rates of SD rats were detected under anaesthesia and normal situation, calculated averages, set rhythms (300beats/min, 350beats/min, 400beats/min); (2) Made sound waves according to setting rhythms with Finale 2011 software; (3) Rats were grouped randomly. Some were received differenet sound waves under anaesthesia or normal situation, some were received nothing as control. (4) Left and right hippocampus were isolated from brains into tubes filled with 0.9% NaCl solution, weighed. Then ultrasonicated tissues and centrifigured. Supernatant was used to serotonin ELISA.
Conclusion: (1) Right and left hippocampus have different responds to same sound wave; (2) Under anaesthesia situation, right hippocampus from group received 300 beats/min sound wave secreted the most serotonin concentration, 0.202 ng/(ml*mg); (3) Under normal situation, right hippocampus from group received 400 beats/min sound wave secreted the most serotonin concentration, 0.128 ng/(ml*mg); (4) Nearer to the current heart rate under certain situation the sound wave rhythm received was, the more serotonin secreted.

P.3.4-043
Glutamate scavenger as a novel neuroprotective treatment for acute spinal cord injury

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Injury to the spinal cord usually results in very limited regeneration of lesioned axons, with subsequent permanent impairment of function. Excitatory amino acids, such as glutamate, released early following injury and lead to altered calcium homeostasis. This excitotoxic process initiates the secondary damage, which includes inflammatory response, neuronal cell death and formation of dense glial scarring that acts as a physical and chemical barrier for axonal regeneration. We have developed a strategy to reduce brain glutamate excitotoxicity by enhancing a natural blood-resident enzymatic system that reduces blood levels of glutamate to produce a brain-to-blood gradient, which then reduces

brain levels of glutamate. We examined the blood-glutamate scavenging (BGS) treatment in a well-established spinal cord hemisection model in mice. Longitudinal sections of the lesioned spinal cord from mice treated or untreated with BGS, one week after spinal cord injury (SCI) were processed immunohistochemically. Different cell markers, such as GFAP, Iba1 and NeuN were used to examine co-localization with proliferation or apoptotic markers in order to determine severity of glial scar formation, microglia activation and neuronal cell death after injury. We showed that five days daily injections of BGS, reduced microglia activation at the lesion site, inhibited astrocyte proliferation, as been demonstrated by co-localization of GFAP with Ki67, and reduced GFAP density at the lesion site. Finally, it increased neuronal survival toward the lesion site, as been determined by NeuN positive cells quantitation and reduced co-localization with caspase3. In addition, we found that our treatment significantly reduced phospho-p38 and increased phospho-Akt levels at the lesion site following SCI 1 hour after injury. Together, these findings indicate that glutamate scavenger is a promising candidate as a neuroprotective treatment of acute SCI condition.

P.3.4-044
Alarmin HMGB1 induces systemic and brain inflammatory exacerbation in post-stroke infection rat model

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Post-Stroke Infection (PSI) is known to worsen functional outcomes of stroke patients and accounts one third of stroke-related deaths in hospital. In our previous reports, we demonstrated that massive release of High mobility group box protein 1 (HMGB1), an endogenous danger signal molecule, during the acute phase in the postischemic brain exacerbates neuronal damage by triggering delayed inflammatory processes. Moreover, augmentation of proinflammatory function of LPS by HMGB1 via direct interaction has also been reported. In the present study, we investigated the role of HMGB1 in the PSI in relation with exacerbating the function of LPS. PSI animal model was produced by administrating a low dose LPS at 24 hrs post-MCAO (middle cerebral artery occlusion). Profound aggravations of brain and systemic inflammation, deterioration of behavioral outcomes, and infarct expansion were observed in LPS-injected MCAO animals, in which serum HMGB1 surge, especially disulfide type, occurred immediately after LPS administration. In contrast, *Rhodobacter sphaeroides* LPS (LPS-RS), a toll like receptor 4 (TLR4)-selective antagonist, failed to exert these effects. Furthermore, blockage of HMGB1 function by delayed administrations of therapeutic peptides known to inhibit HMGB1 (HMGB1 A box, HPep1) at 3 hrs prior to LPS injection, or treatment with LPS after pre-incubation with HMGB1 A box significantly ameliorated damages observed in PSI, demonstrating that HMGB1 plays a crucial role. Together these results indicated that HMGB1 might play an important role in TLR-4-dependent exacerbation of systemic and brain inflammations in a rat model of PSI, and modulation of HMGB1 might provide a valuable therapeutic strategy.

P.3.4-045
Detailed characterization of human neural progenitor cells differentiated from induced pluripotent stem cells

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Most studies focusing on neural development, neural regeneration, neurotoxicity of various compounds, and the mechanism of neurodevelopmental disorders are based on animal (rodent or avian) models or post mortem human samples. Thus, the observations derived from these studies cannot be directly translated into mechanistic insights into human neural development and regeneration. Human pluripotent stem cells, which can potentially be differentiated into any cell types of the body, offer a new perspective to overcome this limitation. In the present study, we differentiated induced pluripotent stem cell lines into toward the neural lineage. Using a multi-step, directed differentiation protocol, specific human neural progenitor cells (NPCs), which are committed to produce PROX-1-positive granule cells of dentate gyrus of the hippocampus, were generated. These NPCs were characterized in terms of morphology, expression of specific cellular markers, growth capacity, and capability of producing mature neurons. Calcium signaling of the human NPCs was also investigated in detail, which demonstrated the functional presence of a numerous receptors, and also the lack of functioning sodium channels. Stable expression of GFP in these cells allowed us to develop a high-content screening-based method to quantitatively assess the neurite outgrowth, the initial step of neural regeneration. Using this approach we studied the internal and external inhibitions of neurite outgrowth in the human NPCs. Our results indicate that NPCs differentiated from human pluripotent stem cells provide a reliable human *in vitro* model system suitable for studying special aspects of human neural development and regeneration.

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Wednesday 13 September 13:00–15:00

Cancer Biology

P.4.1-001
Human embryonal carcinoma stem cells display distinct bioenergetic profile compared to normal embryonic stem cells

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Despite recent advances in characterization of human embryonal cells (hESCs), the bioenergetics of these cells is largely unstudied. Meanwhile, the energy metabolism may be involved in the

regulation of stem cell fate including determination of its tumorigenic potential. Our current work aimed to examine bioenergetic signature of hESCs (H9 cell line) and their malignant counterparts, human embryonal carcinoma stem cells (hECCs, 2102Ep cell line), in order to determine special features that may account for an increased tumorigenic potential of hECCs. We studied the expression level and enzymatical activities of three main enzymes involved in the transport of energy-rich phosphoryl: adenylate kinase (AK), creatine kinase (CK) and hexokinase (HK). High-resolution respirometry was applied to compare abilities of these cell lines to consume oxygen and to estimate the contribution of individual mitochondrial electron transport chain (ETC) complexes to the respiration. Our results showed that hECCs had a distinct expression pattern of key enzymes that are involved in cellular bioenergetics demonstrating a shift in energy distribution towards AK network. The expression level and the activity of AK2 were significantly increased in hECCs while CK system was downregulated. Interestingly, hECCs had reduced levels of oxygen consumption while the mitochondrial content, the level of proton leak and functional activities of ETC complexes were the same in both cell lines. In addition, our data demonstrated that complexes II and IV, as well as ATP synthase and adenine nucleotide transporter (ANT), shared larger control strength over mitochondrial respiration in hECCs than in hESCs. Altogether, the bioenergetic signature of hECCs clearly distinguishes them from normal hESCs and should be further investigated as it may indicate the tumorigenic potential of stem cell. Moreover, these differences should be considered when 2102Ep cell line is used as a reference in a stem cell research.

P.4.1-002
Radiosensitization of resistant (Head and Neck) tumor cells by metal nanoparticles

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Selective targeting of radiation effects to tumors represents a challenge in radiotherapy. Metal nanoparticles (gadolinium, gold, platinum) are preferentially internalized by tumor cells and have been recognized to locally amplify the radiation dose upon irradiation. Nanoparticles delivered in tumor cells might increase tumor-specificity and efficiency of radiotherapy. The physical mechanisms related to the radiation dose amplification by nanoparticles have been already well described; however, cellular structures targeted by nanoparticles remain unknown. The DNA molecule is the most sensitive and critical cell structure in the cell concerning the effects of ionizing radiation. A question remains open of whether a damage to the nucleus is necessary for the radiosensitization exerted by gadolinium and other nanoparticles.

We studied the effect of 3 nm gadolinium based nanoparticles (GdBNs) on the induction and repair of DNA double-strand breaks (DSBs) in the nuclear DNA of U87 tumor cells irradiated with g-rays. We used sensitive method of DSB detection based on confocal fluorescence microscopy coupled with immunodetection of two independent DSB markers, gH2AX and 53BP1.

Our experiments brought surprising results. In the conditions where GdBNs amplify the radiation effects, they remain localized in the cytoplasm and their influence on DSB induction and repair is only insignificant. This suggests that the radiosensitization mediated by GdBNs and potentially other nanoparticles is a cytoplasmic event that is independent of the nuclear DNA breakage (a phenomenon commonly accepted as the explanation of biological radiation effects). Based on recognized intracellular localization of nanoparticles studied, we hypothesize about possible non-DNA targets for (some) nanoparticles. Ministry of Health of CR(16-29835A); Czech Science Foundation (16-12454S); People Programme (Marie Curie Actions) of Eu. Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement 624370.

P.4.1-003
Relationship between chromatin structure and chromosomal rearrangements in myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are group of clonal hematologic disorders. The molecular basis are deletions and/or rearrangements of chromosomes 5, 7 and 8. Large interstitial deletions of the chromosome 5 (5q) appear most frequently. Since the breakpoints are variable but still clustered in specific chromosomal regions, the instability of 5q-arms could not be simply explained by the presence of DNA fragile sites. We hypothesize that the higher-order chromatin (HOC) structure may cause 5q fragility. Chromosomal regions encompassing frequent MDS deletions might adopt specific HOC structure, potentially in MDS precursor cells or some individuals only, which facilitates their damage. We addressed this question by reconstructing 3D HOC structure of the 5q31.1 - 5q32 chromosomal region, which we identified to be deleted with the highest frequency. Using 3D-FISH and high-resolution confocal microscopy, we determined nuclear and mutual positions of 5 BAC probes, hybridizing to individual G-bands between 5q23.3 and 5q32 (in human G0-lymphocytes isolated from healthy donors, CD34 + cells isolated from patients and healthy donors, and human skin fibroblasts). Centromeric, telomeric, and whole chromosome probes were used in combinations with the BAC probes to reveal the arrangement of the affected locus inside the chr. 5 territory. Our results indicate the formation of a large chromatin loop between the 5q23.3 and 5q32 loci that sometimes protrudes out of the chromosome 5 territories. This substantial level of chromatin decondensation and protrusion of the loop into the interchromatin channels might contribute to the region fragility. The flexibility of the loop structure we observed might then explain the variability of MDS deletion breakpoints. In accordance with these results, we suggest that HOC organization of the affected region could contribute to formation of chromosomal aberrations in MDS.

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P.4.1-004
Protein binders as key components of microfluidic chip for selective detection and separation of circulating tumor cells (CTCs) in blood of patients with lung adenocarcinoma

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Lung cancer remains the leading cause of cancer mortality in many countries worldwide. Treatment efficiency and survival rate of patients relies on early clinical diagnosis. Recently, the circulating tumor cells (CTCs) have emerged as a valuable clinical marker for cancer diagnosis and monitoring of patients during the treatment. Unfortunately, the cellular heterogeneity and low abundance of CTCs in blood represent a big analytical challenge. Most detection strategies of CTCs are based on the antibody-based detection of epithelial markers such as EpCAM. However, due to the epithelial mesenchymal transition (EMT) process, many CTCs lose their epithelial phenotype and gain mesenchymal properties. Such assay results in reduction of the already low number of captured cells during the selection. We present an appealing alternative to commonly used antibodies - small artificial protein binders - for both epithelial and mesenchymal membrane markers of CTCs. For the generation of binders targeting epithelial membrane marker EpCAM or mesenchymal membrane marker N-Cadherin, we used assembled highly complex combinatorial library derived from albumin-binding-domain scaffold in combination with Ribosome Display selection. The most promising candidates selected by ELISA are currently being characterized by measuring their binding properties using cell lines of the particular phenotype. Five human cancer cell lines (MCF-7, DU-145, CCD1070Sk, HEK293T, PC-3) were analyzed for the expression of EpCAM and N-Cadherin membrane markers using flow cytometry. The interaction of the binder with cells is measured in real-time mode using the LigandTracer® Green Line. The instrument is particularly beneficial for monitoring of ligand binding to cell-surface receptors on living cells, and allows to determine on- and off-rate kinetics as well as the binding affinity. Protein binders with desirable properties will serve as robust capture proteins for cell-sorting on the unique microfluidic chip.

P.4.1-005
Characterization of Pirh2 ubiquitin ligase as potential target for non-small cell lung cancer therapy

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Targeted therapy design is one of the mainstream approaches in the modern anticancer treatment. Much attention in this field is focused on the development of Mdm2 inhibitors aimed to stabilize p53. A RING-domain containing E3 ligase Pirh2 is considered being the second most important p53-specific ubiquitin ligase and is a promising target for anti-tumor therapy.

Besides p53, Pirh2 interacts and targets for degradation such key regulators of apoptosis, cell cycle and proliferation as c-Myc, p27 and Chk2. It is worth noting that Pirh2 plays controversial role in carcinogenesis. It may function as an oncogene in human lung cancer cells, hepatocellular carcinoma, prostate cancer and others. At the same time the low level of Pirh2 is associated with reduced patient survival in breast cancer, ovarian cancer, and lung squamous cell carcinomas.

Taken together, Pirh2 seems to play an important role in tumor cells both in p53-dependent and independent manner. To better understand the molecular basis of Pirh2 functions in cancer cells we investigated the effect of Pirh2 overexpression and suppression in H1299 non-small lung carcinoma cells that lack p53. We have found that overexpression of Pirh2 in these cells augmented proliferation, migration and resistance to chemotherapeutic agents. Knockdown of Pirh2 in H1299 cells reversed these phenotypes. Collectively, these results highlight the tumorigenic role of Pirh2 in lung cancer. We have also uncovered a potential mechanism underlying the observed effects via activation of c-Myc expression.

Suppression of such multi-targeted enzyme activity can have unpredictable therapeutic effects and depends on many factors within a particular cellular context. Thus, our study strongly points to Pirh2 as a novel target for anti-cancer therapy to treat p53-negative non-small cell lung tumors. Also, it provides a basis for further research aimed at oncogenic E3 ligases.

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P.4.1-006
Arginase and NO-synthase are target enzymes in breast anticancer therapy

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Early reports have been focused on the expression of arginase and NO-synthase (NOS) in murine or human primary cancer tissue as well as malignant cell lines and emphasized its potential role in the promotion of tumour growth via polyamine synthesis or down-regulation of NO-mediated tumor cytotoxicity. In the present work, we have investigated the change of arginase activity and NO quantity in different stages of breast cancer. The study was performed in blood serum patients with breast cancer (women, 48 patients; 1st, 2nd, 3th stages, 37–72 years old) and 8 patients after chemotherapy (2nd stage, 41–67 years old) who were hospitalized in the National centre of oncology after V.A. Fanarjyan (Yerevan). The Tumor-Node-Metastasis staging system was used to describe the stages of breast cancer. Arginase activity was determined spectrophotometrically with a modified method of diacetyl monoxime urea. The quantitative changes of NO were evaluated by determination of nitrite anions concentration by Griess assay. The results have shown that compared with control group (8 women, 34–65 years old) arginase activity was increased for 39.91%, 53.37%, 79.42% and 20.12% in stages 1st, 2nd, 3th and after chemotherapy, respectively. Our data showed statistically correlation for all stages. In post-chemotherapy group arginase activity show 19.7–59.3% decrease compared with breast cancer patients before chemotherapy. This indicates, that before chemotherapy (2nd stage) comparing to the control group, quantity of nitrite anions were increased 2.4 fold, and after chemotherapy 1.5 fold. Thus, for the first time was shown that arginase activity and NO quantity were decreased after chemotherapy. Our study insinuates that arginase and NOS may have important role in disease progression or regression. We suggest that arginase and NOS activity inhibition may have antitumor effects as it inhibits polyamines and NO levels, precursor of cancer cell proliferation, metastasis and tumor angiogenesis.

P.4.1-007
The change of polyamines and NO quantities in different stages of breast and prostate cancers

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Currently is shown the large quantity of polyamines let the oncogenes to overcome the immune system. Increased NO generation in cancer cells may contribute to tumor angiogenesis and metastatic ability by up-regulating vascular endothelial growth factor. The goal of the work was to study the changes of polyamines and NO quantities in different stages of breast and prostate cancers. The study was performed in blood serum patients with breast (women, 48 patients; 1st, 2nd, 3th stages, 37–72 years old) and prostate cancers (men, 26 patients; 1st, 2nd, 3th and 4th stages, 45–78 years old) who were hospitalized in the National centre of oncology after V.A. Fanarjyan (Yerevan). The Tumor-Nodes-Metastasis staging system was used to describe the cancer stages. The chemoradiotherapy wasn't applied to any of patient. Polyamines quantity of serum was determined by thin layer chromatography. The quantitative changes of NO were evaluated by determination of nitrite anions concentration by Griess assay. The increase of total polyamines quantity compared with control (8 men and women, 34–69 years old) was 69.5%, 101% and 131%, respectively in 1st, 2nd, 3th stages of breast cancer. Total polyamines quantity compared was increased by 54.3%, 67.4%, 91.4% and 101.3%, respectively in 1st, 2nd, 3th and 4th stages of prostate cancer. The results indicate, that in blood serum of breast and prostate cancers patients (stage 2) before the chemotherapy comparing to the control group quantity of nitrite anions were increased 2.4 and 2 fold, respectively. The increase of NO and polyamines concentrations in blood serum in earlier stages confirm, that this metabolic pathway of L-arginine has a significant role in malignant processes. We suggest that downstream of polyamines and NO quantities may have antitumor effect. Our further research will be directed to investigate the antitumor potential of arginase and NO-synthase inhibitors on 7,12-dimethylbenz(*a*) anthracene-induced mammary tumors in rats.

P.4.1-008
A twist in the plot: oncolysis by non-productive viral infection

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Attenuation of JAK/STAT signaling and lack of expression of Interferon Stimulated Genes (ISGs) expose tumor cells to oncolytic viruses (OV), which kill cancer cells and harness anti-tumor responses. Gene expression data from prostate cancer (PCa) patients revealed genetic and epigenetic de-regulation of ISGs and IFN signal-mediators, suggesting that PCa tumorigenesis involves the shutdown of IFN/cytokine signaling. As case in study, we showed that LNCaP, an IFN-insensitive PCa cell line, presents both epigenetic silencing of ISGs and bi-allelic truncating mutations to the JAK1 gene. We employed LNCaP cells to adapt the Epizootic Hemorrhagic Disease Virus (EHDV), an orbivirus that naturally infects ruminants, to human PCa cells. The resulting virus (EHDV-TAU) demonstrated enhanced replication and cell killing potential in LNCaP cells, while failing to infect non-transformed prostate epithelial cells. To examine the role of lack of JAK1 expression on the susceptibility of LNCaP cells to viral oncolysis we stably re-expressed JAK1 in LNCaP cells, generating LNCaP-JAK1 cells. As expected, LNCaP-JAK1 cells exhibited IFN-based antiviral responses. JAK1 re-expression also altered IL-6 signaling, resulting in enhanced activation of STAT3, phosphorylation of STAT1 and increased activation of ERK1/2. In these cells, IL-6 potently inhibited proliferation, with dependence on STAT3 expression. Re-expression of JAK1 also revealed a cell-autonomous IL-6-induced anti-viral response, which occurred via activation of STAT1 and expression of ISGs. In IL-6 stimulated LNCaP-JAK1 cells, and in absence of productive infection, EHDV-TAU remained an effective cell killer, inducing Oncolysis by Non-Productive Viral infection (ONPVI). We propose that in addition to oncolysis of tumors by productive infection, OVs may induce ONPVI of infection-resistant tumors, thus expanding the therapeutic range of OIV to tumors residing in inflammatory and infection-refractory microenvironments.

P.4.1-009
Hemi-specific binding by p53 expands the universe of p53 targets

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The tumor suppressor protein p53 is a transcription factor that binds sequence-specifically to defined target genes in the genome in response to DNA damage leading to diverse cellular outcomes such as cell cycle arrest, DNA repair, cell senescence, and apoptosis. The DNA response elements to which p53 binds is made of two 10 base-pair (bp) half-sites with the general form RRRCWWGYYY (R = A, G; W = A, T; Y = C, T), separated by a variable spacer up to 18 bp. When the half-sites are contiguous, one p53 dimer binds to a half-site and another p53 dimer binds to the adjoining half-site, thus forming a functional tetramer via cooperative protein/protein and protein/DNA interactions. However, about 50% of all validated p53 response elements contain spacers between the half-sites and their significance is unclear at present. In this study we show that p53 binds to response elements containing long spacers in two different modes: fully specific and hemi-specific. In the later binding mode, only one p53 dimer is specifically bound to a DNA half-site, whereas the other dimer is bound to the spacer DNA. Nonetheless, both these binding modes have similar binding affinities and the hemi-specific complex competes better than the specific complex with genomic DNA. Furthermore, structural analysis of p53 REs in solution, using cyclization kinetics of DNA mini-circles, show that the REs are not bent in both, their free and p53-bound states, with abutting half-sites or when the half-sites are separated by spacers. These findings provides structural and mechanistic insights into p53/DNA recognition and significantly expand and diversify the network of p53 binding sites in the genome. Moreover, it explains the manner in which p53 binds to clusters of more than one canonical binding site, common in many natural REs.

P.4.1-010
Tetraspanin 6 is a novel regulator of colorectal cancer development

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Tetraspanin 6 (Tspan6) is a poorly studied protein of the tetraspanin family of transmembrane proteins that form specialised membrane microdomains which are implicated in signal transduction and endocytic trafficking. We investigated the expression of Tspan6 in a cohort of genetically profiled colorectal adenocarcinomas and found that Tspan6 expression is significantly reduced in tumours vs. adjacent normal tissues. To illustrate the role of Tspan6 in colorectal cancer we used CaCo-2 cells. Our data show that Tspan6 does not alter cell proliferation nor contributes to growth inhibitory effect of EGFR inhibitor Cetuximab. However, we found that ectopic expression of Tspan6 facilitates lumen formation when CaCo-2 cells were cultured in three-dimensional extracellular matrix (3D-ECM). This effect was linked to the ability of Tspan6 to interfere with the EGFR-dependent signalling network in Caco-2 cells. Furthermore, we have discovered that Tspan6 is present in a complex with EGFR. Our experiments with colonic organoids derived from Tspan6 KO mice confirmed the important role of Tspan6 in EGFR-dependent signalling in colonic epithelium. Interestingly, Tspan6 KO mice develop multiple hyperproliferative lesions in gastrointestinal tract, and present with moderate to severe interstitial oedema in multiple tissues when compared to WT animals. At the molecular level we found that Tspan6 is directly associated with syntenin-1, an adapter protein which is known to play a critical role in biogenesis of multivesicular bodies (MVBs) and exosomal production. Therefore, we hypothesise that Tspan6 may be important in suppressing of EGFR-driven colorectal tumorigenesis by affecting autocrine secretion of EGFR ligands via exosomes.

P.4.1-011
Novel anti-cancer binary system activated by bacteriophage HK022 Integrase

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During the past decade cancer gene therapy has been one of the most promising areas of cancer researches; however the main obstacle of the conventional cancer drugs in use today is the lack of tumor specificity. These conventional treatments also have a narrow therapeutic windows; this is especially the case for locally spread malignancies and for metastases. The main goal of my research is the development of new cancer gene therapy system that will specifically kill only cancer cells, focusing on the Lung cancer due to the disturbing findings that Lung cancer is the leading cause of cancer deaths worldwide, with almost 1.6 million deaths attributed to the disease in 2012 alone. Median survival lies at less than 12 months, largely due to early metastatic dissemination and resistance to therapy. The objective of the research is to develop a new cancer cell specific killing system that is based on a site-specific recombination reaction activated by HK022 bacteriophage Integrase (Int) which in turn will activate the expression of Diphtheria toxin A (DTA) specifically in cancerous cells without affecting neighboring normal cell. As a model, lung cancerous mice is intravenously (IV) tail-injected with two plasmids bound to a DNA delivery carrier. One plasmid expresses Int under the cancer-specific hTERT promoter. The second encodes a silenced DTA gene activated by an Integrase- catalyzed excision reaction that removes a transcription terminator that prevents DTA's expression. The hTERT-promoted Int ensures that the toxin will be expressed in and kill only the cancer cells. My dual anti-cancer system belongs to the toxin gene delivery approach but has the obvious advantages compared to conventional toxin delivery approaches: it is more restrictive and does not trigger any immune response. Moreover, unlike other systems using DNA vectors for mammalian gene manipulations ours is viral-free. It successful will have a strong impact on human cancer curing.

P.4.1-012
Blood-circulating exosomes: differences in proteomic composition during breast cancer development

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It is a known fact that exosomes are circulating in various biological fluids such as blood, urine, saliva and are able to mediate cell-to-cell communication through transfer of RNA and proteins. Despite the fact, that during the transfer of signal from exosomes to recipient cells they are known to bound to cell surface this fraction (cell-surface bound exosomes) remains unstudied in both healthy state and during the development of malignancies.

We developed an efficient method of isolation of plasma and cell-surface bound exosome fractions. Exosomes were pelleted at 100,000 g from 0.1 μm filtered supernatants. Resultants samples were later characterized using current standarts by transmission electron microscopy (TEM), flow cytometry and nanoparticle tracking analysis at Malvern NS-300. Exosomal proteins were further isolated and characterterized with 2-D electrophoresis and MALDI-TOF mass-spectrometry.

TEM demonstrated presence of exosomes of 30–100 nm size as the membrane–wrapped particles in both plasma and blood cell surface eluates. The presence of characteristic tetraspanines: CD9, CD24, CD63, CD81 was confirmed by flow cytometry. Nanoparticle tracking analysis demonstrated that 66% of total blood exosomes are associated with blood cell surface and that exosome concentration do not vary between blood samples of healthy donors and cancer patients. 2D-electrophoresis shown that exosome proteome in both fraction coincides in 40% cases. In the pilot study MALDI-TOF was able to identificate more than 80 proteins, more than half were identificated for the first time. Obtained proteins are involved in the regulation of the humoral immune response, upregulation of lipid metabolism, the formation and excretion proteolipid particles and response to stress and inflammation. The data on the expression of cell surface binding proteins confirm exosomes as interacellular transporters of biomolecules and establishes importance of further research of this fraction.

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P.4.1-013
P2X7 activation – death or rebirth of glioma C6 cells?

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Gliomas are the most common and most aggressive tumors of central nervous system with high metastatic potential and resistance to chemotherapy. $P2X_7$ is the extracellular nucleotide membrane receptor and cation channel activated by ATP in somatic and cancer cells.

$P2X_7$ stimulation leads to fast calcium and sodium influx and potassium efflux and can induce apoptose in somatic cells. Our previous research was focused only on short-term stimulation effect and calcium influx. Therefore, the aim of presented work is to investigate effect of long-term activation of $P2X_7$ receptor in rat glioma C6 cells.

By using MTS cell viability test we observed that intensiveness of cell proliferation significantly increased upon long-term $P2X_7$ stimulation with its synthetic, selective agonist 2'-&3'-O-(4-benzoylbenzoyl)-ATP (BzATP) and level of activated caspase-3 dwindled. These results suggest the proliferative and protective role of the receptor on glioma cells. Further experiments showed that these $P2X_7$-dependent effects were accompanied by increase of phosphorylation of pro-proliferative marker p38 MAPK and level of protein stabilizator HSPA1. Surprisingly, we observed increased mRNA level of IL-8, which can affect tumor microenvironment and leads to appearance of chemoresistant cells. Stimulation of $P2X_7$ in C6 cells decreased the toxicity level of anticancer drugs doxorubicin (100 nM) and carmustine (200 μM) in these cells. Finally, expression of stem cell marker CD133 was observed after 24 hours treatment of glioma cells with BzATP acompanied by phosphorylation of FAK (focal adhesion kinase).

Obtained results suggest that activation of P2X7R may stimulate proliferation and inhibit apoptosis in C6 glioma cells. Also, long-term receptor stimulation leads to drug resistance of cells, most propably, caused by cancer stem cells appearance.

P.4.1-014
Dual effects of chlorophylline on breast cancer cell proliferation in vivo and in vitro

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Glutathione and the related enzymes belong to the defence system of the tissues against chemical and oxidative stress. These enzymes especially Glutathione S-transferase are often overexpressed in tumor cells and are regarded as a contributor to their drug resistance and are thought to play an important role in cancer progression.

The aim of this study is to evaluate the protective effects of chlorophyllin (Chl) as an antioxidant molecule which has inhibitory effects on GST P1-1 in vivo / in vitro. For this aim, we tested the effect of Chl on cell proliferation and cell cycle in MCF-12A, MDA-MB-231 and MDA-MD-468 cell lines. For in vivo studies, N-methyl nitrosourea (MNU) was used for inducing carcinogenesis in female Sprague-Dawley rats. Chlorophylline and MNU solutions were injected intraperitoneally when the rats were 21, 28, 35 and 42 days old. After 5 months, all animals were sacrificed and determined both glutathione levels and related enzymes activities in tumor and liver tissues were analyzed.

As a result, incubation with subtoxic concentrations of docetaxel and Chl have antagonistic effect on MCF-12A and MDA-MB-468 but additive effect on MDA-MB-231. However Chl shows a protective mechanism by increasing viability in cell proliferations results. Our results lead to a conclusion that the cytotoxic effects are not related GST P1-1 inhibition. Cell cycle analysis results confirms these with the cells dying in a nonapoptotic way. In vivo, glutathione levels and related enzyme activities were protected by chlorophylline treatment whereas MNU made them decreased compared to the control group. In conclusion, chlorophylline with antioxidant features decreased the toxic effect of MNU by regeneration of glutathione and enhancement of its related enzyme activities. Therefore, the use of antioxidant molecules, because of proliferative effects and defence-oriented behaviours, should be discussed in cancer therapy.

P.4.1-015
Regulation of CXCR2 receptor signaling in tumor infiltrating T-cells by Lnk adaptor protein

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The small Lnk adaptor protein is a hematopoietic regulator. Through its SH2 domain, it is able to bind a large variety of phosphorylated tyrosine residues on intracellular protein targets. The binding of Lnk to an activated target protein of a cytokine-receptor pathway leads to an inhibition of its downstream signaling cascade.

In humans, an increasing number of Lnk mutations have been demonstrated in myeloproliferative neoplasm (MPN) patients, leading to an increased cellular proliferation, probably due to high activity of the Jak-STAT pathway. Lnk-deficient ($Lnk^{-/-}$) mice exhibit an immune-mediated intestinal inflammation and an MPN-like phenotype, which presents with an enlarged spleen and abnormal blood count.

Using $Lnk^{-/-}$ mice, we have established a model for investigating the role of Lnk regulation at the tumor immune-microenvironment. Subcutaneous injection of melanoma cells into $Lnk^{-/-}$ mice has revealed an intriguing phenotype: tumors of $Lnk^{-/-}$ mice were smaller in mass and volume compared to tumors of wild type (WT) mice. Moreover, tumors derived from $Lnk^{-/-}$ mice presented an increased population of tumor infiltrating lymphocytes (TILs).

We analyzed the immune subpopulations at the tumor microenvironment and spoted an increased population of tumor infiltrating cytotoxic $CD8^+$ T-cells. Further analysis of the cytokine profile at the tumor microenvironment showed an increased presence of Mip-2, which binds the CXCR2 chemokine receptor.

In-vitro stimulation of spleen-derived $Lnk^{-/-}$ $CD8^+$ T-cells, and spleen-derived $Lnk^{-/-}$ $CD4^+$ T-cells with Mip-2 resulted in an enhanced phosphorylation of STAT3 and Erk1/2.

Our findings suggest that Lnk may be a novel regulator of the CXCR2 signaling cascade. This may imply to the involvement of Lnk regulation at the tumor microenvironment, and position Lnk as a possible intracellular immune checkpoint.

P.4.1-016
A more sensitive detection of micrometastases of NSCLC in lymph nodes using the one-step nucleic acid amplification (OSNA) method

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The detection of tumour cells in lymph nodes (LNs) removed during the radical surgical treatment of patients with non-small cell lung cancer (NSCLC) is currently confined by the limits of standard histopathological methods. Micrometastases, clusters of tumour cells or isolated tumour cells may not be revealed by this procedure, if they are located outside the examined section line of the LN, resulting in the underestimation of the stage of the disease. The goal of this study was to obtain a more accurate pTNM status by a more sensitive detection of micrometastases in LNs using a molecular biological assay OSNA (One-Step Nucleic Acid Amplification) which detects mRNA of cytokeratin 19 (CK19) by isothermal amplification (RT-LAMP). The presence of CK19 in a LN is an indicator of the metastatic involvement by tumour cells of epithelial origin. A total of 885 LNs, an average of 13.8 LNs per patient, were removed during 64 surgeries. The sentinel LN concept is not applied in lung cancer. For rational analysis of all surgicaly removed LNs, we applied a new approach. LNs from the same nodal zone were pooled together as a group, 5 groups of LNs were examined in each patient. A total of 320 groups of LNs were examined. OSNA method was compared to standard histopathological examination with haematoxylin-eosin (H&E) staining. Identical results for H&E and OSNA examinations were recorded in 286 groups of LNs (89.4%). In total, positive examinations were recorded in 27 groups of LNs (8.4%) using the OSNA method, which were H&E negative. In 7 groups of LNs (2.2%), the H&E examination was positive, whilst OSNA method produced negative results. The OSNA examination led to a higher pTNM stage classification in 14 (21.9%) patients and what is important this should result in different therapeutic approach. The clinical significance remains the subject of follow-up research.

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P.4.1-017
Effects of usnea florida ethanol extracts on cytotoxic activity in MCF-7 and L929 cells

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The aim of this study was to investigate different cytotoxic activities of the ethanol extracts of *Usnea florida* (L.) F.H.Wigg. on human breast adenocarcinoma cell line (MCF-7) and mouse fibroblast cell line (L929). *U. florida* is a lichen which synthesizes important secondary metabolites such as usnic acid. It shows a potentially valuable of biological actvities including antibacterial, antiviral, antiproliferative effects. Dried lichen samples were extracted by ethanol at a ratio of 1:20 (w/v) with the help of soxhlet extractor (26°C, 2 hr). Then, solvent was removed from samples by rotary extractor (250 Rpm, 50°C, 1 hr.) and lyophilization for 12 hours. Phenolic compounds found in *U. florida* extracts were determined with the help of DPPH and HPLC methods. MCF-7 and L929 were cultured in the presence various concentrations of extracts for 72 hr. *U. florida* inhibited the survival of MCF-7 and L929 cells in a concentration and time dependent manner, shown by XTT and real time cell analysis in Xcelligence device. According to cytotoxic analysis, IC_{50} values of extracts were calculated as 29 μg/ml on MCF-7. Although, *U. florida* effected MCF-7 in low doses, it did not show same activity on L929 cells. IC_{50} values of extracts were determined 458 μg/ml on L929. These finding suggest that *U. florida* effects on breast cancer cells at low doses is cytotoxic but there is no activity on healthy cell lines. So, presented results support further investigations of *U. florida* as a prospective therapeutic agent with potential relevance in the traetment cancer. This work was financially supported 15101018 project number by *Selçuk University Scientific Research Projects Coordination Unit*. Thanks to *Selcuk University Advanced Technology Research&Application Center* and *Directorate of Mushroom Research And Application Center* for using their laboratories.

P.4.1-018
Characterization of a lymphosarcoma retrovirus in northern pike Esox lucius l., isolated in Ukraine

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The occurrence of tumours with a suspected viral etiology is well documented in a number of fish species. Thirteen proliferative diseases in fish have been associated with retroviruses. Typically, these occur as seasonal epizootics affecting farmed and wild fish, and most lesions resolve spontaneously. Only 6 tumorigenic piscine retroviruses have been fully or partially sequenced. These include WDSV, WEHV-1, WEHV-2, perch epidermal hyperplasia viruses 1 and 2, and salmon swim bladder sarcoma virus.

Esocid lymphosarcoma or lymphoma is a disease of adult northern pike (*Esox lucius*) in Europe and North America. Recently, cases of the esocid lymphosarcoma in Dnipro reservoirs of Ukraine are observed more often and therefore the purpose of our study was to determine the viral etiology of the disease.

In this report, a retrovirus was detected in adults northern pike, during a fish health inspection in 2015 of Dnipro reservoirs in the north region of Ukraine near Kyiv. Preliminary examination of infected fish revealed a range of cutaneous lesions that were characterized by expansive, soft, pale-tan, knobby plaques with irregular margins. The plaques were located on the lateral parts of the body. The age of infected fish was 2 to 5 years. At this age pikes are actively involved in the spawning and it was hypothesized that horizontal transmission of the disease may occur in the spring during the spawning season. Water temperature may also affect the replication of a virus. According to our research, the incidence of tumors ranged from 17 to 30% of total pike population. In addition, the nucleotide sequences of about 140 bp of retroviral RNA-depend DNA polymerase gene fragments were analyzed and the identity of the detected retrovirus to strain TM-4 from Ireland was revealed. The identity of nucleotide sequences was 97%–99%. Also the Ukrainian isolate of pike retrovirus was closely related to the WDSV, WEHV1 and WEHV2 that were found in the USA.

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P.4.1-019
Transcriptome-based analysis of allele-specific gene expression in hepatocellular carcinoma

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Allele-specific expression (ASE) accounts for phenotypic variation and has been reported to be associated with some hereditary disorders including colon neoplasms. Hepatocellular carcinoma (HCC) that ranks as 2nd leading cause of cancer-related mortality is characterized by prominent somatic mutations heterogeneity and a lack of frequently mutated potent drivers and druggable targets. Thus identification of recurrent ASE genes and associated germline single nucleotide polymorphisms (SNPs) that are tumor-specific might provide additional insights into hepatocarcinogenesis mechanisms and possible approaches for HCC therapy. We performed whole transcriptome sequencing data analysis of 45 paired liver and non-viral HCC samples which revealed tumor-specific allelic expression of 839 genes harboring 1031 unique SNPs ($P < 0.05$, Fisher's exact test) including common up-regulated variants with shared ASE pattern across different samples, minor alleles with frequencies that exceed global minor allele frequencies and might be associated with elevated risk of developing HCC, reported and putatively deleterious variants. Overrepresentation analysis of ASE genes revealed enrichment in genes encoding components of PDGF, TGF-beta, Ras, PI3 kinase, IGF and several other signaling pathways critical for HCC development. ASE genes were evenly distributed across the genome and enriched with transcriptional targets of HIF1A, ESR1 and STAT1 factors that are differentially expressed in examined tumors. We also identified a set of recurrent tumor-specific ASE genes that comprised RCBTB1 (cell cycle control), ETAA1 (DNA damage response), CFLAR (apoptosis regulation), PEAK1 and FAM195B (cell migration), ABCC9 (multi-drug resistance), FADS1 and several pseudogenes. Our results imply that ASE of germline SNPs may be an additional mechanism to confer susceptibility to HCC and favor tumor progression.

P.4.1-020
Biological outcome of genetic modifications in the p66Shc level in MCF-7 and MDA-MB-231 human breast cancer cells

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Mitochondria perform a substantial diversity of interconnected functions, including reactive oxygen species (ROS) production, as well as apoptosis. Thus, it is not surprising that mitochondrial dysfunctions in cancer cells appear to be potential targets in chemotherapy. Substantial evidence indicate that apoptosis and the ROS production process involve a small p66Shc adaptor protein that plays a dual role in cells depending on its phosphorylation pattern (acts as a negative regulator of proliferation or participates in ROS production). These facts make p66Shc pathway a potential target concerning cancer proliferation or tumor progression especially taking into account differences in p66Shc signaling pathway and ROS production between human breast cancer cells and corresponding controls Going further, we aim to determine how p66Shc genetic modifications (overexpression and knockout of p66Shc) may affect mitochondrial bioenergetics and p66Shc signaling pathway in human breast cancer cells.

To achieve the goal of our studies human breast cancer cell lines (MDA-MB-231 and MCF-7) with overexpressed and knocked out p66Shc level has been used. Proliferation rate, p66Shc signaling pathway as well as mitochondrial bioenergetics parameters have been estimated.

Obtained results may indicate that genetic modifications in p66Shc level demonstrate different effect in MDA-MB-231 and MCF-7 human breast cancer cell lines.

Detailed analysis of the p66Shc signaling pathway and p66Shc-related oxidative stress, as well as mitochondrial bioenergetic parameters may provide a general overview of the p66Shc-dependent mechanisms and answer the question regarding whether negative or positive modulation of this pathway may improve recent therapeutic strategies in the case of breast cancer. This work was supported by grant from the National Science Centre, Poland (Grant 2016/21/N/NZ1/00278).

P.4.1-021
A phosphoproteomic screen reveals how to exploit adaptive response to targeted therapies towards cell signalling for a better response of PI3K-hitting drugs in the clinic

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An active form of a major downstream target of PI3K, pAkt, is increased in 50% of pancreatic cancer and correlated with a poor prognostic. So far, the use of PI3K-targeting drugs presents a limited clinical benefit in highly resistant solid cancers. We hypothesize that the shutdown of such a critical signal forces the cancer cells to change its signalling network, reducing the efficiency of drugs.

PI3K class I activity is performed in Vertebrate cells by four isoforms. We analysed the importance of PI3K isoform-signal nodes in pancreatic cancer by studying their downstream signal networks and their regulatory pathways.

To demonstrate for the first time that there is a differential adaptive response to the constant inhibition of each PI3K isoforms or all PI3K, we performed a time course large scale phosphoproteomic screening using SILAC followed by enrichment of phosphopeptides, their identification/quantification by LC-MS/MS, and statistical/bioinformatics analysis of the phosphoproteins levels and mapped PI3K isoform-specific signalling that were triggered by serum activation.

By pharmacological inactivation of one PI3K isoform or all PI3K, we observed phosphopeptides which were induced by p110γ inactivation only or p110α-only or induced by the inhibition of all the isoforms of PI3Ks. We now aim to validate these early modifications of signalling networks upon PI3K inhibition. These data make the rational for the combinative use of PI3K isoform-specific drugs in pancreatic cancer patients for a better response in the clinic.

P.4.1-022
High-throughput sequencing of immunoglobulin genes rearrangements for diagnostics of minimal residual disease in acute lymphoblastic leukemia

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Diagnostics of minimal residual disease (MRD) is the most powerful prognostic tool in different hematological malignancies including acute lymphoblastic leukemia (ALL). MRD testing allows to determine risk of relapse and guides treatment decision. Here we describe a new method for MRD detection based on targeted high-throughput sequencing (HTS) of rearranged immunoglobulin genes specific for leukemic clones of patients with ALL.

Initial detection of immunoglobulin genes rearrangements was performed on DNA from bone marrow taken from patients before the treatment. The amplicons for sequencing were obtained by 8 multiplex PCRs with the primers covering all possible combinations of V- D- and J-genes from TCRB, TCRG, TCRD, IGH, IGK and IGL loci. The amplicons were then sequenced on Illumina MiSeq platform. Rearrangements specific for leukemic cells were identified by analysis of their frequencies in obtained TCR/BCR genes repertoire.

For further MRD monitoring genomic DNA was extracted from bone marrow cells taken from patients on the day 36 after the beginning of treatment. Libraries for HTS were generated in 32 multiplex PCRs with different ammount of DNA input (3, 30 and 300 ng) and the primer combinations specific for BCR/TCR genes rearrangements identified in the initial sample. The amplicons were also sequenced on Illumina MiSeq.

Using described method, we analyzed 32 initial samples and identified over 100 rearrangements of immunoglobulin genes. MRD analysis was performed for 10 follow-up samples. Detected MRD levels were consistent with the results obtained by flow cytometry or real-time PCR analysis of fusion ETV6-RUNX1 gene transcripts.

Thus, HTS based methods are a prospective tool for sensitive and accurate MRD diagnostics without limitations on the depth of analysis.

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P.4.1-023
Regulation of c-met signaling by neddylation in hepatocellular carcinoma cells

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Neddylation is one of the ubiquitin-like modifiers and its mechanism is deteriorated in cancer cells. Neddylation inhibitor MLN4924 is being used in phase I clinical trials. In our previous studies, we have shown that activation of c-Met pathway has an important role in the development and progression of hepatocellular carcinoma (HCC) and, c-Met is usually activated in a ligand-independent fashion. In this study; firstly the change in total neddylation levels of HCC cells expressing low or high c-Met was identified. We have used cells grown in 2% fetal bovine serum (FBS) and 10% FBS: THLE-2 to represent normal liver cells; HuH-7, HepG2, Hep3B, Mahlavu, SK-Hep-1, SNU (398/449/475/182/423/387), PLC and Focus representing HCC cells. Total neddylation was found to be higher in 10% FBS, suggesting that cell metabolism affects neddylation pattern. All SNU and Focus cells have higher neddylation pattern in 10% FBS condition whereas in 2% FBS, epithelial like HCC cells and normal liver cells have slightly higher total neddylation pattern. When c-Met is immunoprecipitated from HCC cell lysates that are DMSO treated, hepatocyte growth factor (HGF) treated or HGF and SU11274 (c-Met inhibitor) treated, c-Met neddylation was observed in all conditions. In addition c-Met neddylation levels were elevated in c-Met inhibited conditions. We have further identified the effects of treatment combination of HGF or SU11274 with MLN4924 on cell motility and invasion capacity of HCC cells. Finally, c-Met and NEDD8 protein expression profiles were immunohistochemically identified during hepatocarcinogenesis using commercially available HCC tissue array. As a result of this study, neddylation of c-Met and inhibitory roles in c-Met signaling in HCC was revealed for the first time.

P.4.1-024
5FU loaded silk fibroin nanocarriers for colon cancer targeted therapy

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Nanocarriers offer several advantages over conventional administration of drugs such as protection of the encapsulates against degradation, improvement of the therapeutic concentration, controlled retention time, bioavailability, decrease of toxicity, etc. Furthermore, nanoparticles (NPs) are ideal for tumor targeting due to their ability to penetrate the neo-vasculatures. The aim of the current study was to develop and validate *in vitro* a nanosized carrier based on silk fibroin (SF) for the targeted delivery of 5FU in colon cancer (CC) therapy.

After synthesis, the silk fibroin nanoparticles (SF NPs) were characterized in terms of morphology, size and size distribution. The 5FU uptake was determined using UV-VIS spectrophotometry and then the drug release was investigated for several days by using UV-VIS spectroscopy. Next, the toxicity of the unloaded and 5FU loaded SF NPs was evaluated on a highly malignant CC cell line (HT-29) in terms of: cell viability and proliferation potential, apoptosis, morphology and NPs cytotoxic potential on cells. Cell viability and proliferation potential was investigated by using the MTT spectrophotometric quantitative assay and Live/Dead fluorescence microscopy assay. Additionally, HT-29 cell cycle after the treatment with the unloaded and 5FU loaded SF NPs was assessed by flow cytometry.

Our results showed that we obtained SF NPs of approximatively 100 nm. The treatment with the unloaded SF NPs did not exert any cytotoxic effects on HT-29 cells as it did not affect HT-29 cell viability, proliferation potential and morphology. Unloaded SF NPs did not induce HT-29 cells apoptosis.

However, the treatment of the same cells with 5FU loaded SF NPs dramatically decreased cell viability and proliferation potential, triggered morphological changes in cells shape and induced apoptosis.

In conclusion, these NPs could be further used for *in vivo* studies on animal models in order to determine their distribution, effect and clearance.

P.4.1-025
Development of Anticalins binding prostate-specific membrane antigen (PSMA) for theranostic applications

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Prostate-specific membrane antigen (PSMA) is a validated target for the imaging and therapy of prostate carcinoma (PCa), by far the most commonly diagnosed neoplasia in men. Anticalins, engineered binding proteins based on a non-immunoglobulin scaffold, were selected from a naïve library against this tumor target via phage display. Subsequent affinity maturation using bacterial surface display yielded Anticalins with extraordinary affinity (K_D ~ 500 pM) as measured in BIAcore. In order to improve plasma half-life and imaging contrast in animal studies, the Anticalin was modified by PASylation technology. Radiolabeling was achieved by coupling the metal chelator NODA-GA either via maleimide chemistry to a free Cys side chain or via aminooxy conjugation to the non-natural amino acid p-acetyl-L-phenylalanine (Apa). The conjugated protein was purified and charged with radioactive ^{68}Gallium. *In vivo* tumor imaging of mice bearing PSMA-positive xenografts using positron emission tomography (PET) will be presented.

P.4.1-026
Role of NLRP3 inflammasome in cancer cells

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We investigated P2X7 receptor and NLRP3 expression, and their role in oral cancer cells. We found that the P2X7R and all NLRP3 inflammasome components were upregulated in head and neck squamous cell carcinoma tissues biopsied from human patients. Similarly, in A253 cells derived from the epidermoid carcinoma, expression of P2X7R, apoptosis-associated speck-like protein containing CARD (ASC), and pro-form caspase 1 were highly upregulated compared to normal Human Salivary Gland (HSG) cell line. Active caspase-1 and its final product, active interleukin-1β, were both increased when primed A253 cell were stimulated with P2X7 agonists, and this elevated NLRP3 inflammasome activity was suppressed by P2X7 antagonist. However, none of those phenomena was observed in HSG cells. Inhibition of both NLRP3 inflammasome and P2X7R led to the significant cell death of primed A253 cells, but viability of primed HSG cells or the primary cultured human fibroblast cells was unaffected. Furthermore, inhibition of either P2X7R or NLRP3 inflammasome decreased invasiveness of A253, and this effect became more evident when both P2X7R and NLRP3 inflammasome were simultaneously blocked. Therefore, it is concluded that the P2X7R and the activation of NLRP3 inflammasome play important roles in the survival and invasiveness of head and neck squamous cell carcinoma in human.

P.4.1-027
DEGUELIN, a novel anti-cancer agent, inhibits tumor aggressiveness and increases docetaxel efficacy in lung cancer cells

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Lung cancer is leading cause of mortality due to its aggressive behavior and commonly diagnosed at advanced stages. Standard drug regimens are not satisfactory. Previous studies has been demonstrated that vasculogenic mimicry (VM) and increased tumor cell motility are associated with poor prognosis, high tumor grade and the indicators of tumor aggressiveness. Vasculogenic mimicry is known as vessel-like network formation by tumor cells. As a rotenone derivative, deguelin has been demonstrated to have several anti-cancer efficacies including angiogenesis and potentially VM inhibition. The present study investigated anti-VM activity of deguelin, docetaxel, and their combination in Lewis Lung Cancer (LLC) cells. Tumor cell motility alterations after the treatment modalities were also investigated. The results of both assay suggested that deguelin had strong anti-VM and anti cell migration activity against LLC cells. Synergism was also exist between deguelin and docetaxel. In conclusion, deguelin has able to reduce tumor aggressiveness and metastatic potential when used alone or combine with docetaxel in lung cancer.

P.4.1-028
Innovative therapeutic modalities for solid EpCAM-positive tumours

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The epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein expressed on healthy and adenomatous epithelia, where it plays a role in homophilic cell adhesion. Furthermore, it was shown that this protein has an impact on cell proliferation. EpCAM is strongly overexpressed by carcinomas, which makes it an attractive target for diagnosis and therapy of tumours. In 2009, the EpCAM trispecific antibody catumaxomab (Removab) was authorized for treatment of cancer patients. A new approach would be to target the intracellular EpCAM signalling itself.

We established and performed a High-Content Screen to identify compounds, which specifically inhibit the EpCAM signaling cascade. The obtained and verified hits were then further characterized by cellular and biochemical assays with regards to their effects on cytotoxicity, target gene expression, regulated intramembrane proteolysis (RIP), and cell proliferation.

P.4.1-029
Inhibition of cancer cell proliferation by a novel regulatory mechanism that renders RB insensitive to CDK inhibition

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Control of the G1/S phase transition by the Retinoblastoma (RB) tumor suppressor is critical for the proliferation of normal cells in tissues, and its inactivation is one of the most fundamental events leading to cancer. RB is inactivated by Cyclin-dependent kinases (CDK) to promote cell cycle-regulated gene expression and progression into S phase. In our studies trying to understand the molecular basis of cell adaptation to stress, we have found that the p38 stress-activated protein kinase (SAPK) maximizes cell survival to stress by delaying the G1/S transition of the cell cycle. The p38 SAPK controls cell cycle progression by targeting the p57 CDK inhibitor (Joaquin et al., 2012) and also through the down-regulation of cyclin expression. The down-regulation of cyclin expression by p38 is achieved by the direct phosphorylation of RB at its N-terminus. These phosphorylations promote the repressing activity of RB by increasing its interaction towards the E2F transcription factors. Remarkably, the N-terminal phosphorylation of RB by the p38 makes RB insensitive to cyclin-dependent kinase (CDK)-Cyclin inhibition. Correspondingly, introduction of a RB phosphomimetic mutant in cancer cells reduces colony formation and decreases their proliferative and tumorigenic potential in mice (Gubern et al., 2016; Joaquin et al., 2016). This novel mechanism of RB regulation opens up a window for developing new cancer drug treatments for tumors harbouring high CDK-Cyclin activity.

P.4.1-030
Expression profiles of HERV-R and HERV-K Env proteins in various cancerous tissues

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Human endogenous retroviruses (HERVs) have been proposed to be strongly related with certain cancers including breast cancer, leukemia, colon cancer, and melanoma. Here, we analyzed the expression profiles of HERV-K and HERV-R Env proteins in 11 different kinds of cancer tissue microarrays. We compared the expression levels of HERV Env proteins in cancers with surrounding normal tissues and also analyzed the correlation with various clinical data. In addition, we investigated the co-localization and expression pattern relationship between HERV-K and HERV-R Env proteins.

The expression of HERV-K Env protein in tumors was usually higher than normal tissues and specifically high in breast, melanoma, liver, stomach, kidney, prostate, cervical, esophagus and colon cancer. The expression of HERV-R Env protein in tumors was also higher than normal tissues and specifically high in melanoma, liver, stomach, ovarian, kidney, prostate, cervical, esophagus, and colon cancer. General expression patterns of HERV-K and HERV-R Env proteins were various in each individual but showed similar pattern between HERV-K and HERV-R Env in same individual. Correlations between HERV Env expressions and clinical investigations were vary depending on HERV types and cancers. HERV-K Env protein levels are up-regulated in old age group of liver cancer patients. HERV-R Env protein expression was peaked in tumor stage 2 group of colon cancer patients. Furthermore, the expression pattern of HERV-K and HERV-R Env proteins are significantly relevant. These results suggest that the expression of HERV-K and HERV-R Env may be regulated in the same manner as epigenetical regulations and the expressions of HERV Env proteins are correlated with certain cancers.

P.4.1-031
Hypermethylation of 5 tumor suppressor miRNA genes (MIR-124a-2, -125b-1, 129-2, -137, -193a) is associated with metastasis of epithelial ovarian cancer

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DNA methylation plays a systemic role in deregulation of miRNA genes and, consequently, target genes of corresponding miRNAs in malignant tumors. Hypermethylation was shown for the large group of tumor-suppressor miRNA genes in various malignancies, but in epithelial ovarian cancer (EOC), it was not studied extensively yet. In the present work, DNA methylation of 10 cancer-associated miRNA genes was analyzed in EOC for the first time.

A representative set of 54 paired (tumor/normal) tissue samples of EOC patients and methylation-specific PCR were used.

The methylation frequencies of 9 tumor suppressor miRNA genes (*MIR-124a-1/2/3, -127, -125b-1, 129-2, -132, -137, -193a*) were found significantly higher ($P \leq 0.01$, Fisher's exact test) in tumor samples in comparison with matched histologically normal samples. The highest frequency of hypermethylation (48–61% *vs.* 4–9%) was noted for 4 genes: *MIR-124-3, -127, -129-2, -193a.* Conversely, hypomethylation was revealed for *MIR-191:* 13% *vs.* 59%, $P \leq 0.01$. For a number of miRNA genes, a significant ($P \leq 0.05$) correlation of hypermethylation with EOC progression was revealed, including strong association of hypermethylation of 5 tumor suppressor miRNAs (*MIR-124a-2, -125b-1, 129-2, -137, -193a*) with the presence of metastases in lymph nodes and/or distant tissues ($P \leq 0.05$). *MIR-137* hypermethylation was found as the most perspective biomarker for EOC metastasis prediction ($P = 5 \times 10^{-5}$). Using ROC analysis, the marker systems for the diagnosis of EOC and prediction of metastasis were compiled: sensitivity and specificity on the examined sampling were in the range 85-100%, AUC value >0.9.

Thus, we identified 10 hyper- and hypo-methylated miRNA genes associated with pathogenesis and metastasis of EOC and suggested novel potential biomarkers. This work was financially supported by the Russian Science Foundation grant 14-15-00654.

P.4.1-032
Novel miRNAs involved in the regulation of apoptosis via DAPK1, APAF1, BCL2, and RASSF1(A) genes in breast cancer

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The interaction between miRNAs and mRNAs at the post-transcriptional level is considered as the most delicate and dynamic mechanism of expression regulation. The aim of the study was to identify novel regulatory miRNAs for pro-apoptotic *DAPK1* and *APAF1* genes, anti-apoptotic *BCL2* gene, and *RASSF1(A)* gene, which is involved in the induction of apoptosis, in breast cancer (BC).

We used 38 paired BC samples and qPCR with 2 references (*RNU6* and *RNU48*) for miRNA level evaluation and *B2M* as reference gene for mRNA level study. The screening for miRNAs, which are potentially involved in the regulation of expression of examined genes, was carried out with algorithms of miRWalk 2.0 database. For statistics we used the IBM SPSS Statistics 22.

The analysis of expression level of 20 miRNAs in BC samples showed a significant decrease correlation ($P \leq 0.05$, Fisher's exact test) of the level of 10 miRNAs and increase of miR-375 level. Among 4 protein-coding genes, significantly frequent ($P \leq 0.05$) expression decrease was shown for *DAPK1, APAF1*, and *RASSF1(A)* and increase – for *BCL2*. The negative correlations were revealed between expression level alterations of 3 genes and 6 potential regulatory miRNAs for the following pairs: *BCL2* – miR-124-3p, -212-3p, -24.2-5p (*Rs*: −0.32, −0.36, −0.36, P = 0.03–0.05); *DAPK1* – miR-127-5p (*Rs* = −0.43, $P \leq 0.01$), miR-9 (*Rs* = −0.37, P = 0.02); *RASSF1(A)* – miR-375 (*Rs* = −0.39, P = 0.02); and at the level of a trend for pairs: *APAF1* – miR-132, -193a-5p (*Rs* = −0.28, −0.27, $P \leq 0.1$). The results of transfection of MCF7 cell line with miR-124-3p duplex oligonucleotide analogues strengthened the hypothesis on the direct or indirect interaction of this miRNA with mRNA of the *BCL2* gene.

Thus, novel interactions of *DAPK1, APAF1*, *BCL2*, and *RASSF1(A)* mRNAs with a number of miRNAs were suggested that could be useful as missing chains in signaling pathways and potential targets in complex BC therapy. This work was financially supported by the Russian Science Foundation grant 14-15-00654.

P.4.1-033
The impact of DNA structure and flexibility of p53 response elements with long spacers on p53 recognition of its binding sites

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The transcription factor p53 is a well-known regulator of cellular stress response. p53 acts as a tumor suppressor through activation of numerous DNA targets, which can lead to cell cycle arrest, DNA repair, senescence, or apoptosis pathways, among others. It has been established that p53 binds its target sites through sequence specific recognition of response elements (REs). These REs have a general consensus sequence of two decamer half-sites ($(RRRCWWGYYY)_2$, where R = A, G; W = A, T; Y = C, T), separated by spacer sequences of 0–18 bp. The mechanism by which p53 assembles on the RE as a homo-tetramer is known. However, despite understanding p53's mechanism of DNA binding, the process by which p53 discriminates between its target sites is still obscured. Recently, some indications have appeared that the spacers between p53 RE repeats are responsible for additional levels of regulation to p53 transcription. This is especially meaningful for the more than 30 validated p53 REs with long spacers (equal or greater than 10 bp). As it is already established that the flexibility of the RE has a significant influence on p53's binding affinity, we will show that for REs with long spacers the affinity of p53 to the RE and the cooperativity of p53 assembly is determined through the combined effect of the RE's and the spacer's flexibility. Determining the relationship between the RE flexibility and p53 RE recognition will allow us to expand our understanding of p53's target selection.

P.4.1-034
Expression of STING in peripheral blood lymphocytes from patients with preinvasive and microinvasive cervical cancer

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The role of STING (Stimulator of Interferon Genes)-mediated signaling pathway is one of the central issues of current research on regulation of antitumor immunity, with STING protein expression being mostly considered in the context of sensing of DNA from dying tumor cells or cells infected with oncogenic agents by antigen-presenting cells, however little is known about functional significance of STING expression in other leukocyte populations, particularly T cells. We focused our study on early-stage cervical carcinoma, assuming that during the course of neoplastic development STING is able to receive signals both from HPV-DNA and tumor-derived DNA. Using flow cytometry assay we compared the level of STING protein expression in major subsets of peripheral blood T-lymphocytes from women with preinvasive or microinvasive cervical cancer and healthy controls. To assess STING expression levels, relative Mean Fluorescence Intensity values (ΔMFI) normalized to the isotype control and autofluorescence of corresponding T-cell subsets were analyzed. According to the results, STING levels did not differ between CD3CD4 and CD3CD8 cells obtained from healthy controls or cancer patients suggesting that the expression of STING is equally associated with both CD4 and CD8 T-cell subset. However, in the patient group an increase in ΔMFI(CD4CD25)/ΔMFI (CD3CD8) ratio was observed, implying that STING expression was more pronounced in CD4CD25 lymphocytes. There was no significant difference in STING expression in total circulating lymphocyte population between patients and controls. Thus, STING being a key-player in modulation of innate immune reactions may have an essential role in T-cell functions; during the development of infection-related cancer, the importance of this role can be realized through redistribution of the level of STING signaling in different T-cell subsets, which may affect the processes of T-cell activation. The work was supported by the RSF grant No. 17-15-01024.

P.4.1-035
Therapy for folate receptor 1 (FOLR1) positive gastric cancer using chimeric antigen receptor (CAR) T cell

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Chimeric antigen receptor (CAR) T cell therapy is an immunotherapy that destroys cancer cells using genetically engineered patient's T cells targeting specific cancer antigen. Since folate receptor 1 (FOLR1) is often overexpressed in many cancer cells, it is considered as a good target for CAR T cell therapy. We constructed a CAR vector expressing a fusion protein composed of scFv of FOLR1 antibody, and signaling domains of CD28 and CD3 zeta that are required for T cell activation. Anti-FOLR1 CAR was expressed in Jurkat cells, CD4 positive T cell line, by transient transfection. When anti-FOLR1 CAR-Jurkat cells were co-cultured with FOLR1-overexpressing K562 cells, they were activated and secreted high level of IL-2. This indicates that our CAR construct functions properly. Then, FOLR1 positive or negative gastric cancer cell lines were selected by the fluorescence-activated cell sorting (FACS). FACS data shows that MKN1, MKN7, SNU484, GCIY are FOLR1 positive and SNU216, SNU601, SNU668 are FOLR1 negative. CAR-Jurkat cells were activated when co-cultured with FOLR1 positive gastric cancer cells, but were not activated by FOLR1 negative gastric cancer cells. Then, we made anti FOLR1 CAR T cells using human PBMC, and tested if they are effective against gastric cancer. As a result, our anti FOLR1 CAR T cells specifically induce cell death of FOLR1 positive gastric cancer cell. Therefore, we expect CAR T cells targeting FOLR1 to be a good selective therapy for FOLR1 positive gastric cancer cells.

P.4.1-036
Coumarin derivatives in the pharmacotherapy of anticancer diseases

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Coumarin and its derivatives are found widely throughout nature, and many of these compounds exhibit a broad range of useful biological activities. The naturally occurring coumarin ring system displays a number of interesting pharmacological properties and has intrigued chemists and pharmacologists for decades. It is therefore conceivable that these hybrid compounds could also be used as potential drug candidates for multifactorial diseases. In this study, a series of novel coumarin-tacrine hybrids (*1–4*) were biologically evaluated for their potential inhibitory effect on topoisomerase I enzyme. Our research investigated the nature of the interactions of these new coumarin derivatives with DNA and the ability of these hybrid molecules to inhibit topoisomerase I was studied. The derivatives were analysed against A549 adherent lung adenocarcinoma cells. The presence of the particles was determined through observations of their fluorescence in the green channel. According to our results, the presence of derivatives was detectable predominantly in sample *4*. In other samples, the fluorescence of the derivatives was not istinguishable from the autofluorescence of the cancer cells. In cells, the derivatives were distributed in cytoplasm and displayed no signs of interference with the cell nuclei. Based on mitochondrial staining and overall distribution of the signal, we were unable to confirm the accumulation of derivatives in mitochondria or in other organelles or membranes. Flow cytometric analysis of derivatives content in A549 cells revealed the cumulative fluorescence of derivatives *1-4* from the green (FL-1) to the red (FL-3) channel. Derivative *3* was found to display the highest level of fluorescence. The obtained results could be of benefit in the design and development of new coumarin based agents.

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P.4.1-037
Cytotoxic bacterial ribonuclease (binase) immobilized on halloysite nanotubes for targeted delivery to tumor cells

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Nowadays the scientists are actively engaged in the development of methods for targeted delivery of anticancer drugs. In our study we used biocompatible halloysite clay nanotubes (HNTs) as a potential system for immobilization of antitumor ribonuclease of Bacillus pumilus (binase) which possess a selective cytotoxicity toward some tumor cell lines, causing their apoptotic death. The expression of oncogenes such as Ras, Kit, AML1-ETO and FLT3 determines the sensitivity of model cells to the toxic effects of binase. The specific antitumor effect of binase towards Ras-transformed cells is explained by the direct binding and inhibition of Ras protein activity. Mutations in RAS are found in approximately 30% of all human cancers, including a colorectal cancer which is known as one of the most widely distributed cancers in the world. It was shown that the K-RAS oncogene is mutated in about 40% of colorectal cancers.

The aim of our study was to prove a possibility to use binase as an anticancer agent with prolonged action towards colon cancer cells. We assessed Colo320 cell line viability by MTT-analysis. We treated cells by binase, nanotubes, and binase immobilized on nanotubes. The obtained results showed that the addition of 100 μg/ml binase immobilized on the HNTs reduced the viability of the Colo320 cells by 60%, which is twice as much as the cytotoxic effect induced by binase alone at the same concentration. The HNTs themselves demonstrate no cytotoxic effect on cells at concentrations up to 600 μg/ml.

The application of HNTs as a carrier provides the possibility of their use as a rectal suppositories for colorectal cancer treatment. We believe that the immobilization of binase on HNTs will allow avoiding its degradation by cellular proteases and will provide a sustained release of the enzyme.

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P.4.1-038
Effect of overexpression of the pancreatic master gene PDX1 on the embryonic master genes expression in pancreatic cancer cells

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PDX1 is often referred to as the "master regulator" of pancreogenesis. It initiates a hierarchical cascade of events during development. Upon neoplastic transformation, the role of PDX1

changes from tumor-suppressive to oncogenic. At early stages of carcinogenesis PDX1 inhibits Kras-driven dedifferentiation of acinar cells, at the later malignant stage PDX1 supported cell proliferation. PDX1 expression is lost in malignant cells in the epithelial-mesenchymal transition during metastatic process.

In our study PANC1 and BxPC3 cells stably expressed *PDX1* were generated with the use of lentiviral vectors. The expression of the *NKX6.1, NR5A2, KLF5,* and *ONECUT1* genes was 2-, 2,5-, 1,8-, 3- and 1,3-fold increased in $BxPC3^{PDX+}$ cells as compared to $BxPC3^{PDX-}$ cells. At the same time, the expression in $BxPC3^{PDX+}$of *MUC1* and *SLUG* was 3- and 2-fold decreased. In $PANC1^{PDX+}$ cells we observed 2-fold decrease in the expression of *ISL1*, 2-fold increase in the expression of *KRT8*, and 1,3-fold increase in the expression of *MUC1* as compared to $PANC1^{PDX-}$ cells.

The most likely cause of the diverse effects of overexpression of PDX1 may be a different status of the enhancers regulating these genes. To test this hypothesis, we analyzed the distribution of histones with specific post-translational modifications characterizing various functional states of enhancer and promoter chromatin at the loci of the studied genes. We demonstrated that the total content of the regions with histone modifications characteristic for active enhancers tended to be higher in highly differentiated cells (BxPC3) than in low differentiated "quasimesenchymal" cells (PANC1). The diverse effects observed in $BxPC3^{PDX+}$ and $PANC1^{PDX+}$ are most likely the result of a combination of cell-specific traits, such as local chromatin conformation of the PDX1 recognition sites and the specific sets of PDX1 partner proteins.

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P.4.1-039

Withdrawn

P.4.1-040
Search for cell lines to be used as models of cancer-associated fibroblasts

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Cancer-associated fibroblasts (CAFs) are the most abundant cells of the tumor stroma of different tumor types. Expression of the fibroblast activation protein (FAP) is one of the most characteristic features of CAFs. FAP is a serine protease selectively expressed in CAFs. It was found in the stroma of over 90% of human malignant tumor types, being almost undetectable in most normal tissues. Studies have shown that elevated levels of FAP-positive CAFs in a tumor often correlate with tumor progression and poor outcome. Thus, FAP represents an attractive object for targeted impact on the tumor. However, CAFs are not readily available as reproducibly renewable cell material, which hampers the studies. Therefore, our aim was to find model cell lines that stably express FAP.

To this end, we evaluated the transcription level of the *FAP* gene in 25 cell lines (pancreatic, lung, liver cancer cells; lymphoproliferative disease cells; sarcoma, neuroblastoma, melanoma, epidermoid carcinoma cells; etc.) and in 8 tumor stroma primary cultures of pancreatic and lung cancer. The highest transcription level was detected in sarcoma cells lines RMS 13 and SJSA-1, and neuroblastoma cells NGP-127, where it was comparable to the transcription level in lung CAFs. A Western blot analysis of FAP expression in cultured fibroblasts and the indicated cell lines confirmed that the expression was observed only in those cells where transcription was detected.

It is known that the FAP protein has transmembrane and soluble forms. To be used as a model, cells should express a transmembrane form of FAP. Therefore, we determined FAP location in the FAP-positive cell lines using immunofluorescent staining and confocal microscopy. The results suggested possible cell surface location of FAP in case of RMS 13, SJSA-1, and NGP-127 cells. We believe that these cell lines might be used as models of FAP-positive CAFs.

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P.4.1-041
Analysis of antioxidant and antiproliferative activities of three different molecular sizes silk protein sericin extracted from the cocoon of *Antheraea mylitta*

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Sericin, a natural polymer produced by the silkworm, *A. mylitta*, binds two fibroin filaments together into silk fibers that ultimately construct a cocoon. Sericin was extracted from the cocoon and fractionated by ultrafiltration into three different molecular sizes: fraction 1 (F1) contains the proteins ranging from 50–200 kDa, fraction 2 (F2) 30–50 kDa and fraction 3 (F3) 10–30 kDa. The antioxidant and antiproliferative activity of these fractions were determined *in vitro* against human breast cancer (MDA-MB 231) cell and normal human keratinocyte (HaCaT) cell using different concentrations (200, 100 and 20 μg/ml) of proteins. The results showed free radical scavenging of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) by sericin F1 at 200 μg/ml was more (71–75%) in comparison to F3 (54–59%) and F2 (27–30%). Similarly, at the same concentration, inhibition of reactive oxygen species (ROS) generation and lipid peroxidation were found to be more by sericin F1 (70–72%) than F3 (57–63%) and F2 (38–42%). Sericin also showed its notable reducing power (72% by F1, 31% by F2 and 56% by F3), ability to reduce hydroxyl radical-induced oxidative DNA damage (60% by F1, 28% by F2 and 52% by F3) and prominent anti-apoptotic activity (76% by F1, 47% by F2 and 68% by F3). Antiproliferative assay on MDA-MB 231 cells showed that F2 (89%), F3 (82%) and F1 (50%) inhibited cancer cell growth more effectively than normal cells (28% by F2, 24% by F3 and 15% by F1). Further, analysis of cell cycle by flow cytometry showed that F2 arrested the cell growth in S phase. At lower concentrations (100 and 20 μg/ml), different sericin fractions showed a similar type of activity but at the reduced level. Overall these data suggest that different sericin fractions possess different biological activities which can be used accordingly in specific application and may have potential application as cancer chemopreventive agent.

P.4.1-042
SPRR2A is a potential mediator of therapeutic resistance and poor prognosis following definitive radiotherapy in head and neck cancer

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Small proline rich protein 2A (SPRR2A) is involved in epithelial homeostasis and potentially in development of metastasis and resistance to cellular stress. In a comprehensive sequencing approach performed on metastatic and recurrent head and neck cancer (HNC) cell lines, SPRR2A was identified as one of the most consistently differentially-regulated genes. Here we sought to characterize the relevance of SPRR2A in HNC. qPCR of *SPRR2A* was performed using RNA extracted from 51 matched primary tumors and lymph node metastases (LNMs) from HNC. Immunohistochemistry (IHC) for SPRR2A was equally performed in matched primaries and LNMs from 147 patients with full clinical data. All these patients had regional LNMs, underwent neck-dissection, and received postoperative radiotherapy (+/- chemotherapy). Univariate survival analysis was performed using the log-rank test. Multivariate analysis was performed using Cox proportional hazards regression analysis. *SPRR2A* RNA as well as SPRR2A protein levels were significantly downregulated in LNMs when compared to primaries ($P < 0.001$ and $P < 0.01$, respectively). More specifically, SPRR2A positivity in IHC was detected in 61.91% of primaries vs. 31.30% in LNMs. Univariate survival analysis demonstrated that high SPRR2A expression in LNMs carried a significantly poorer regional recurrence-free survival (RRFS, log-rank p-value: 0.02). On multivariate analysis, SPRR2A high expression in LNMs (HR 2.80, 95% CI 1.16–6.79, $P = 0.02$) was a significant independent predictor of RRFS. Expression of SPRR2A in primary tumors did not have a significant impact on survival. Our current results suggest that SPRR2A may be involved in the radiation response after metastatic colonization of regional lymph nodes in HNC, and its expression may be potentially used as a marker of risk stratification in patients with loco-regionally advanced disease treated with radiotherapy.

P.4.1-043
Fenofibrate augments the sensitivity of drug-resistant human prostate cancer DU145 cells to docetaxel

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Microevolution of drug-resistant cancer cell populations is the most prominent adverse effect of currently available chemotherapeutic regimens. The interference of fenofibrate (FF) with the growth and survival of numerous tumor cell lineages in vitro prompted us to estimate whether FF can be applied to reduce the effective doses of docetaxel (DCX) and mitoxantrone (MTX) in the therapy of drug-resistant prostate cancers.

Using a long-term adaptation regime, we have established 2 sub-lines of DCX-resistant DU145 cells (DU145_DCX20 and DU145_DCX50) and subjected them to the combined FF/DCX and FF/MTX treatment. 2.5 and 10 nM DCX did not affect the proliferation and invasive potential of these cells, although it exerted a significant cytostatic effect on wildtype DU145 cells.

Drug-resistance of DU145_DCX20 and DU145_DCX50 cells was correlated with their slightly less effective proliferation and lower invasive potential in comparison to wildtype DU145 cells, and with the higher activity of multi-drug resistance (MDR) transporters. FF (25 mM) considerably inhibited MDR activity in DCX-resistant DU145 cell populations as demonstrated by dye efflux approach. It also augmented the cytostatic and anti-invasive activity of DCX in drug-resistant DU145 cell populations through the induction of autophagy. A corresponding effect of FF was observed in the presence of MTX.

Our observations suggest that FF can be applied to reduce the effective doses of chemotherapeutic drugs in the therapy of drug-resistant prostate cancers. It can be considered as the potential metronomic agent that may enhance the efficiency of long-term palliative prostate cancer chemotherapy and attenuate its adverse effects. *This work was financially supported by the Polish National Science Centre (2015/17/B/NZ3/01040).

P.4.1-044
Screening breast cancer by joint detection of tumor marker carbohydrate antigen 15-3 and carbohydrate antigen 72-4 with biosensor based on imaging ellipsometry

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Breast cancer developing from breast tissue is the leading type of cancer in women worldwide, and screening high risk population and concluding a clinical diagnosis in the early stage act as a pivotal factor to cure breast cancer. Carbohydrate Antigen 15-3 (CA 15-3) is considered as a specific tumor marker for breast cancer carcinogenesis. However, its detection specificity is insufficient in clinic. Almost one third of those who test positive in CA 15-3 are excluded after further examinations. In order to increase the detection specificity, joint detection of several related tumor markers for breast cancer has been proposed to gain the improved feedback. Thus, a need for joint detection of multiple indicators has been required in clinic. The biosensor based on imaging ellipsometry (BIE) to visualize biomolecular interactions was reported in 1995 and the present model of BIE is mainly composed of a multi-throughput biomolecule array reactor and an imaging ellipsometry reader. With these properties, BIE can detect multiple indicators simultaneously, which completely meets the requirement for joint detection of tumor markers in clinic. By introducing control groups, CA 15-3 and Carbohydrate Antigen 72-4 (CA 72-4) as two breast cancer related tumor markers have been realized with BIE, respectively, and then their joint detection has been performed with BIE to increase the detection specificity for breast cancer screening. 149 serum samples composed of both the healthy people and patients have been analysed by BIE. Compared with the results obtained by standard approaches in clinic, the correlation analysis indicates the BIE are highly consistent with the clinical methods. In order to estimate the BIE performance for tumor markers detection, receiver operating characteristic (ROC) curve analysis has been introduced. Its result suggests that the joint detection of CA 15-3 and CA 72-4 plays a positive role in the improvement of the diagnosis specificity of breast cancer.

P.4.1-045
Ubiquitin specific protease 7 – a new additive to the HIF-1α stability maintenance system

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The uncontrolled growth of cancer cells is associated with the development of hypoxia, which is followed by activation of the HIF-1α signaling pathway. HIF-1α stability is partially regulated by ubiquitin ligase Fbw7, which degrades HIF-1α. Moreover, analysis of mass spectrometry data of Fbw7-interacting partners, revealed us about 10 deubiquitinating enzymes. One of them is USP7, a protein that is reported to be involved in HIF-1α stability maintenance. Our aim was to find if USP7 is a hypoxia-regulated enzyme and whether its action towards HIF-1α can be caused via its interaction with Fbw7 or certain Fbw7-interacting partners.

Materials and methods: Experiments were conducted with human cell lines - embryonic kidney HEK293, colorectal carcinoma HCT116 with Fbw7 shRNA knockdown, and respective scrambled control cells. All the cells were cultured under normoxia or when indicated under hypoxia (1% O_2) at 37°C in DMEM supplemented with 10% FBS. Gene expression was studied by qRTPCR and the protein levels were detected by Western blot. For protein-protein interaction studies, co-immunoprecipitation experiments were performed.

Results: We found that hypoxia causes the decrease of the USP7 mRNA level, but no significant changes were detected on the USP7 protein level under hypoxia, which might indicate that the USP7 protein is stabilized under hypoxic conditions, which is yet to be studied. Our data obtained from co-immunoprecipitation experiments showed that the USP7 protein could be an additional player in Fbw7-HIF1a regulating system. Moreover, we also found that lack of another HIF-1α-regulating ubiquitin specific protease induces the level of USP7 protein, supporting its involvement in the Fbw7 system.

Altogether, these findings suggest that USP7 is a novel partner of in the HIF-1α complex, which strengthens its role in hypoxia signaling, and therefore – in cancer development.

P.4.1-046
The UDP-glucose ceramide glucosyltransferase (UGCG) impacts cell energy metabolism in breast cancer cells

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The UDP-glucose ceramide glucosyltransferase (UGCG) is a golgi apparatus located membrane protein, which transfers UDP-glucose to the lipid ceramide resulting in glucosylceramide (GlcCer) generation. GlcCer is an important membrane component, which maintains the water permeability barrier of the skin, but a discussion arised whether or not GlcCer are also regulators of cell signaling cascades or even signaling molecules in physiological and pathophysiological processes. Alterations in UGCG expression and GlcCer levels are linked to several pathophysiological processes like diabetes, cancer and multi drug resistance (MDR) development in several cancer types. The molecular mechanisms of these UGCG-asscociated processes are rarely known wherefore it is important to investigate the role of the UGCG and their product GlcCer in the context of the cell metabolism. In first studies we could show that an UGCG overexpression in cancer cells leads to alterations of the glycosphingolipid-enriched microdomain composition in the plasma membrane resulting in membrane protein activation. Subsequently, energy uptake out of the media seems to be more efficient and pro-anabolic processes are induced leading to inreased proliferation rates of UGCG overexpressing cells.

P.4.1-047
microRNAs link obesity and cancer

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Obesity is a risk factor for several types of cancer, suggesting shared molecular mechanisms. Several microRNAs implicated in metabolic dysfunction are also oncogenes or tumor suppressors. We aimed to identify cancer-relevant microRNAs that respond to metabolic hormone signaling in cultured cells or show altered levels in obese human subjects, and to explore their functions. We previously reported elevated miR-221 in the fat tissue of obese subjects. MiR-221 was downregulated by leptin and TNF-α in cultured human pre-adipocytes; miR-221 overexpression upregulated several proteins involved in fat metabolism, mimicking PPAR activation. MiR-221 directly targeted adiponectin receptor 1, important for insulin sensitivity, and the angiogenesis-promoting transcription factor ETS1. Thus, miR-221 may contribute to the development of the insulin resistance and cancer risk that accompany obesity.

Recently, we found that miR-4443 was upregulated by leptin and insulin in cultured colon cancer cells, concomitantly with a decrease in cell invasion. This up-regulation of miR-4443 was MEK1/2-dependent. MiR-4443 overexpression decreased invasion and proliferation, and directly downregulated NCOA1 and TRAF4, genes with known roles in cancer metastasis. Thus, miR-4443 may act in a tumor-suppressive manner downstream of leptin and insulin signaling, and insulin and/or leptin resistance (e.g. in obesity) may suppress this pathway and increase cancer risk. Supporting this notion, the miR-4443 locus is frequently deleted in several types of cancer.

Our new data shows that miR-10b, involved in breast cancer progression, is more strongly downregulated in the primary tumors of obese patients, suggesting that the metabolic state of the organism can lead to a significant difference in the molecular pathology of a tumor.

Our research aims to improve the understanding of the mechanisms of cancer pathology, enabling better informed, more personalized treatment of metabolism-dependent cancers.

P.4.1-048
Can boric acid application change the cytotoxic, antioxidant, antiproliferative and apoptotic effects of camellia sinensis on MCF 7 cells?

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Tea (*Camellia sinensis* L.) is one of the most consumed beverages today. Boric acid deficiency or toxicity in soil affects product yield and quality. The objective of the study is to investigate whether applied boric acid in different concentrations to *C. sinensis* has alterations of apoptotic, antiproliferative and antioxidative effects on human breast cancer (MCF 7) cells.

C. sinensis was grown up in Rize, Turkey. The land was divided into four groups. Each group was occurred five areas (10 m^2). The first group is control (A). Boric acid in concentration ranges of 100 (B), 300 (C), 500 (D) mg/m^2 in sodium tetraborate buffer were applied as a single dose on the second, third and fourth groups, respectively. MCF 7 cells were treated with different concentrations of *C. sinensis* extracts for 48 h. Cytotoxicity was tested by MTT. Proliferation and apoptosis were examined with immunocytochemistry and TUNEL methods, respectively. Malondialdehyde (MDA), glutathione (GSH) and protein carbonyl (PCO) levels were measured.

IC_{50} values of MCF 7 cells treated with *C. sinensis* extract in A-D groups were found to be 2.79, 2.69, 2.66 and 2.45 mg/ml, respectively. The optimal dose of the extract for inducing apoptosis and reducing proliferation in MCF 7 cells was determined to be 3 mg/ml of *C. sinensis* leaf extracts applied with boric acid in D group. Boric acid treated *C. sinensis* extracts in D group was found to increase GSH level in MCF 7 cells when compared to untreated *C. sinensis* extracts. MDA level in MCF 7 cells treated with boric acid applied *C. sinensis* extracts decreased inversely proportional to the amount of boric acid applied. PCO level in MCF 7 cells treated with 2.6 and 3 mg/ml concentrations of *C. sinensis* extracts in B group decreased when compared to untreated control MCF 7 cells.

Leaf extracts obtained from *C. sinensis* grown in soil treated with boric acid have more antiproliferative, apoptotic and antioxidant effect on MCF 7 cells.

P.4.1-049
Downregulation of NR0B2 gene in hilar cholangiocarcinoma

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Hilar cholangiocarcinoma (HC) is the most common malignant tumor affecting the extrahepatic bile duct. The incidence of HC is increasing worldwide. HC has high mortality rate owing to its nonspecific symptoms at early stages of the disease and aggressive surgical treatment. Therefore, the search of biomarkers for HC diagnostics and the development of novel therapeutic strategies are of great value. Using CrossHub software we performed bioinformatics analysis of The Cancer Genome Atlas (TCGA) project RNA-seq data for cholangiocarcinoma samples. A potential tumor suppressor gene, *NR0B2*, was found. We carried out the analysis of *NR0B2* gene expression in 15 paired HC samples by qPCR. More than 100-fold downregulation of *NR0B2* was revealed in 73% (11 from 15) of cases. *NR0B2* gene encodes structurally unique nuclear receptor, transcription factor, which plays an important role in drug metabolism, transport, and cellular signaling pathways. Alterations in *NR0B2* expression were reported to be associated with renal, breast, and colorectal cancers, hepatocellular carcinoma, and lymphoma. In the present study, we demonstrated that *NR0B2* gene is related to bile duct carcinogenesis and its expression level could be a promising biomarker of the disease. This work was financially supported by grant MK-8047.2016.4 from the President of the Russian Federation and grant 15-04-08731 a from the Russian Foundation for Basic Research. The work was performed using the equipment of EIMB RAS "Genome" center.

P.4.1-050
CTLs redirected by autocrine-selected ligands specifically engage non-Hodgkin's lymphoma B cells

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Current theraphy of follicular lymphoma (FL) includes administration of monoclonal antibodies specific for the surface molecules of B cells in combination with chemotheraphy. Despite dramatic clinical progress and increased survival of patients suffering from FL, novel approaches of FL treatment are highly demanded. In the present study we developed a novel platform that may significantly enhance efficacy and safety of FL treatment. Elaborated strategy includes determination of specific ligands of B cell receptor (BCR) exposed on the surface of FL tumor cells utilizing combinatorial autocrine-based selection. Selected polypeptide ligands are implemented into the universal cassete bearing chimeric antigenic T cell receptor and immideatly after selection may be used for redirection of human cytotoxic lymphocytes (hCTLs). Single case proof-of-concept study accomplished herein demonstrated ability of generated CAR-redirected hCTLs to specifically eliminate FL tumors *ex vivo* and *in vivo*. Universality of created platform rise possibility of personalized treatment of non-Hodgkin's lymphomas that may be realized in less than three weeks in a translational medicine fasion. This work was supported by RFMEFI60716X0145.

P.4.1-051
Tumor cell derived cystatin F as mediator of NK and T cell cytotoxicity

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Cysteine cathepsins are lysosomal peptidases involved in the processes of immune response. Among them the regulation of granule dependent cytotoxicity of cytotoxic T lymphocytes (CTL) and natural killing (NK) cells during tumor progression deserved a lot of attention in recent time. The activity of cysteine cathepsins is controlled by cystatins. Of these, cystatin F is the only cystatin that is localized in endosomal/lysosomal vesicles and thus capable of direct regulation of cathepsins' activity. It is translocated to vesicles as an inactive disulphide-linked dimer. After proteolytic removal of its N terminal peptide, it becomes a monomer and a potent inhibitor of cathepsins C, H and L with the potential to down regulate progranzyme and perforin processing. Using cystatin F mutants we demonstrated that the levels of active cathepsin C and granzyme B in NK cells directly depend on the presence of cystatin F and its activation from dimeric to monomeric form. Target cells, like tumor cells, may secrete inactive dimeric cystatin F which is internalized to cytotoxic cells and inhibits granzyme and perforin activation in secretory granules inducing in this way NK cell anergy. We showed that the glycosylation pattern determines its secretion, internalization to NK cells and trafficking to endosomal/lysosomal vesicles. A similar cystatin F dependent regulatory mechanism we demonstrated also for CTL. These cells showed significantly reduced cytotoxicity against target cells and increased levels of cystatin F. In addition, using proximity ligation assay and confocal microscopy we found cystatin F to be co-localized with cathepsin C and granzyme B. Our results suggest cystatin F as an important mediator

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used by tumor cells to impair NK and T cell cytotoxicity and to escape antitumor immune response. By disabling internalization or activation of cystatin F we could increase the cytotoxic potential of NK and T cells and improve the immunotherapy of cancer patients.

P.4.1-052
The progression of glioblastoma multiforme (GBM) is regulated by TGF-beta/Snail-1/Cx43 signaling axis

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Phenotypic heterogeneity of glioblastoma multiforme (GBM) cells is crucial for GBM progression and for the formation of its invasive front. Because the development of invasive cancer cell sub-populations is governed by TGF-beta/Snail-1/Cx43 signaling axis, we focused on the involvement of this system in the phenotypic diversification of GBM cells. In T98G cell populations, stable lineages of "epithelioid" are accompanied by rear-polarized "fibrobastoid" cells that spontaneously form T98G invasive front in vitro and in vivo. Short-term TGF-beta treatment recruited "epithelioid" T98G cells to GBM invasive front through their "secular" transitions towards the rear-front polarized, "fibroblastoid" phenotype, as demonstrated by time-lapse videomicroscopy, fluorescence microscopy and transmigration assays. Furthermore, permanent phenotypic reprogramming towards "fibroblastoid" phenotype was seen in "epithelioid" T98G cell lineages after long-term TGF-beta stimulation. Ectopic expression of Snail-1 led to Cx43 up-regulation and increased the invasiveness of "epithelioid" T98G cells, whereas the opposite cell reactions were observed upon ectopic Snail-1 down-regulation. Corresponding effects were seen upon the manipulation of Cx43 levels in these cells, whereas "fibroblastoid" T98G cells were less sensitive to Snail-1/Cx43 up-regulation. Our observations indicate that TGF-beta regulates the microevolution of invasive GBM cell subpopulations, thus determining the formation of GBM invasive front in a Snail-1/Cx43-dependent manner. Our data illustrate the role of EMT-related TGF-beta/Snail-1/Cx43 signaling axis in secular and permanent cell reprogramming of GBM cells and show the interrelations between Snail-1 and Cx43 in GBM progression.

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P.4.1-053
Snail-1/Cx43 signalling axis regulates the sensitivity of glioblastoma multiforme cells to temozolomide

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Temozolomide (TMZ) is an alkylating agent, which easily penetrates blood-brain barrier and isindependent of hepatic metabolism. Therefore, it is commonly used in the chemotherapy of glioblastoma multiforme (GBM), where it is suspected to exert a cytostatic effect by the methylation ofguanine and the induction of base-pair mismatches during DNA replication. However, the preciseconsequences of anti-tumorigenic TMZ effects in GBM chemotherapy remain unclear, especially in thelight of the reports on GBM cell resistance to TMZ. To estimate the mechanisms of the tolerance ofGBM cells to TMZ, we investigated a short and long-term impact of TMZ on the invasive properties ofT98G cells, including their motility and transmigration potential in vitro. A cytostatic/pro-apoptotic effectof TMZ on T98G cells was accompanied by a relatively high invasive potential of T98G cells thatmanaged to survive a pulse TMZ treatment. Furthermore, a permanent reprogramming of TMZ-resistant T98G cells towards an aggressive, Snail-1/Cx43- positive, fibroblastic-like phenotype wasobserved after a long-term TMZ treatment. These cells maintained increased invasive properties afterTMZ ablation, as confirmed by the invasion tests ex vivo and in vivo. Ectopic down-regulation of Snail-1 or Cx43 sensitized these cells to TMZ, whereas Snail-1/Cx43 up-regulation by expression vectorsincreased the resistance of wildtype T98G cells to this drug. These observations suggest thatTGFβ/Snail-1/Cx43 signaling axis regulates the resistance of GBM cells to TMZ and confirm theinterrelations between Snail-1 and Cx43 in GBM progression.

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P.4.1-054
Aptamers as a new tool for identification of cancer-related markers

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Advances in the research on various types of cancer provide new opportunities in the clinical development of cancer therapy. Aptamers - single stranded oligonucleotides are novel tools in this area, mainly because of their unique properties. They can bind to various proteins, thus changing their properties and influence their 3D structures. Aptamers-based targeted cancer therapy has been evolving in recent years, with a primary focus on aptamers as potential diagnostic tools.

Identification of proteomic targets for selected aptamers A26 and A33 in prostate cancer cells was a crucial aspect of present research. Application of targeted methodology with atto488-labeled aptamers allowed for recognition of specific aptamer-protein interactions. Changes in hydrophobic properties of fluorescently labeled aptamer-protein complexes enabled analysis using RP HPLC with fluorescence detector combined with mass spectrometry analysis.

Comparison of proteins identified after incubation with aptamers A26; A33, and A33sc as a control, revealed specific targets. Proteins KI67, CAD23, and PPARD were characteristic for interactions with A26 aptamer. Proteins: CC120, SYNE1, and MACF1 were found to be specific for A33 aptamer.

Moreover, the proteins selected in this experimental set-up represent an attractive target for further studies on aptamers and their possible role in cancer treatment and diagnosis.

The approach based on fluorescently labeled aptamers for fishing out the aptamer-protein complexes gives unequivocal results in contrast to application of biotinylated aptamers, because of the large number of nonspecific interactions. Designing and

optimization of the robust proteomic approach to study aptamers and their action, allowed for providing the results of great interest and importance in cancer investigations.

P.4.1-055
The redox status and migratory potential of newly synthetised hydantoin derivatives 3-Benzyl-5-isoprpyl-5-phenylhydantoin in human colon cancer cell line HCT-116

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Cancer treatments have been aimed to finding effective newely or modified chemicals, which should increase anti-tumor activity, be highly selective, have low toxicity and minimum side effects. The hydantoin and its derivates are effective in controlling of a variety of inflammatory disorders and are one of the oldest used anticonvulsives, but recently their antitumor activities have been indicated. In this study anti-proliferative, antioxidative and migration effects of hydantoin derivate 3-Benzyl-5-isoprpyl-5-phenylhydantoin were tested on human colon cancer cell line HCT-116. The cells were exposed to an increasing concentrations, from 0.01 μM up to 100 μM. Anti-proliferative index and nitrite levels of this hydantoin derivate against HCT-116 cell line were determined spectrophotometrically after short-term (24 h) and long-term (72 h) treatments. The effects on migration potential were tested for concentrations of 1 μM and 10 μM after 24 h and 72 h exposure also. Migration index was determined by Boyden chamber transwell migration assay. The results obtained in this study showed that all used concentrations of 3-Benzyl-5-isoprpyl-5-phenylhydantoin exhibited strong antiproliferative activity on HCT-116 cells. The results also showed increased concentrations of nitrites (indicator of NO) at supernatants of cultivated cells, which indicate significant antixidative role of this derivate. These data indicate that elevated NO production could be one of the causes of decreased migration of this cell line induced by hydantoin derivate, since NO plays significant role in cell mobility. Based on these results, it can be concluded that the tested hydantoin derivate has a strong antiproliferative effects and reduces the migration index of HCT-116 cell line, suggesting significant potential antitumor role of this molecule. In general, these results indicate antitumor activity of 3-Benzyl-5-isoprpyl-5-phenylhydantoin and suggest its potential antitumor applications.

P.4.1-056
Targeting Upr mechanism by dasatinib as a promising therapeutic approach in chronic myeloid leukemia

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Unfolded protein responses (UPR) is a cytoprotective mechanism which is activated by ER stress. Although UPR maintances balance by providing control of proteins in the cell, UPR can protect tumor cells from inappropriate condition in many different human cancers including chronic myeloid leukemia (CML). Due to its adaptive mechanism, tumor cells can resist to stressful conditions. Many studies showed that activation of this adaptive mechanism is required for cancer cells to maintain malignancy and therapy resistance. Therefore, targeting this adaptive mechanism might be promosing approach to treat cancer such as CML. In this study, we determined the effects of dasatinib on CML cell model K562, with determining the expression levels of specific genes related with UPR.

The cytotoxic effects of dasatinib were determined by WST-1 analysis in time and dose manner of K562 cell line. Total RNA was isolated from K562 cells treated with dasatinib and untreated cells were accepted as control group. Reverse transcription procedure was performed for cDNA synthesis. The RT-qPCR is used for gene expression analysis. Gene expression levels were evaluated by using RT^2 Profiler PCR Array. IC_{50} value of dasatinib, 4.6 nM for 48th hour, changed expressions of targeted-genes related with UPR.

As a result, ATF6, transcriptional regulator of some ER-chaperones, was downregulated 3.01 fold. HSPA5 (GRP78) is known overexpressed in CML involved in the folding and assembly of proteins in the ER was downregulated 4.83 fold. HSPH1 gene (Hsp110) which prevents the aggregation of denatured proteins under stress conditions showed 3.13 fold decrease. HERPUD1 gene is related to the accumulation of unfolded proteins in the ER showed 3.50 fold decrease.

In conclusion, the results show that dasatinib distrupts the UPR mechanism that play significant role in cancer progression and therapy-resistance in CML. Thus, dasatinib-induced dysregulation of UPR mechanism may promise encouraging therapy for CML.

P.4.1-057
Neuropeptide Y stimulates proliferation and inhibits migration capacity by downregulating iNOS gene expression in human trophoblast cell line JEG-3

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Trophoblast migration is an essential step in implantation/placentation and failure in this process could result in poor placental perfusion and oxidative stress that contribute to several pregnancy disorders. Neuropeptide Y (NPY) is a bioactive peptide involved in various physiological processes and recently was shown to be indicative for several pregnancy complications connected with diminished trophoblast migration. In this study we have investigated the role of NPY in different aspects of trophoblas functions, important during placentation process. Human choriocarcinoma cells JEG-3 were exposed to NPY in short-term (24 h) and long-term (72 h) treatments, respectively in concentrations of 0.1 nM (physiological concentration) and 1 nM (pathological concentration corresponding to NPY levels in pregnancy disorders). The aim of this study was to determine the effects of NPY on proliferation, nitrite production and migration capacity of JEG 3 cells. Proliferation ratio was measured by using trypan blue staining and MTT assay, changes in migration index were determined by using Boyden chamber transwell migration assay, nitrites (indicator of NO) by using Griess assay and iNOS gene expression by qRT PCR protocol. The examinated parameters were detected spectrofotometrically using ELISA microplate reader and by gene expression software. The obtained results suggest that NPY induces proliferation in both used concentrations, and nitrite levels were decreased in 1 nM NPY treatment. The migration index was unaltered in concentrations of 0.1 nM, and significantly decreased after 1 nM NPY treatment. Also, the data showed significant decrease in iNOS iRNA levels after 72 h of 1 nM NPY treatment. These results

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suggest that NPY at elevated levels could reduce trophoblast migration by inhibiting the expression of iNOS gene, but is useful in its physiological concentrations inducing significant trophoblast proliferation, an important aspect of migration on maternal tissue.

P.4.1-058
Fenofibrate attenuates pro-tumorigenic potential of cancer stem cell-like cells in drug-resistant prostate cancer cell populations

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Multipotent "cancer stem cells" account for the progression and drug-resistance of prostate cancer. Therefore, they represent an attractive target for novel palliative therapies of advanced, drug-resistant prostate cancers. Due to its excellent tolerability profile and anti-cancer activity, fenofibrate (FF) has been pinpointed as a potential metronomic agent that could augment the sensitivity of cancer cells to chemotherapeutic drugs. We hypothesized that FF can interfere with the persistence of the drug-resistant phenotype of prostate cancer cell lineages through the interference with the functions of multipotent cancer stem cell-like (SCL) cells. FACS analyses of wildtype and docetaxel(DCX)-resistant DU145 cells revealed minute sub-populations of the drug-resistant and multipotent $CD133^{+}/CD44^{low}$, $CD133^{+}/CD44^{high}$ and $CD133^{-}/CD44^{high}$ SCL cells. Upon prolonged propagation in vitro, SCL cells were capable of forming the aggregates of round and loosely attached cells, surrounded by morphologically diverse cellular clusters. The abundances of SCL cells were elevated within DCX-resistant DU145 cell populations. DCX increased the fraction of SCL cells within the wildtype DU145 cell populations, whereas FF considerably reduced the ability of both wildtype and DCX-resistant DU145 cells to generate $CD133^{+}/CD44^{low}$SCL cells. The progeny of $CD133^{+}/CD44^{high}$ SCL cells derived from DCX-resistant DU145 cells was less sensitive to DCX and FF but retained high sensitivity to the combined DCX/FF treatment. These observations confirm that the phenotypic plasticity of SCL cells and/or of their progenies participates in the maintenance of prostate cancer cell heterogeneity and drug-resistance. They also indicate that FF can enhance the efficiency of palliative prostate cancer therapies through the interference with the function of cancer stem cells. *Project financially supported by the Polish National Science Centre (2015/17/B/NZ3/01040).

P.4.1-059
Determination of AURK A and AURK B expression levels in dasatinib-treated K562 cells

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Chronic myeloid leukemia (CML), genetically characterized by a reciprocal translocation between chromosomes 9 and 22, is a chronic blood disorder that causes the creation of a chimeric gene encoding BCR-ABL fusion protein with a constitutive tyrosine kinase activity. Patients are generally treated by tyrosine kinase inhibitors (TKIs). Dasatinib is the second-generation tyrosine kinase inhibitor that is used for inhibition of Bcr-Abl.

Mitotic catastrophe is a type of cell death that occurs during or immediately after an erroneous mitosis. Escape of cells from apoptosis via mitotic catastrophe is clinically important. Aurora kinases (AURKA and AURKB) have important function in chromosome alignment, segregation and cytokinesis during mitosis. İn addition, BCR-ABL1 activation induces AURKA and AURKB expression and inhibition of these molecules are associated with mitotic catastrophe. Our aim is to evaluate whether dasatinib contributes mitotic catastrophism on CML cells or not. The cytotoxic effect of the dasatinib in K562 cell line was found to be 4.6 nM and evaluated by WST-1 analysis. The effect of dastinib on expression levels of AURKA and AURKB in K562 was determined by quantitative RT-PCR relatively. After the 48 hours treatment with dasatinib, expression levels of mitotic catastrophe related AURKA and AURKB genes were downregulated 2.1 and 2.3 fold in K562 cells, respectively.

In conclusion, we determined that dasatinib, may also be effective in mitotic catastrophy as well as in apoptosis induction. We also suggest that the use of dasatinib with Aurora kinase inhibitors will be of clinical benefit.

P.4.1-060
Assessment of cytokines level in management of patients with prostate cancer

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Background: Prostate cancer (PCa) is a major health problem in modern society, and its prevalence is continuously increasing. Furthermore, PCa is currently the second most common cause of cancer death in men. To improve the outcome prediction for patients treated with radical prostatectomy, a personalized medicine strategy, starting from proteomic and genomic approach for tumor and patient must be applied.
Methods: We determined cytokine levels in sera from 23 patients with prostate cancer, 5 with benign prostate hyperplasia and 9 healthy controls. Using Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Merck-Millipore) on a Luminex 200 system, we analyzed 12 analyte-specific bead sets: IL-2, IL-10, IL-1β, IL-12, IL-4, IL-6, IL-8, TNFα, VEGF, FGF-2, G-CSF, IFNγ. Multiplex data acquisition and analysis were performed using xPONENT 3.1 software.
Results: The assessed serum level of cytokines/chemokines/angiogenesis factors was increased in Pca patients compared to the control group (IL-6 – 3.5, IL-8 – 3.9, TNF-α – 1.2, FGF-2 – 4.1, VEGF –4.5; GM-CSF –6.7; IFNɤ - 3.7 fold higher). It has also been observed increases in level expression of these biomarkers in benign prostate hyperplasia versus control.
Conclusions: Proteomics represents a promising approach for the discovery of new biomarkers able to improve the management of PCa patients, an important tool to identify new molecular targets for PCa tailored therapy. Identification of markers associated with multi-stage PCa will provide greater scientific understanding of possible causes and underlying mechanisms, and important insights needed for improving life expectancy.
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P.4.1-061
Role of therapy-induced cellular secretomes in resistance of cancer cells

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Tumors easily develop resistance to chemotherapeutic agents. One of the well-known examples is the development of ovarian cancer resistance to the platinum-based agents. This is explained as follows: the small number of tumor cells having survived chemotherapy treatment rapidly form new tumor that is not susceptible to the effect of agents already having been used. The phenomenon is well known in clinical practice but is poorly studied at the molecular level. There is evidence, that cells dying under the effect of the therapy secrete signaling molecules into the extracellular medium to promote survival of neighbor cells. This study was aimed on search for the molecules responsible for development of resistance to chemotherapeutic drugs. We performed LC-MS/MS analysis of secretomes of ovarian cancer cells before and after cisplatin treatment. We also analyzed proteomes of malignant ascites from several ovarian cancer patients prior to and after chemotherapy. We found an exciting phenomenon: therapy-induced secretomes were enriched with spliceosomal components. However, under normal conditions, most spliceosomal proteins and snRNAs are localized in the nucleus of the cell. To address this we performed proteomic analysis of extracts from cytoplasmic and nuclear fractions derived from the same samples that were used to generate secretomes. Interestingly, according to our data, after treatment of cells with cisplatin, spliceosomal proteins significantly increased in the cytoplasmic fraction. This indicates the relocalization of spliceosomal proteins from the nucleus into the cytoplasm under the influence of therapeutic stress. The splicing factors identified in therapy-induced secretomes were linked to induction of epithelial-to-mesenchymal transition leading to more aggressive phenotype of cancer cells. These data may provide potential prognostic markers predicting the success of treatment. The work was supported by the RSF 14-50-00131 and RFBR 16-04-01414, 16-34-60136.

P.4.1-062
Post-transcriptional modulation of ABC transporters by human papillomavirus proteins E6 and E7 in head and neck squamous cell carcinoma (HNSCC). Impact on multidrug resistance

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HNSCC represents the sixth most frequent malignancy worldwide, HPV infection being a major risk factor. HPV^+ HNSCC leads to a different disease pattern than HPV^- HNSCC. E6 and E7 are responsible for the oncogenic potential of HPV and interact with several signalling pathways of the host cell. ABC transporters are proteins mediating the efflux of chemotherapeutic agents and cancer multidrug resistance. The aim of this work was to evaluate the effect of E6 and E7 from high risk HPV types on the expression of the transporters ABCB1, ABCC2 and ABCG2, the molecular mechanisms and the impact on drug resistance in HNSCC. Oropharyngeal cancer cell lines HNO206 and HNO413 were transfected with plasmids codifying E6/E7 from HPV16, -18, or without insert (control). Transporter expression was assessed at protein level by western blot and, for treatments exhibiting a significant effect, also at mRNA level by qRT-PCR. HPV18 E6/E7 up-regulated the protein levels of ABCB1, ABCC2 and ABCG2 (+88%, +130% and +165%, respectively) in HNO206 cells and ABCG2 in HNO413 cells (+53%). At mRNA level, HPV18 E6/E7 up-regulated *ABCC2* and *ABCG2* (+44% and +87%, respectively) and down-regulated *ABCB1* (−31%) in HNO206 cells. HNO413 cells exhibited an *ABCG2* mRNA up-regulation by HPV18 E6/E7 (+121%). Assays with actinomycin D indicated stabilization of *ABCC2* mRNA ($t_{1/2}$ = 9.7 h vs. 5.8 h) in HNO206 cells and *ABCG2* mRNA in HNO206 and HNO413 cells by HPV18 E6/E7 ($t_{1/2}$ = 22.5 h vs. 6.7 h and 48.7 vs. 17.8 h, respectively). Protein half-life analysis showed no stabilization of ABCB1 protein by E6/E7, rather suggesting a translational regulation. In addition, HPV18 E6/E7 increased the resistance to the ABCB1 substrate paclitaxel (+95%), the ABCC2 substrate cisplatin (+107%) in HNO206 cells and the resistance to the ABCG2 substrate 5-fluorouracil in HNO206 and HNO413 cells (+269% and +56%, respectively). These results suggest a differential response to HNSCC chemotherapy due to HPV status of the tumors.

P.4.1-063
Structure-activity relationships in polymorphic G-quadruplex forming segment of c-MYC promoter

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One of the driving forces of malignant transformation is activation of a proto-oncogene that is converted into oncogene by a mutation, which changes the protein function or expression. Misregulation of the proto-oncogene c-Myc has been identified in many human cancers. Therefore, the detailed knowledge of c-Myc regulation is necessary to develop appropriate therapies. Expression of c-Myc is co-regulated by a G-quadruplex that can be formed from a G-rich motif Pu27 within the c-Myc promoter. Pu27 consists of five G-tracts separated by one A or T nucleotide. Formation of a G-quadruplex requires only four G-tracts. Hence, the Pu27 segment give rise to several distinct G-quadruplex conformations. It is not known, which particular G-quadruplex conformation bears the biological role. To address this point, we have prepared a set of Pu27 oligonucleotides with naturally occurring nucleotide substitutions in different G-tracts that limit the number of possible G-quadruplex conformations. Using single particle FRET, we have identified populations of the G-quadruplex topologies that the Pu27 variants form *in vitro*. By performing pull-downs from nuclear lysates, we have revealed proteins that bind to the particular G-quadruplexes. To test the function, Pu27 element in the human c-Myc promoter has been mutated and the activity examined in a luciferase reporter assay. Preliminary data from our experiments indicate that there are several structural topologies of G-quadruplex formed from Pu27 sequence, which are functionally equivalent.

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P.4.1-064
Evolutionary trade-offs in tumor development

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The mutational theory of cancer postulates that genome alterations confer a proliferative advantage to a cell, allowing it to expand into a tumor. Tumors need to perform other biological tasks in addition to proliferating, such as escaping the immune system, recruiting blood vessels, metastasizing, etc. These tasks require different gene expression profiles and no single gene expression profile can be optimal for all tasks. This leads to a trade-off situation. Different tumors may make different trade-offs and prioritize some tasks over others, causing inter-tumor variability. Such variability in genome alterations, gene expression and drug sensitivity across tumors has been well documented by large-scale tumor profiling efforts like METABRIC or TCGA. If tumors face trade-offs, multi-task evolution theory predicts that tumors should fall on a low-dimensional polytope in gene expression space. We find that gene expression profiles from 2000 + breast tumors from the METABRIC cohort are well described by a polytope with four vertices. The vertices of the polytope represent 'archetypes' which are specialists at a certain task. The task of an archetype can be inferred from statistically enriched clinical and biological properties of tumors closest to this archetype. This enrichment analysis suggests that primary breast tumors face trade-offs between proliferating, signaling, growing biomasss, and differentiating into a healthy tissue. These tumor archetypes are characterized by different driving genome alterations. Finally, the sensitivity of tumors to drugs depends on their position relative to the archetypes. These results suggest that multi-task evolution could serve as a theoretical framework to explain the diversity of gene expression, genome alterations and drug sensitivities across tumors.

P.4.1-065
Regulation of cell-to-cell communications by urokinase system during angiogenesis

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It was shown that during tumor growth mesenchymal stem cells are integrated into the tumor-associated stroma of the cancer. However, the mechanisms of participation of these cells in tumor angiogenesis have not been fully understood. In many cancer tumors dramatic increase in the activity of the urokinase system was found. Urokinase system – urokinase-type plasminogen activator (uPA), its high affinity receptor (uPAR; CD87) and plasminogen activator inhibitor 1 (PAI-1) - is implicated in regulation of several steps of angiogenesis - endothelial cells division, migration, degradation and invasion of the abluminal basement membrane and formation and stabilization of vascular network. uPA promotes pro-angiogenic signaling upon binding to several surface receptors, including uPAR, low-density lipoprotein-related receptor (LRP/a2MR) and integrins. uPAR-bound uPA is typically localized on the leading edge of the migrating endothelial and other cells. In this study, we used a two-dimensional co-culture model of human umbilical vein endothelial cells (HUVEC) with human adipose stromal cells (ADSC) without exogenous matrix addition to study self-assembled network formation(SNF) by HUVEC. We observed that co-culture results in up-regulation of expression of uPA, uPAR and PAI-1. Upon direct HUVEC –ADSC co-culture uPAR was upregulated on the surface of HUVEC and the concentration of uPA increased in conditioned medium. uPAR is essential for SNF since blocking of uPAR by specific antibodies abrogated this process. We also found that endocytosis machinery is important for SNF by HUVEC in the presence of ADSC by using the LRP antagonist - RAP, inhibitor of intracellular protein transport - monensine, or inhibitor of microtubule polymerization – colhicine. Thus we found that urokinase system is critical for cell-to-cell interactions between HUVECs and ADSC during SNF. *The work was supported by Russian Science Foundation grant # 17-15-01368.*

P.4.1-066
Targeting cancer chemoresistance via mitochondrial dysfunction and autophagy inhibition

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It is believed that majority of death in cancer patients can be due to acquisition of radio- or chemoresistance by a small fraction of cells called cancer initiating cells (CIS) which are more tumorigenic than the rest of the cancer cells. However, unlike majority of cancer cells CIS resemble normal ones as their bioenergetic pathways rely more on oxidative phosphorylation rather than on glycolysis. They also have moderate level of oxidatives stress and more functional mitochondria which may provide a different approach for targeting cancer resistance. Recently the idea of targeting mitochondria with antibiotics to induce mitochondrial dysfunction (MDF) and to perform cellular caloric restriction has been proposed. Based on in vitro and in vivo models, here we report our recent findings on exploring the possibility that antibiotics of several classes affecting mitochondrial functions can also target CIS as well as chemoresistant cancer cells. In addition, we provide compelling evidence that inhibiting autophagy is essential to specifically amplify effects of antibiotic-induced MDF. We conclude that there is a therapeutic potential for eradicating cancer chemoresistance by reducing autophagy and combinatorial treatment with specific antibiotics.

P.4.1-067
Differential expression of genes involved in energy metabolism in colorectal cancer

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Energy metabolism change is one of the main cancer hallmarks, and it characterized by glycolysis activation. More than 30 enzymes are involved in glycolysis but causes, and mechanisms of its activation in different cancer types are still not completely

uncovered. Some genes encoding the glycolytic enzymes demonstrate tumor-specific activation or inactivation. Aberrant expression by a different set of genes is specific for different types of cancer. We studied the expression of these genes in colorectal cancer (CRC) using bioinformatics analysis of transcriptome database and qRT-PCR.

For the preliminary screening of gene expression in the CRC, we used "The Cancer Genome Atlas" database. We identified 21 genes involved in energy metabolism and their regulation were characterized by differential expression in CRC: HK1, HK2, HK3, PFKL, PFKP, PFKM, ALDOA, ALDOB, PGK1, PGAM1, PGAM2, BPGM, ENO1, ENO2, ENO3, PKM2, PKLR, OGDHL, TP53, PRKAA2 and HIF1A. Significant expression changes were observed in genes involved in phosphorylation of glucose to glucose-6-phosphate (HKs). Expression level of HK2 was decreased, while HK2 expression is slightly decreased, and for HK3 it was significantly increased. Decreased expression level was observed for PFKP gene involved in phosphorylation of fructose-6-phosphate. Changes in PFKL and PFKM expression level were not found. In addition, deregulation of genes involved in synthesis of phosphoglycerate was identified. Expression level of PGAM2 was decreased whereas PGAM1 expression was stable. Moreover, we observed decrease in the expression of OGDHL gene, which encodes a subunit of OGDC complex involved in the TCA-cycle. We revealed also increased expression level of HIF1A gene, encoding a factor induced by hypoxia. Therefore, we have detected several specific features which provide deregulation of energy metabolism in CRC.

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P.4.1-068
The differential antitumor effects of Cannabis extracts on various cancer cell types

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Cannabinoid compounds and their analogs are known to have palliative effects in oncology. There is accumulating evidence of cannabinoids' antitumor effects as several studies have shown a regression of different cancer types *in vivo* in response to cannabinoids. Several other studies revealed a proapoptotic and antiproliferative response to some cannabinoids *in vitro*, as well as inhibitive effects on the invasion and migration of cancer cells. Our results demonstrated that different *Cannabis* extracts produce a variety of antitumor effects: specifically, induction of apoptosis and inhibition of proliferation. In order to demonstrate differential antitumor effects of differing *Cannabis* extracts, we established a method for screening the varying antitumor effects of cannabinoid compounds on various cancer subtypes *in-vitro*. Our results show that several *Cannabis* extracts are very potent in killing tumor cells. We found that different strains (each with a distinct cannabinoid composition) have differing yet deleterious effects on various tumor cell lines. Depending on the cancer-driving mutation, we suggest that specific pathways are activated or inhibited by the application of *Cannabis* extracts.

P.4.1-069
L-canavanine selectively impaires motility of human glioblastoma cells under arginine deprivation

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Glioblastoma is one of the most devastating cancers, characterized by a poor prognosis and high rates of recurrence. It is hardly amenable to conventional chemotherapies because of its high motility and invasive nature, and the problem of brain-blood barrier permeability for a drug. Single amino acid starvation is increasingly recognized as a potentially efficient strategy for cancer treatment. We propose arginine deprivation-based combinational treatment with the arginine antimetabolite L-canavanine. We compared the cytotoxic effect of L-canavanine for human U251MG and U87MG glioblastoma cells as experimental models.

Many cell biological processes, such as migration, morphogenesis, cytokinesis, and endocytosis, rely on a dynamic of actin cytoskeleton. Combinational treatment caused significant changes in actin polymerization. Reduction in F-actin-associated fluorescence was accompanied by a decrease in filamentous actin polymer level as well as symptoms of apoptotic cell death. It should be noted that no substantial changes were observed neither in the morphology nor in the actin cytoskeleton organization on the control isolated rat glia cells subjected to the same experimental conditions as the glioblastoma cells.

Cell migration and invasion have an important role in cancer metastasis. We observed the disassembly of focal adhesions under combination of arginine deprivation with canavanine treatment. But expression of the proteins involved in adhesion contact formation did not change with respect to control cells except of the levels of focal adhesion kinase (FAK).

Thus our observations seem to indicate that lack of arginine in combination with canavanine profoundly and selectively impaired motility and adhesion of human glioblastoma cells lines and showed that these effects were due to specific alterations in the actin cytoskeleton organization.

P.4.1-070
The integrin beta3 Leu33Pro polymorphism and cancer risk

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Platelets and their fibrinogen receptors may be central to haematogenous cancer spread, in addition to various adhesive proteins on both platelets and tumor cells. The Leu33Pro polymorphism of the beta3 subunit modulates the function of alpha (IIb) beta3a integrin receptor on platelets. We examined whether this polymorphism influences cancer risk and progression. Using participants from the Moscow population, we assessed the risk of prostate cancer (PC) and bladder cancer (BC) in individuals with the Leu33Pro polymorphism relative to those without the polymorphism (non-carriers). Integrin Leu33Pro polymorphism was genotyped using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. Concerning the metastases group for BC the frequency of Leu33Pro polymorphism carriers was higher than that in non-invasive group

($P < 0.02$). For the Leu33Pro polymorphism carriers the frequency of local PC is 2.8 times higher and the one of metastases PC is 2.4 times higher than that for non-carriers patients. We conclude that the integrin Leu33Pro polymorphism does not modify the cancer risk but it may influence the metastasis spread and the malignant potential of BC and PC. Our data suggest that tumor progression for the patients with Leu33Pro polymorphism has a greater rate of local invasion and metastases. Our findings are compatible with laboratory observations that integrin Leu33-Pro polymorphism is associated with increased reactivity and aggregability of platelets in vitro and with increased cell cycle progression or tumor cells proliferation through extracellular signal–related kinase, which has an impact on metastasis and malignant potential of tumor BC. The Leu33Pro polymorphism seems to be a novel prognostic factor for BC and PC and is useful and convenient for high risk patients screening and treatment.

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P.4.1-071
The role of tumor-associated inflammation in brain gliomas pathogenesis

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The investigation of tumor-associated inflammation (TAI) as the main pathogenetic factor of brain gliomas growth and progression was carried out. This process is realized on systemic level through decreasing of membrane potential, which is mediated by blood cells aggregation promotion. The red blood cells (RBC) aggregation rate was evaluated with the method of surface plasmon resonance. The connection between blood cells aggregation rate and glioma cells compactness was detected as the main event in tumor progression, because tumor stem cells are accumulated in more solid cell layers. RBC membrane targets were investigated for single-minded influence on aggregation processes with an aim of inflammation inhibition. High and low concentrations of verapamil and ketamine showed the mechanism of development and peculiarities of changing in RBC aggregation in patients with brain gliomas. As a result a pre-surgical subsidiary differential diagnostics between inflammations and tumors processes, between low and high malignant gliomas was developed. A link was found between RBC aggregation modifications under influence of ionotropic NMDA-receptors blocators (ketamine and verapamil), and possibility of peripheral blood lymphocytes to support genome stabilization. Dependences were discovered between proliferative function of lymphocytes and peripheral RBC aggregation rates, as well as between the last one and activity of polyamine oxidation enzymes diamino- and polyamine-oxidases (DAO and PAO). The verification of TAI role in glioma pathogenesis was found in a correlation between RBC aggregation rate under impact of verapamil and ketamine in low doses and its antitumor activity in the experiments on the rats with high malignant rat glioma culture 101.8, wich is an analogue of human glioblastoma. *The publication was financially supported by the Ministry of Education and Science of the Russian Federation (the Agreement No.02.A03.21.0008).*

P.4.1-072
Mechanical properties and molecular regulation of invadopodia formation in melanoma cells

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Invadopodia are actin-rich protrusions of the plasma membrane through which cells adhere to the extracellular matrix and degrade it. They are commonly formed by cancer cells and believed to promote tumor invasion and metastasis.

In this study, we explored the mechanical properties that enable invadopodia penetration into the extracellular matrix (ECM), and the regulation of their formation in melanoma cells. We show that the core actin bundle of invadopodia interact with integrin-mediated matrix adhesions at thier basal end, extends through a microtubule-rich cytoplasm, and interact, at thier apical end, with the nuclear envelope and indent it. We show that these mechanical interactions are important for invadopodia stability as well as for their invasive function. In addition, we explored the molecular regulation of invadopodia formation, focusing on the involvement of protein tyrosine kinases in their assembly. We would like to report here on a particular involvement of AXL receptor tyrosin kinase in invadopodia formation, which is known to be a mechanical sensor in cells. We show here that AXL is physically associated with the actin cores of invadopodia and regulates their development. Knock down of AXL significantlly increases invadopodia formation, function and actin dynamics, but, interestingly, over expression of the protein also leads to a similar effect. In contrast, knock down of AXL reduces invasion and migration in transwell, as suggested previously, suggesting that invadopodia-mediated adhesion not necessarily enhance invasion, but may, rather, restrain cell invasion when they are not regulated. Using AXL specific inhibitor we show that there is a "window" of AXL activity that is optimal for invadopodia function. This window might have to do with the mechanical state of the cells. Overall we propose that fine tuning of AXL levels is involved in the regulation of invadpodia formation and activity.

P.4.1-073
microRNA-mediated regulation of human papillomavirus type 16 oncogene expression

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Persistent infection with high- risk human papillomaviruses (HR-HPVs) can cause cervical cancer at various sites of the anogenital tract, including the cervix, as well as a subset of head and neck cancers. Overexpression of the viral oncogenes E6 and E7 triggers initiation of epithelial cell transformation. However, the molecular mechanisms that control the viral gene expression are obscure. Recent data suggest that host microRNAs may contribute to regulation of viral carcinogenesis by targeting viral genome. The purpose of this study was to detect the microRNAs differentially expressed in cervical squamous cell carcinomas samples and normal squamous epithelium of the cervix, with a focus on the cellular microRNAs with potential to regulate HPV16 viral gene expression. Using the next generation sequencing, we identified 52 cellular microRNAs with aberrant expression in cervical cancer. Six down-regulated microRNAs have the potential binding sites in the E6/E7 mRNA as well as in upstream

regulatory region (URR) HPV16. Four microRNAs (miR-135a, -135b, -196a and 224) with putative binding sequences on E6 mRNA HPV16, showed statistically significant downregulation of the E6 mRNA in transfection experiments. Two microRNAs (miR-135a and -224), may effect expression of the viral oncogenes through the viral long non-coding RNA (lncRNA) transcribed in the URR HPV16. Knockdown of the viral lncRNA using siRNAs modulates the viral oncogene expression in cervical cell lines (Siha and Caski). In conclusion, we demonstrated, for the first time, that host miRNA-135a, b, -196a and 224 negatively regulate expression of the E6 HPV16. These data suggest that cellular microRNAs may play an important role in the human papillomavirus 16 regulation and may serve as potential targets for future antiviral drug development and treatment of HPV-associated lesions.

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P.4.1-074
Isoprenoids responsible for protein prenylation modulate the biological effects of statins on pancreatic cancer cells

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Statin treatment of hypercholesterolemia is accompanied also with depletion of the mevalonate intermediates, including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) necessary for proper function of small GTPases. These include Ras proteins, prevalently mutated in pancreatic cancer. In our study, we evaluated the effect of three key intermediates of the mevalonate pathway (mevalonate/FPP/GGPP) on GFP-K-Ras protein localization in MiaPaCa-2 human pancreatic cancer cells carrying a K-Ras activating mutation (G12C) after exposure to individual statins (20 μM) using fluorescence microscopy. The changes in gene expression induced in MiaPaCa-2 cells treated with simvastatin, FPP, GGPP, and their combinations with simvastatin were examined by whole genome DNA microarray analysis. All tested statins efficiently inhibited K-Ras protein trafficking from cytoplasm to the cell membrane of the MiaPaCa-2 cells. The inhibitory effect of statins on GFP-K-Ras protein trafficking was partially prevented by addition of any of the mevalonate pathway's intermediates tested. Expressions of genes involved in metabolic and signaling pathways modulated by simvastatin treatment was normalized by the concurrent addition of FPP or GGPP. K-Ras protein trafficking within the pancreatic cancer cells is effectively inhibited by the majority of statins; the inhibition is eliminated by isoprenoid intermediates of the mevalonate pathway.

P.4.1-075
A VDAC1-based peptide as a therapeutic agent for liver cancer

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Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related deaths. The incidence of HCC is increasing, despite progress in identifying risk factors. Therefore, innovative therapeutic strategies are urgently required. A multi-functional mitochondrial protein, the voltage-dependent anion channel (VDAC1), is identified here as a highly innovative target for liver cancer therapy. VDAC1 serves as the "gate" for metabolic and survival signals, thus regulating cellular energy and metabolism. VDAC1 is also involves in mitochondria-mediated apoptosis, and interacting with the anti-apoptotic proteins as Bcl2, Bcl-xL and hexokinase, over-expressed in cancers. Recently we designed and tested a cell-penetrating VDAC1-based peptide, Tf-D-LP4, that bind to anti-apoptotic proteins and induce cell death in a panel of cancerous cells and inhibits tumor growth in several liver cancer mouse models Here, we validated the effect of the peptide in liver cancer cell lines and mouse models; diethylnitrosamine (DEN)-induced liver cancer in C57BL/6 mice and metabolically-induced HCC. Intravenous injection of the peptide into DEN-treated mice resulted in concentration-dependent inhibition of tumor formation up to complete elimination. Immunostaining of liver sections revealed that peptide treatment led to cell death and to decreased levels of metabolic enzymes. Furthermore, haematoxylin/eosin stained liver sections obtained from untreated DEN mice showed the pathology of liver steatohepatitis, reflected by a fat liver, inflammation and ballooning degeneration and fibrosis. In contrast, the livers from peptide-treated DEN mice appeared normal, like those of healthy mice. These results show that the VDAC1-based peptide, Tf-D-LP4, triggers cell death and impair cell energy and metabolism homeostasis, and thus represents a promising therapeutic approach for liver cancer.

P.4.1-076
How to convince your microorganism to turn into a bioactive small molecule factory

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Bioactive Small Molecules (BSM), produced by natural resources such as microorganisms and plants, are widely used in research and development in academia, in the biotechnology and pharmaceutical industry and in diagnostics. Many BSM are associated with cancerous bio-molecular processes, some are used as anti-tumor agents whereas some are carcinogenic. Development of BSM is a complex task, which begins with identification of the ideal microorganism, understanding its nature and optimal culture conditions, to eventually allow production of the BSM of interest. Even then, a BSM can often be described as a needle in a haystack and needs to be pulled out, identified and purified. Most of the processes described herein end up in relatively small scales (a few liters) yielding at the most gram quantities of the BSM of interest. Here we describe the long course of challenging development and up-scaling process of a few BSM associated with cancer (Apoptolidin, Cyclosporin A and Fumonisin B) which ended up with great deal of success.

P.4.1-077
Overproduction of Bacillus licheniformis ribonuclease and its inhibitor YrdF

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Despite the enormous number of studies in the field of antitumor therapy, the number of deaths from oncology is continuously growing. The problem of low efficiency of chemical medicines is associated with the lack of selectivity and high toxicity of them, that's why the search for new antitumor agents with targeted action, selectivity against cancer cells and low immunogenicity is very important. It is known that ribonucleases belonging to the N1/T1 RNase superfamily possess a selective cytotoxic effect on

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cancer cells. For binase, RNase from *Bacillus pumilus,* the selective growth inhibition of the cells expressing specific oncogenes (*ras*, *kit* and AML1/ETO) was shown. The mechanisms of biological action and the nature of selectivity of these enzymes are not fully studied. Earlier, a three-stage ion-exchange chromatography-based purification technique was proposed for three homologous RNases from *B. pumilus* (binase), *B. altitudinis* (balnase) and *B. licheniformis* (balifase) but it proved to be time-consuming and didn't give a large protein yield. Here, we report on the construction of the pET26b/pET15b-based plasmids carrying the genes for extracellular N- and C-terminal His-tagged balifase and its intracellular C-terminal His-tagged inhibitor YrdF which is needed to compensate RNase cytotoxicity. The constructs obtained allow overexpression of balifase and YrdF and are helpful for study of the biological activities of purified proteins as well as the assessment of the roles of protein termini in balifase dimerization and spectrum of YrdF action.

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P.4.1-078
S6K2 promises an important therapeutic potential for cancer

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S6 kinase 1 (S6K1) and S6 kinase 2 (S6K2), which are crucial modulators of Akt/mTOR signaling pathway, belong to the family of AGC kinases which play vital roles in cellular growth and survival. Therefore, these kinases are the foci of cancer therapy studies. Due to their high sequence similarity between themselves, S6K2 was regarded to have the same functions as S6K1. In earlier times, S6K1 was extensively studied, also as a result of its earlier discovery. However, recent studies unraveled distinct functions of S6K2.

Some studies revealed the important role of S6K2 in cell survival. Pardo OE et al. (2006) reported that fibroblast growth factor-2 (FGF-2) stimulated non-small cell lung cancer cells (NSCLC) escaped from apoptosis through formation of a complex involving S6K2 and anti-apoptotic proteins BRAF and protein kinase C-epsilon (PKCε). Another study by Liwak U at al. (2012) revealed that S6K2 derepresses the translation of anti-apoptotic proteins Bcl-XL and XIAP via phosphorylation and subsequent degradation of the tumor suppressor protein PDCD4 in HEK293T cells.

Sridharan S and Basu A (2011) investigated how S6K2 contributed to the prevention of the apoptosis of breast cancer cells. They found out that downregulation of S6K2, increased TNF-induced apoptosis in MCF-7 cells. They also discovered that this contribution of S6K2 to cell survival involves phosphorylation and thus activation of Akt. This study also displayed the involvement of proapoptotic protein Bid, which is deactivated by S6K2 to maintain cell survival.

All these studies point out the promising role of therapeutic approaches aiming solely at S6K2. A specific S6K1 inhibitor, PF-4708671, was characterized by Pearce et al. (2010). However, no specific S6K2 inhibitor is currently present. Studies targeting subcellular localization of S6K2 or interaction of S6K2 with other proteins promise important development for cancer therapy.

P.4.1-079
Sub-cytotoxic doses of anti-neoplastic drugs increase caveolin-1-dependent migration, invasion and metastasis of cancer cells

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Expression of the scaffolding protein Caveolin-1 (CAV1) enhances migration and invasion of metastatic cancer cells. Yet, CAV1 also functions as a tumor suppressor in early stages of cancer, where expression is suppressed by epigenetic mechanisms. Thus, we sought to identify stimuli/mechanisms that revert epigenetic CAV1 silencing in cancer cells and evaluate how this affects their metastatic potential. We reasoned that restricted tissue availability of anti-neoplastic drugs during chemotherapy might expose cancer cells to sub-therapeutic concentrations, which activate signaling pathways and the expression of CAV1 to favor the acquisition of more aggressive traits. Here, we used *in vitro* [2D, invasion] and *in vivo* (metastasis) assays, as well as genetic and biochemical approaches to address this question. Colon and breast cancer cells were identified where CAV1 levels were low due to epigenetic suppression and could be reverted by treatment with the methyltransferase inhibitor 5′-azacytidine. Exposure of these cells to anti-neoplastic drugs for short periods of time (24–48 h) increased CAV1 expression through ROS production and MEK/ERK activation. In colon cancer cells, increased CAV1 expression enhanced migration and invasion *in vitro* via pathways requiring Src-family kinases, as well as Rac-1 activity. Finally, elevated CAV1 expression in colon cancer cells following exposure *in vitro* to sub-cytotoxic drug concentrations increased their metastatic potential *in vivo*. Therefore exposure of cancer cells to anti-neoplastic drugs at non-lethal drug concentrations induces signaling events and changes in transcription that favor CAV1-dependent migration, invasion and metastasis. Importantly, this may occur in the absence of selection for drug-resistance.

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P.4.1-080
Self-assembling multifunctional nanostructures for the controlled delivery to cancer cells

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A significant attention of researchers in nanobiotechnology is being directed toward the problems of targeted drug delivery and the development of effective theranostic nanoagents. The aim of this work was the design and synthesis of multifunctional nanostructures as promising agents for effective diagnostics and targeted therapy of cancer. To create such nanoagents, we have developed new universal ways of nanoparticle biofunctionalisation (with preservation of the orientation and functional activity of the molecules being attached) using self-assembly and genetic engineering approaches. In particular, we have used peptides that bind the solid phase (in this case, the surface of nanoparticles) and high affinity protein pair Barnase-Barstar as a "molecular glue" between the combined components. The design of fusion proteins of one component of this pair (e.g., Barstar) with peptide that binds the surface of nanoparticles and the use of fusion proteins of another component (e.g., Barnase) with recognizing or fluorescent molecules (e.g., antibody or fluorescent protein)

makes it possible to obtain universal nanostructures with specified characteristics. We have previously shown that Barnase-Barstar protein pair being incorporated into the inteface between nanoparticles of different nature exhibits suprising resistance to dissassembly under severe conditions (8 M GdmHCl, 8 M Urea, 5 M NaCl, pH 1.5), thus providing very strong bond in any environment. The effectiveness of these approaches was demonstrated both in vitro a cell-free mode and by specific cancer cell labelling using the flow cytometry and our original MPQ-cytometry methods. This work was partially supported by Russian Foundation for Basic Research and by the National Intellectual Development Foundation (NIDF) according to the research project No.17-34-80105 "mol_ev_a" (nanoparticle modification, cell culture) and the Russian Science Foundation grant No.17-74-20146 (protein modification, nanoparticle synthesis).

P.4.1-081
Transforming growth factor-beta 1 is a major mediator of radioactive iodine therapy-induced anti-tumor immunity

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Although radiotherapy has been thought to induce tumor cell death via direct cytotoxic effects, various studies have shown that delivery of localized radiation to tumors often leads to systemic responses at distant sites, a phenomenon known as the abscopal effect which has been attributed to the induction and enhancement of the endogenous anti-tumor innate and adaptive immune response. TGF-β1 is involved in the regulation of numerous aspects of the immune response, including T cell homeostasis and can modulate the response to exposure to ionizing radiation. The aim of this study was to evaluate the effects of therapeutic irradiation with radioiodine (I-131) on TGF-β1, normal T and B lymphocytes and on imbalance between expression of MMP-9 and TIMP-1, in 54 patients (8M/46F) with papillary thyroid cancer (PTC) and 41 (3M/38F) with PTC associated with Hashimoto's thyroiditis (PTC+HT). Peripheral blood samples were collected just before and, subsequently, at 4 days after I-131 administration (3.7 GBq). PTC+HT patients had positive titers of anti-thyroglobulin autoantibodies (TgAb). The lymphocyte subpopulations were measured by flow cytometry and the serum levels of TgAb, TGF-β1, MMP-9, TIMP-1 by ELISA. In PTC patients, I-131 therapy resulted in a reduction with 15% in TGF-β1 level ($P = 0.001$) and an increase with 11% in CD8 + T-cells count number. TGF-β1 inhibition enhanced anti-tumor immunity mediated by CD8 + T-cells ($P = 0.004$) and decreased with 44% the imbalance between MMP-9 and TIMP-1 ($P < 0.001$). In PTC+HT patients, I-131 administration led to an increase with 18% in TgAb level ($P = 0.001$), 9% in CD19 + B-lymphocytes ($P = 0.03$), 27% in TGF-β1 ($P = 0.001$) and a reduction with 8% in CD8 + T-cells count number ($P = 0.02$). Elevated TgAb titers have been associated with increased TGF-β1 concentrations ($P < 0.001$). TGF-β1 activation decreased tumor-specific CD8 + T-cell response ($P < 0.001$). Our data suggest that TGF-β1 is a major mediator of I-131 therapy-induced anti-tumor immunity.

P.4.1-082
Role of Tim 3 in acute myeloid leukemia

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Background: The immune receptor T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) is often highly expressed in human acute myeloid leukaemia (hAML) cells compared to leukocytes isolated from healthy individuals. This suggests Tim-3 as a promising target for both leukaemia diagnostics and therapy. However, biochemical activity of the Tim-3 and its functional role in leukaemia progression remains to be elucidated.

Aim: To investigate the pathophysiological role of Tim-3 in hAML cells

Methods/Design: THP-1 and U-937 cell lines as well as primary hAML cells were used in thus study. Western blot analysis, ELISA, confocal laser microscopy, qRP-PCR and on cell assay/in-cell Western were used to conduct the experiments.

Results: Our data have shown that Tim-3 mediates ligand-induced activation of phosphatidylinositol-3-kinase (PI-3K)/mammalian target of rapamycin (mTOR) intracellular signalling pathway. Importantly, it acts as a trafficker for galectin-9 thus controlling its secretion.

Conclusion: Tim-3 is required for secretion of galectin-9 in hAML cells. Importantly, galectin-9 is capable of impairing functional activity of cytotoxic T cells and natural killer cells and thus might allow malignant cells to escape host immune attack.

P.4.1-083
Understanding a mechanism of incomplete penetrance of human tumor suppressor gene PTEN by adaptive evolution of *C. elegance*

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Phosphatase and tensin homolog *(PTEN)* is a tumor suppressor gene that's frequently deleted or mutated in several human cancers, with medium to high penetrance. The frequency of monoallelic mutations of the protein is about 50%–80% in sporadic tumors and 30%–50% in breast, colon, and lung tumors. In this work we focused on the *Caenorhabditis elegans* orhtolog of the human PTEN, *daf-18*. Previous studies showed that human PTEN can functionally replace DAF-18 in *C. elegans*, suggesting that human PTEN and DAF-18 are functionally similar and that the regulation of PTEN is highly conserved in *C. elegans*. This makes *C. elegans* an excellent choice as a model organism, as it has both cellular complexity and conservation of disease pathways, while being much simpler than other animal models. Despite many types of diseases manifesting incomplete penetrance, its mechanism isn't clear. Our hypothesis is that employment of adaptive or enforced evolution will allow us to find an alternative pathway or point mutation which will allow *C. elegans* to overcome the depletion of *daf-18*.

According to previous studies; there is about 70% penetrance after starvation of 72 hours. The worms were starved in every generation. We've started to see a change in penetrance after only two generations, and at about generation 13 there was a decrease of 20% in penetrance. We plan to keep the evolutionary process until the penetrance will decrease to the wt phenotype of about 5%. Since the change was very fast we hypothesize some epigenetic modifications involved. To further study the mechanism, we plan to sequence both DNA and RNA from the different generations, and also perform an EMS screen as an enforced version of evolution. The understanding of incomplete penetrance of PTEN,

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and later other oncogenes, will enable to distinguish between mutation carriers. It will be possible to eliminate unnecessary suffering from those who are not at risk, and to pay more attention for those who are at high risk.

P.4.1-084
Cathepsin L is necessary for survival of colorectal cancer cells under metabolic stress

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Tumor cells can survive under metabolic stress by evasion of programmed cell death or by promoting autophagy process. Some studies have shown that cathepsin L, a lysosomal protease, is involved in these processes. Thus, we investigated whether cathepsin L is involved in cell survival of colorectal cancer cells exposed to metabolic stress. For this study, COLO32O and SW620 colorectal cancer cell lines were cultured in serum-free medium or medium without glucose to induce metabolic stress for 24 hours. Cathepsin L activity was inhibited by siRNA-mediated silencing or by drug inhibition. Subsequently, we analyzed the role of cathepsin L in cell viability, complemented with detection of specific markers of apoptosis and autophagy, and analysis of DNA integrity. To determine variations in the location and expression of cathepsin L, we performed immunofluorescence assays of cathepsin L expression, along with detection of cytoskeleton and lysosome markers. Furthermore, to analyze changes in cathepsin L activity, we used a specific substrate; this experiment was supplemented with analysis of the active forms of cathepsin L by zymography. Finally, to analyze changes on the expression of other lysosomal proteases after cathepsin L inhibition, we performed PCR analysis of cathepsin D and B. Our results showed that cathepsin L is necessary for colorectal cancer cells survival under metabolic stress. Cathepsin L inhibition leads to a defect in cell autophagy, which would be reflected in an increase in cellular apoptosis.

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P.4.1-085
Inhibition of cathepsin S induces mitochondrial ROS that sensitizes TRAIL-mediated apoptosis through p53-mediated downregulation of Bcl-2 and c-FLIP

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Cathepsin S is highly expressed in various cancer cells, and it has protumoral effects, including promotion of migration, invasion, and neovascularization. In this study, we show that inhibition of cathepsin S could sensitize cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis. An inhibitor of cathepsin S (Z-FL-COCHO; ZFL) markedly induced apoptosis in human renal cancer cells treated with TRAIL. In contrast, combined treatment with ZFL and TRAIL had no effect on normal cells. ZFL downregulated Bcl-2 expression at the transcriptional level in a p53-dependent manner, and overexpression of Bcl-2 also markedly blocked apoptosis induced by combined treatment with ZFL and TRAIL. In addition, ZFL induced downregulation of c-FLIP, and overexpression of c-FLIP blocked the apoptosis induced by ZFL plus TRAIL. Moreover, ZFL increased the expression of Cbl, an E3 ligase of c-FLIP, in a p53-dependent manner, and knockdown of Cbl markedly prevented c-FLIP downregulation and the apoptosis induced by ZFL plus TRAIL. Interestingly, ZFL induced p53 expression via production of mitochondrial reactive oxygen species (ROS). We also demonstrated that downregulation of cathepsin S by small interfering RNA sensitized TRAIL-mediated apoptosis in Caki cells. Our results indicated that inhibition of cathepsin S stimulates TRAIL-induced apoptosis through downregulation of Bcl-2 and Cbl-mediated c-FLIP by ROS-mediated p53 expression. These results reveal the importance of cathepsin S on resistance against TRAIL, and inhibition of cathepsin S activity plays a crucial role in TRAIL-mediated cell death of cancer cells.

P.4.1-086
Hyperthermic chemotherapy inhibits respiration, glutamate dehydrogenase and increases susceptibility to cisplatin of ovarian cancer cells

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Hyperthermic chemotherapy is used aiming to overcome the resistance of cancer cells to cisplatin. Mitochondria in tumour cells are dependent on glutamate metabolism and activity glutamate dehydrogenase (GDH). We investigated effects of mild hyperthermia (40°C and 43°C for 1 h), cisplatin or combination of both treatments on GDH activity, respiration and cell viability immediately after hyperthermic treatment (0 h) and after 24 and 48 h recovery at 37°C in ovarian tumour Ovcar-3 cell line. GDH was activated under hyperthermic conditions at starting point (0 h), however in the presence of cisplatin hyperthermia induced opposite effect – GDH activity decreased by 6 and 26% at 40 and 43°C, respectively). After 24 h recovery from hyperthermia GDH activity was reduced in both groups, and GDH inhibition was stronger in cells treated with cisplatin. Inhibitory effect remained after 48 h recovery only in cisplatin treated cells (GDH activity decreased by 61% at 37°C and by more than by 70% after 40 or 43°C hyperthermia. Hyperthermia alone (40°C and 43°C) had no statistically significant effect on respiration as compared to 37°C and did not affect cell survival after 48 h recovery. Pretreatment of cells with cisplatin at hyperthermic conditions (43°C) decreased State 2 and State 3 respiration rates by 28% and 42%, respectively. Combination of hyperthermia and cisplatin reduced cell viability more than cisplatin alone (viability of cisplatin treated cells was 21%, meanwhile combination with both hyperthermic conditions resulted in 10% of viable cells after 48 h). Signs of early apoptosis in hyperthermia alone treated cells appeared after 24 h, but were absent after 48 h. Cisplatin induced early apoptotic signs after 24 and 48 h recovery both under normothermic and hyperthermic conditions. We conclude that hyperthermia enhanced sensitivity of Ovcar-3 cells to cisplatin: the combinatory treatment inhibited respiration, GDH and reduced cell viability more than cisplatin alone.

P.4.1-087
Bioenergetic comparison of human colorectal and breast cancer clinical patients

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Bioenergetics is a fast growing field in cancer research, where many promising outcomes could provide targeted cancer treatment. Energy metabolism specific literature is characterized by many contradictions, concluding that cancer cells metabolize their increased glucose uptake via glycolysis rather than more energy efficient oxidative phosphorylation. Furthermore, the majority of these conclusions are the outcome of *in vitro* studies on cell culture models, without taking into consideration the factors arising from the tumor microenvironment giving significant effects *in vivo*. We have conducted quantitative cellular respiration analysis on human breast (HBC) and colorectal cancer (HCC) clinical tissue samples and cell cultures. Our results displayed that both HBC and HCC showed significant rates of oxidative phosphorylation and respiratory capacity was not lost due to tumor activity. This supports our previous findings that HBC and HCC are not fully pure glycolytic tumors. In addition, the functional deficiency of Complex I in the electron transport chain is characteristic for HCC but not for HBC tissue samples and clear differences were seen in the Km values of ADP measurements, while comparing clinical samples with cell cultures. Additionally, two distinct groups of mitochondria were detected in HBC tissue samples, which might be linked to two-compartment metabolism, where tumor acts as a metabolic parasite on stromal cells. These results show that tumor clinical samples are not directly comparable to cell cultures and comparing HBC to HCC refers to differences in mitochondrial respiration and in mitochondria population. Further research is in progress to generate a full cancer development model consisting of cell cultures, clinical polyps and malignant versus healthy tissue samples.

P.4.1-088
Phospholipase D1 Acts through Akt/TopBP1 and RB1 to regulate the E2F1-dependent apoptotic program in cancer cells

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The RB1/E2F1 signaling pathway is frequently deregulated in colorectal cancer and has been suggested to intersect with Wnt/β-catenin and PI3K/Akt pathways, but molecular evidence for this link is lacking. In this study, we demonstrate that phospholipase D1 (PLD1), a transcriptional target of β-catenin/TCF4, orchestrates functional interactions between these pathways during intestinal tumor development. Overexpression of PLD1 in intestinal epithelial cells protected cells from apoptosis induced by PLD1 ablation in the $Apc^{min/+}$ mouse model of intestinal tumorigenesis. Mechanistic investigations revealed that genetic and pharmacologic targeting of PLD1 promote the E2F1-dependent apoptotic program via both miR-192/4465-mediated downregulation of RB1 and inhibition of Akt-TopBP1 pathways. Moreover, the miRNA-RB1 axis and Akt pathway also contributed to the PLD1-mediated self-renewal capacity of colon cancer-initiating cells. Finally, PLD1-driven E2F1 target gene expression positively correlated with tumor stage in patients with colorectal cancer. Overall, our findings suggest that PLD1 mediates cross-talk between multiple major signaling pathways to promote the survival and malignancy of colon cancer cells and may therefore represent an ideal signaling node for therapeutic targeting.

P.4.1-089
The ubiquitin ligase RNF5 in acute myeloid leukemia (AML) development and stress response

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AML is a hematologic cancer with heterogeneous nature characterized by accumulation of somatic genetic alterations in hematopoietic progenitor cells that alter mechanisms of self-renewal, proliferation and differentiation. Despite the development of new investigational therapies, the majority of patients who achieve complete remission with initial chemotherapy will eventually relapse.

The ER stress response play a pivotal role in normal cellular homeostasis by governing cell commitment to survival or death in response to internal and external cues. Several dedicated ubiquitin ligases play critical role in the ERS and unfolded protein response. One of those is RNF5, an ER-associated E3 ubiquitin ligase and part of the UBC6e/p97 network, which are key components of ER-associated degradation. RNF5 regulates the stability and clearance of misfolded proteins functioning in various cellular processes, affecting proliferation and chemo-resistance of tumor cells, as shown for breast cancer and melanoma.

Importantly, RNF5 expression is elevated in AML cell lines and patient samples. Using AML-derived cell lines, we found that RNF5 is required for AML cell proliferation and clonogenicity. Correspondingly, inhibition of RNF5 expression in human AML cells attenuated their growth and induced cell death. Further, loss of RNF5 sensitized AML cells, while its overexpression protected them from ER stress and Bortezomib treatment. These data suggest that RNF5 plays important roles in AML growth and response to therapy. Possible mechanisms underlying RNF5 regulation and function in AML biology will be discussed.

P.4.1-090
DNA-PK regulates the radiosensitivity and oncogenicity of MET-addicted cancer cell lines via a novel MET phosphosite

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MET, the receptor tyrosine kinase for hepatocyte growth factor, is a proto-oncogene mainly expressed in epithelial cells and involved in development, homeostasis and tissue regeneration. Deregulation of MET signaling has been reported in numerous cancers, leading to a broad interest in MET targeting for therapy. Additionally, there is emerging evidence that MET signaling extends to the DNA damage response (DDR) machinery and protects tumors from DNA-damaging agents. A study of post-translational changes in a MET-addicted cancer cell line upon MET inhibition and ionizing radiation revealed a yet unreported phosphorylation site on MET that is part of a consensus motif recognized by master DDR kinases. Within this study we aimed to investigate the function of this novel MET phosphosite in the context of radioresistance and oncogenesis. Our results show that this site can be phosphorylated by DNA-PK and that its phosphorylation fluctuates in response to MET inhibition. Interestingly, MET-addicted transformed cells expressing the phosphodeficient (Ser to Ala mutation) form of active MET are more radiosensitive than their nonmutated counterparts, both *in vitro* and in a mouse xenograft model. Additionally, we show that preventing this phosphorylation has an impact on the oncogenicity of MET by affecting the migration and anchorage-independent growth capacity of cells. Altogether, our results describe a novel MET phosphosite that bears an important function for radiosensitivity and oncogenicity of MET-addicted cancer cell lines, providing new insights into the crosstalk linking MET and the DDR.

P.4.1-091
Arginyl-tRNA-protein transferase 1 (ATE1) in melanoma growth and drug response

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Arginyl-tRNA-protein transferase 1 (ATE1) transfers arginine from arginyl-tRNA to protein N-termini, resulting in protein arginylation. According to the N-end rule pathway, arginylation occurs on aspartate, glutamate or oxidized cysteine and affects protein stability. Loss of ATE1 resulted in decreased cell migration and invasion, which were associated with impaired actin arginylation. Conversely, mouse embryo fibroblasts (MEFs) obtained from ATE1 knock-out mice exhibit increased tumorigenic potential, compared to their wild-type counterparts. We set to study possible role for ATE1 in melanoma.

ATE1 expression was found to inversely correlate with melanoma patient survival and deregulated ATE1 expression was noted in 20% of melanoma specimens. Of those, ATE1 expression was found to be higher in NRAS mutant melanomas. Correspondingly, inhibition of ATE1 expression attenuated growth of melanoma in culture, and sensitized melanoma to serum starvation. Surprisingly, ATE1 knock-down conferred greater degree of drug resistance to NRAS- compared with BRAF- mutant melanoma cells. Elevated level of ATE1 mRNA was identified in metastatic compared to primary melanoma samples. Correspondingly, inhibition of ATE1 decreased melanoma migration in Boyden chamber assay. These data suggest that ATE1 plays distinct roles in melanoma growth and response to therapy. Possible mechanisms underlying ATE1 regulation and function in melanoma will be discussed.

P.4.1-092
The BRAF mutation analysis in circulating tumor DNA and tumor tissue DNA using biochip-based assay

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Melanoma is the most dangerous type of skin cancer. Assessment of *BRAF* status is mandatory in melanoma patients before applying targeted therapy. Commonly, the DNA extracted from tumor tissue is used for diagnostics of mutations. The circulating tumor DNA (ctDNA) analysis presents great potential in diagnosis, prognosis and patient follow-up due to low invasiveness, rapid data turnaround and cost effectiveness. We developed a specific biochip for detection *BRAF* mutations (V600E [c.1799T>A], V600M, V600K, V600R, V600D, V600E [c.1799_1800delTGinsAA]) in both ctDNA and tumor tissue DNA. We used nested LNA clamp PCR for preferable amplification of mutated over WT DNA. The length of PCR product was about 80 bp allowing the successful analysis of highly degraded DNA. Amplified fragments were labeled via incorporation of fluorescently labeled dTTP during the second round of PCR and hybridized with specific oligonucleotides immobilized on a biochip. The 30 control DNA samples extracted from tumor tissue and 17 ctDNA samples were included in the analysis. *BRAF* mutations in the tumor samples were previously determined using Sanger sequencing. The ctDNA was isolated from 2 ml of plasma collected prior to surgical intervention. Genotypes of all control samples were determined correctly. Moreover, we tested 17 plasma samples from metastatic melanoma patients with known *BRAF* status: 10 tumors were mutated and 7 were WT. We detect *BRAF* mutations in 8/10 ctDNA samples from *BRAF*-positive patients and none of 7 ctDNA samples from *BRAF* WT patients. The analytical sensitivity is 0.2% mutated DNA in a background of WT DNA. The biochip-based assay is a simple and sensitive method for *BRAF* mutation analysis in both tumor tissue DNA and ctDNA. This method can be useful in primarily diagnosis and treatment management of melanoma patients. This work was supported by Grant of the President of the Russian Federation (# MK-2519.2017.4).

P.4.1-093
New method of Irisin levels identification in gastric cancer patients with cachexia

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The latter findings describe a myokine Irisin, product of a transmembrane protein FNDC5 (Fibronectin type III domain containing 5) precursor cleavage, as a very important factor affecting human metabolism, also playing essential role in endothelial dysfunction, atherosclerosis, inflammation, cachexia and gastrointestinal system cancer. However, these are studies based on ELISA approach, which is characterized by the low specificity and selectivity. We have established a new method of Irisin level identification based on antibody immunodetection, validated in human serum by mass spectrometry method. This promising technique has been used in studies of cachexia level among gastric cancer patients. Cachexia, a condition of simultaneous loss of both fat and skeletal muscle tissue might be associated with Irisin levels. There are evidences of even up to 50% lower occurrence of cancer in individuals who exercise and possess high Irisin serum levels. These studies may bring the answer to the problem of evidence of circulating Irisin in human serum and give rise to the novel gastric cancer therapy.

P.4.1-094
Baicalein reverses cisplatin-induced multi-drug resistance in human bladder T24 cells via regulating drug transporters and NF-kB signaling pathway

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Scutellaria baicalensis is widely used in Korean medicine as a traditional adjuvant in the chemotherapy of various tumors. Baicalein is one of the bioactive flavonoid components isolated from the root of *S. baicalensis*. Although baicalein has been reported to be used in combination with chemotherapy drugs to improve the proliferation of cancer cells, the scientific evidences to explain this complexity are lacking. This study aims to demonstrate that baicalein can overcome drug resistance in cisplatin (CDDP)-resistant human bladder T24 (T24/CDDP) cells. Our data indicated that the application of baicalein exhibits a synergistic enhancement of CDDP-induced cytotoxicity as well as of the expression of apoptotic signaling molecules in (T24/CDDP) cells. However, CDDP-induced expression of the multi-drug resistance efflux transporters was markedly reduced in the presence of baicalein, resulting in a higher intracellular concentration of CDDP. In addition, the application of baicalein increased CDDP-induced accumulation of reactive oxygen species and loss of mitochondrial membrane potential ($\Delta\Psi m$), and decreased nuclear factor κB (NF-κB) signaling pathway in CDDP-treated T24/CDDP cells. These results indicate that baicalein can notably improve the CDDP-suppressed cancer effect, which may be a consequence of the elevation of intracellular CDDP via the drug transporters as well as the down regulation of NF-κB signaling pathway. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korea government (2016R1C 1B1014724 & 2015R1A2A2A01004633)].

P.4.1-095
A metabolomic discovery approach for identification of metabolic changes associated with MET inhibition and DNA-damaging agents in MET-addicted and non-MET-addicted cellular systems

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Overexpression of the MET receptor tyrosine kinase is often associated with poor prognosis and furthermore, HGF/MET signaling confers resistance to cancer treatments by protecting tumors from DNA-damaging agents. In order to gain insight into the link between MET and the DNA damage response, we carried out MS-based metabolomics analysis to study metabolites and metabolic pathways in various cell lines overexpressing the MET receptor. In the current work, several MET-addicted and non-addicted cell lines have been used. The cell line panel includes the gastric carcinoma cell lines GTL-16, MKN45, SNU5, KATOII, SNU638, Hs746T and the non-small cell lung cancer cell lines EBC-1, H1993, H1648, H820 and HCC827. We inhibited MET in these cell lines using the novel specific small molecule tyrosine kinase inhibitor tepotinib, which is currently under clinical evaluation. The phosphorylation levels of MET and the activation of its downstream signalling pathways were assessed by Western blotting. Preliminary experiments showed alterations in energy metabolism, tricarboxylic acid (TCA) cycle and amino acid metabolism. We identified one metabolite that is consistently altered in all MET-addicted cellular systems: 5′-Phosphoribosyl-N-formylglycinamide, involved in the *de novo* purine synthesis pathway. Interestingly, the complementary transcriptomics analysis of EBC-1 and GTL-16 cells showed that critical purine synthesis enzymes are downregulated upon MET inhibition (METi), a finding confirmed also by quantitative Real-Time PCR. Currently, we are focusing on transcription factors known to be involved in the purine synthesis pathway (e.g., E2F1 that is being downregulated in the transcriptomics dataset upon METi). Given the data obtained so far, we hypothesize that E2F1 overexpression could potentially lead to a rescue of MET-addicted cancer cells after METi, thus deepening our understanding over the role of the MET oncogene in cancer pathogenesis.

P.4.1-096
1,2,3-triazole-Jaspine B hybrids induces apoptosis of human bladder cancer T24 cells through activation of JNK signaling pathway

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1,2,3-triazole-Jaspine B hybrids is a Jaspine B derivant isolated from marine sponges and has been known to have cytotoxic activity in some cancer cell lines. However, the molecular mechanisms of its anti-cancer activity have not been clearly elucidated yet. In the present study, we investigated the pro-apoptotic effects of 1,2,3-triazole-Jaspine B hybrids in human bladder cancer cell lines (T24, EJ, J82 and 5637), and found that the growth inhibitory effect of 1,2,3-triazole-Jaspine B hybrids was more sensitive in T24 cells than in EJ, J82 and 5637 cells. The anti-proliferative effect of 1,2,3-triazole-Jaspine B hybrids in T24 cells was associated with the induction of apoptotic cell death. The induction of apoptotic cell death by 1,2,3-triazole-Jaspine B hybrids was connected with an up-regulation of Fas and death receptor 5 (DR5), and down-regulation of cellular inhibitor of apoptosis protein 2 (cIAP-2); however the expression of Bcl-2 family proteins and levels of mitochondrial membrane potential (MMP, $\Delta\psi m$) were not relevant. 1,2,3-triazole-Jaspine B hybrids induced the proteolytic activation of caspases (-3 and -8), and degradation of caspase-3 substrate proteins, such as poly(ADP-ribose) polymerase (PARP), β-catenin and phospholipase C-γ1 (PLCγ1). In addition, 1,2,3-triazole-Jaspine B hybrids selectively increased the phosphorylation of c-Jun N-terminal kinase (JNK); however, a JNK-specific inhibitor, SP600125, significantly reduced 1,2,3-triazole-Jaspine B hybrids-induced up-regulation of DR5 and apoptosis. Collectively, these findings indicate that the apoptotic activity of 1,2,3-triazole-Jaspine B hybrids in T24 cells may be associated with the activation of extrinsic signaling pathway through up-regulation of DR5 connected with JNK signaling pathway. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korea government (2015R1A2A2A01004633 & 2016R1C1B1014724)]

P.4.1-097
Driver-mutation specific CNV patterns of distant metastatic melanomas

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Malignant melanoma is the most life-threatening skin neoplasm, responsible for almost all skin cancer deaths. It is known that primary tumors with specific driver mutations show different biological behavior. However, a little is known about their role in distant metastatic process. Our goal was to determine driver mutation-specific CNV patterns of metastatic melanomas in brain, lung and liver. A total of 11 primary and 31 corresponding metastatic melanomas were included in the analysis, representing *BRAF*, *NRAS* mutant or double wild-type (WT) molecular groups. CNV/LOH patterns were defined with Affymetrix OncoScan and CytoScan HD arrays. Nexus Copy Number 8.0 software was applied for raw data analysis. Pathway analysis was carried out by DAVID 6.8 database. Generally, more one-copy loss was observed in WT, and more LOH in *NRAS* metastases. In case of brain, *NRAS* samples showed CN gains and LOHs enriched in ECM organization. All the examined lung metastases were *BRAF*, including 4 samples genetically discordant with their WT primary pair. Regarding liver, *NRAS* melanomas exhibited gains of genes responsible for methylation and histone modification. Proportion of LOH was 3–10 fold higher in *NRAS* samples compared to *BRAF* and WT. Different distant organ metastases bearing the same driver mutation were also examined. Altered CNV patterns was only observed in *BRAF* samples. Gain of genes involved in homologous recombination repair and cell adhesion was characteristic for brain metastases. In lung metastases mismatch repair genes were significantly gained. Whereas *BRAF* liver metastatic melanomas were featured by loss of genes involved in methyltranferase activity. In conclusion, based on our results analysis of the molecular drivers of metastatic melanoma, and not only the primary tumor, may also be of great importance in the future therapeutic decision-making process. Different CNV pattern of *BRAF* organ metastases may be caused by distinct microenvironment of the host organ.

P.4.1-098
CNV patterns of distant metastatic melanomas revealed by aCGH

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Distant metastatic process is the predominant cause of melanoma specific death. Despite intensive research, there is still no effective therapy for patients with advanced staged disease. Our primary goal was to determine alterations in copy number variaton (CNV) pattern of cutaneous melanomas with different oncogenic driver mutations (NRAS, BRAF or double wild type) and matched distant metastases (brain, lung and liver). Affymetrix OncoScan and CytoScan HD platforms were used to analyze CNV patterns. Raw data analysis was performed with Nexus Copy Number 8.0 and ChAs softwares. Pathway analysis was established by web-accessed DAVID 6.8 database. Increased number of genomic alteration was observed in distant metastases (DM) compared to primary tumors (average 21.7% vs. 14.3%). Generally, CN gain of genes involved in cell adhesion and motility pathways was observed in DMs. Comparing CNV patterns of different DM sites, brain showed higher copy of defensine genes. Liver metastases were characterized by amplification of scramblases, genes in Ca- and cadherin dependent cell adhesion and WNT signaling, and loss of protein phosphatase and metallopeptidase coding genes. Lung metastases were featured by overrepresentation of genes in transcription regulation, cell cycle and TNF pathways, whereas CN loss was observed in methyltransferase activity and spectrin associated microfilament development. In concert with literature, our results revealed increasing chromosomal instability during metastatic process. Moreover, differently altered pathways in distinct organ metastases bearing different driver mutations suggest that

metastatic process is not uniform, and should depend on the microenvironment of host organ. This may have future consequences in management of advanced disease.

P.4.1-099
Mild doses of doxorubicin alters apoptotic response and cell cycle arrest on both luminal A and triple negative breast cancer cell lines

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Breast cancer is one of most common cancers and is the leading cause of cancer-related mortality amongst women. It is divided into five subtypes in accordance with their expression profiles of certain genes as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Triple negative breast cancer (TNBC) is the most heterogeneous subtype of breast cancer which is characterized by elevated risk of relapse and low prognosis. Currently the only effective therapy against TNBC is chemotherapy. In the present study, it was aimed to evaluate the difference between apoptosis rate and cell cycle distribution in MCF-7 and TNBC cancer cell line MDA-MB-231 upon exposure to 50, 200 and 800 nM of Doxorubicin (DOX). After cells were treated with various doses of DOX for 48 h, cell viability was measured by sulforhodamine B (SRB) assay. Subsequently, Tali® apoptosis and cell cycle assays were performed in order to examine the percentage of apoptotic cells and to define which cell phase the cells were gone under cell cycle arrest. According to the results, DOX caused cytotoxicity and reduced viability rates of both cell lines and the reduction was more sigificant on MDA-MB-231. Data obtained by apoptosis assay demonstrated that DOX triggered apoptosis along with the dose applied and the percentage of apoptotic cells were to be 2.29 and 16.5 fold, respectively when compared to the following the application of 800 nM of the drug. Cell cycle assay pointed out that DOX induced cell cycle arrest predominantly at G2/M checkpoint in a dose dependent manner in both of the cell lines although the effect was more evident in MDA-MB-231 cells. Findings suggest that, DOX induces apoptosis in both ER, PR and/or HER2 possessing cells (MCF-7) and the cells which do not own any of these receptors (MDA-MB-231). The influence of the drug is believed to be more intense on MDA-MB-231 due to its gene expression profile and capacity to resist to the drug.

P.4.1-100
Systematic analysis of BCR-ABL interactome in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a myeloproliferative cancer that is caused by "Philadelphia chromosome" translocation that results in a formation of fusion protein BCR-ABL. This constitutively active tyrosine kinase is necessary and sufficient to cause CML. Several small molecule tyrosine kinase inhibitors (TKI) targeting BCR-ABL kinase activity had been developed and greatly improved CML prognosis. However, significant number of patients develops resistance to TKIs and relapse. Growing evidence shows the importance of other BCR-ABL interaction partners in CML pathogenesis. Precise elucidation of the interactome can lead to design of conceptually new drug targeting different pathways and overcoming TKI resistance. One of our goals is to elucidate precise binding interface among BCR-ABL and it's "core complex" interaction partners. Our approach involves use of peptide microarrays, which allow us to map the binding interface with single amino acid resolution. Binding motifs discovered in unstructured parts of BCR-ABL can be used to generate synthetic peptide abolishing particular protein interaction. Furthermore, we created large pallet of BCR-ABL deletion/substitution mutants in order to verify interaction boundaries and co-immunoprecipitation experiments have yielded potential new binding sites for some of the core complex interactors. The realization of this project was allowed due to financial support from Ministry of Health of the Czech Republic (15-33232A, 15-34405A); National Program of Sustainability II (MEYS CR: LQ1605 and LQ1601) and European Union ICRC-ERA-HumanBridge (No. 316345).

P.4.1-101
Advanced oxidation protein products and reduced glutation levels in bladder cancer

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Bladder cancer is potentially lethal and is the most costly urologic malignancy to manage. Risk factors associated with bladder cancer include carcinogens in tobacco smoke and environmental and/or occupational exposure to chemical compounds. More than 380,000 new cases of bladder cancer are diagnosed worldwide, accounting for $\sim$150,200 deaths each year. GSH is an essential cellular thiol which has important antioxidant functions in detoxication of xenobiotics, in a number of isomerization reactions, and storage and transport of cysteine. In addition, GSH is essential for cell proliferation and maintaining the thiol redox potential in cells. Advanced oxidation protein products (AOPP) were also proposed as one of the possible markers of oxidative injury, which originates under oxidative and carbonyl stress and increase global inflammatory activity. The aim of this study is to compare the GSH and AOPP levels between control and bladder cancer samples. The blood samples were collected from 13 patients who were diagnosed with bladder cancer in Kocaeli University, Urology Department before transurethral resection of bladder tumor (TUR-BT) operation and 8 samples collected from healthy individuals. These samples were used to compare GSH levels which were measured with Ellman manual method. AOPP levels were examined by Human AOPP ELISA kit(SUNRED, Shanghai) in 16 control and 15 patient urine samples before TUR-BT. Statistical analysis was done with SPSS 20.0.In our study, GSH levels in patients with bladder carcinoma were found to be significantly lower than the control group ($P < 0.05$). AOPP levels in patients with bladder carcinoma were not statistically significant ($P > 0.05$). Decreased levels of essential antioxidants in the circulation have been found to be associated with an increased risk of cancer.

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P.4.1-102
The role of MAD2L2 in early stages of tumorigenesis

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The protein complex called Anaphase Promoting Complex/Cyclosome (APC/C) is responsible for controlling cell division. The APC/C allows cells to divide only when the DNA is well condensed and arranged into chromosomes shapes, aligned at the cell equator and attached correctly to microtubule fibers.

Our protein of interest, MAD2L2, has an important role in preventing premature APC/C activation, hence preventing premature cell division that could cause mitotic aberrations.

We have recently shown that MAD2L2 binds another key APC/C activator protein called CDH1, and prevents it from binding and activating the APC/C prematurely. As the role of MAD2L2 in mitotic regulation becomes important, we aim to understand the mechanisms by which MAD2L2 inhibits the APC/C and its involvement in early stages of tumorigenesis. We will study tissue culture model of human cancer cell with MAD2L2 overexpression. To ensure using the most relevant cells, we performed a bioinformatics data mining to find cancers types with high MAD2L2 expression levels and found that some triple negative breast cancer cells, have high levels of MAD2L2. Focusing on the MDAMB157 cell line we found that their APC/C regulation is abnormal and they suffer from multiple mitotic defects.

Understanding the link between mitosis and tumorigenesis seems obvious, however not much is known about the link between theses events. Our research will help to understand the fate of cells with abnormal APC/C function and the link to early tumerogenesis. We hope to develop MAD2L2 as a potential diagnostic and prognostic bio-marker and in the future, as our understanding on the mitotic influence of MAD2L2 improves, MAD2L2 could be a target for developing therapeutic agents.

P.4.1-103
Sphingosine-1-phosphate gradient and Spns2 transporter in lymphocyte trafficking and breast cancer metastasis

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Sphingosine 1-phosphate (S1P) is a pleiotropic bioactive sphingolipid metabolite that regulates numerous processes important for immune responses and cancer. S1P is generated within cells and is exported out of cells to exert its effects through activation of five specific S1P receptors in autocrine or paracrine manners (1). We have previously shown that Spns2 transports S1P out of cells (2), and its deletion in mice alters immune cells trafficking and protects against the initiation and progression of several inflammatory diseases (3). More recently, a genome-wide in vivo screen of more than 800 mutant mice by the Sanger institute identified Spns2 as a key host microenvironmental regulator of metastatic colonization. Deletion of Spns2, either globally or specifically in lymphatic endothelial cells enhanced cancer cell killing by effector T cells and natural killer cells, thereby suppressing pulmonary metastatic colonization (4). Similarly, interfering with the S1P gradient by inhibition of S1P lyase also drastically reduced breast cancer metastasis. Further studies are needed to understand how Spns2 and S1P lyase control levels of S1P in the circulation and lymph to regulate the S1P gradient and lymphocyte trafficking. Metastasis is the leading cause of death for cancer patients and this work suggests that targeting Spns2 and the S1P gradient is a potentially promising option for suppressing breast cancer metastasis.

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P.4.1-104
Inactivation of VHL in kidney cancer cells affects genome methylation

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VHL inactivation is a key oncogenic event for renal carcinomas. In normoxia, VHL suppresses HIF1a-mediated response to hypoxia. In this work, we performed VHL inactivation by CRISPR/Cas9 in Caki-1 kidney cancer cell line and studied its effects on gene expression and DNA methylation. We performed RNA-seq and reduced representation bisulfite sequencing (RRBS) for intact Caki-1 cells, hypoxia-treated cells and cells after VHL inactivation. Similarly to hypoxic conditions, VHL inactivation caused transcriptional upregulataion of hypoxia-associated genes, targets of Hif1a transcription factor and genes involved in glycolysis. We discovered differentially methylated regions (DMRs) associated with VHL inactivation and showed that they were localized in promoters of important chromatin modifiers and transcriptional regulators including BRD4. The observed promoter hypermethylation of several transcriptional regulators was associated with decreased gene expression. It has previously been shown that hypoxic conditions inhibit TET-dependent hydroxymethylation of cytosines and cause DNA hypermethylation at gene promoters. We showed that even without hypoxia, VHL inactivation led to hypermethylation of the genome. To attribute the observed DNA methylation changes to certain epigenetic mechanisms, we explored DNA methylation changes within different genomic and epigenomic markups. We showed that hypermethylation mainly occurred in AP-1 and TRIM28 binding sites, while HDAC6 sites tend to be hypomethylated after VHL inactivation.

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P.4.1-105
Reducing mitochondrial VDAC1 levels in cancer cells leads to reprogramed tumorigenic properties via the metabolism–epigenetics axis

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Epigenetics refers to heritable changes in gene expression without alteration in DNA sequence and is essential for normal cell development and maintenance. Alterations in the epigenetic landscape network are a hallmark of cancer. Mutations in epigenetic regulators are involved in cancer cellular plasticity and chemoresistance. Epigenetic modifications are realized by chromatin- and histone-modifying enzymes, with their activities being directly dependent on metabolites and the cellular energetic status. Cancer cells undergo metabolic reprograming associated with proliferation, tumor growth, and stemness, with the metabolism-epigenetics link having been explored in tumorigenesis.

In this study, we targeted cancer cell metabolism via silencing the expression of the voltage-dependent anion channel 1 (VDAC1), a mitochondrial protein controlling cell energy and metabolic homeostasis. Our previous study demonstrated that silencing VDAC1 using specific si-RNA in glioblastoma (GBM)

resulted in marked decreases in VDAC1 levels and cell and tumor growth. VDAC1 silencing not only inhibited GBM tumor growth and reversed oncogenic properties, such as reprogrammed metabolism, angiogenesis and stemness, but also resulted in alteration expression of 3,000 genes, as analyzed using DNA microarray. Among these, about 20 genes were associated with epigenetic processes, including acetylation (HATs/KATs), deacetylation (HDACs, SIRTs), DNA methylation (DNMTs) and demethylation (KDMs). The epigenetics link to metabolism was demonstrated via metabolic reprograming as induced by VDAC1 silencing and further shown by a decrease in the expression of enzymes generation methylated and acetylated substrates. These results clearly show that depletion of the mitochondrial gatekeeper VDAC1 lead to a rewiring of cancer cell metabolism, thereby affecting the metabolism–epigenetics axis and further demonstrating the interplay between metabolism and oncogenic signaling networks.

P.4.1-106
Enhanced down-regulation of BIRC2 and BIRC3 antiapoptotic proteins with NO-donor DETA NONOate in prostate DU-145 CSCs: potential for combination therapy

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Cancer stem cells (CSCs) are a subset of tumor cells responsible for tumor initiation and exert a high self-renewal potential. They also exhibit increased resistance to chemotherapy and radiation. Evidence shows that nitric oxide (NO)-donors such as DETA NONOate possess chemo-sensitizing effects. Our previous observations of apoptotic effects of third generation bisphosphonate zoledronic acid (ZA) in human prostate DU-145CSCs through over-expression of some pro-apoptotic and under-expression of some anti-apoptotic proteins prompted us to investigate the impact of DETA NONOate on cell survival and ZA-induced apoptosis in prostate cancer. CSCs and non-CSCs isolated from human prostate cancer DU-145 cell line were first treated with 500 microM of DETA NONOate for 24 hours and then with IC_{50} doses of ZA for an additional 72 hours. Cell viability tests and Annexin V assays were performed by Muse™ cell analyzer. Expressions of 84 apoptosis-related genes were analyzed by qRT-PCR array. Sequential treatment with DETA NONOate demonstrated significantly greater cytotoxicity through decreased cell viability and enhanced levels of total apoptosis in prostate DU-145 CSCs compared to our previous results of treatment with ZA only. Expressions of inhibitor of apoptosis protein (IAP) family *BIRC2* and *BIRC3* genes decreased significantly in CSCs pretreated with DETA NONOate compared with those treated with ZA alone. These effects of DETA NONOate were not observed in non-CSCs. Our findings indicate that combined treatment with ZA and DETA NONOate has more cytotoxic and apoptotic effect on CSCs than non-CSCs and that nitric oxide sensitizes CSCs to ZA-induced apoptosis through down-regulation of IAP proteins BIRC2 and BIRC3. Therefore, sequential treatment with nitric oxide donor DETA NONOate and drugs specifically targeting key survival pathways in prostate CSCs warrant testing in prospective clinical trials for treatment of aggressive prostate cancer.

P.4.1-107
The effect of deuterium depleted water on apoptosis and growth rate of cancer cells

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According to recent data, during apoptosis cells produce various signals that promote growth of neighboring surviving tumor cells. In addition, these signals enhance therapy resistance of recipient cells. We investigated the effect of deuterium depleted water on the level of spontaneous apoptosis in different cancer cell lines- A 549 cell lines (lung adenocarcinoma), ZR-75-1 (metastasis of breast cancer), HT29 (colorectal adenocarcinoma). According to our data deuterium depleted water did not affect the level of spontaneous apoptosis in all tested experimental conditions.

In our experiments, we used DMEM medium that was dissolved in DDW (deuterium depleted water) with the addition of 10% FBS. To test viability of cells we first plated 6000 cells in the wells of 96 well plate and 24 hours later various concentrations of DDW-containing medium were added and cells were incubated for another 24 h. Next cells were treated with MTT reagent to determine viability. We showed that DDW does not influence the survival of cells. This result was confirmed when we used propidium iodide/ Annexin V Labeling kit and subsequent FACS analysis. According to these data the level of spontaneous apoptosis does not exceed 10% in both normal nutrient media and in the medium prepared with DDW. Previously, the antitumorigenic effect of DDW was demonstrated using mice cancer models. Also, there is a number of evidence indicating that DDW can improve patient survival and quality of life when used in combination with standard therapy. However the mechanism how DDW affects tumors is still unknown. Our previous studies showed that DDW decreases cell motility in vitro. All together these data indicate that DDW may be a promising tool for treatment of various aggressive and highly metastatic tumors. The publication was financially supported by the Ministry of Education and Science of the Russian Federation (the Agreement number 02.a03.0008).

P.4.1-108
Evaluation of anti-cancer activity of new stilbene and methoxydibenzo[b, f]oxepin derivatives

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Stilbenes, 1,2-diphenylethen derivatives, including resveratrol and combretastatins, are compounds of anticancer feature especially active against tumor angiogenesis. Fosbretabulin, disodium phosphate salt of combretastatin 4 (CA-4), in combination with carboplatin, is on the last stages of clinical tests in patients with thyroid cancer. The mode of action of these compounds includes suppression of angiogenesis through interfering with tubulin (de) polymerization, thus making them vascular targeting agents (VTAs).

We have previously synthesized new five E-2-hydroxystilbenes and seven dibenzo[b, f]oxepins in Z configuration, with methyl or nitro groups at varied positions. The aim of the present work was to evaluate the anticancer activity and molecular mechanism of action of these compounds. Four cell lines: fibroblasts EUFA30 and human embryonic kidney HEK293, representing

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healthy tissues, and cervical cancer HeLa and glioblastoma U87, representing cancer cells, were subjected to four stilbenes and seven oxepins. Two of this compounds, JJR5 and JJR6, showed relatively high activity against cancerous cells tested and were selected for further investigations. They induced apoptosis with sub-G1 or S cell cycle arrest and PARP cleavage, with no visible activation of caspases 3 and 7. Proteomic differential analysis of stilbene-treated cells led to the identification of several proteins involved almost exclusively in cell cycle management, apoptosis, DNA repair, and stress response, e.g. oxidative stress, like γ-glutamylcyclotransferase, UV excision repair protein RAD23 homolog B, or heme oxidase HO-1. We conclude that JJR5 and JJR6 compounds are worth further investigation and can be a base for structure modification(s) to obtain even more active compounds. This work was supported by the National Science Centre, Poland, UMO-2012/07/B/ST5/03194 and UMO-2011/03/B/NZ4/02425 for ALKBH studies.

P.4.1-109

Withdrawn

P.4.1-110
New inhibitors of ALKBH dioxygenases overexpressed in neck and head cancer

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AlkB protein from *E. coli* (EcAlkB) is a profoundly studied member of 2-oxoglutarate and Fe(II) – dependent dioxygenase superfamily that removes alkyl lesions from DNA bases via oxidative demethylation restoring native bases in DNA. In human cells, there are nine EcAlkB homologs, ALKBH1-8 and FTO. These dioxygenases, repair alkylation lesions to DNA and RNA but also show other biological functions. Cancerous tissues develop greater efficiency of DNA repair systems in comparison to healthy ones. Overexpression of DNA repair proteins in tumor leads to the removal of DNA lesions before they become toxic to cancer cell, in this way assuring tumor welfare and creating major mechanism of resistance to anticancer therapy. Our main approach was to determine the levels of expression of particular ALKBHs in cancer tissues by Western-blot in head and neck cancer tissues. We have demonstrated that dioxygenases ALKBH 1, 3, 4, 5, and FTO are highly expressed in these tumors. Statistical analysis allowed to create protein expression tree of ALKBHs tested. We have found that FTO and ALKBH5, and ALKBH1 and ALKBH3 show similar expression patterns in healthy and cancerous tissues. To answer the question whether ALKBHs under study form any oligomers, we performed size exclusion chromatography and observed monomeric forms of ALKBH3, 4 while ALKBH1, 5, and FTO formed protein complexes. Natural substances, rhein and emodin, show anti-cancer, anti-inflammatory, and anti-microbial activities. Rhein inhibits activity of EcAlkB, ALKBH2, ALKBH3, and FTO. We synthesized and investigated new rhein and emodin derivatives as a potential inhibitors of ALKBHs and found that chloridoemodins in the form of mono- and di-chlorides are the most active inhibitors of ALKBHs among compounds tested. Moreover, they were more selective against cancerous than healthy cells and seem to be a promising anthraquinones in anti-cancer therapy.Funding: Pol/Nor/196258/59/2013.

P.4.1-111
Adenosine A3 receptor regulates stemness of glioblastoma stem-like cells under hypoxic conditions

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Introduction: Glioblastoma stem-like cells (GSCs) are a cellular subpopulation of glioblastoma (GBM) which, due to their tumorigenic capacity and resistance to therapy, have been proposed as the main responsible for tumor recurrence. It has been suggested that GSC differentiation could eventually eliminate these cells; however, hypoxic niches, where GSCs are found, promote their stemness and protect their undifferentiated phenotype. Hypoxic niches induce elevated adenosine levels. Therefore, our aim was determine the role of adenosine receptors on the stemness of the GSC phenotype.
Methodology: Control and Adenosine A_3 Receptor (A_3AR) knockout GSCs were exposed to the A_3AR antagonist MRS1220 (10 μM) under normoxia (21% O_2) or hypoxia (0.5% O_2) for 24 hours. Basal levels of extracellular adenosine were quantified by HPLC. The A_3AR expression was evaluated by Western blot and cells positive for CD133, nestin and CD44 were determined by cytometry. The self-renewal capacity of GSCs was evaluated by the formation of neurospheres.
Results: Both extracellular adenosine levels and A_3AR expression in GSCs were increased under hypoxia. The A_3AR/KO GSCs exhibited decreased expression of CD133 and nestin stem markers compared to control cells. Pharmacological antagonism of the A_3AR decreased CD133 positive cells (~50%) and the CD44 positive population (~40%) demonstrating a loss of stemness markers. Furthermore, A_3AR antagonism markedly reduced neurosphere formation.
Conclusions: The adenosine A_3 receptor subtype mediates stemness and the self-renew capability of GSCs under hypoxic conditions.

P.4.1-112
The role of microtubule-associated protein TPX2 in sensitization of head and neck cancer cells to ionizing radiation

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Head and Neck cancer is the sixth most common non-skin cancer worldwide, leading to more than 350'000 deaths per year. The important physiological role of the affected anatomical regions can have a functional impact such as impaired speech and breathing, as well as complicate the complete removal of the cancer by surgery. Thus, radiotherapy-based approaches remain a prevalent therapeutic modality in head and neck squamous cell carcinoma (HNSCC) and tumor radioresistance constitutes a major source of treatment failure, underlying the necessity to explore and implement effective radiosensitization strategies for HNSCC. In this study, we examine the role of the Targeting protein for Xklp2 (TPX2) in sensitizing HNSCC cell lines to

radiation therapy. TPX2 is a microtubule-associated protein which plays a key role in spindle formation and function by recruiting and activating mitotic regulators such as Aurora-A. Furthermore, TPX2 has been shown to associate with DNA damage response factors, such as ATM and MDC1. To investigate radiosensitivity of HNSCC depending on TPX2 expression levels, we have employed an unique cell line panel consisting of patient-matched primary and metastatic/recurrent HNSCC cell lines originating from the University of Michigan SCC cell line collection. Our data indicate that TPX2 knockdown leads to a prolonged expression of irradiation-dependent phosphorylation of H2AX and has a negative impact on cell proliferation and survival. Moreover, we could show a direct interaction of TPX2 with the histone acetyltransferase Males absent on the first (MOF; also known as MYST1 or KAT8) known to acetylate histone H4 on lysine 16 (H4K16). Importantly, as the depletion of MOF leads to decreased H4K16 and both MOF and H4K16 are important for an effective DNA damage repair, their crosstalk with TPX2 supported by our current data might have important implications in HNSCC responses to DNA-damaging agents.

P.4.1-113
Expression of putative protein markers related to the sensitivity or resistance to retinoids in neuroblastoma tumor tissue samples and tumor-derived cell lines

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Our study is aimed at the analysis of expression of selected putative biomarkers (PBX1, HMGA1, HMGA2, NF1, HOXC9 and DDX39A) related to the sensitivity or resistance of neuroblastoma cells to retinoids. Retinoids are used in therapy of pediatric patients suffering with high-risk neuroblastomas: at the end of intensive multimodal treatment, administration of retinoids in patients with minimal residual disease was proved as effective and it could delay or prevent tumor relapse after myeloablative therapy. Nevertheless, about 50% of these patients were resistant to this treatment or developed resistance during therapy. 19 cell lines derived from the tumor samples taken from patients surgically treated for NBL were included in this study. Expression of selected putative markers listed above was analyzed both on mRNA and protein levels in these neuroblastoma cell lines. In the next step, sensitivity of these cell lines to the natural (ATRA, 13-*cis*-RA, 9-*cis*-RA) and synthetic (4-HPR, bexarotene) retinoids was evaluated using MTT assay. Simultaneously, results achieved using this panel of neuroblastoma cells lines were compared with expression of these protein markers in tumor tissue samples, from which these cell lines were derived, and with outcome of patients treated with retinoids. This unique experimental design allowed us to compare the sensitivity of these markers under in vitro conditions as well as the usefulness of them in giving precision in prediction of patient's clinical outcome.

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P.4.1-114
Mitochondrial VDAC1-based peptides as a potential therapeutics for Glioblastoma

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Glioblastoma multiforme (GBM), a common primary brain malignancy characterized by high morbidity, relapse rate and mortality, is highly invasive, proliferative, and resistant to chemo- and radiotherapies and for which effective therapeutic options are lacking. GBMs undergo metabolic reprograming and develop cell survival strategies, involving anti-apoptotic defense mechanisms, hallmarks of numerous cancers. As such, tumor metabolism and apoptosis resistance are emerging avenues for cancer therapy. The outer mitochondrial membrane protein voltage-dependent anion channel 1 (VDAC1) is a central player in cell energy metabolism and assumes a key role in mitochondria-mediated apoptosis. Here, we demonstrate that VDAC1-based cell-penetrating peptides induce massive cell death in several brain cancer-derived cell lines, including GBM and GBM-derived stem cells. The mode of action of the VDAC1-based peptides involved induction of mitochondria-mediated apoptosis and impairment of energy and metabolic homeostasis. In subcutaneous and intracranial xenograft GBM mouse models, VDAC1-based peptides, markedly inhibited tumor growth and induce apoptosis. Strikingly, peptide-treated tumors showed down-regulation of the glucose transporter, glycolytic enzymes and VDAC1 expression. In contrast, the levels of pro-apoptotic proteins, such as p53, cytochrome c and caspases were increased. Moreover, peptide tumor treatment induced dramatic decreases in cell proliferation and eliminated stem cells. These findings show that VDAC1-based peptides dramatically inhibit cancer cell growth and tumor development, eliminate cancer stem cells and trigger apoptosis, raising the possibility of a more effective pipeline of anti-glioblastoma drugs designed to overcome GBM stemness, invasiveness and relapse.

P.4.1-115
Microenvironment of tumors and wounds and its pharmacological modulation

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Clinical observation of existing anti-tumor therapies have revealed that our current focus on the possibility to modulate the incipient cancer cells has not resulted in significantly improved survival. Therefore, it is now suggested that the inhibition of biological programs that are associated with the tumor microenvironment (TME) may be critical to the diagnostics, prevention and treatment of cancer. The TME is composed of cellular and non-cellular components (extracellular matrix - ECM) and has become a research hallmark. Although, a growing body of evidence has revealed that the TME differs distinctly from the corresponding normal stroma, remarkable similarities between the connective tissue reaction in wounds and in tumors have been reported by Harold Dvorak's article "Tumors: wounds that do not heal" published 30 years ago. Further studies comparing tissue repair and regeneration with aspects of malignancy revealed that these two process cascades do have even more in common. A suitable wound microenvironment would accelerate tissue repair and prevent extensive scar formation. In present study we define key signaling molecules (growth factors, cytokines, chemokines, and galectins) involved in the formation of the tumor microenvironment that decrease overall survival and

increase drug resistance in cancer suffering patients. Additional attention will also be given to show whether targeted modulation of these regulators will promote tissue regeneration and wound management in patients.

P.4.1-116
Cancer associated fibroblast are not form by epithelial-mesenchymal transition in nu/nu mice

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Cancer-associated fibroblasts are very important players to form specific cancer microenvironmnet and bioactive elements influencing the biological properties of malignant tumors. Their origin from different cell types has been established. It is suppose and under debate that exist three ways of the possibility of their formation: a) by epithelial-to-mesenchymal transition from cancer cells b) by activation of local mesenchymal cells c) by MSC (mesenchymal stem cell), which migrate from bone marrow to tumor. We focused on epithelial-to-mesenchymal transition. This study shows that human cancer cells lines FaDu FaDu (human squamous cell carcinoma isolated from pharynx; HTB-43), Sw620 (human colorectal adenocarcinoma; CCL-227) and HT-29 (human colorectal adenocarcinoma; HTB-38) grafted to nu/nu CD-1 mice induced formation of tumor stroma with the presence of typical smooth muscle actin-containing cancer-associated fibroblasts. These cells seem to be of the host origin because they are not recognized by an antibody specific for human vimentin, as was also verified in vitro. These results suggest that cancer-associated stromal fibroblasts are not formed by epithelial-to-mesenchymal transition from cancer cells.

P.4.1-117
Highly specific targeting of human acute myeloid leukaemia cells using gold nanoparticle-based conjugates

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Highly specific targeting of human malignant cells with the purpose of delivery of cytotoxic drugs into them is a very promising but not well developed therapeutic strategy. It is a major focus of current Nanomedicine, Biotechnology, and Synthetic Biology.

In this study we demonstrated for the first time a new approach for highly specific targeting of human acute myeloid leukaemia (AML) cells by functionalised gold nanoconjugates made on the basis of citrate-stabilised gold nanoparticles (AuNPs). We constructed a complex, where 5 nm AuNPs carry a 27 KDa single chain antibody against T cell immunoglobulin and mucin domain 3 (Tim-3) in the ratio – 1 AuNP: 1 antobody molecule. The rest of the gold surface is covered with rapamycin immobilised in the form of glutathione-SH ester. The immune receptor Tim-3 is highly expressed in human AML cells and thus can be used as a target for recognition. Rapamycin inhibits activity of mammalian target of rapamycin (mTOR), a master regulator of translational pathways in AML cells. Inhibiting mTOR leads to a rapid killing of AML cells. Using these nanoconjugates we managed to successfully deliver rapamycin into the AML cells reaching attenuation of the mTOR activity. Concentration of rapamycin required to reach such an effect is at least 50 times lower compared to the one of free rapamycin required to achivce similar effect. We therefore concluded that our technology is of potential use for highly specific targeting of AML cells.

P.4.1-118
ADME-related genes in breast cancer patients of Kazakhstan

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Background: Breast cancer (BC) still remains one of the most common cancer pathology in many countries, including Kazakhstan. The potency of therapeutic strategies is strongly depend not only on the expression of the drug targets, but also on pharmacogenes involved in drug absorption, distribution, metabolism and excretion (ADME). The altered primary structure resulting from genetic polymorphisms can generally affect reduction or absence of chemotherapy perception in BC patients. Methods: Single nucleotide polymorphisms in 26 ADME genes were tested in 757 breast cancer patients and healthy individuals, including 446 Kazakh and 311 Russian women. The SNPs were chosen from the 1000 Genomes Project database and further pruned using Tagger option of Haploview ($r^2 = 0.95$). Genotyping of 768 SNPs selected was performed using a custom GoldenGate 768-plex microarray manufactured by Illumina.

Results: SNPs in *CYP3A4* (rs3735451), *UGT2B7* (rs6600892), *ABCC1* (rs4148378), *AKR1C3* (rs11252906) and *MAP3K1* (rs62358073) demonstrated statistically significant differences in genotype distribution in case and control groups. Assessing the risk of the development of breast cancer the heterozygous genotypes of four SNPs were found to be risk-related in both Kazakh (*rs*6600892 – OR = 3.53; *rs*3735451 – OR = 4.68; *rs*11252906 – OR = 20.64; *rs*62358073 – OR = 1.67) and Russian groups (*rs*6600892 – OR = 3.28; *rs*3735451 – OR = 9.84; *rs*11252906 – OR = 28.12; *rs*62358073 – OR = 3.63). Additionally, an SNP *rs*4148378 was determined as a protective in both populations (OR = 0.13 and OR = 0.04 respectively).

Conclusions: The exploration of ethnically specified Kazakh and Russian BC patients and controls from Kazakhstan determined the same polymorphisms in *CYP3A4*, *UGT2B7*, *ABCC1*, *AKR1C3* and *MAP3K1* genes as risk loci in both ethnic groups. The results suggest similarity in molecular and genetic mechanisms of BC pathogenesis in Kazakh and Russian populations.

P.4.1-119
L1CAM induces perineural invasion of pancreas cancer cells by upregulation of metalloproteinase expression

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Pancreas cancer cells have the tendency to invade along nerves. Such cancerous nerve invasion (CNI) is associated with poor outcome; however the exact mechanism that drives cancer cells to disseminate along nerves is unknown. Immunohistochemical analysis of human pancreatic ductal adenocarcinoma (PDAC) specimens showed overexpression of L1CAM in cancer cells and in adjacent Schwann cells (SC) in invaded nerves. Using migration, invasion and wound healing assays, we found that L1CAM secreted from SCs acts as a strong chemoattractant to cancer cells. L1CAM secreted by SCs induced MAPK signaling activation. L1CAM secretion also upregulated the expression of metalloproteinase-2 (MMP-2) and MMP-9 by PDAC cells by STAT3 activation, further facilitated CNI. Blocking of L1CAM secretion by SCs impaired PDAC invasion in a nerve invasion model. Using a transgenic KPC mouse model, we show that treatment with anti-L1CAM Ab significantly reduces CNI in vivo. Our results identify a paracrine response between SCs and cancer cells in the neural niche, which promotes cancer invasion via L1CAM secretion.

P.4.1-120
What to consider for sex-balanced researches using cell lines

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Background and purpose: With the recognition of sex as an important biological variable, sex-balanced researches are gradually increasing in clinical and animal studies. Recently, funding agencies began asking researchers to sex balance in cell experiments as well. As commercial vendors do not always provide sex information for their cells, researchers need to figure it out by themselves. We examined sex of the cells obtained from commercial venders using PCR-based methods and compared the results with what was provided.

Method: Six male and six female human gastric cancer cell lines were obtained from Korean Cell Line Bank (KCLB). To test the sex of the cell lines, PCR amplifications of SRY, Amelogenin, and STS were performed using genomic DNA. The three genes are located on sex chromosomes and their lengths are different depending on whether the genes are located on X or Y chromosomes. Fluorescence in situ hybridization (FISH) analysis was also performed using CEPX/CEPY probe.

Result: The PCR products from all six female cells showed single bands. Unexpectedly, the PCR products from four of the six male cell lines showed single bands rather than double bands. Results from FISH experiments using three of the male cell lines confirmed our PCR results.

Conclusion: There were some discordance between the sexes determined by PCR and what was provided by vendors, which could be because cancer cells have abnormal sex chromosomes. Simple sex-determining PCR methods may not suitable to verify sex of cancer cells.

P.4.1-121
Post translational regulation of radioactive iodine uptake in papillary carcinoma of the thyroid

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Background: Radioactive iodine (RAI) is the mainstay of treatment for differentiated thyroid carcinoma (DTC). Nevertheless, the mechanism of RAI resistance that occurs in many patients with DTC remains unknown. We aimed to elucidate the role of post-translational regulation of radioiodine uptake.

Methods: We analyzed the expression pattern of the ribosomal glycosylphosphatidylinositol transamidase (GPIT) complex in DTC. We used functional RAI uptake assays to assess the role of GPIT in iodine uptake both in vivo and in vitro. The effects of MEK inhibition on the GPIT subunit PIGU and the sodium iodide symporter (NIS) were assessed in three DTC cell lines and in human DTC biopsies. We used a multivariate logistic regression model to study the role of PIGU in the response to RAI treatment in advanced DTC. All statistics were two sided.

Results: Expression profiling of different GPIT complex subunits revealed significantly lower expression of PIGU in papillary carcinomas than in matched normal thyroid tissue ($P < 0.001$). Expression of PIGU in the K1 human papillary carcinoma cell line resulted in a robust increase in NIS glycosylation and trafficking to the cell membrane, accompanied by a robust increase in I125 uptake both in-vitro ($P < 0.001$) and in-vivo ($P < 0.001$). Treatment with the MEK inhibitors U0126 and PD302 rescued PIGU expression. Finally, the PIGU expression level in tumors of 18 patients with recurrent DTC correlated with biochemical response to RAI treatment.

Conclusions: We showed that downregulation of PIGU in DTC determines NIS function and RAI avidity. This represents a novel mechanism for RAI resistance.

P.4.1-122
Effect of ciprofloxacin on TRAIL-induced apoptosis through up- regulation of death receptors by CHOP expression and protein stability in cancer cells

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Ciprofloxacin (CPX) are potent anti-microbial agents with multiple effects on host cells and tissues. Previous studies have highlighted their pro-apoptotic effect on human cancer cells. Here, we show that subtoxic doses of CPX effectively sensitize multiple cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)- induced apoptosis. Although TRAIL alone mediated partial proteolytic processing of procaspase-3 in lung cancer cells, cotreatment with CPX and TRAIL efficiently restored complete activation of caspases. We found that

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treatment of lung cancer with CPX significantly upregulated death receptor (DR) expression and protein stability of DR5. The induction of death receptor was mediated through regulation of a transcription factor CCAT enhancer- binding protein homologous protein (CHOP); as silencing of these signaling molecules abrogated the effect of CPX. Taken together, these results indicate that the activity of CPX toward death receptors and protein stability contributes to the amplification of death receptor expression, thereby restoring TRAIL sensitivity in lung cancer cells.

P.4.1-123
Post-transcriptional regulation of immune checkpoint genes by mir-16 in melanoma

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The complex interface between T lymphocytes and cancer ('the immunological synapse') comprises of both co-stimulatory and co-inhibitory proteins that modulate the lymphocyte towards activation or anergy. 'Checkpoint inhibitors' have impressive activity in melanoma, but not all patients respond and drug resistance often develops. Micro-RNAs are master regulators of gene expression. Bioinformatic analyses of expression of 15 checkpoint mRNAs and 8 miRNAs from 451 samples from the melanoma TCGA database showed a statistically significant positive correlation between the expression of 9 checkpoint mRNAs to each other and to mir-16. These results were corroborated in vitro. Bioinformatic analysis suggested that mir-16 may potentially target the 3'UTR of 3 of these mRNAs. Using luciferase reporter assays, we found that CD80 (B7.1) is a direct target of mir16 in vitro. Overexpression of mir-16 in melanoma cell lines led to downregulation of CD80, CD274 and CD40, while downregulation of mir-16 increased the expression of these genes. Survival data from 163 stage III melanoma patients show that high levels of mir-16 and low levels of any of six checkpoint mRNAs (CD80, CD86, ICOSLG, TNFSF4,TNFRSF18 or CD274) is significantly associated with poor prognosis. Our results suggest that mir-16 targets checkpoint mRNAs and is generally under a strict joint transcriptional regulation with them. Its ability to modulate CD80 expression suggests that it serves as a key regulator of the immunological sample. We hypothesize that an aberrantly high expression of mir-16 decreases the expression of the co-stimulatory checkpoint CD80 in melanoma, and is associated with a low expression of additional checkpoint mRNAs, leading to a net effect of decreased immunogenicity and to immune evasion. Further elucidation of both transcriptional and post-transcriptional regulation of the immunological synapse may help point to novel targets for immune modulation and may improve immunotherapeutic drugs.

Translational Control and mRNA Localization

P.4.2-001
Tomato SEP3-like LeMADS5 transcription factors have diverse activity during reproductive development and fruit ripening in cultivated and wild Solanum section Lycopersicon accessions

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MADS-domain transcription factors are involved in specifying meristem and organ identity. Tomato LeMADS5 is the Arabidopsis SEP3 homologue with a putative significant role in fruit development and ripening. The present work is focused on the natural LeMADS5 diversity in wild and cultivated tomato species. Using designed LeMADS5-specific primers, complete *LeMADS5* genes of *S. pimpinellifolium* and *S. peruvianum* were identified. As compared to *S. lycopersicum* cv. Heinz *LeMADS5*, genes contain indels and synonymous and non-synonymous SNPs. Based on obtained LeMADS5 sequences, RT-PCR primers were designed, and *LeMADS5* expression was examined in flowers, mature green (MG) and ripe (RF) fruits of cultivated and wild tomato species differing in fruit morphophysiology. *S. lycopersicum* var. *humboldtii* and *S. lycopersicum* cv. Silvestre recordo *LeMADS5* transcription levels were relatively uniform with the peak in flowers and minimum in RF fruits that was similar to the only described cv. Micro-Tom. In heirloom species *S. pimpinellifolium*, *LeMADS5* was equally expressed in flowers and RF fruits, with maximum in MG fruits. In other wild species *S. cheesmaniae*, *S. neorickii*, *S. peruvianum*, *S. peruvianum* var. *dentatum*, *S. arcanum*, and *S. habrochaites*, *LeMADS5* transcription was 6–20-times higher in fruits compared to flower-specific expression, which was 5-times lower than in *S. lycopersicum*. Furthermore, *S. neorickii* and *S. habrochaites LeMADS5* expression was highest in RF fruits, while in other species the peak of *LeMADS5* expression was in MG fruits. Polymorphism of *LeMADS5* genomic and derived protein sequences, as well as different *LeMADS5* expression mode in wild and cultivated tomatoes besides conserved role for LeMADS5 in fruit development and ripening may suggest but with some distinctive properties linked to the diverse flower and fruit characteristics. The study was supported by the Russian Science Foundation grant 16-16-10022.

P.4.2-002
The metabolic pathway of D-Tyr and editing of D-Tyr-misaminoacylated substrates in Thermus thermophilus

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Homochirality of proteins is important for cell survival. L-enantiomers of amino acids are predominant in all living systems, but D-amino acids also can be included in a variety of physiological behaviors such as spore germination, cell wall reorganization etc. To prevent inclusion of D-amino acids into polypeptides, protein biosynthesis machinery requires precise control and editing check-points. The metabolic pathway of D-Tyr includes 2 main enzymes: TyrRS (tyrosyl-tRNA-synthetase), which exists as a *cis*-editing factor and DTD (D-aminoacyl-tRNA-deacylase) – *trans*-

editing factor that hydrolyzes D-aminoacyl-tRNA, mistakenly formed during aminoacylation by TyrRS. However, the mechanisms of deacylation still require deep analysis and investigations. Steady-state kinetic parameters for the reaction of L- and D-Tyr binding to tRNA was determined by thin-layer chromatography with α-[^{32}P]-radiolabelled-tRNA^{Tyr}. Km for both enantiomers did not differ significantly (3.34 ± 0.8 μM for D-Tyr and 3.59 ± 0.8 μM for L-Tyr), confirming the fact of absent discrimination between substrates at the binding step. The constant of velocity of L-Tyr incorporation is only 11-fold higher than for D-Tyr, also pointing to the weak discriminating properties of TyrRS toward Tyr enantiomers. To study the mechanism of DTD' editing function we performed molecular modelling by AUTODOCK and Modeller, molecular dynamics simulations (MD) by Gromacs and VMD. The structural model of DTD from *T. thermophilus* bound to its substrate was generated using the reported crystal structure of *Plasmodium falciparum* DTD. The results of MD simulations after 5 ns allowed choosing amino acids for site-directed mutagenesis studies. 12 substitution mutants were created and checked for their editing activity. Summarizing, the catalytic constants for wild-type and some mutant forms of deacylase were determined. Based on the biochemical and MD data the deacylation mechanism of D-Tyr-tRNA in thermophilic bacteria was proposed.

P.4.2-003
Real-time luciferase measurement in living cells and its application for investigation of protein biosynthesis

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Luciferase encoding reporters are widely used for gene expression analysis. The standard procedure involves accumulation of the reporter protein in living cells followed by cell lysis and a single measurement of luciferase activity in the lysate. However, this approach does not allow tracing a dynamics of protein accumulation with high resolution, and thus is not suitable for studying fine details of gene expression and its regulation. In our laboratory, we established the method of continuous luciferase measurement in cultured mammalian cells and applied it for studying the expression of reporter constructs at the translational level in real time. We compared the translation efficiency and kinetics for several mRNAs encoding two luciferases - firefly and Renilla, depending on substrate variants and concentrations, and tested a number of transfection reagents. Then we investigated how the mRNA translational properties changed under conditions of various cellular stresses (oxidative stress, ultraviolet irradiation etc.) The method further allowed us to show that in vitro synthesized intron containing mRNAs can be efficiently spliced after their transfection into proliferating cells. We also tracked the expression kinetics of the luciferase gene delivered by a plasmid DNA transfection and compared it to the direct mRNA delivery. Thus, we developed a highly sensitive method for the continuous measurement of the reporter activity in cultured mammalian cells and showed its applicability for studying different aspects of the gene expression. This work was supported by the grant of the Russian Federation government No.2016-220-05-308 (14.W03.31.0012).

P.4.2-004
Functional role of ribosomal protein S6 oligoglutamylation in *Escherichia coli*

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Ribosome is the major molecular machine of all living cells, so it is not a surprise that such a complex object requires an extensive set of "maintaining" proteins, which are involved in ribosome maturation and regulation. One of the most interesting of these modifications is the oligoglutamylation of the ribosomal protein S6 that is performed by a protein RimK, yet the physiological role of it has remained obscure. We demonstrated that this modification is growth phase dependent and irreversible. To understand a nature of modification regulation we have created a reporter plasmid, which encodes a fluorescent timer protein under control of *rimK* promoter. By flow cytometry we have shown that modification's growth phase dependence is primarily based on RimK expression profile. An influence of this modification on *E. coli* cells was investigated by comparative proteomics and different phenotype-related assays, including: cell survival, ability to compete for nutrients and resistance to stress.

We have tried dozens of stress conditions to induce S6 modification, but it seems to us that this kind of modification is induced by growth arrest itself, rather than any specific stress conditions of growth. During our studies, we have noticed that a protein RaiA, which is believed to be a ribosome hibernation factor, have a C-terminal domain, which is very similar to CTD of the ribosomal protein S6, and it is located in the same part of a ribosome as the S6. To check a possibility of RaiA modification we have overexpressed RaiA in wild type and *ΔrimK* cells and shown that it is modified in a similar way to S6 and this modification is RimK-dependent. Functional role of this modification will be discussed. This work was supported by grants from the Russian Foundation for Basic Research 16-04-01100 A and Russian Science Foundation grant 14-14-00072.

P.4.2-005
Effect of anoxia and polyscias filicifolia bailey biomass tincture on the protein synthesis process in the isolated pig heart

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The aim of this study is to investigate what components of the cell-free protein synthesis system are influenced by anoxia and *Polyscias filicifolia* Bailey biomass tincture in the isolated pig heart. The effect of anoxia was evaluated after 20 minutes and 90 minutes of anoxic perfusion. With the aim to determine the effect of *Polyscias*, pig hearts were perfused with a buffer containing the *Polyscias filicifolia* Bailey biomass tincture. The protein synthesis level decreased by 19% in the cell-free system containing cytosol and ribosomal fraction from the anoxic heart as compared with the control heart. The analogous results were observed when the cell-free system contained cytosol from the anoxic heart and ribosomal fraction from the control heart. In the case of 90 minutes anoxia, the protein synthesis level diminished by 45% when cytosol from the anoxic heart was used and only by 16% when ribosomal fraction from the anoxic heart was used. Protein synthesis level in the translation system containing

cytosol from the heart after 20 minutes of anoxic perfusion with the buffer containing *Polyscias* was the same as in the control. After 90 minutes of anoxic perfusion with the buffer containing *Polyscias filicifolia* Bailey tincture the protein synthesis level did not reach control values and was less than in the control. The protein synthesis level in the cell-free translation system under oxygen deprivation is related to changes in cytosol and ribosomal fraction. We can conclude that one of the causes influencing protein synthesis process under anoxia may be the changes in the tRNA and aminoacyl-tRNA synthetases activity under anoxia. In the case of anoxic perfusion with buffer containing *Polyscias* tincture, the protecting effect of *Polyscias filicifolia* Bailey on protein synthesis was observed. This effect was determined by its influence on the activities of components of cytosol and ribosomes.

P.4.2-006
Similarities between the bacterial and eukaryotic translation initiation pathways

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During translation initiation the small ribosomal subunit positions the initiator tRNA and correct start codon of mRNA at the P site with the help of multiple initiation factors. It is known that the initiation pathway requires multiple steps where the ribosome and factors undergo conformational changes. Previously we reported single particle cryo-electron microscopy (cryo-EM) reconstructions of pre-initiation complexes (PICs) from yeast in an open as well as closed conformation of the small ribosomal subunit. We have also provided 11 single-particle cryo-electron microscopy (cryoEM) reconstructions of the complex of bacterial 30S subunit with initiator tRNA, mRNA, and IFs 1–3, representing different steps along the initiation pathway. All these PICs provide insights into key events during eukaryotic and bacterial translation initiation and into the roles played by the different initiation factors, showing that despite the large differences in the complexity and regulation of initiation in bacteria and eukaryotes, both seem to employ a common core mechanism with the three factors that are common to both kingdoms.

P.4.2-007
Translationally-active tetramolecular RNA G-quadruplexes derived from transfer RNAs

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RNA G-quadruplex (RG4) structures are thought to be involved in multiple biological processes. Recent genome-wide analyses of human mRNA transcriptome identified thousands of putative intramolecular RG4s that readily assemble *in vitro* but shown to be unfolded *in vivo*. Previously, we have shown that mature cytoplasmic tRNAs are cleaved during stress response to produce tRNA fragments that function to repress translation *in vivo*. We report here that these bioactive tRNA fragments assemble into intermolecular RG4s. We provide evidences for the formation of uniquely stable tetramolecular RG4 structures consisting of five tetrad layers formed by 5'-terminal oligoguanine motifs of an individual tRNA fragment. RG4 is absolutely required for functions of tRNA fragments in the regulation of mRNA translation and stress responses, and its disruption abrogates their bioactivities both *in vitro* and *in vivo*. Collectively, our data rationalize the existence of naturally occurring RG4-assembling tRNA fragments and emphasize their regulatory roles.

P.4.2-008
Characterizing mRNA export at high resolution in individual nuclear pores in single cells

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The export of mRNA from the cell nucleus is one of the pillars of the gene expression pathway in eukaryotes. Conventional light microscopy does not allow high resolution analysis of mRNA export in intact cells nor does it enable the examination of specific and functional interactions that exported molecules undergo as they pass from the nuclear side of the nuclear pore complex (NPC), through the inner channel of the pore, and then out to the cytoplasmic side. Such limitations hinder our understanding of the biology of mRNA export within the context of gene expression and its regulation, and require the innovation of new approaches. A key factor involved in the passage of the transcript through the nuclear pore complex is Nuclear Export Factor 1 (NXF1/Tap). We have performed measurements within individual nuclear pores using super-resolution STED microscopy, a FLIM-FRET approach, as well as single mRNA tracking in living human cells. These approaches have allowed the detection and measurements of specific interactions taking place between NXF1 and mRNAs, and between NXF1 and proteins within the NPCs, in intact cells. We are able to discriminate between specific NXF1-NPC interactions under regular conditions and when mRNA export is blocked, and characterize interactions involved in the various stages of mRNA transition through the nuclear pore.

P.4.2-009
Splicing efficiency and sub-cellular localization of coding and long non-coding RNAs

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Splicing of eukaryotic pre-mRNAs occurs in most expressed transcripts, and is required for subsequent nuclear export. In our study, we aim to assess the importance of the splicing process in determining sub-cellular localization of transcripts at global scale, and to explore possible mechanisms of regulation of sub-cellular localization by splicing. To address these questions, we analyzed RNA-seq data from 10 ENCODE human cell-lines, which allows quantification of splicing and cytosol/nucleus expression levels. Our analysis reveals a strong correlation between splicing and cytoplasmic localization on a genome-wide scale in all examined cell-lines. In addition, differential splicing and differential localization between cell-lines are significantly associated in most possible pairs of cell-lines, suggesting that the splicing process positively regulates nuclear export in steady state and can be used to predict sub-cellular localization. To further explore the long-term splicing-localization relationship in the transcriptome, we compared mRNAs with long non-coding RNAs (lncRNAs), which are relatively inefficiently-spliced transcripts. Sub-cellular

localization differs significantly between mRNAs and lncRNAs and is associated with the difference in splicing efficiency. Processing of pre-mRNAs is typically rapid and co-transcriptional, however it has been shown that some poorly-spliced transcripts can be spliced post-transcriptionally and exported to the cytoplasm upon stimulation. Therefore, we hypothesized that rapid splicing upon stimulus is important in regulating rapid nuclear export in immediate-early genes. Indeed, splicing and localization changes upon EGF stimulation of MCF7 cells are correlated in some genes, supporting a role for splicing induction in dynamic, short-term regulation of nuclear export, overall contributing to a rapid increase in protein production.

P.4.2-010
Peptidyl transferase inhibitors arrest the ribosome at specific amino acid codons: insights from an integrated approach

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Antibiotics that target the eukaryotic ribosome represent potential antitumor and anti-inflammatory drugs. For many of them, the functional details of their activity remain incompletely understood. Using toe-printing technique, we analyzed effects of several translation inhibitors in mammalian cell extract. When preincubated with the lysate before mRNA addition, both aminoglycoside antibiotics and translocation inhibitors predictably stopped elongating ribosome at the very beginning of the coding region. In contrast, antibiotics that affected the peptidyl transferase center (PTC) of the 60S subunit demonstrated complex patterns of toe-print signals along the mRNA. We investigated this phenomenon in detail in a case of antitumor drug omacetaxine mepesuccinate (homoharringtonine) and its derivative harringtonine, which had been used recently in ribosome profiling studies. We showed that both drugs specifically halted elongating ribosomes at Lys, Arg or Tyr codons positioned in the P-site. Statistical analysis of ribosome profiling data generally confirmed this conclusion in a transcriptome-wide scale. Molecular modeling suggested that such specificity was dictated by additional contacts of the antibiotic side chain with peptidyl moiety of the P-tRNA. We unexpectedly found the same toe-print pattern for a chemically distinct trichothecene antibiotic, T-2 toxin, while another inhibitor of this group, diacetoxyscirpenol, produced a completely distinct picture of ribosome stops. Furthermore, the Fleeting RNA Transfection (FLERT) of living mammalian cells revealed an intriguing specificity of the PTC inhibitors toward reporter mRNAs with different 5' untranslated regions. Our data suggests that the mechanism of inhibition of the protein synthesis by PTC inhibitors is more complex than it was originally proposed. The work was supported by RFBR (grant no. 16-04-01271) and the grant of the Russian Federation government No.2016-220-05-308 (14.W03.31.0012).

P.4.2-011
Dynamic regulation of the tRNA pool

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The abundance of available mature transfer RNAs (tRNA) determines the efficiency, throughput, and accuracy of conversion of the protein-coding transcriptome into the proteome. Several disorders arise from tRNA aberrations, such as tRNAs mutations, or alterations in tRNAs levels, processing or modifications. Even "silent" mutations, which do not change protein sequences, but change the identity of the tRNA molecules that deliver the amino acid to the ribosome, can lead to protein misfolding and disease. Exact balance of the tRNA pool is critical in multicellular organisms: the tRNA pool is unique in different tissue and, as we demonstrated, in proliferative cancerous cells and arrested/differentiated cells, recognizing pro-proliferation and pro- differentiation tRNAs.

The gap in knowledge regarding tRNAs regulation stems largely from the requirement for additional methods that enable dynamic measurements of tRNAs expression. tRNA levels are difficult to measure, since tRNAs are short molecules, due to the abundance of tRNA base modifications, and because of the presence of rigid secondary structures. Using new method for tRNA deep sequencing, and development of a novel fluorescence reported for tRNA genes we were able to deciper the exact expression of tRNA individual genes. We have uncovered a new regulation system for controlling tRNA abundance in different cells, and demonstrated the saptio- temporal- regulation of the previously considered "transparent" tRNAs.

P.4.2-012
Novel small (non)coding RNAs with an extended Shine-Dalgarno sequence

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A great variety of small non-coding RNAs are known to play different regulatory roles in bacteria. While mRNAs rarely are involved in any regulation and mostly act as templates for protein synthesis. In our previous research [1], we identified a 165-mer RNA, which is conserved in *Bradyrhizobiaceae* and harbors a unique Shine-Dalgarno (SD) sequence with a length of 17 nucleotides (nt). This RNA is weakly synthesized under different growth conditions in *B. japonicum* USDA 110, but strongly interacts with 16S rRNA of 30S ribosomal subunits showing ability to be a potential translational inhibitor. Its coding gene was named *rreB* (ribosome-regulated expression in *Bradyrhizobiaceae*). On the other hand, RreB RNA possesses a small ORF that encodes a polypeptide with 14 amino acid residues, which miserable translation was shown using fusions with eGFP. Thus, it's unclear up now whether RreB RNA displays its function as non-coding regulatory RNA and inhibits ribosome or it could be translated and gives rise to a small peptide with unknown function. Finally, it could possess a double function. RreB analogues also were predicted in other families of alpha-proteobacteria. Four putative candidates with 10–14 nt SD sequence followed by small ORFs were found in *S. meliloti*. In the present research, we aimed identification of those RNAs by RT-PCR and Northern blotting as well as verification of their ability to bind 30S subunit and serve as templates for synthesis of the corresponding peptides. To check translation activity of those RNAs, we substituted ORFs by mCherry gene saving original 5'-UTRs and

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cloned constructs in a vector under control of a strong ribosomal promoter. We also obtained vectors for protein superexpression in which original 5′-UTRs were substituted by the canonical one and put constructs under IPTG control. This work was supported by the Russian Scientific Foundation (grant N14-24-00061).

1. Hahn J. *et al.* RNA Biol., 2016, in print.

P.4.2-013
Investigation of relation between miR-196 expression and probiotics

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Introduction: Probiotics are known as microbial nourishment supplements that beneficially affect the host by improving its intestinal microbial balance. miRNAs are noncoding RNAs of approximately 22 nucleotides that act as post-trancriptional regulators of gene expression. more than 600 miRNA have been annotated in the human genome thus far. The miR-196 miRNA gene family located within the Hox gene clusters has been shown to function during embryogenesis and abnormally expressed in various malignancies, including leukaemia, melanoma, and colorectal cancer. Despite its involvement in numerous biological processes, the control of miR-196 expression is still poorly defined. So we aimed to investigation of whether miR-196 associated with dietary supplement probiotics.

Material and Method: This study was conducted with 20 volunteers (11 males, 9 females) were healthy and aged between 18–65 years, who applied to Diet Clinic of Necmettin Erbakan University Meram Medical Faculty Hospital for control. İndividuals took dietary supplement probiotics for 10 days. Plasma was obtained from blood samples of individuals-before and after using probiotic-. Samples were analyzed by RT-PCR. The level of miR-196 was evaluated.

Results: In this study, U6 snRNA was used as an internal control to calculate the expression of selected miRNA following the formula: $\Delta Ct_{target}miRNA = Ct_{target}miRNA–Ct_{U6}$. Lower ΔCt represent higher miRNA abundance level and higher ΔCt represent lower miRNA abundance level. T-test was used to compare difference between group.miR-196 were identified fold change ≥1.5 but according to t-test; miR-196 values of $P > 0.05$.

Conclusions: Expression levels of miR-196 are very low in various diseases, especially in cancer. Consequently, our study provides new insights into the pathways that the miR-196 targets.

P.4.2-014
Effects of dietary supplement probiotics on human miRNAs

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Introduction: Probiotics are defined as "live microorganisms that beneficially affect the host once consumed in adequate amounts". Probiotics have been reported to prevent inflammatory bowel disease, exhibit antimicrobial and anticolorectal cancer activities. MicroRNAs (miRNAs) are small noncoding RNAs 17–25 nucleotides long that can be seen as master-coordinators, regulating fundamental cellular processes such as proliferation, apoptosis, and development. Completion of the Human Genome Project has been one of the most important turning point in nutrition science, as in some other sciences. There is much evidence that, nutrition is important in gene expression and effect of genetic variations on disease prevention. So we were aimed to investigate the effects of probiotics which are taken as dietary supplement on the human miRNAs.

Material and Method: This study was conducted with 20 healthy individuals (11 males, 9 females) aged between 18–65 years. Individuals took dietary supplement probiotics for 10 days. Plasma is obtained from blood samples of individuals-before and after using probiotic – was analyzed by RT-PCR. We evaluated levels of miR-15, miR-16 and miR-29.

Results: In this study, U6 snRNA was used as an internal control to calculate the expression of selected miRNAs following the formula: ΔCttargetmiRNA =CttargetmiRNA–CtU6. Lower ΔCt represent higher miRNA abundance level and higher ΔCt represent lower miRNA abundance level. T-test was used to compare difference between group. 3 miRNA groups were identified fold change ≥ 1.5 and according to t-test; miR-15, miR-16 and miR-29 values are significantly increased.

Conclusions: While expression levels of miR-15, miR-16 and miR-29 are very low in various diseases, especially in cancer; the expression of miR-29, miR-15 and miR-16 were found high in using probiotics. Consequently, further studies are mandatory to a better understand and confirm probiotics effect mechanism on miRNAs.

Protein Degradation

P.4.3.A-001
Modulation of protein traffic networks to rescue F508del-CFTR from the endoplasmic reticulum

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Background: The most common cystic fibrosis-causing mutation (F508del, present in ~85% of CF patients) leads to CFTR misfolding which is recognized by the endoplasmic reticulum (ER) quality control (ERQC) resulting in ER retention and early degradation. It is known that the CFTR traffic from the ER is mediated by specific sorting motifs that include the 4 retention motifs AFTs (arginine-framed –RXR-tripeptides) and of the diacidic (DAD) exit code that controls the interaction with the COPII machinery, as previously described.

Aim: Here, we aim to identify traffic factors that regulate CFTR exit from the ER at these specific QC checkpoints.

Methods: We performed pull-down assay by co-immunoprecipitation of F508del-CFTR (with and without mutated AFT motifs) as well as of wt-CFTR (with and without abrogation of the

diacidic code) to identify and isolate the factors that interact specifically with each of these variants. The respective protein profiles were analysed by LC-MS/MS and proteins showing differential interactions were selected.

Results and Discussion: A high number of interacting proteins (~800) was identified. In those with stronger interaction with F508del-CFTR, there is an enrichment in proteins involved in RNA processing and complex organization and a decrease in those related to epithelial integrity when compared to the interactome of F508del-CFTR with abrogated AFT motifs. A subset of 52 proteins was identified as potentially involved in F508del-CFTR rescue. Several of these interactors are involved in protein trafficking and processing as well as in cell integrity and homeostasis and not previously directly associated with CFTR regulation, being currently under validation. The identification of the specific CFTR interactors/regulators, and its validation which is in progress, will likely identify novel therapeutic targets that could be ultimately used to the benefit of CF patients.

P.4.3.A-002
Dia2-mediated control of Ctf4 in the context of replication complex integrity

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Dia2 from *S. cerevisiae* is a F-box protein that is associated with the modular ubiquitin ligase SCF (Skp1/cullin/F box) to form an E3 ubiquitin ligase. Two key replication proteins – Mrc1 and Ctf4, are involved in SCF^{Dia2} association to replication complex. They physically interact with Dia2 and this interaction is responsible for destabilization of Mrc1 in a proteasome-dependent manner during S-phase checkpoint recovery. Our previous data demonstrated that the S-phase checkpoint escape (a process named adaptation) is also associated with detachment of Mrc1 from chromatin. As we have also shown, Mrc1 plays a key role in the harmonization of polymerase and helicase in the Replication pausing complex. Moreover, recent data shows that Ctf4 is necessary for the stable Pol α association to chromatin and, alike Mrc1, is important for its connection to DNA helicase. That is why our aim is to study the Dia2-mediated protein level control of Ctf4 in the context of replication complex integrity. Our results demonstrate that Ctf4 is destabilized in a Dia2-dependent manner after the completion of S-phase. We show that Dia2 is responsible to keep the levels of Ctf4 low during G2/M phase of the cell cycle. As Dia2 deficient strains seem to have difficulties when entering G2/M phase and also exhibit prolonged G2/M phase, it can be speculated that decreasing Ctf4 levels in a Dia2 dependent manner, after completion of DNA synthesis is an important event, related to replication complex disassembly and G2/M phase entry.

P.4.3.A-003
HSP90 regulates the G1/S transition and cell cycle progression in a PLK1-dependent manner

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Most of the studies on PLK1 have focused on different aspects of late G2 and mitosis. However, increasing numbers of publications are appearing on the role of PLK1 in DNA replication. These reports claim an essential role of PLK1 during G1 and S phases. In a similar vein, Wei and coworkers emphasize the role of PLK1 in CDH1 degradation for cell cycle progression from G1 to S phases. In the present work, we study the stability of PLK1 in relation to the G0/G1 to S phase transition. Starting with the analysis of the effect of geldanamycin (GA) on PLK1, we discovered the implication of a new ubiquitin ligase on PLK1 degradation. GA specifically inhibits chaperone HSP90 activity, thereby destabilizing its client proteins, including PLK1. We found that $SCF^{\beta TrCP}$ ubiquitinates cytoplasmic PLK1 for its degradation via the proteasome from G1- to S-phases of the cell cycle, and we determined the kinases that regulate this process. Our results demonstrate that the degradation of PLK1 is dependent on CDK1 and GSK3β under normal growing conditions, and that CDK1 is a major kinase involved in PLK1 degradation by $SCF^{\beta TrCP}$/proteasome after GA treatment. PLK1, in addition to cyclin A, is an upstream modifying enzyme that promotes CDH1 phosphorylation to trigger its ubiquitination and degradation. The consequence of this pathway is the accumulation of CDH1 substrates allowing cell cycle progression from G1-to-S phase. To elucidate the effect of PLK1 degradation on the G1/S transition, we arrested HeLa cells in early G1 phase and analyzed the cell cycle progression after GA treatment. Our results strongly support the notion that cells delay cell cycle progression when PLK1 does not have the proper conformation due to HSP90 inactivation, destroying it via the βTrCP/proteasome, which, in turn, prevents CDH1 degradation. In these conditions, CDH1 substrates do not accumulate and cell cycle arrests, providing a novel pathway for regulation of the cell cycle at the G1-to-S boundary.

P.4.3.A-004
Development of a treatment based on proteasomal inhibition and antioxidant usage in protein wasting observed in diabetes

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The presence of muscle atrophy especially in skeletal and cardiac muscles is one of the common complication of diabetes. The most important mechanism considered to be responsible of muscular atrophy is ubiquitin-proteasome system. This study aimed to develop a therapeutic strategy based on proteasomal inhibition by using bortezomib (BTZ) as a specific proteasome inhibitor and resveratrol (RSV) as an antioxidant. Besides, a new model that mimics *in vivo* conditions was developed in muscle cells. Differentiated rat skeletal (L6) and cardiac (H9c2) cell lines were used. Viability tests, western blot and RT-qPCR, enzymatic chymotrypsin-like activity (CLA) methods were used. Streptozotocin-induced Wistar rats also included as an *in vivo* model. The β5 subunit of proteasome and its CLA, NFkB, muscle specific ubiquitin ligases gene and protein expressions were evaluated in tissues. Muscle weight and diameter, inflammation and tissue damage were measured in gastrocnemius, soleus, heart and extensor digitorum longus muscles of animals. A new hypercatabolic model including inflammation and high glucose was created in

L6 cells. CLA and NFkB expression were increased. It was found that BTZ is a promising therapeutic agent for diabetic patients. Additionally, muscle atrophy in diabetic animal model was clearly revealed by macroscopic and molecular data. Fiber diameters were reduced and CLA was increased as an underlying mechanism. NFkB nuclear translocation and leukocyte infiltration showed inflammation in tissues. β5 expression and its CLA were repressed by BTZ. RSV prevented muscle atrophy in diabetes was shown by proteasomal inhibition. It also increased muscle diameters of gastrocnemius and heart muscles. In conclusion, a candidate therapy model was occurred both *in vivo* and *in vitro*. The effect of RSV for preventing diabetic cachexia was shown first time by proteasomal pathway. Whole results promoted antidiabetic usage potential of BTZ and RSV.

P.4.3.A-005
Molecular and functional insights into mitochondrial intermembrane space proteins degradation by ubiquitin-proteasome system

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Majority of mitochondrial proteins are synthetized in the cytosol and undergo an active import to specific compartments of mitochondria. To maintain cellular protein homeostasis, mechanisms of protein synthesis, transport and folding are accompanied by the quality control mechanisms that degrade misfolded or mislocalised proteins. Our results indicate that proteins of the mitochondrial intermembrane space (IMS) are substrates of the major cytosolic system for specific protein degradation – the ubiquitin-proteasome system (UPS). Proteins destined to the IMS can be ubiquitinated and degraded by the proteasome not only prior to their import but also following their retro-translocation to the cytosol. The goal of the current project is to find molecular factors (internal and external) important for the degradation of the IMS proteins by the UPS. In *S. cerevisiae* specificity of ubiquitination process is governed by several ubiquitin-conjugating enzymes (E2) and more than 60 ubiquitin ligases (E3). To identify enzymes involved in the IMS protein turnover, we have used a functional screening. Hits from this procedure were characterized by the *in vivo* degradation and ubiquitination assays. We have also assessed internal determinants of the IMS protein turnover using directed mutagenesis. Our results confirmed the *in vivo* conjugation of ubiquitin to lysine residues within IMS protein. As expected, the removal of ubiquitination sites proved to have a strong stabilizing effect for our IMS model protein. Conversely, mutations that disturb protein import or folding resulted in the increased ubiquitination and degradation. The results obtained in this study broaden the knowledge about degradation of mitochondrial proteins by the UPS by providing a mechanistic insight into the specificity of the process. We also elucidate mutual relations of ubiquitination process and protein import to mitochondria. The work was founded by National Science Centre, Poland, DEC-2013/11/D/NZ1/02294.

P.4.3.A-006
A novel non-canonical binding mode for serine proteases on plant Kunitz inhibitors

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Protease inhibitors from the Kunitz family are widely distributed in plant kingdom. They share a conserved b-trefoil fold in which variable loops are involved in interactions with proteases. The majority of the Kunitz inhibitors are targeting serine proteases; however, inhibitors of cysteine and aspartic proteases have also been described. Our work is focused on structural diversity of Kunitz inhibitors in potato, encoding a large set of isoinhibitors of various inhibitory specificities. PDI and PTI are wound inducible Kunitz isoinhibitors with potential defense role in potato against insect herbivores and pathogens. We analyzed their interaction with serine proteases using crystal structures of the inhibitor complexes with trypsin at 1.9 Å and 1.7 Å resolutions. We show that PDI and PTI evolved two types of reactive centers that differ in the location on the inhibitor molecule as well as in their binding mode, demonstrating canonical and novel non-canonical inhibition mechanisms.

P.4.3.A-007
Reciprocal effects of Hsp70 on the activity of different proteasome forms; 20S proteasome degrades Hsp70 without ubiquitination

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Ubiquitin-proteasome system (UPS) degrades most cellular proteins and contributes to the protein quality control (PQC). Cell adaptation to stress such as heat shock (HS) demands flexibility of UPS and is associated with the changes in cellular proteasome pool, which encompasses different forms of proteasomes. However, the reason for such diversity is unclear. In frames of PQC heat shock protein 70 (Hsp70) cooperates with the UPS. Hsp70 together with its co-chaperones determines the fate of damaged proteins. If the client-protein cannot be repaired, it is ubiquitinated and escorted for degradation by the UPS. After stress relief Hsp70 is itself ubiquitinated and rapidly hydrolyzed by the 26S proteasome. Although interplay between the UPS and Hsp70 is of special interest the effect of Hsp70 on the functional state of different forms of proteasomes is poorly investigated. Here we tested the effects of recombinant human Hsp70 on the activity of different forms of proteasomes: constitutive 20S (c20S) proteasome, immune 20S (i20S) proteasome and constitutive 20S proteasome with attached 19S regulator (26S) proteasome. We have shown that recombinant human Hsp70 modulates the activity of different forms of proteasomes in different ways. Thus, following the incubation with Hsp70 the activity of purified c20S and i20S proteasomes was decreased two-fold, while 26S proteasomes were activated. Furthermore, we demonstrated that Hsp70 can be degraded by the 20S proteasome independent of ubiquitination, this could explain the observed reduction of the 20S proteasome activity. Interestingly, Hsp70 degradation is likely promoted by disordered C-terminus and resulted in production of a distinct cleavage product with MW of 30 kDa. This study demonstrated an essential role of proteasome regulators in the interplay between Hsp70 and the different proteasome forms. Besides, it revealed a novel route for Hsp70 degradation that could be important under various conditions including stress.

P.4.3.A-008
Stress conditions result in down-regulation of RNA polymerase III activity and degradation of its largest subunit by ubiquitine/proteasome system

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In yeast *Saccharomyces cerevisiae* transcription by RNA polymerase III (Pol III) is repressed during stress conditions. This regulation is known to be mediated by Maf1, a global negative repressor of Pol III. Another possible mechanism which may contribute to Pol III repression independently on Maf1 is degradation of Rpc160, the largest subunit of Pol III. We observed degradation of Rpc160 in yeast treated with rapamycin, 6-azauracil (6-AU) or mycophenolic acid (MPA) and upon transfer of yeast from glucose to the medium with a non-fermentable carbon source. Instability of Rpc160 was confirmed by cycloheximide chase experiment. We demonstrated that the degradation of the Rpc160 subunit is proteasome dependent by using proteasome inhibitor MG132 or a mutant with the deletion of *UMP1* chaperone required for correct maturation of the 20S proteasome. Finally, ubiquitinated form of Rpc160 was identified by SDS-PAGE when isolated by two-step purification method in strain encoding TAP-tagged Rpc160 and overproducing His-tagged ubiquitin. Next, we examined the effect of decreased nucleotide pool on the Pol III activity. Yeast were treated with MPA which inhibits *de novo* biosynthesis of guanine nucleotides and is used as an immunosuppressant and anti-cancer drug. tRNA transcription is rapidly down regulated by MPA in both control and *maf1Δ* yeast mutant. Longer MPA treatment resulted in reduced interaction between Pol III subunits followed by proteasome degradation of Rpc160. Obtained results show global consequence of reduction of nucleotide pool leading to disassembly of Pol III complex.

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P.4.3.A-009
The ubiquitin conjugating enzyme UBE2O induces myeloma cell apoptosis by degrading the transcription factor c-Maf

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The transcription factor c-Maf is a key player in the development of multiple myeloma (MM), an incurable malignancy of plasma cells. c-Maf can be processed via the ubiquitin-proteasome pathway but the specific ubiquitinating enzymes are not known yet. In the present study, we identified c-Maf interacting proteins by tandem mass spectrometry and found a series of ubiquitination and proteasome-associated proteins, of which UBE2O was the only ubiquitin conjugating enzyme. In the subsequent studies, we found UBE2O interacted with c-Maf and mediated typical K48-chain ubiquitination. The in-tube ubiquitination assay showed that UBE2O directly mediated c-Maf ubiquitination in the absence of ubiquitin ligases. Consistent with c-Maf degradation, UBE2O inhibited the transcriptional activity of c-Maf and down-regulated the expression of c-Maf downstream genes including cyclin D2, integrin beta 7 and CCR1. Because c-Maf promotes MM cell proliferation, we next investigated the roles of UBE2O in MM biology. DNA microarray analysis revealed that UBE2O was expressed in normal bone marrow cells but was downregulated in monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (MM) and MM cells, suggesting UBE2O was downregulated in MM cells. More importantly, when UBE2O was re-expressed in MM cell lines, c-Maf protein was decreased, and MM cells underwent apoptosis. Notably, UBE2O-induced MM cell apoptosis was c-Maf dependent because UBE2O suppressed the proliferation and induced apoptosis of c-Maf-expressing MM cell lines such as RPMI-8226 and LP1 but it failed in NCI-H929 and KMS12 that lacked c-Maf protein. Furthermore, enforced expression of UBE2O delayed the growth of myeloma xenografts in nude mice. Therefore, the present study demonstrated that UBE2O mediates c-Maf polyubiquitination and degradation, induces MM cell apoptosis and suppresses myeloma tumor growth. These findings provide a novel therapeutic strategy against MM.

P.4.3.A-010
Locomotor activity and rheoreaction type in Atlantic salmon, *Salmo salar* L., depend on protein degradation in their skeletal muscles

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It is known that protein degradation in fish muscles relies mostly on three distinct pathways such as lysosomal digestion by cathepsins, calcium-dependent proteolysis by calpains, and the ubiquitin-proteasome system. The calcium-dependent proteolysis considered to be a major pathway regulating muscle turnover in fish, while cathepsins and ubiquitin-targeted protein digestion by the proteasome are primarily responsible for bulk protein degradation. Proteins (particularly, myofibrillar) and lipids prevail in fish metabolism as the energetic substrates. Thus, the proteolytic systems mentioned are responsible for both basic protein metabolism in fish muscles and mobilization of energetic resources in the situations requiring high energy consumption. The locomotor activity and rheoreaction type in fish supposed to relate with the activities of protein degradation systems in Atlantic salmon, Salmo salar L., skeletal muscles. Atlantic salmon parr retain their position in the water current possessing static type of rheoreaction, while smolts prefer passive downstream migration described as negative type of rheoreaction. Protein degradation systems activities in the skeletal muscles occur to be higher in parr as compared with smolts. Thus, parr possess high level of physical activity following high intensity of intracellular proteolysis, in contrast to passively moving smolts. The data obtained indicate contribution of free amino acids released from the skeletal muscles proteins by proteolysis to the energy production in fish metabolism.

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P.4.3.A-011
Novel structural mechanism of allosteric regulation of aspartic peptidases via evolutionary conserved exosite

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Pepsin-family aspartic peptidases are biosynthesized as inactive zymogens in which the propeptide blocks the active site until it is proteolytically removed upon enzyme activation. Here we describe a novel dual regulatory function of the propeptide using a set of crystal structures of a parasite cathepsin D, IrCD1. In the IrCD1 zymogen, the intramolecular autoinhibition by the intact propeptide is mediated by an evolutionary conserved exosite on the enzyme core. After activation, the mature enzyme employs the same exosite to rebind a small fragment derived from the cleaved propeptide. This fragment functions as an effective natural inhibitor of mature IrCD1 that operates in a pH-dependent manner and through a unique allosteric inhibition mechanism. The study uncovers the propeptide-binding exosite as a target for the regulation of pepsin-family aspartic peptidases and defines the structural requirements for exosite inhibition.

P.4.3.A-012
Investigating the regulation of 20S proteasome mediated protein degradation

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Protein degradation by 26S proteasome and its regulation is known for many years, but relatively little is known about the regulation of ATP and ubiquitin independent 20S mediated proteolysis. The recent studies showed that NQO1 and DJ-1, which share many structural similarities, bind to 20S proteasome and regulate protein degradation. However, a comprehensive molecular mechanism underlying this regulation is unknown. In order to investigate the 20S proteasomal regulation by NQO1 and DJ-1, we made an attempt to map the interaction sites between the NQO1/DJ-1 and 20S proteasome. Our native mass spectrometry studies revealed the binding of NQO1 and DJ-1 to the 20S proteasome from different species, suggesting for an evolutionarily conserved regulatory mechanism. Further, we applied peptide array screening and found that binding sites of archaeal 20S proteasome within the NQO1 and DJ-1 share a common secondary structure, implying a role for exposed binding sites in regulating the 20S proteasome.

P.4.3.A-013
Further characterization of compound 8o, a potent inhibitor of human prolyl carboxypeptidase

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Prolyl carboxypeptidase (PRCP) is a lysosomal serine protease that is implicated in metabolic disorders. It is particularly known for its role in body weight control by inactivating so-called 'anorexigenic' peptides, neuropeptides that cause a loss of appetite. Although there is considerable knowledge about the synthesis and the release of these peptides, the enzymes that degrade them are poorly understood. The purpose of this study was to produce recombinant human PRCP (rhPRCP) and further characterize compound 8o (Zhou, 2010) as inhibitor of human PRCP (hPRCP) for use in experiments investigating PRCP's role in body weight control. The BaculoDirect baculovirus expression system was used for the expression and production of rhPRCP. First, a plasmid containing the sequences for the HoneyBee mellitin secretion signal and hPRCP was recombined with linear baculovirus DNA. Sf9 insect cells were then transfected with this recombinant baculovirus DNA resulting in the production of rhPRCP. Subsequently, a three-step purification was performed to purify the produced rhPRCP. The purified rhPRCP was used, in comparison with purified natural hPRCP, to further characterize compound 8o by use of two different assays: a RP-HPLC and a continuous fluorometric assay. The reversibility and potency of compound 8o were determined using synthetic and natural substrates. Also the selectivity of the compound was tested against the closely homologous serine proteases (DPP2, FAP, PREP, DPPIV, DPP8 and DPP9). rhPRCP was purified 3369-fold and successfully identified as human lysosomal prolyl carboxypeptidase. Compound 8o was characterized as a potent, reversible and selective inhibitor of rhPRCP and hPRCP. These results show that the produced rhPRCP and the characterized compound 8o can be used in further experiments to increase knowledge about PRCP's role in body weight control.

P.4.3.A-014
Immunoproteasomes equipped by REG alpha/beta heptamers coordinate autoimmune attack on the myelin sheaths ab intra

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Absolute majority of substrates are recognized by proteasome only being conjugated with ubiquitin (Ub) molecule, representing universal degradation signal operated by ubiquitination system. Ub-independent proteasome targeting is rationalized by existence of two types of direct proteasome signals (DPS) – specific amino acid sequence or posttranslational modification that are recognized by proteasome regulatory subunits. Historically, the first one was shown to be existed in ornithine decarboxylase (ODC), whereas acetylation of core histones was recently reported as second type of DPS. Here we demonstrate the third one, representing charge-mediated DPS. This type of degradation signals, initially discovered in multiple sclerosis (MS) autoantigen, myelin basic protein (MBP), and further artificially reconstructed in Basic Elementary Autonomous Degrons (BEADs), is most efficiently engaged by REGα or REGγ-capped proteasomes in ATP-independent manner. REGα/β regulatory particles are classical IFNγ-inducible proteins, directly associated with immunoproteasome, and involved in antigen presentation in inflammation conditions. In line with this we showed that brain-derived immunoproteasomes from SJL mice with experimental autoimmune encephalomyelitis (EAE) in a ubiquitin-independent manner generate significantly increased amounts of myelin basic protein (MBP) peptides that induces cytotoxic lymphocytes to target mature oligodendrocytes *ex vivo*. Ten times enhanced release of immunogenic peptides by cerebral proteasomes from EAE SJL mice is caused by a dramatic shift in the balance between constitutive and $b1i^{high}$ immunoproteasomes in the central nervous system (CNS) of SJL mice with EAE. Therefore, our findings uncover novel insights into myelin metabolism in pathophysiological conditions and suggest that immunoproteasomes equipped by REGα/β heptamers became deadly machines coordinating autoimmune attack on the myelin sheaths *ab intra*.

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P.4.3.A-015
Proteasome-mediated hydrolysis of myelin basic protein: charge instead of ubiquitination

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Proteasome hydrolyzes more than half of the intracellular proteins utilizing the polyubiquitin chain as a degradation signal. Nevertheless, there are unique exceptions – proteins that do not require ubiquitination for the proteasomal degradation, while a protein site with a specific amino acid sequence (degron) or an auxiliary protein are responsible for the proteasome binding. The currently known degrons are two-component and consist of the proteasome recognition site and the unstructured fragment, from which the protein unfolding and its subsequent translocation into the catalytic chamber of the proteasome are initiated. Previously we established that myelin basic protein (MBP), one of the major components of the myelin sheath covering neuronal axons in the central nervous system and at the same time a dominant autoantigen in multiple sclerosis, is degraded by proteasome without ubiquitination. We next found that this protein has a unique extended ubiquitin-independent degron that is uniformly distributed over its amino acid sequence. Based on the composition of amino acids composing MBP, an artificial sequence consisting of several repeats of seven amino acid cluster has been created, which has been shown to be an effective ubiquitin-independent degron. We studied the dependence of the rate of hydrolysis of model substrates fused with artificial degron on its various parameters, including charge, length, and topology. Our observations suggest that designed degron is flexible in terms of its length and positioning but retains functionality only bearing a significant basic charge. Concluding, discovered charge-mediated hydrolysis of the myelin basic protein by the proteasome may have important physiological meaning in the etiology and pathogenesis of multiple sclerosis.

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P.4.3.A-016
Analysis of degradation mechanism of mistargeted tail-anchored proteins on mitochondrial outer membrane

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Correct targeting of newly synthesized proteins to their destination organelles is essential for proper cellular functions. Hundreds of eukaryotic membrane proteins are anchored to membranes by a single transmembrane domain at their C-terminus. Many of these tail-anchored (TA) proteins are post-translationally targeted to the endoplasmic reticulum (ER) membrane or mitochondrial outer membrane (OMM). For the ER membrane, TA proteins use an ATP-dependent targeting pathway called the guided-entry of TA protein insertion (GET) pathway. In yeast cells, some TA proteins targeted to the ER membrane are mislocalized to the OMM by the defects in the GET pathway functions. Recently, the mitochondrial AAA-ATPase Msp1 was found to clear such mistarged TA proteins from the OMM (EMBO J. 2014. 33(14): 1548–1564., PNAS. 2014. 111(22): 8019–8024.). However, the molecular mechanism of the Msp1-dependent degradation is largely unclear. To address this issue, we used a Pex15 variant lacking the C-terminal 30 residues (Pex15-Δ30) as a model Msp1-mediated degradation substrate. We first confirmed that Pex15-Δ30 is degraded by 26S proteasome. In order to investigate the substrate selection mechanism by Msp1, we constructed fusion proteins between wild type DHFR (DHFRwt) or structurally unstable DHFR variant (DHFRds) with the transmembrane domain derived from Pex15-Δ30. We found that the DHFRds fusion protein, not the DHFRwt fusion protein, was degraded in an Msp1-dependent manner. Thus, Msp1 monitors the folding state of TA proteins as a merkmal of the mistargeted proteins for proteasomal degradation. Currently, we are searching for the E3 ubiquitin ligase responsible for the Msp1-mediated degradation of Pex15-Δ30.

P.4.3.A-017
Hydrogen peroxide-induced degradation of type I collagen fibers and influence on cell function

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The main responsibility of human skin is to communicate the organism with the surrounding environment and to protect against different physical and chemical factors. Collagen fibers and fibroblasts which form skin are exposed to a number of factors every day. This factors lead to collagen fibers degradation and disturbances in the fibroblast metabolism. One of the main chemical stress factors for the human skin is hydrogen peroxide. To protect collagen fibers and fibroblasts against damaging effects of chemical external factors such us hydrogen peroxide hyaluronic acid and collagen solution can be used. The aim of this study was to investigate the efficiency of native component of extra cellular matrix such as hyaluronic acid and collagen solution in preventing the collagen fibers degradation and influence on human skin fibroblasts functions after hydrogen peroxide treatment. Human skin fibroblasts were obtained from skin fragments collected after plastic surgery. The structure collagen substrates before and after hydrogen peroxide treatment were analyzed by Atomic force microscopy and Scanning electron microscopy. Skin cells viability and interaction with collagen fiber substrates before and after hydrogen peroxide effect and hyaluronic acid, collagen solution treatments were analyzed by Confocal microscopy and MTT analysis. Our studies have shown that treatment of collagen fibers by 0.3% hydrogen peroxide solution lead to collagen degradation. After this hydrogen peroxide condition treatment collagen structure substrate is restored by fibroblast cultivation in the presence hyaluronic acid and collagen solution on this substrate. Increase concentration of hydrogen peroxide in solution higher 0.3% lead to non-restored collagen fibers substrates. Hyaluronic acid promotes the type I collagen synthesis by fibroblasts.

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P.4.3.A-018
Regulation of PafA, the prokaryotic ubiquitin-like protein ligase by PafB and PafC

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Intracellular proteolysis is fundamental for the regulation of diverse cellular processes, for protein quality control and for amino-acid recycling under starvation conditions. While regulated intracellular protein degradation in eukaryotes occurs primarily via the ubiquitin-proteasome system (UPS), most bacterial species are devoid of proteasomes, and do not rely on ubiquitin-like tagging for the targeting of proteins to degradation. However, bacterial species belonging to the phyla Actinobacteria and Nitrospira possess, a proteolytic system analogous to the UPS exists. In this system, initially discovered as important for the full virulence of *Mycobacterium tuberculosis*, a prokaryotic ubiquitin-like protein (Pup) is conjugated to lysine side chains of protein substrates, thereby targeting them for degradation by a proteasome. An enzyme termed PafA is responsible for Pup conjugation to hundreds of protein targets. Being the only Pup ligase, the regulation of PafA activity is central in the Pup-proteasome system. Moreover, its promiscuous nature necessitates careful control in order to avoid excessive tagging and degradation of cellular proteins. As the regulation of PafA expression is currently poorly understood, my work focuses on this topic. The *pafA* gene was previously reported to be part of an operon with two additional genes of unknown function, *pafB* and *pafC*. These genes are strictly conserved across bacterial species that possess the Pup tagging system and they both present a WYL domain. This domain often associated with DNA-binding domains, thus supporting a hypothesis of PafBC being *pafA* transcriptional regulators. Our findings indeed support this hypothesis, and further indicate that transcriptional regulation by PafBC requires *cis* elements in the *pafA* upstream and downstream regions. This demonstrates for the first time transcriptional regulation of PafA, and sheds new light on the function and role of PafBC in the Pup-proteasome system.

P.4.3.A-019
Insights into the molecular mechanism that coordinates the activities of the proteasome regulatory complex and the 20S core

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Preliminary results from our lab demonstrate the tight coordination between the 19S deubiquitination activities, the nucleotide cycle within the 19S and the 20S proteasome catalytic activities. We thus propose that the proteasomal DUBs serve as the master regulatory module of proteasomal function, owing to their irreversible action (removing of the ubiquitin chain) that must be timed with substrate commitment. We further demonstrate a role for the sensing of a loose domain upon unfolding by the proteasomal ATPase ring, in timing substrate deubiquitination and in signaling the onset of downstream events. To study and better understand the regulatory loops synchronizing the transfer of the processed substrate from the 19S regulatory complex into the 20S proteasome, we searched for mutants in PAN, the archaeal proteasome regulatory ATPase that enhanced or diminished it's activation upon association with the 20S proteasome. Based on our preliminary findings, we propose a molecular mechanism by which the association between the proteasome regulatory ATPase ring and the 20S core particle affect the conformation of the Walker A and the Walker B modules within the P loop in a manner that regulates ATP hydrolysis and the associated functions of the proteasome regulatory complex.

P.4.3.A-020
Pex13p degradation facilitates peroxisomal matrix protein import in Hansenula polymorpha

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Peroxisomes are highly versatile eukaryotic organelles that play a vital role in regulating cellular metabolism, providing compartments where metabolic pathways can be contained and controlled (Gabaldon, 2010). Some well-known peroxisomal processes include the oxidation of fatty acids and the biosynthesis of plasmalogens and penicillin, but many more exist (Pieuchot & Jedd, 2012). Their importance in cell vitality is underscored by a number of inherited developmental brain disorders caused by defects in peroxisome biogenesis (Waterham et al, 2016). Because all peroxisomal membrane proteins (PMPs) are made in the cytosol and post-translationally imported, PMP targeting to peroxisomes is a critical part of peroxisome function and cellular metabolism. However, little is known about how PMPs are removed from the peroxisomal membrane and degraded. Here, we have studied the degradation of Pex13p, a PMP involved in peroxisomal matrix protein import. We report that the PMP Pex13p undergoes rapid degradation via the ubiquitin-proteasome system (UPS). Pex13p is ubiquitinated in a Pex2p dependent manner while deletion of *PEX2* causes Pex13p to build up on the peroxisomal membrane. Finally, we demonstrate that Pex13p degradation facilitates peroxisomal protein import, uncovering a novel form of peroxisome regulation via the UPS. Taken together, our demonstrate the existence of a ubiquitin-dependent pathway that targets PMPs for cytosolic degradation, which we have termed Peroxisomal Membrane Associated Degradation (PMAD). Future research aimed at investigating the molecular mechanisms and underlying functions of the PMAD pathway will undoubtedly reveal fascinating insights how protein degradation regulates additional aspects of peroxisome function and cell vitality.

P.4.3.A-021
The mechanism of proteasomal substrate appending by the ubiquitin chain

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The 26S proteasome is a 2.5MDa complex responsible for the selective, ATP-dependent degradation of ubiquitinated proteins. Engagement of a substrate protein triggers conformational changes within the proteasome, which drive substrate unfolding, deubiquitination and translocation into the 20S proteolytic core. Premature deubiquitination may interfere with substrate degradation by dissociating it from the proteasome. Conversely, failure to remove or trim the polyubiquitin chain would prevent further processing and may even clog the proteasome. Thus, as deubiquitination must occur prior to unfolding and translocation into the proteasome catalytic core (20S), a mechanism that keeps the deubiquitinated substrate appended to the proteasome regulatory complex (19S) must exist. We established a unique mono- and polyubiquitination system that combines enzymatic and chemical ubiquitination. This system allows us to better characterize the

efficient ubiquitin tag and to follow the fate of the ubiquitin moieties upon degradation of polyubiquitinated substrates. Based on our ongoing findings we hope to shed light on the biological mechanism that coordinate the removal of the polyubiquitin chains from the substrate *en route* to degradation, preventing premature release of deubiquitinated substrates from the proteasome.

P.4.3.A-022
Structural and functional analysis of the DJ-1 minifamily members in *Saccharomyces cerevisiae*

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Proper regulation of proteasomal degradation pathways is crucial for cell viability and proliferation and impairment of these regulatory pathways in humans can lead to a variety of neurodegenerative diseases. We recently discovered that the oxidative stress response protein, DJ-1, which is a Parkinson-associated protein, is a regulator of the 20S proteasome. However, the mechanism underlying its ability to inhibit the 20S proteasome is still unclear. *Saccharomyces cerevisiae* contains four DJ-1 orthologues: Hsp31, Hsp32, Hsp33 and Hsp34 referred to as the Hsp31 minifamily. Among this family, Hsp32, Hsp33 and Hsp34 sequences are almost identical while Hsp31 sequence is slightly different, and probably this is the ancestral gene that gave rise to the other three duplications. Studying the differential functionality and structural differences between these proteins will help clarify DJ1's mechanism of action. We discovered that the Hsp31 minifamily members exert different ability to inhibit the 20S proteasome, and a phenotypic analysis of yeast strains containing individual and combined deletions of these genes showed a suppressive effect of Δ*hsp31* over Δ*hsp32*, Δ*hsp33*, Δ*hsp34* group under oxidative and methylglyoxal stress. Overall our results suggest a different functions for the Hsp31 minifamily members in yeast which will be useful for establishing DJ-1's mechanism of action.

P.4.3.A-023
Multiple-reaction monitoring for detection of recombinant secreted proteinases from *Bacillus*

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Quantitative measurement of proteins is one of the important tasks in molecular biology. Multiple-reaction monitoring (MRM) technique was applied in this study to measure expression of secreted proteinases. In optimized by signal peptides (SP_{Pac}, SP_{Yngk} from *Bacillus megaterium*) LIKE system, genes of secreted serine proteinases from *Bacillus pumilus* (subtilisin like proteinase – AprBp and glutamyl endopeptidase – GseBp) were cloned. The proteinases deficient strain *Bacillus subtilis* BG20-36 was used as a host of all constructed vectors (pLIKE-rep+SP_{Pac}-*aprBp*, pLIKE-rep+SP_{Pac}-*gseBp*, pLIKE-rep+SP_{Yngk}-*aprBp*, pLIKE-rep+SP_{Yngk}-*gseBp*). Initially, total proteolytic activity was determined on skim milk agar plates. As an inducer for protein production in LIKE system an antibiotic bacitracin was used. After addition of bacitracin to the plate media, clear zones of skim milk hydrolysis around the colonies were appeared. Next, for MRM analysis, selection of the unique peptides of proteinases was based on their theoretical digestion by trypsin using Skyline 3.6.0 software. For each enzyme, at least 3 unique peptides with minimum of 2 transitions were forecasted. Purified AprBp and GseBp were subjected to trypsin digestion and their analysis was carried out on the AB SCIEX QTRAP 6500 instrument. Peak areas for each peptide were calculated using MultiQuant 3.0.2 software. Quantitative increase of AprBp and GseBp proteins in recombinant strains containing SP_{Yngk} signal peptide after bacitracin induction was observed. However, peak areas for both enzymes were significantly higher in the strain *B. pumilus* 3–19. Our results suggest that: 1) LIKE system is suitable for the expression of secreted proteins; 2) (heterologous) signal peptides screening is a crucial step for optimal production of secreted proteins; 3) MRM workflow allows relative quantification of secreted proteins (*e.g.* proteinases).

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P.4.3.A-024
Post-translation regulations can induce correlated fluctuations of protein levels in single cells

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Isogenic cells living in the same environment naturally show noisy expression. While part of this noise is intrinsic to cells and unpredictable, another part, termed extrinsic noise, can be predicted by the state of circuits regulating gene and protein levels. Mechanisms that effect extrinsic noise have been extensively characterized at the transcriptional level where, for example, the concentration of a transcription factor can predict the concentration of its targets. Here, we ask whether post-transcriptional regulation also bears the potential to effect expression noise significantly. We posit that proteins subjected to identical post-transcriptional regulation should be affected in the same way, and therefore, their concentrations should co-vary across cells. To investigate this question we expressed pairs of yellow and red fluorescent proteins (YFP and RFP), subjected them to specific post-transcriptional regulation, and measured the co-variation of their concentrations across cells. Subjecting the proteins to a translation bottleneck did not significantly impact their extrinsic noise. In contrast, fusion of RFP to a misfolded protein (RFP-misP) nearly doubled the extrinsic noise and abolished the cell-to-cell co-variation between RFP-misP and YFP. Cell-to-cell co-variation in protein levels was restored when both YFP and RFP carried the same misfolded tag, which confirmed the noise it added was extrinsic. Numeric simulations revealed that cell-specific degradation rates and coupling of degradation to production were sufficient to reproduce the experimental data. Thus, our results show that post-transcriptional regulation can shape expression noise dramatically, and suggest that two fundamental cellular processes, protein production and degradation, are coupled in single cells.

P.4.3.A-025
Acr3 arsenite transporter undergoes Rsp5-dependent degradation through MVB pathway

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Acr3 is a plasma membrane transporter, a member of the bile/arsenite/riboflavin transporter (BART) superfamily, that confers high-level resistance to arsenicals in the yeast *Saccharomyces cerevisiae*. We have previously shown that the yeast Acr3 acts as a low affinity As(III)/H+ antiporter and exhibits 10

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transmembrane span topology with cytoplasmically oriented N- and C-terminal domains. Quality control of membrane proteins is crucial for membrane maintenance. We examined Acr3 degradation and showed that Acr3 transporter undergoes endocytosis and vacuolar degradation through MVB pathway. Degradation of Acr3 does not require a functional proteasome. Acr3 degradation is Rsp5 ubiquitin ligase and Doa4 deubiquitinase dependent. Based on the 10-transmembrane model of Acr3 we selected 11 cytoplasmically oriented lysine residues potentially subjected to a posttranslational modification by ubiquitination and replaced each residue with arginine. Single K to R substitution did not increase Acr3 stability, however, triple mutants accumulated in endosome and vacuolar membranes. We also observed that ubiquitination is indispensable for proper vacuolar degradation of Acr3 through MVB pathway.

Autophagy

P.4.3.B-001
Macrophage migration inhibitory factor-induced autophagy is involved in dengue virus replication

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Dengue virus (DENV) infection is the most common mosquito-borne viral infection, which is common in tropical and subtropical countries. DENV infection can cause mild dengue fever and life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Cytokine storm plays a vital pathogenic role in DHF/DSS. However, currently there is no effective antiviral drugs available. Previous studies have shown that DENV infection can induce autophagy of infected cells which facilitate the replication of virus. In addition, the amount of a pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) in dengue patients' sera is correlated with the severity of the disease. Since MIF is able to induce autophagy formation of cells, we propose and test the hypothesis that MIF-induced autophagy is involved in DENV replication. We found that MIF secretion was increased in DENV-infected human hepatoma cell line (HuH-7) in a time dependent manner. Utilizing shRNA to knock down endogenous MIF, we found that autophagy, virus replication and viral titer were all inhibited. Moreover, after treating with MIF inhibitors, ISO-1 and p425, both autophagy and DENV infection were inhibited. In conclusion, we demonstrated in this study that DENV infection induces MIF release, thus facilitating DENV replication via autophagy formation. Further study by targeting MIF may prevent not only inflammation but also inhibit DENV replication, which may develop into a new strategy to treat DENV infection.

P.4.3.B-002
Cardiomyocyte BECN1-dependent autophagy in acute overload of the left ventricle

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Hemodynamic overload of the left ventricle of the heart is accompanied by activation of some mechanisms responsible for regulated cell death including apoptosis. In the recent years there has been an increasing interest to cardiomyocyte autophagy, which may be a phenomenon involved in programmed (or regulated) death in some cases, but might also have reparative potential in other circumstances providing cell survival at the expense of its internal resource mobilization. In this work BECN1-dependent cardiomyocyte autophagy was evaluated by the content of Beclin 1 (BECN1) in cardiomyocytes without morphological evidence of plasma membrane damage in rabbit left ventricular myocardium on 1, 3 and 5 days of acute hemodynamic overload caused by narrowing of the ascending aorta by 1/3 of its initial diameter. Activity of cardiomyocyte autophagy was assessed on the basis of BECN1 content estimation immunochemically with the use of primary goat polyclonal antibodies (SantaCruzBiotechnology, Inc., USA). It was shown that the content of BECN1 was rather low in the control group (2.77 vol.%) and significantly decreased (0.54 vol.%) on day 1 after the onset of cardiac overload compared with the controls. On day 3 there was a further decrease in BECN1 in cardiomyocytes (0.20 vol.%). On day 5 this index negligibly increased (0.38 vol.%), but still remained significantly lower in comparison with controls. It was also found that intensity of positive stain was much higher in the myocardial sites with denser vascular tree. It might be suggested that cardiomyocyte autophagy is a mechanism of cell resource mobilization in the intact myocardium rather than regulated cell death. Its inhibition under acute cardiac overload is most probably due to a severe energy deficit.

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P.4.3.B-003
Activity and distribution of senescence-associated β-galactosidase in aging oocytes and eggs

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Senescence in somatic cells is commonly characterized by enhanced activity of senescence-associated β-galactosidase (SA-β-gal), reflecting alteration of autophagocytosis. However, little is known about the autophagy-associated changes of SA-β-gal activity in aging gamete cells. To address this issue, in the present work, we measured intracellular activity and distribution of SA-β-gal in aging oocytes and eggs of the African clawed frog *Xenopus laevis*. Database mining revealed the presence of three homologous β-galactosidase isoforms in the annotated *Xenopus* genome. Their transcripts were abundant in the ovarian tissue and eggs. The protein products were predicted to contain an N-terminal signal peptide sequence, suggesting enzyme translocation to the cellular organelle fraction. Biochemical analysis of SA-β-gal activity confirmed its localization mainly in a particulate fraction of oocytes and eggs, with the optimum of catalytic activity at pH 4.0–5.0. These data indicate that SA-β-gal is a largely lysosomal enzyme in *Xenopus* oocytes and eggs, so the changes in its activity may reflect autophagy dynamics. Further analysis revealed gradual increase of SA-β-gal activity in *Xenopus* oocytes and eggs aged *in vitro* over 72 h. The activity increase observed in the eggs was significantly higher than that in the oocytes, suggesting faster aging of eggs vs. oocytes. We further used the novel cell-permeable fluorescent substrate of SA-β-gal, SPiDER-β-Gal, to visualize the lysosomal compartment in *Xenopus* oocytes and eggs. Strong fluorescent signal was observed in a fraction of dense cytoplasmic granules of the average size 8.9 ± 3.6 μm. It colocalized with a subpopulation of yolk platelets, specialized late endosomes that accumulate and store processed vitellogenin in frog oocytes, eggs and early embryos. Altogether, our results demonstrate that detecting SA-β-gal

activity can be used to monitor autophagy in the gamete cells, such as *Xenopus* oocytes and eggs.

P.4.3.B-004
The differences of microRNA and mRNA in occupational silicosis patients

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Silica is one of the most important occupational harmful factors in China. In 1997, the International Agency for Research on Cancer announced crystalline silica is carcinogenic to humans. Epigenetic regulation of gene expression has been widely studied in cancer, and aberrant microRNA plays a role in the development of various diseases. But the reports about relation silica and aberrant microRNA have been limited and focus on certain gene, rats model and blood from silicosis patient. We performed genome-scale microRNA and mRNA profile of lung tissues from 7 silicosis patients to identify the microRNA and mRNA patterns in silicosis through Affymetrix Gene Chip microRNA4.0 Array and HTA 2.0 Array. We found 241 significantly different microRNAs, 1417 mRNA and 326 lncRNA in early-stage and advanced-stage compared with normal lung, respectively. Using the strategy for STC gene expression data, we defined some profiles. The expression model profiles are related to the actual or the expected number of genes assigned to each model profile. We worked out 231 differential microRNA trends, and 1289 differential mRNA trends. Through the STC analysis and the relationship of microRNA and mRNa, we chose the correlated microRNA and mRNAfor the following validity experiments. 404 silicosis patients and 177 control groups were picked. And through several times of checking of the microRNA, mRNA and lncR\NA in the subjects blood, we finally found that microRNA 8063 and 181b-5p, and GNAI3 and PTEN mRNA were changed significantly between silicosis patients and controls. It is the first time we identified the differences of microRNA and mRNA in the blood of the silicosis patients. It will provide the clue for the further studies of the mechanisms of silicosis.

P.4.3.B-005
IRE1-JNK branch of ER stress response promotes inflammation and autophagy in heart tissue

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Endoplasmic reticulum (ER) associated functions, such as proper synthesis and functional folding of proteins, are reported to be key factors for the normal function of heart, especially for cardiomyocytes. About 30% of total proteins in eukaryotic cells takes place in the secretory pathway and initial maturation steps of these proteins held in ER. Under stress conditions, accumulation of misfolded/unfolded proteins in ER lumen removes the inhibitory effect of Grp78 on UPR regulatory proteins; IRE1, PERK and ATF6. Activation of IRE-JNK branch of ER stress response is known to enhance inflammation and autophagy by inducing AP-1 and Beclin-1/Bcl-2 disintegration, respectively. While a number of proinflammatory cytokines were regulated by AP-1, free Beclin-1 interacts with other autophagy related proteins involved in autophagosome formation. Either of these pathways may increase viability under stress conditions by limiting damage or excessive autophagic activity may lead to death of cells. In this direction, we have investigated Grp78, Grp94, IRE1, PERK, JNK, IL-6, TNF-α, TGF-β, bcl-2, beclin-1 and LC3-II, well known markers of ER stress, inflamation and autophagy, in the heart tissue of rabbits. Autophagic activity in heart tissue is also observed in hypercholesterolemic vs control rabbits by electron microscopy. Role of ER stress on apoptotic cell death is evaluated by TUNEL method.

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P.4.3.B-006
Monitoring the kinetics of extracellular vesicle uptake by imaging flow cytometry

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Extracellular vesicles are essential compartments for long distance cell-cell communication. They function as carriers of different compounds including proteins, lipids and nucleic acids. Pathogens excel in vesicle release to mediate cell communication in diverse processes, particularly in manipulating the host response. Establishing research tools to study the interface between pathogen derived vesicles and their host recipient cells will be particularly useful. Multi-parametric, high-throughput Imaging Flow Cytometry (IFC) combines the speed and multiparametric quantification of flow cytometry with the information-rich imagery of microscopy. Here we utilized IFC for developing a method to monitor the uptake of malaria-derived vesicles by host immune cells. By staining different cargo components, we were able to directly track the cargo's internalization over time in live cells. Interestingly, we found that transferred RNA accumulates in distinct cellular localizations during the course of the uptake. This method can be used to measure the kinetics of cargo delivery and to track the cellular destination of specific types of cargo components. Our method hereby provides a tool to study the dynamics of vesicle uptake in different host-pathogen and pathogen-pathogen systems.

P.4.3.B-007
A systems biological view of life-and-death decision with respect to endoplasmic reticulum stress – the role of PERK pathway

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One of the most important tasks of a cellular system is to maintain its intrinsic homeostasis against external stimuli. The proper balance of both secreted and membrane proteins is controlled in the endoplasmic reticulum (ER). Accumulation of misfolded proteins due to different ER stress events leads to the activation of three branches (PERK, IRE-1 and ATF6, respectively) of unfolded protein response (UPR). The primer role of UPR is to reduce the bulk of damages and try to drive back the system to the former or a new homeostatic state by self-eating dependent autophagy, while excessive level of ER stress results in apoptotic cell death. Our study focuses on the PERK-induced branch of UPR. Hereby we confirm that silencing of PERK extends

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autophagy-dependent survival during ER stress. We also claim that the proper order of surviving and self-killing mechanisms is controlled by positive feedback loops between PERK and IRE-1 branches. This regulatory network makes possible smooth, continuous activation of autophagy during ER stress, while the induction of apoptosis is irreversible and switch-like. Using our knowledge in molecular biological techniques and systems biological tools our goal is to give a qualitative description about the dynamical behaviour of the system by exploring the key regulatory motifs.

P.4.3.B-008
Endoplasmic reticulum stress is activated after epibrassinolide treatment leading autophagy in wild type and Atg5−/− mouse embryonic fibroblasts: a survival mechanism in non-malignant cells

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Autophagy is a process of cellular self-degradation during which macromolecules, damaged/aged organelles and proteins are delivered to the lysosome by engulfment within double-membrane vesicles. The accumulation of un/misfolded proteins in the ER can activate ER stress leading unfolded protein response (UPR) resulting autophagy. Beclin-1, LC3 and Autophagy Related Genes (Atg) play key roles in formation of autophagosome membrane, which are triggered by Ulk-1 signaling through AMPK (AMP-activated protein kinase). Conversely, autophagy is inhibited by the mammalian target of rapamycin (mTOR), a central cell-growth regulator that integrates growth factor and nutrient signals. Epibrassinolide (EBR) is a member of brassinostreoids plant hormones with a structural similarity to mammalian steroids. Our previous work demostrated that EBR treatment caused ER stress and UPR which resulted in caspase-dependent apoptosis in SW480, DLD-1, HT-29 and HCT116 colon cancinoma cell lines. Here, we demostrate that EBR treatment activates UPR by inducing ER stress-related proteins in wild type (wt) MEF (mouse embryonic fibroblast) cells. In contrary to our previous results in malignant cells, MEF cells do not undergo apoptosis after EBR treatment in stead autophagy is activated causing cell survival. We used $Atg5^{-/-}$ MEF cells to understand the correlation between ER stress and autophagy. Suprisingly, we observed a significant downregulation of p62/SQSTM1 protein, which is known as one of the autophagy marker, in $Atg5^{-/-}$ MEF cells. ER stress was also triggered in these cells. Therefore, we conclude that autophagy is a key mechanism to promote cell survival after EBR treatment against ER stress in both wt and $Atg5^{-/-}$ MEF cells. These findings suggested that EBR as an ER stress inducer, is able to activate autophagy in order to promote cell survival in non-malignant cells.

Structural Computational Biology

P.4.4.A-001
Unveiling the role of the mutation F508del in cystic fibrosis

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Cystic fibrosis is a genetic disease that causes the accumulation of mucus in epithelia, mainly affecting the airways of the lungs. These secretions not only cause obstruction, but also inflammation and infections by Pseudomonas and Staphylococcus organisms. (1)(2) The mutated gene in this disease encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is a chloride channel. (1) Although there are over 1500 mutations related to cystic fibrosis, the most common is the deletion of phenylalanine 508 (F508del). F508del causes the misfolding of the CFTR polypeptide leading to its degradation in the ER. Nevertheless, a small amount of defective channels is still able to reach the cell membrane, but display an impaired function leading to minimal chloride transport. (3) The CFTR channel belongs to the class of ABC transporters. These proteins have been extensively studied using computational methods in our lab (4–6) in order to clarify their mechanism and mode of action. (5) (6) In the present work, we derived a new model for the NBD1-NBD2 association of human CFTR based on existing data using comparative modelling techniques and performed molecular dynamics simulations of the CFTR protein in both mutant and wild-type forms with the goal of studying the conformational consequences of ATP hydrolysis in the mutant and wild-type forms.

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P.4.4.A-002
Study of interaction between D–mannitol and ribonucleosides by fluorescent probe

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Natural and synthetic oligoribonucleotides which are modified with D–mannitol have biological activities and are used as antiviral drug. The aim of our research was to investigate stack interaction between ribonucleosides and D–mannitol which can be experimentally detected by fluorescent probe. During the research we have observed the decrease of fluorescence intensity of the dye water solution with ribonucleosides; this point to stack interaction between dye molecule and ribonucleosides base. Increasing the nucleosides concentration was accompanied by regular decreasing of fluorescence intensity to saturation of nucleosides molecules. The saturated decreasing of fluorescence intensity depends on nucleosides type and are different for solutions of A, C, U and G. Maximum effect was observed for fluorescent sensor solution with riboadenosine. Additional decrease of intensity was observed in complexes with D–mannitol. The largest difference in fluorescence intensity was observed in riboadenosine and his complex with D–mannitol. Basing on quantum-chemical calculations, it is established that the hydrogen bonds between D–mannitol molecule and two centers of nucleosides can be generated. Also, the calculations show appreciable changes in charge distribution in base molecules, especially at those atoms which can generate hydrogen bonds. This can be accompanied by change in stack interaction between sensor and ribonucleoside molecules. The spectral study of water solutions of mixture of dye probe with ribonuleosides has shown about stack interaction between

investigated molecules. In studied solutions the D–manitol causes the additional spectral effect which point to interaction between ribonucleosides and mannitol by generating of two hydrogen bonds.

P.4.4.A-003
Classifiers of DNA sequences based on physical characteristics profiles of DNA

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Nowadays the pace of genome sequencing outruns the rate of their annotation partially due to the classical method of sequence analysis are not able to account for DNA-protein interactions for detection of the regulatory DNA sites. The recent discovery of the phenomenon of molecular mimicry corroborates that proteins do not read DNA as text, which means that DNA-protein recognition and interaction is governed by physical characteristics of DNA and proteins. Because of this, it seems natural to use distributions of physical characteristics of the DNA molecule for solving the tasks of prediction of regulatory DNA sites. It was previously shown that such approach performs better compare to sequence text analysis. To improve the accuracy of the predictions we propose to combine properties of a different kind into one profile. We have chosen characteristics representing different properties of the DNA molecule: dynamic characteristics of the DNA open states – their activation energy and size, the electrostatic potential on the surface of the DNA and GC-content. For dimensionality reduction, we applied principal component analysis to calculated profiles. Two classifiers were trained in reduced feature space with Naive Bayes and Random Forest algorithms on five different sets of the *E. coli* genome DNA sequences: experimentally found promoters, genes, antipromoters, promoter islands and DNA sites located 300 bp and more from proved promoters (non-promoters). The classifiers were trained to distinguishing between promoters and one of the other chosen DNA sequence types. Random Forest classifier showed the highest rates of sensitivity and specificity, with the accuracy rates exceeding 90%.

P.4.4.A-004
AIMP1/p43 protein structural topography studies using bis-ANS fluorescence probe

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Aminoacyl tRNA synthase complex-interacting multifunctional protein 1 (AIMP1/p43) is an auxiliary component of mammalian aminoacyl tRNA synthetase complex (MARS). Outside of the complex it shows pleiotropic cytokine activity, modulates the proliferation of different types of cells, suppress angiogenesis and stimulate apoptosis and inflammation. Since the spatial structure of full-length protein is still unknown, the specific physical nature of protein conformational changes and their contribution to the functional activity of the AIMP1/p43 protein remain largely unknown. The purpose of this work is the AIMP1/p43 protein structural topography studies using combination of experimental techniques and computational methods for investigation of bis-ANS interaction with AIMP1/p43. The investigation of AIMP1/p43 structural topography was performed by steady state fluorescence spectroscopy technique based on bis-ANS fluorescence quantum yield increasing upon binding to the hydrophobic regions of proteins. In these experiments we observed the obvious increase in the fluorescence quantum yield of bis-ANS with temperature increasing. In order to identify hydrophobic potential binding site for bis-ANS on the molecular surface of AIMP1/p43, accuracy flexible docking was performed. As a result, it was found that binding of bis-ANS to AIMP1/p43 occurs in a several hydrophobic pockets with a different binding affinity. Our research has shown the key role of both the hydrophobic and ionic interactions involved in the binding sites formation. A significant temperature-dependent increase in fluorescence quantum yield is the result of additional hydrophobic regions exposure on the protein surface as a consequence of the conformational fluctuations.

P.4.4.A-005
The FAD synthetase in the spotlight: new antimicrobials targeting the biosynthesis of flavin cofactors

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Bifunctional FAD synthetases (FADS), prokaryotic enzymes that catalyze the biosynthesis of the cofactors flavin mononnucleotide (FMN) and flavin adenine dinucleotide (FAD), are essential in flavin and flavoproteome homeostasis. This crucial role in cellular metabolism, together with the significant differences with their eukaryotic counterparts (monofunctional riboflavin kinases and FMN adenylyltransferases), converts the FADS in a promising drug target for the development of inhibitors with antimicrobial activity. We have identified 40 potential inhibitors of *Ca*FADS and *Spn*FADS (FADS from *Corynebacterium ammoniagenes* and *Streptococcus pneumoniae,* representative members of the prokaryotic FADS faimily) by the activity-based high-throughput screening of chemical libraries. These hits have been thoroughly characterized by the determination of their antimicrobial spectrum and their cytotoxic effect on eukaryotic cells. Only 5 of the hits show antimicrobial activity against Gram-positives, and one of them against Gram-negative microorganisms as well. Nevertheless, their therapeutic application could be limited due to their cytotoxicity on eukaryotic cells at the concentrations required for the antimicrobial activity. Both antibacterial and cytotoxic effects may be associated with the off-target alteration of other essential flavoproteins and flavoenzymes. In a further step, the molecular information of the antimicrobial mechanism will be used for the optimization of these compounds to generate second-generation antimicrobials with higher efficacy and less toxicity.

P.4.4.A-006
Structural basis of chloramphenicol-metabolizing enzyme

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Chloramphenicol (Cm) and florfenicol (Ff) are broad-spectrum antibiotics that inhibit protein synthesis. Both antibiotics are a bacteriostat but differ in their chemical structure in that a fluoro group is attached to C3 of Ff. Specifically, both antibiotics prevent a formation of the peptide bond by irreversibly binding to a

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receptor site on the 50S subunit of the bacterial ribosome. It is well established that chloramphenicol acetyltransferase inactivates Cm by first recognizing the hydroxyl group of C3 in Cm and then specifically acetylating the hydroxyl group. Due to this specificity, Ff cannot serve as a substrate of chloramphenicol acetyltransferase. Interestingly, a novel esterase, which was recently characterized by metagenome screening, inactivates both antibiotics possibly by hydrolyzing those antibiotics, suggesting that this esterase could be a novel enzyme in inactivating both Cm and Ff. Sequence analysis indicates that its sequence is highly similar to those of microbial hormone sensitive lipase. Consistent with this comparison, a newly identified esterase contains Ser156 in the GxSxG motif and Asp252 and His282, the catalytic triad conserved in the family of microbial hormone sensitive lipase. In order to understand this novel enzymatic feature, we are carrying out X-ray crystallographic analysis. Our studies will provide structural insights into antibiotics hydrolysis.

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P.4.4.A-007
Statistical model for the synthetic DNA adaptation to the environmental conditions

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The increasing usability of synthetic genetic constructs ranging from biotechnology and gene therapy strategies to its role as an alternative information carrier increases the importance of DNA sequence adaptation to environmental factors and genetic machinery of the host. We suggest an improved reverse translation algorithm that optimizes the genetic sequence encoding a given protein to the host organism's biochemical features which significantly depend on the biophysical properties of their living environment. The optimization strategy is based on the adjustment of the local GC content variations according to a recently suggested superstatistical model which represents a long DNA molecule by a series of consecutive $\sim$150 bp DNA segments where nucleotides are allocated randomly, corresponding to the local equilibrium scenario, while the fractions of different nucleotides in consecutive segments alternate in a long-range correlated manner. To adjust the synthetic patch to the neighboring DNA fragments, the local GC content variability in the vicinity of the patching point is also taken into account and could be solved by adding linker sequence(s) either up- or downstream of the patch, or both. In case when the synthetic DNA patch has to encode functional genes on both strands the optimization problem could be solved only iteratively because each of directions from 5′ to 3′ and from 3′ to 5′ should be adjusted separately and thus affect each other. We tested our algorithm computationally using 130 complete genome sequences of both pro- and eukaryotic organisms. We believe that the suggested algorithm could contribute to the further improvement of stability and the adaptation abilities of long synthetic DNA patches in various heterologous systems.

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P.4.4.A-008
Structural basis for antibody targeting of a broadly expressed microbial polysaccharide

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Poly-*N*-acetyl-D-glucosamine (PNAG) is a polysaccharide that is produced by many microbes, including bacteria, fungi, and protozoan parasites. PNAG is abundant on the cell surfaces and in biofilms of most hospital-acquired antibiotic resistant infections such as *Staphylococcus aureus*, for which new treatments are actively being pursued. Importantly, PNAG is not expressed in humans (or mammals), making it a promising target for the development of monoclonal antibodies (mAbs) and vaccines with potential efficacy against a variety of PNAG-expressing microbes. A human IgG1 mAb (F598) that binds PNAG has shown activity against gram-positive and gram-negative bacteria, fungal pathogens, and some protozoan parasites. F598 is both opsonic and protective in a variety of *in vitro* and *in vivo* models and is undergoing pre-clinical and clinical assessment for several microbial pathogens. To understand how F598 targets PNAG, we have determined crystal structures of the unliganded F598 antigen binding fragment (Fab) and its complexes with a PNAG oligosaccharide (9-mer) and with *N*-acetyl-D-glucosamine (GlcNAc). Recognition of PNAG occurs in a large groove-shaped binding site that traverses the entire light and heavy chain interface and accommodates at least 5 GlcNAc residues. The Fab-GlcNAc complex revealed a near identical binding mode of the monosaccharide and a core GlcNAc of the oligosaccharide suggesting an anchored binding mechanism of PNAG by the F598 mAb. Although intact F598 IgG is required for effector functions, the Fab used for structural studies retained binding to PNAG on the surface of an antibiotic-resistant biofilm forming strain of *S. aureus*. The structures of F598 Fab and its complexes with target glycans presented here have facilitated modeling of bivalent F598 IgG binding to PNAG, which is the first step in Fc-dependent mAb protection to PNAG-expressing pathogens.

P.4.4.A-009
Structural insight into molecular mechanism of poly(ethylene terephthalate) degradation

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Poly(ethylene terephthalate) (PET) PET is the most widely used plastic in the world and its non-degradable properties cause global environmental problems. Recent identification of a *superior PET-degrading* bacterium *Ideonella sakaiensis* has become a promising strategy for the recycling of PET through its enzymatic biodegradation. Here we determined the crystal structure of *Ideonella sakaiensis* PETase (*Is*PETase) at a 1.5 Å resolution. *Is*PETase has an optimal substrate binding site to accommodate two MHET moieties of PET polymers as substrates, and the catalytic triad are located at the center of the substrate binding site. Based on the structural and site-directed mutagenesis experiments of *Is*PETase, we also proposed the detailed PET degradation process into terephthalic acid and ethylene glycol. Moreover, the phylogenetic tree analysis of 69 PETase-like proteins was performed to suggest candidate PETases that might have similar level of PET-degrading activity to *Is*PETase.

P.4.4.A-010
Two new crystal structures of zinc-finger-associated domains from *D. melanogaster* reveal structural similarity to the only known structure of this protein family

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The spatial genome organization is controlled by a set of special proteins having an architectural function. These proteins establish long-range genomic interactions, through a formation of various multi-protein complexes, and usually have multidomain structure with several zinc-finger domains required for DNA binding and additional effector domains with different functions. Among these additional domains are, in particular, zinc finger-associated domain (ZAD) is a ubiquitous motif of C2H2 zinc finger proteins of *Drosophila*. Genes that encode ZAD-containing proteins are specific for Arthropoda genomes. Only a few ZAD-encoding genes have known functions, and the role of ZAD is being discussed. They are known to form dimers and it is hypothesised that they are responsible for the specific interactions and complex formation in long-range genome interactions. Up to date there was only one known structure of ZAD-domain from *Drosophila* transcription factor Grauzone (GrauZAD). Since amino-acid sequences of these domains are low-conserved beyond zinc-coordinating cysteines it was unclear whether they possess the similar fold. Here, we present novel structures of two ZAD-domains of Serendipity-delta and CG2712 proteins from *D. melanogaster*. Both structures were solved at 3.0Å and 2.0Å, accordingly by MAD technique based on the anomalous scattering of Zn atom. Both domains have dimeric state in a crystal and similar overall fold. However, some unique spatial features of the proteins were found, compared to each other and to GrauZAD. Revealed features may reflect specificity in protein-protein interactions occurring between these types of domains. Despite low sequence similarity these domains have very similar spatial structure, which can be regarded as a 3D template in possible recruiting of other yet unknown regulatory complexes.

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P.4.4.A-011
Discovery of selective inhibitors of imidazole glycerol phosphate dehydratase from *Mycobacterium tuberculosis* by docking-based virtual screening

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Imidazole glycerol phosphate dehydratase (IGPD) which catalysis the conversion of imidazole glycerol phosphate (IGP) to imidazole acetol-phosphate in the histidine biosynthesis pathway in bacteria, lower eucaryotes and plants is absent in mammals. Histidine biosynthesis pathway is essential for optimal growth of *Mycobacterium tuberculosis*. Due to this IGPD is an attractive target for anti-tuberculosis agents development. The 3D-structures of apo *Mt* IGPD and its complex with substrate IPG are known at 2.02 and 2.1 Å correspondingly. In this study we carried out virtual screening and molecular dynamics simulations to identify potent inhibitors of IGPD using the Mcule online drug discovery platform. The fragment of the enzyme molecule composed of three subunits forming the whole active site was served as a target for docking. The potency of compounds as inhibitors was assessed using the Vina scoring function and the accordance to Lepinski's rules. Four best-scoring compounds L1-L4 were evaluated by molecular dynamic simulation with 5 ns trajectory. The interface area between the ligands and enzyme is studied using PISA program, the hydrogen bonds and polar contacts were analyzed using Coot program. It was shown that each of L1-L4 ligands covers the active site area and prevents the penetration of the substrate into the active site. The hydrogen bonds and polar contacts between ligands and active site amino acid residues were identified. These findings allow suggest that chosen ligands can serve as leading compounds for the development of selective inhibitors of IGPD.

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P.4.4.A-012
Kinetic and structural changes in HsmtPheRS, induced by pathogenic mutations in human FARS2

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Mutations in the mitochondrial aminoacyl-tRNA synthetases (mtaaRSs) can cause profound clinical presentations, and have manifested as diseases with very selective tissue specificity. To date most of the mtaaRS mutations could be phenotypically recognized, such that clinicians could identify the affected mtaaRS from the symptoms alone. Among the recently reported pathogenic variants are point mutations in FARS2 gene, encoding the human mitochondrial PheRS. Patient symptoms range from spastic paraplegia to fatal infantile Alpers encephalopathy. How clinical manifestations of these mutations relate to the changes in 3D-structures and kinetic characteristics remains unclear, although impaired aminoacylation has been proposed as possible aetiology of diseases. We determined four crystal structures of HsmtPheRS mutants, and carried out extensive MD simulations for wild type and nine mutants to reveal the structural changes on dynamic trajectories of HsmtPheRS. Using steady-state kinetic measurements of phenylalanine activation and tRNAPhe aminoacylation, we gained insight into the structural and kinetic effects of mitochondrial disease-related mutations in FARS2 gene.

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P.4.4.A-013
Structural modeling of liposomes in a free state and with adsorbed Influenza virus M1 protein from synchrotron SAXS data

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We conducted synchrotron-based small-angle X-ray scattering studies of protein-free and protein-coated (with M1 protein) liposomes of different lipid composition. The sizes of the bilayer vesicles and the radial electron density profiles of the lipid bilayers were restored. We demonstrated that the thickness and the density profile of the lipid bilayer depend on the chemical structure of lipids, e.g. the ratio between cholesterol and negatively charged phospholipids. Liposomes with adsorbed M1 protein provide an additional fingerprint on the electron density profile of the system. This effect became more pronounced with the incubation time of the protein. However, in the case of raft-forming lipid compositions (comprising four types of lipids, including sphyngomyelin and cholesterol) the interactions between M1 protein and liposomes perturbed the vesicle structure and lead to the significant decrease of their size. To complement the polydispersity size analysis with three-dimensional structural models, variety of double-layered hollow ellipsoid-like structures with distinct semiaxes were generated based on the atomic coordinates of a single lipid molecule. To account for possible perturbations of the liposome interface, the radial distance of each monomer was randomized with the maximum deviation of 1 nm from the ideal ellipsoid surface. Semiaxes values of the quasi-ellipsoids in the generated pool varied from 45 to 80 nm. Theoretical scattering intensities from such set of models were computed by the program Crysol and screened against the experimental SAXS data to find the optimal configurations. The optimal fitting was obtained by the model with the semiaxes values of 51, 58 and 60 nm, that is in agreement with the model independent calculations.

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P.4.4.A-014
Multidisciplinary structural characterization of helical plant viruses by small angle X-ray scattering and tritium planigraphy data

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A virion of flexible Potato virus X (PVX) was studied using combination of tritium planigraphy (TP) and small angle X-ray scattering (SAXS). Homological modeling of coat protein (CP) structure in PVX virion was based on the available high-resolution structure of CP from the other member of the Potexvirus – Papaya Mosaic virus. We propose an algorithm for determining the orientation of the CPs and refinement the coordinates of amino acids atoms in PVX virion by TP data. The search of optimal correlation between the maximum inclusion of tritium labels and the side of molecule forming the terminal face of the helical assembly permits to restore a detailed image of the quaternary structure of the virus, and to determine the interfaces between the CPs. Additionally, low resolution *ab initio* shapes were reconstructed using bead modelling approach against solution scattering data. Given the structural parameters of the solute, which pointed to the extended architecture of the viruses and their CPs, the initial round of the shape determination was performed without symmetry restrictions using elongated cylinders as the search volumes. As the resulting shapes possessed quazi-helical symmetry, corresponding solid helical models were generated yielding the best agreement with the typical P1 reconstructions and a refinement cycle was launched within such search volume. The reconstructed models were still able to neatly fit the experimental SAXS data confirming the helical arrangement of the particles and revealed sparse bead occupancies in the core region of the helix in a full agreement with the expected virus architecture. Both structural reconstructions, on the base of TP and SAXS, complement each other giving the detailed spatial PVX virion organization. This work was supported in part by Russian Foundation for Basic Researches (projects 15-54-74002 EMBL, 16-03-00375, 15-04-01406).

P.4.4.A-015
New linear interaction between charged lysine side chain and carbonyl group in protein structures

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Regular hydrogen bonds involving lysine side-chain NH3+ group and various uncharged acceptors as well as salt bridges between lysine and negatively charged residues are well documented in the literature. Unexpectedly, screening of Protein Data Bank (PDB) also reveals another type of interaction between lysine side chain and polar carbonyl group. This interaction is characterized by linear atom arrangement, where carbonyl oxygen is positioned on the three-fold symmetry axis of the NH3+ group. Automated analysis of all high-quality crystallographic structures in the PDB (resolution 1.5 Å or better) produced more than 10,000 examples of this interaction. We termed it linear lysine-carbonyl interaction and modeled its properties in gas phase and in aqueous environment typical of protein surface sites. wB97x-d/cc-pVQZ quantum chemical computations using polarizable continuum solvent model have been performed to scan potential energy in the model system methylammonium – N-acetamide along the selected coordinates (N^{ζ}-O distance and C^{ε}-N^{ζ}-O angle). At 2.7 Å and 180° we have found a saddle point with the depth of 2 kcal/mol. This saddle point is transformed into a global minimum when NH3+ group is hydrogen bonded on all three of its protons; analysis of PDB indicates that this is indeed the case in most protein structures. Linear lysine-carbonyl interaction is almost entirely electrostatic in nature, as confirmed by the energy decomposition analysis; at the same time it gives rise to potentially measurable J-coupling, which opens a path for its direct observation by NMR. Analysis of linear lysine-carbonyl interactions in the PDB indicates that carbonyl

frequently occurs in turns and coils and relatively infrequently in β-sheet. In a number of cases we have found that linear lysine-carbonyl interaction caps the C-terminal end of α-helices, providing an alternative to hydrogen bond at the respective carbonyl site.

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P.4.4.A-016
Structural characteristics of CBS-pyrophosphatase from Desulfitobacterium hafniense in solution: SAXS study

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Inorganic pyrophosphatases (PPases) are enzymes that exist in all living organisms. Pyrophosphatases play an important role in cell metabolism shifting the equilibrium of such reactions as the protein, RNA and DNA synthesis [1]. Soluble PPases of family II found in prokaryotes are the most active and structurally diverse. Although three-dimensional structures have been obtained for several families II of PPases detailed structural information is not available for pyrophosphatses contain a regulatory insert comprising a pair of cystathionine β-synthase (CBS) domains (CBS-PPases) [2]. Crystallization attempts of full-length CBS domain-containing PPase is a significant challenge, and have not been successful to date. The aim of the present study is to investigate the behavior of CBS-PPase from *Desulfitobacterium hafniense* in solution, i.e. under near-physiological conditions by small-angle X-ray scattering (SAXS), and complementary methods. CBS-PPase is a homodimeric protein, and its structure was determined by molecular docking using known crystal structures of regulatory and catalytic domains. Using obtained dimer of CBS-PPase and modern techniques of SAXS data interpretation and modeling we found out that in solution the protein exists as an equilibrium mixture of two oligomeric forms. The obtained results will provide structural new structural insight into the mechanism of CBS-PPase regulation by nucleotides.

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P.4.4.A-017
Prediction of transmembrane protein crystallization, purification and solubilization

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Transmembrane proteins play vital roles in the life of cells, anchoring cells to the extracellular matrix, joining cells and acting as gatekeepers and receptors in the membranes. They make up to 25–30% of the human proteome and are targeted by around 50% of marketed drugs. However, only 2% of the structures deposited in structure databases belong to transmembrane proteins. One of the most widely used methods for solving protein structures is X-ray crystallography. Transmembrane proteins, due to their special physical-chemical properties, are hard to be solubilized and crystallized, thus making the determination of their structures difficult. The process is time and money consuming, thus prediction for the success or failure of the crystallization may help the crystallization projects. Our aim was to develop a prediction method for the crystallization process of transmembrane proteins. So far, no method is available that would be specific for transmembrane proteins. We used the TargetTrack and PDBTM databases to create training and test sets for all the steps of the crystallization process. Machine learning algorithms were used to train and optimize models for all steps. Prediction results would be incorporated into the recently developed TSTMP database (www.tstmp.enzim.ttk.mta.hu), to help the target selection of human transmembrane proteins.

P.4.4.A-018
Martini coarse-grained force field: extension for lipidated amino acids

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Peripheral membrane proteins may be modified post-translationally by the covalent addition of a fatty acid to a cysteine or a glycine respectively, through S-acetylation or N-acetylation. Such post translational modifications alter the lipophilicity of the modified proteins and allow them to anchor into biological membranes. Members from both the Src and Ras super families such as K-Ras, N-Ras, Rab, Src, Lck and Fyn require such modifications to modulate various functions which are related to apoptosis, cell signaling and cell cycle functions. In this work, we present a new set of parameters for the Martini force field for four of the most common membrane anchors which are used by peripheral membrane proteins: palmitoylated cysteine, farnesylated cysteine, geranylgeranylated cysteine and myristoylated glycine. The parameterization follows the guidelines that were previously presented for lipids, proteins, carbohydrates and DNA and focuses on the reproduction of the free energy of partitioning between 1-octanol and water and the structural dynamics that characterize these molecules when embedded in a lipid bilayer. Bonded and non-bonded parameters have been reproduced from all-atom simulations using the CHARMM36 force field. Examination of the obtained atomistic simulations has revealed the following: 1) The modified cysteines obtain a kinked rod conformation within the bilayer while glycine myristoyl occupies a curved bow-like conformation. 2) cysteine geranylgeranyl has a secondary stable conformation in the bilayer in which it curls back towards the peptide backbone while interacting with the bilayer as a closed loop. We have successfully reproduced these results in our coarse-grained simulations which currently offer a new opportunity to study lipid-protein interactions of peripheral membrane proteins that utilize these membrane anchors.

P.4.4.A-019
Spatial structure of full-length NRADD, 25 kDa single-span integral membrane protein

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NRADD (also called p45) is a 25 kDa co-receptor of the p75 neurotrophin receptor. It associates with the p75 and inhibits its activity. In this work, we solved the spatial structure and studied mobility of the NRADD in lipidic bicelles using solution nuclear magnetic resonance spectroscopy techniques. The structure is

homologues to the p75NTR and represents relatively short unstructured N-terminal segment, followed by the α-helical transmembrane domain, extended intrinsically disordered chopper domain and C-terminal classic death domain. The flexibility of the juxtamembrane chopper domain does not impede free motion of the death domain, the fact that is required for the protein activity and should be explained. This study represents the first of its kind spatial structure of the full-length type I receptor, i.e. single-span membrane protein, obtained using nuclear magnetic resonance spectroscopy in solution.

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P.4.4.A-020
Codons and amino acids biases help to maintain proper electrostatics around transcription factors binding sites

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DNA is highly charged and its electrostatic and other physical properties define its shape in the functional space and greatly influence its interactions with different proteins, especially regulating transcription. Electrostatic potential is distributed non-uniformly along DNA and correlates with GC content, strongly depending on the sequence arrangement and its context (flanking regions). Binding sites of transcription factors of different protein families in different taxa are located in long areas of high electrostatic potential. Electrostatic potential distribution on transcription factors protein surface reflects that of binding sites. Promoters in average have high value of electrostatic potential profile. It is known, although not quite commonly realized, that some transcription factors binding sites lie in protein coding areas. The overall percentage of them is not very high but significant. Moreover, some protein coding areas host also promoters for the genes mainly located on the opposite strand. We found that there is codons and moreover even amino acids bias around promoters and transcription factors binding sites spanning for some hundred of codons and that these biases are due to electrostatic properties of the considered codons. The data obtained demand serious rethinking of the concept of molecular evolution. The apparent nonsynonymy of synonymous substitutions may lead to different wrong estimations of the sequences fate, including misuse of the molecular clock and mistaken evaluations of specific mutations biomedical importance. The amino acids bias leads to even more important shift in the conception of natural selection in proteins. It substantiates the view on the DNA as not only the text of the first step in realizing the Central Dogma, but a complex organ of heredity that fulfills different and sometimes contradictory demands.

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P.4.4.A-021
Phosphoenol pyruvate increases the stability of *Escherichia coli* and Bacillus subtillis FtsZ polymers by inhibition of the GTPase activity

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Bacterial division is a key process with at least a dozen of proteins involved, among them FtsZ and FtsA or ZipA that form the Z-ring in the middle of the cell. Z-ring is the scaffold that recruits the rest of the proteins to form a complex called the *divisome*. FtsZ, a GTPase, responds to several modulatory inputs. Cell division is regulated at the level of FtsZ assembly because no significant changes seem to occur in the concentration of FtsZ, either throughout the cell cycle or under different growth conditions. Regulatory proteins are ZapA, ZapB and SepF that stabilize the Z-ring by inhibition of the GTPase activity, and this process is reverted by ClpX and EzrA. UgtP, involved in glucolipid biosynthesis in *B. subtilis*, acts as a metabolic sensor to detect nutrient status and transmits this information to the division machinery. Beside these regulatory mechanisms we propose that phosphoenol pyruvate (PEP) could be an excellent candidate for a direct modulation of the Z-ring formation. Specifically, our results of light scattering, electron microscopy and GTP hydrolysis showed that the presence of PEP in the range of intracellular concentrations enhances polymerization and increases the stability of FtsZ polymers of *E. coli* and *B. subtilis*, by inhibition of its GTPase activity. PEP binds to a site close to the gamma-phosphate of GTP as shown from our docking bioinformatics results. The effect of PEP was specific because acetyl phosphate, 2-phosphoglycerate and pyruvate had no effect. However, 3-phosphoglycerate induced polymerization in the absence of GTP probably by the structural similarity with PEP. The results as a whole indicated that the *in vitro* FtsZ polymerization is enhanced by PEP in a specific manner, favoring the stability and elongation of the polymers. This finding is of great interest, as there is little knowledge of regulation by intermediate glycolytic metabolites on cell division that could be related with nutrient availability. DIVINOCELL 223431.

P.4.4.A-022
QM/MM maturation in silico of an immunoglobulin repertoire to generate highly developed functionality

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Synergetic applications of combinatorial and rational design allowed bringing a broad functionality to immunoglobulin molecule both in binding and catalysis. These achievements may have tremendous therapeutic applications because of potential successful implementation of novel technologies of Ig engineering and expression. The prediction of catalytic efficiency *in silico* using QM/MM approach becomes realistic now, with the help of supercomputer facilities. This allowed making the most reasonable mutations in Ig combining site and improve thermodynamic and kinetic characteristics of biocatalyst. A wild type clone was chosen initially by selection from a combinatorial library using the activated aryl-phosphonate. One of the selected clones, reactibody A17, demonstrated Ig-mediated hydrolysis of the organophosphorus pesticide paraoxon, in a process proven to

proceed via covalent catalysis and became a potential bioscavanger. Here we made the attempt to make artificially-tailored prototype of a dead-end bioscavenger toward organophosphorus compounds. Molecular docking and cluster analysis of paraoxon in the active site of WT Ig-paraoxonase revealed five possible orientations of the substrate. *In silico* analysis of 167,538 mutants identified the optimum structural mutagenesis in the antibody active center for converting wild type reactibody into an improved pesticide bioscavenger. Mutations of S-L35 offered the best substrate positioning for nucleophilic attack of phosphate group on the active center, and S-L35R was predicted to improve the rate of phosphotyrosine formation by more than two orders of magnitude. QM/MM molecular simulations may thus serve as a valuable tool for virtual Ig maturation to deliver a novel, desired functionality.

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P.4.4.A-023
PDB-wide annotation of biological assemblies from full quaternary structure superposition

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The Protein Data Bank (PDB) provides an ever increasingly rich source of information on macromolecular structures and complexes. An important challenge associated is to discriminate crystal contacts from biological interfaces forming the quaternary structure. In its current state, PDB provides users with alternative quaternary structures for each protein without pointing to the most likely biological form. PISA is currently the most reliable server to predict biological assemblies, but the challenging nature of these predictions results in 15 to 20% errors. Moreover, physico-chemical and evolutionary properties of interfaces that are used to predict their biological relevance results in datasets may bias downstream analyses. These limitations motivated us to develop a novel approach to infer biological assemblies by structural superposition of full quaternary structures. Our premise was that similar interfaces found in homologous proteins are likely biological. Structural alignments of full quaternary structures involving up to sixty subunits allowed us to annotate over a half of all oligomers present in PDB. A benchmark revealed that biological assemblies annotated as being correct showed an error rate as low as 2%. Our method also allowed us to correct the biological assembly of 11,119 structures, with 89% of these corrections being accurate. Overall, our approach yields an exceptionally low overall error rate of 4%, which is three times lower than that of the best current methods. This provides a gold standard dataset of previously unmatched size, yields annotations with an error rate comparable to that of manual curation; thus facilitating research in structural systems biology.

P.4.4.A-024
Global flexibility of a unique arabinanase suggests a "harpoon" mode of action

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α-L-arabinanases are key enzymes in the breakdown of arabinan, one of the main polysaccharides constituting the plant cell-wall, and hence present a wide range of important potential biotechnological applications. AbnA is an extracellular α-L-arabinanase from the thermophilic bacterium *Geobacillus stearothermophilus-T6*, belonging to the GH43 family. AbnA is larger than most other related arabinanases, possessing 848 residues (94 kDa). The 3D structure of AbnA has recently been determined by X-ray crystallography, revealing a unique and novel multi-domain architecture, the largest structure reported so far in the GH43 family. The enzyme is built of four different domains, arranged in a pincer-like structure. Two of these domains are unique to AbnA, where specifically, one of them represents a new carbohydrate-binding module (CBM) family. Three different crystallographic conformational states have been determined for AbnA, two "closed" states and one "open" state, differentiated by approximately 13 Å movement in location of the third and fourth domains. Normal mode analysis (NMA), small angle X-ray scattering (SAXS) and dynamic light scattering (DLS) experiments suggest additional conformational changes for AbnA, involving movement of up to 100 Å in the positions of the third and fourth domains. Substrate-binding structural experiments demonstrate binding of an arabinopentaose substrate to the new CBM domain, in addition to the catalytic domain. These findings, together with complementary isothermal titration calorimetry (ITC), mutagenesis, and kinetics experiments, suggest a novel "harpoon" catalytic mechanism for arabinan degradation by AbnA.

P.4.4.A-025
Computer-based rational design of improved functionality for antibody catalysts toward organophosphorus compounds

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The elimination of pathological effects of organophosphorus (OP) nerve agents has a great biomedical importance. Engineered Ig molecule was proven to be one of the preferable biological antidotes due to their potential capability to act *in vivo* as covalent OP trap. This scavenger Ig activity might be achieved by combinatorial approaches vs/or in addition to rational design of antigen combining sites. Previously we selected A 17 "reactibody" molecule from semi-synthetic phage display library of human immunoglobulins displaying hydrolyzing activity toward pesticide paraoxon throw stage of covalent intermediated state of phosphotyrosine adduct. We made succeeded to improve this parent activity by virtual computer screening. Here we demonstrating the refined rational design of parent reactibody by increasing the probability of reaction using computer – based predicted conditions to stabilize tyrosine residue in pre-attack state. We suggest that origin of residue may change the mechanism of substrate binding from uniform binding to differential binding. Based on crystal structure of A17 we performed the molecular docking, metadynamics calculations analysis of several active site key residues changes and constructed panel of A17 mutants. The mutant L-L47K was demonstrated 240-fold higher catalytic efficiency for paraoxon in comparison with wild type A17. The refined 3D structure and steady state kinetics of this mutant experimentally proved our computer based predictions.

The work was supported by RSF Grant #16-14-00191.

P.4.4.A-026
Substrate discrimination, catalysis and inhibition in the intramembrane rhomboid proteases

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Rhomboid serine proteases, a family of enzymes embedded within the cell membrane of all forms of life, play regulatory roles in various cell pathways. The membrane environment in which they function and unique mechanistic features distinct them from their soluble counterparts. Several challenging issues regarding their catalytic mechanism include: Are proton transfer from the catalytic Ser to His and nucleophilic attack on the substrate concerted or stepwise? How are intra-membrane substrates discriminated by the enzyme exosite? What is the mechanism of inhibition by the non-competitive peptidyl aldehyde inhibitors bound in the enzyme active site? What is the underlying mechanism that explains how rhomboid protease are adopted for the hydrolysis of only long peptides that contain a transmembrane domain (TMD)? We applied our MD-QM/SCRF (VS) method on molecular clusters simulating the enzyme active site and molecular dynamics simulations to address these key structural and mechanistic issues. Two fundamental features of rhomboid catalysis: the enzyme recognition and discrimination of substrates by TMD interactions in the exosite, and the concerted mechanism of non-covalent pre-catalytic complex to covalent tetrahedral complex (TC) conversion, provide the grounds for understanding of these questions at the molecular level.

P.4.4.A-027
Predicting sublimation entropy and dissociation constants on a basis of quantitative evaluation of movements of molecules in crystals

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To predict dissociation and association constants (for a protein and a ligand, for a molecule and a crystal, for a monomer and an amyloid fibril, etc.), it is important to be able to calculate both terms of binding free energy, that is, enthalpy and entropy of binding. Both these terms can be calculated by molecular dynamics simulations, but this method is very computationally expensive, and entropy calculations are especially slow. We have developed an alternative, very fast method of calculation of binding entropy and dissociation constants. Our approach is based on a quantitative evaluation of range of movements of molecules in a bound state. In this work, we consider the process of reversible dissociation of molecules from crystals. The range of movements of molecules in the bound state (here, in the crystal) can be calculated knowing geometrical parameters of the considered molecules as well as experimental data on saturated vapor pressure and enthalpies of sublimation of the crystals. At first, we have calculated the range (and the corresponding amplitude) of the movements, and then the averaged value was used for prediction of sublimation entropies and dissociation constants. The calculated values have shown a good agreement with the corresponding experimental values. Thus, the proposed method can be successfully used for predictions of both sublimation entropies and dissociation constants for molecular crystals.

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P.4.4.A-028
Transient kinetic analysis of ATP hydrolysis by the CCT/TRiC chaperone

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Chaperonins are large ATP-fueled molecular machines, which assist folding of both newly synthesized and denatured proteins. The eukaryotic chaperonin CCT/TRiC is built from two identical back-to-back stacked rings that are composed of 8 different subunits. The arrangement of the subunits was established more recently but the exact function of every different subunit and the reaction cycle of CCT remain unclear. CCT displays positive intra-ring and negative inter-ring cooperativities with respect to ATP binding and hydrolysis, but many aspects of the allosteric mechanism are still unknown. Here, the transient kinetics of ATP hydrolysis by wild-type CCT/TRiC and several mutants were studied. The reaction was found to have at least four phases: two burst phases, a lag phase and a steady-state phase. The traces were well fitted to an equation comprising one linear and three exponential terms. The phases were assigned by (i) determining their dependence on ATP and K+ concentrations and (ii) by measuring their sensitivity to the mutation Gly345→Asp in subunit CCT4, which decreases cooperativity in ATP binding. The values of the observed rate constants corresponding to the burst phases are found to decrease with increasing ATP and K+ concentrations, thereby indicating that the apo-state of CCT/TRiC is in equilibrium between several conformations and that "conformational selection" by ATP takes place before hydrolysis. The amplitude of the lag phase, which follows, decreases with increasing ATP concentrations, thus indicating that it reflects a transition between states with low affinity for ATP and a state with high affinity for ATP that is predominant under steady-state conditions. A kinetic model based on the data is suggested, in which CCT/TRiC is in equilibrium between a relatively large number of states that are distinguished kinetically, in agreement with its proposed sequential allosteric mechanism.

P.4.4.A-029
Comparison of methods to calculate conformational entropies from crystal structures

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Conformational entropy is of fundamental importance in ligand binding, but its role is not often addressed, mainly because it is hard to measure or calculate in an accurate way. Experimentally, conformational entropies are usually estimated through NMR relaxation experiments, but in theory, they could also be estimated directly from protein crystal structures, because there is information on protein dynamics in the crystal structure, available as atomic displacement parameters (B factors). We have compared several ways to obtain information about the atomic motions in protein structures, including traditional molecular dynamics (MD) simulations, MD simulations in the crystallographic unit cells, ensemble refinement simulations and B factors from various traditional refinements of crystal structures. From these sets of structures, we calculated entropies by different methods, such as dihedral angle histogramming, quasi-harmonic analysis or directly from B factors, using the approach of Zagrovic and coworkers. To minimize errors that could derive from structural differences, all the calculations were done on two diastereomeric ligands bound to galectin-3C. Relative conformational

entropies calculated from atomic fluctuations in MD simulation, whether traditional or in a crystal unit cell, give consistent results which are close to the experimental estimate. In comparison, calculating entropies directly from B factors, either from crystallographic refinements or MD simulations, yielded results that are 6–8 times too large, both for relative and absolute conformational entropies. One of the possible sources of error is that the B factor of each atom includes translational and rotational movements of the entire protein, thus not accounting for correlated motions. These results suggest that conformational entropy cannot be estimated directly from crystal structures through B factor analysis, but further corrections can be made by including correlation from MD simulations.

P.4.4.A-030
Inter-domain contact between tandem WW1 and WW2 domains of WWOX

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The domain is the smallest functional unit of a protein. Since many WW domain-containing proteins compete with one another in various cellular pathways, presumably via WW domains binding, the dynamics of this competition are of great interest. How do WW domain proteins compete over the same classical motifs? What provides the specificity and affinity necessary for competitive target binding? We have begun to answer this question by examining WW domain interactions with short peptide sequences derived from known partner proteins. Isothermal Titration Calorimetry (ITC) experiments confirm that several individual class I WW domains display micromolar affinity for their partner peptides, and nuclear magnetic resonance spectroscopy (NMR) structures suggest this is due to additional contacts between the domain and peptide flanking regions. Thus it seems that certain WW-domains have been given a competitive edge through evolutionary customization of peptide flanking regions. However, examples of high affinity binding by individual WW domains are unusual. Among WW domains, high affinity binding more commonly occurs as a coordinated effort between tandem WW domain units, as is evidenced from ITC data and NMR models. As such, our next step has been to analyze tandem WW domains and their mutual binding of target peptides. Drawing from Rosetta-based methodology for domain assembly, we have modeled WWOX WW1 and WW2 tandem domains on the molecular level. We have represented these two domains while in complex with partner peptide ErbB4 PY3, as well as while unbound. In this way we have been able to determine the inter-domain interactions by which WWOX WW2 – a "non-functional" WW domain that inherently lacks binding ability when in isolation – structurally stabilizes and augments WWOX WW1 affinity for WWOX binders.

P.4.4.A-031
Loop truncation can increase protein stability: computational and NMR approach

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In our research, we are interested in understanding the molecular mechanisms of the effects exerted by changes in the length of a loop region on protein stability. Previous studies conducted on flexible loop regions revealed that the energetic consequences of changing loop length are predominantly related to the entropic cost of ordering a loop during protein folding. In our collaborative study of human acylphosphatase (AcP) using experimental tools and all-atom molecular dynamics simulations we showed that the stabilization due to loop deletion was of an entropic nature and the thermodynamic stabilization can mainly be attributed to the stabilization of the folded state. The motivation for our present research is to support computationally observed mechanism of the effect of loop truncation on stability of AcP protein using solution NMR spectroscopy. Using multidimensional NMR spectroscopy we will study the effect of loop truncation on its structure and conformational dynamics. In addition, to generalize the effect of loop length shortening on the conformational entropy of the native state we studied four different proteins in which we gradually deleted residues in selected loops and quantified the dynamics of the wild-type and the loop-shortened variants. Using all-atom computational approach we showed that an increase in the native state entropy following loop shortening is not unique to the AcP protein, yet nor is it a general rule that applies to all proteins following the truncation of any loop.

P.4.4.A-032
Reversed hexagonal phase in a model thylakoid membrane – a molecular dynamics study

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The thylakoid membranes of higher plants and green algae form an internal system of interconnected membranes where light-dependent reactions of photosynthesis occur. Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are two main galactolipid constituents of the thylakoid membrane. Rod-like shaped DGDG forms bilayers (Lα phase), while wedge-shaped MGDG forms a reversed hexagonal-phase (H_{II}) structures. Nonlammelar phases are important in biogenesis of thylakoids and are assumed to be required in violaxanthin de-epoxidation to zeaxanthin in the xanthophyll cycle. We have built a computer model consisting of 2880 molecules of MGDG in reversed hexagonal phase. Molecular dynamics simulation at 295 K and pressure of 1 bar was carried out for 1 μs to elucidate structural parameters of the MGDG H_{II} phase. This research was supported in part by PLGrid Infrastructure.

P.4.4.A-033
Molecular factors determining the binding specificity of proteins to single-stranded DNA

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Recognition of single-stranded DNA (ssDNA) is important for many fundamental cellular functions, and a variety of proteins (SSB) have evolved for sequence specific binding with ssDNA to form stable complexes. Structural studies of these complexes provide key insights into their recognition mechanism. However, computational modeling of the specific recognition process and predicting their structure is challenging primarily due to inherent flexibility of ssDNA and heterogeneity in their binding energy landscape. Consequently, protein-ssDNA interactions have not been explored much by computational studies, unlike protein-dsDNA. Here, we report a newly developed coarse-grained model to predict the structure of SSB-ssDNA complexes and to assess

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their sequence specific binding energy. We tuned three factors, namely base-aromatic stacking strength, balance between electrostatic and stacking strengths, and ssDNA flexibility that can modulate specific recognition. The model is successfully applied to predict the binding conformations of six distinct SSB folds of different cellular functions with ssDNA strands of variant sequences. Estimated binding energies agreed well with their experimental binding affinities. We found that a right balance of electrostatic and aromatic energies is needed for ssDNA recognition, the extent of which varies for different systems. Bound conformations from the simulation show funnel shaped binding energy distribution where the native-like conformations correspond to the energy minima. Coarse-grained simulations are faster, and our findings suggest that this approach is appropriate to predict the complex structure of SSBs and ssDNA, and can be used to study more complex systems such as RecA.

P.4.4.A-034
High-resolution modeling of peptide-protein interactions using a fragment-based global docking approach, PIPER-FlexPepDock

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Peptide-protein interactions contribute a significant fraction of the protein-protein interactome. Accurate modeling of these interactions is challenging due to the vast conformational space associated with the interactions of highly flexible peptides with large receptor surfaces. To address this challenge we have a developed a fragment based high-resolution peptide-protein docking protocol. By streamlining the Rosetta fragment picker for accurate peptide fragment ensemble generation, the PIPER docking algorithm for exhaustive fragment-receptor rigid-body docking and Rosetta FlexPepDock for flexible full-atom refinement of PIPER docked models, we have successfully addressed the challenge of accurate and efficient global peptide-protein docking at high-resolution with remarkable accuracy. Validation on a representative set of solved peptide-protein complex structures demonstrates the accuracy and robustness of our approach, and opens up the way to high-resolution modeling of many more peptide-protein interactions and to the detailed study of peptide-protein association in general.

P.4.4.A-035
Conformational dynamics of GanP – the carbohydrate-binding protein of the GanPST bacterial three-component sensing system

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Two-component sensing systems (TCSs) are crucial for bacteria to adapt to changes in their environment, regulated mainly *via* conjugation between an external stimulus and a cellular response. These systems usually consist of two proteins, typically a transmembrane histidne kinase (HK) that autophosphorylates upon receiving an extracellular input, and a response regulator (RR) that receives the phosphoryl group from the HK and leads to the appropriate cellular response. Recently, the existence of similar three-component sensing systems have been reported. These unique systems contain in addition a substrate-binding protein (SBP) that binds the signal molecule and interacts with the HK to initiate its autophosphorylation. GanP is the SBP of the newly discovered GanPST three-component system of the thermophilic bacterium *Geobacillus stearothermophilus*, putatively responsible for the binding of extracellular galactose and activation of the galactan utilization system of the bacterium. In order to study the structural basis of the GanP fuction, its 3D structure in complex with galactose has been recently determined by X-ray crystallography at 1.8 Å resolution. The GanP structure reveals the presence of two distinct domains (marked in blue and green in the figure below), connected by a three-stranded hinge, with a galactose molecule trapped in the inter-domain interface (yellow). This atomic-resolution structure enables identification of the residues involved in the specific binding of galactose. The tight binding of galactose between the two domains raises the possibility of domain conformational changes upon ligand binding, which, in turn (via the HK and RR proteins) leads to the activation of the galactan utilization system. Small-angle X-ray scattering (SAXS) measurements on GanP, conducted in the presence and absence of galactose, validate these global conformational changes, which are further supported by theoretical Molecular Dynamics (MD) simulations.

P.4.4.A-036
The 3D structure of ctAlkD – a HEAT-like repeat family alkypurine glycosylase from *Clostridium thermocellum*

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Methylating compounds are a common cause of DNA damage, especially in soil dwelling bacteria. To counter such environmental effects, bacteria such as *C. thermocellum* possess a class of DNA alkylation repair enzymes, which excise methylated purines from the genome, thereby ensuring genomic stability and preventing mutations. Recently the same class of enzymes was implicated in conferring resistance to antibiotics by excising bulky inter-strand crosslinks. *C. thermocellum* is an anaerobic, gram-positive, soil-dwelling bacterium. In its environment it is exposed to toxic exogenous substances, among these are compounds that cause methylation of DNA bases. Under these conditions similar bacteria have evolved a special class of DNA glycosylases that remove methylated purines and as of recently, are known to remove bulky inter-strand crosslinks inflicted by antibiotics. Analysis of the *C. thermocellum* genome revealed a distant homolog, *ct*AlkD, that shares only up to 21% sequence identity with known members of the HEAT-like repeat glycosylase family. To investigate the structural and functional relationships of this distant homolog, the gene was cloned, over-expressed, the protein purified, and subsequently subject to crystallization. After optimization, protein crystals grew within several hours, shaped as elogated hexagonal rods in crystallization solution that contained solely 20% PEG 8K and water. Diffraction data collected at the ESRF reached 2.4 Å and identified the crystals as belonging to a rombohedral space group, with two protein monomers in the crystallographic asymmetric unit. While similar to other HLR homologs in some parts of the protein, the structure of *ct*AlkD reveals some unique structural features, which will be presented and discussed.

P.4.4.A-037
Calcium sparks in cardiac cells in silico

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Local spontaneous Ca^{2+} releases (calcium sparks) from sarcoplasmic reticulum (SR) through RyR-channels play an important role in excitation-contraction coupling in a ventricular cardiac cell and in the action potential formation in a cardiac pacemaker sell. Spontaneous sparks initiating extra Ca^{2+} releases can be a reason of the rhythm disturbances in ventricular cardiomyocytes. Earlier our workgroup have proposed a simple physically reasonable electron-conformational model (ECM) (Moskvin, PBMB 2006) to describe gating both of the isolated RyRs and the RyR clusters in a cardiac cell. The RyR dynamics includes fast electronic transitions triggered by Ca^{2+}, tunneling effects and slow conformational Langevin dynamics which implies both effective friction and thermal fluctuation forces. Temperature stimulation/suppression of the open/close RyR tunnelling can be considered as a main contributor to temperature effects on the RyR gating (Moskvin, Biophysics 2016). At this phase we describe local Ca^{2+} releases in a single Ca^{2+}-release unit (RU), which consists of a junctional release compartment (SR lumen), a cluster of coupled RyR-channels (10×10) and a dyadic space between SR and membrane. The behavior of the RyR-channels cluster is described by ECM stochastic equations, which consider that RyR's activation/deactivation probability depends on the Ca^{2+} concentrations in the dyadic space as well as in the SR lumen. In the computer experiments we observed the process of sparks spontaneous formation and termination. A frequency of sparks initiation increased with the SR lumen Ca^{2+} refill rate. A sparks termination process accrued due to lumen local depletion, which leads to RyR's opening probability decrease and to RyR's stochastic attrition to the closed state. We show that incidence and frequency of Ca^{2+} sparks decreased dramatically with a temperature increase from 22 to 37°C. This results correlate well with experimental data (Ferrier, AJP-H&Circ Phys 2004).

P.4.4.A-038
Atomistic view into structure-function relationships of functional amyloids in *Staphylococcus aureus*

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Amyloids are aggregates of misfolded proteins, forming various structures from small soluble oligomers to large plaques of insoluble fibrils. Amyloids have been associated with many human neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. In recent years, microbial amyloids were found to be ubiquitous and preform many physiological functions, including being the major component in biofilm which leads to resilient microbes and antibiotics resistance, mediating host-pathogen interactions, affecting microbial-ecology and anti-microbial activity. In this study we shed light on the poorly characterized amyloid peptides called Phenol Soluble Modulins (PSMs) in the pathogenic *Staphylococcus aureus* and their functional roles. PSMs are key virulence determinants, particularly in highly virulent *Staphylococcus aureus* strains such as the community-associated methicillin-resistant *S. aureus* (CA-MRSA), which are the most prominent causes of nosocomial infection in the US. This bacterium is notable for its propensity to form biofilms on implanted medical devices leading to persistent and aggressive infections. As a means of pathogenesis, PSMs cause lysis of human cells including leukocytes and erythrocytes, stimulate inflammatory responses, and contribute to biofilm development. Atomic molecular structures of amyloid forming proteins are necessary to understand and describe their functions. Yet, structural studies of amyloid proteins are particularly challenging due to their unstructured and partially disordered nature. Therefore, a reductionist approach for structural investigation was developed employing the search for and use of the structured region of the amyloid. These "amyloid spines" are formed via dedicated segments within the amyloid protein. Such segments can form well-ordered amyloidal structures, and they serve as good models of their parent proteins both structural and functional manners.

P.4.4.A-039
Discovery of selective inhibitors of purine nucleoside phosphorylase and thymidine phosphorylase by docking-based virtual screening

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Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of purine ribo- or 2'-deoxyribonucleosides to the purine and ribose- or 2-deoxyribose-a-1-phosphate. Interest in PNP as a drug target arises from its ability to rapidly metabolize purine nucleosides and from its role in the T-cell branch of the immune system. PNP inhibitors have potential therapeutic value since children lacking PNP activity exhibit severe T-cell immunodeficiency while maintaining normal B-cell function. Thymidine phosphorylase (TP) is an enzyme that catalyzes the reversible conversion of thymidine into thymine and 2-deoxyribose-1-phosphate, the first reaction is essential in the salvage pathway of pyrimidine nucleosides. Thymidine phosphorylase is an angiogenic factor that exerts its angiogenic effect by stimulating the endothelial cell migration. This has been identified as a potential target in the development of anti-cancer drugs. In this paper, we report the usage of crystallographic and modeling methods for the structure-based drug-design of new competitive inhibitors of PNP and TP. The grown crystals had such kind of quality that the three-dimension structures of PNP and TP were achieved at high-resolution from X-ray crystallography. Model of PNP, which was used for docking procedure, were selected as center of docking field. The positions of atoms of these systems were optimized, and then all atoms were refined by the classical MM method of the molecular simulation program package GROMACS. After the refinements of positions of systems we identified amino acid residues, which are responsible for bind between the chosen ligands and the active sites of these proteins. The selected ligands demonstrate their binding association by the atomistic MD simulation method.

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P.4.4.A-040
A computational screen for RNA editing alterations in autoimmune diseases

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In many autoimmune diseases, the immune system identifies ones own cells as malignant or infected and attacks them. Usually, MHC molecules are used to differ such cell from healthy ones on the cleansing of the host. However, during autoimmune disease, peptides derived from normal self proteins are targeted by the body defenses. We suggest that some of the factors leading to inflammation and even to the body to mistaken self for non-self are originated by the post transcriptional event of RNA editing. RNA editing usually occurs in introns, however part of the phenomena effects exons as well. By altering the sequence of the exon, miss-sense RNA mutation can occur, of whom some might be presented on the MHC marking the cell as defected. The magnitude of both editing and MHC loading is greatly enhanced by exposure to interferons, further increasing the putative immunogenic properties of the editing affected proteins and possibly contributing to recognition of other self derived peptides as antigens, as they are presented next to the edited version, thus creating a putative positive feed back. We created a robust computational pipeline for the purpose of measuring the effect of the above from RNA seq data. We measure various patterns of A to I editing in the sequences to get a clean signal in order to measure the extent of editing in a sample, as well as deducing a list of putative edited sites. All This, in order to measure editing levels and editing derived peptides, as well of their affinity to the host HLAs, some of which might putatively trigger the cascade described above.

P.4.4.A-041
Contemplate suggests possible alternative conformations for a query protein of known structure

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Protein function involves conformational changes, but often, for a given protein, only some of these conformations are known. The missing conformations could be predicted using the wealth of data in the PDB. Most PDB proteins have multiple structures, and proteins sharing one similar conformation often share others as well. The ConTemplate web server (http://bental.tau.ac.il/contemplate) exploits these observations to suggest conformations for a query protein with at least one known conformation (or model thereof). We demonstrate ConTemplate on a ribose-binding protein that undergoes significant conformational changes upon substrate binding. Querying ConTemplate with the ligand-free (or bound) structure of the protein produces the ligand-bound (or free) conformation with a root-mean-square deviation of 1.7 Å (or 2.2 Å); the models are derived from conformations of other sugar-binding proteins, sharing approximately 30% sequence identity with the query. The calculation also suggests intermediate conformations and a pathway between the bound and free conformations.

P.4.4.A-042
The double life of PHDV-C5HCH tandem domain within the NSD family

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The Nuclear Receptor-Binding SET Domain protein (NSD) is a family of three HMT proteins playing a critical part in chromatin integrity. In particular the tandem domain constituted by the fifth PHD finger and the adjacent C_5HCH domain has context dependent activation/repression activities with different pathophysiological outcomes. In fact, despite the high sequence similarity shared by the PHDV-C5HCH tandem domain within the NSD family (60% of Sequence Identity), it appears to have evolved to exert different functions. To investigate the PHDV-C5HCH role as structural platform for multiple interactions within the NSD family, we took advantages of ITC, NMR experiments and computational techniques. In line with the fact that PHD fingers usually interpret histones post-translational modifications in a modification and context-specific fashion, thus promoting chromatin changes and/or protein recruitment, we found that PHDV-C5HCH$_{NSD3}$ behaves as classical histone marks reader recognizing H3K9me3 through the canonical interactions. On the contrary, PHDV-C5HCH$_{NSD1}$ interacts only very weakly with histone H3 via non-specific electrostatic forces, irrespectively of histone methylation and involves a distinct surface with respect to the classical histone binding groove. Moreover, our results demonstrate a μM interaction between PHDV-C5HCH$_{NSD1}$ and the C2HR domain of the transcriptional repressor NSD1 Interacting Zinc Finger Protein (Nizp1), supporting the role of PHD fingers as structural scaffolds working as versatile non-histone binding domains. Interestingly, PHDV-C5HCH$_{NSD3}$ interacts only weakly with C2HR-Nizp1, thus confirming the notion that PHDV-C5HCH has evolved to exert different functions.

P.4.4.A-043
Long non-coding RNAs and genome structural organisation

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Recently, it has been observed that long noncoding RNAs (lncRNAs) could play an important role in shaping the genome architecture in interphase and mitotic cells. However, the exact mechanism by which lncRNAs interact with chromatin and modulate the overall organization of the chromatin structure is beginning to emerge. Here, we used an integrative approach based on restrained-based 3D modeling of human chromosomes, 3D co-localisation analysis using genome-wide chromosome conformation capture and sequence analysis to investigate this mechanism. We identify a class of lncRNA named staple-RNAs (stpRNA) that modulate the genome structure by directly "reading" the genomic sequence via RNA-DNA triplexes formation. We observed that the stpRNAs organize the genome in a non-specific manner mainly interacting with repetitive elements.

The Structural Organization of the Cell

P.4.4.B-001
One library to make them all: streamlining yeast library creation by a SWAp-Tag (SWAT) strategy

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The yeast *Saccharomyces cerevisiae* is ideally suited for systematic studies relying on collections of modified strains (libraries). Despite the rich toolbox of genomic modifications, only a few have given rise to libraries, attesting to the difficulty of manufacturing such collections. To overcome this obstacle we developed a swap-tag method (SWAT), in which one parental collection is easily and efficiently modified to give rise to any library of choice. We showcase this approach by constructing a genome-wide collection of ~5500 strains carrying a SWAT-GFP module at the amino termini of proteins, and using it to create both a seamlessly N-tagged GFP library and an mCherry-tagged library. These libraries enabled us to characterize hundreds of proteins for the first time, in addition to the investigation of proteome wide phenomena. The SWAT strategy supports fast and effortless creation of yeast libraries, opening the door for endless new ways to systematically study cell biology.

P.4.4.B-002
Superparamagnetic nanoparticle based subcellular fractionation for plasma membrane, endosome and lysosome isolation

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Here, we elaborate the methodology for SuperParaMagnetic NanoParticle (SPMNP) based subcellular fractionation for plasma membrane, endosomes and lysosomes isolation. SPMNP based subcellular fractionation combines classical biochemistry with nanobiotechnology towards high pure and high yield organelle isolation. Particularly, methodologies for isolating plasma membrane (SPMNP-PM^{capture}), early or late endosomes (SPMNP-Endo^{capture}) and lysosomes (SPMNP -LYS^{capture}) are elucidated in this study. Depending on the target organelle, appropriate SPMNPs (SPMNP 1.0, 2.0 and 3.0) are synthesized, functionalized and applied to living eukaryotic cell for subcellular fractionation. SPMNP based fractionation strategy has several advantages compared to existing fractionation methodologies in terms of purity, yield, and isolating intact organelles under physiological conditions. Isolated magnetic organelles are available for generating its protein (proteomics), lipid (lipidomics), and glycan (glycomics) composition using mass spectrometry (MS) platform. Isolated organelles can be extended to protein/protein complex purification for biochemical and structural biology studies. SPMNP based subcellular fractionation is the technology that can complement existing tools for cell biology research.

P.4.4.B-003
Fluorescent protein from *Dendronephthya* sp. as a ratiometric green/red emitting pH sensor

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Fluorescent protein-based biosensors are now widely applicable for monitoring live cell processes. Here, we show that a fluorescent protein from *Dendronephthya* sp. (DendFP) exhibits a noticeable pH-sensitivity. Unlike most of known genetically encoded pH-sensors, fluorescence of the protein shifts from the red to green spectral range upon medium acidification. Therefore, quantitative measurements of intracellular pH are feasible by ratiometric comparison of emission intensities in the red and green spectral ranges, which makes DendFP advantageous compared with other genetically encoded pH-sensors.

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P.4.4.B-004
Nuclear speckles can buffer the availability of splicing factors in the nucleoplasm to regulate rates of gene expression

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Splicing factors (SFs) are concentrated in nuclear structures known as nuclear speckles, of unclear function. The movement of SFs between nuclear speckles and transcribing genes, in order to participate in the splicing reaction, is regulated by kinases and phosphatases. To test the effect of nuclear speckle structure on transcription and splicing kinetics in living cells, nuclear speckles were disassembled by Clk1 kinase overexpression, and SF dynamics were measured in the nucleus using FRAP and FCS. Disassembly of the nuclear speckles increased the diffusing fraction of SFs in the nucleoplasm and reduced their residence times on an active gene. Moreover, an mRNA undergoing many splicing events was rapidly released from the transcribing genes without a splicing delay. In order to understand if the high abundance of diffusing SFs in the nucleoplasm after nuclear speckle disassembly was leading to the more rapid processing of the mRNA, we overexpressed certain SFs in these cells and identified several SFs that also drove the rapid release of the mRNA from the gene, without disassembly of the nuclear speckles. We suggest that nuclear speckles can buffer the availability of splicing factors in the nucleoplasm, thereby regulating the kinetics of mRNA release from the gene after processing.

P.4.4.B-005
Phosphate buffered saline induced single cell dissociation, blebbing and death of human embryonic stem cells

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Phosphate-buffered saline without Ca^{+2} and Mg^{+2} ($PBS^{Ca\text{-}Mg\text{-}}$) is a buffer solution commonly used in biological research. $PBS^{Ca\text{-}Mg\text{-}}$ has many uses because it is isotonic and non-toxic to most cells. It

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is often used for rinsing cells from culture medium and different reagents and to preparing living cell samples for different types of analyses. Here we have shown that $PBS^{Ca\text{-}Mg\text{-}}$ is not suitable for all cells. Exposure of the cultured pluripotent human embryonic stem cells (hESCs) to $PBS^{Ca\text{-}Mg\text{-}}$ for 20 min resulted in extensive dissociation, blebbing and cell death. hESCs in standard culture conditions form dense flat colonies consisting of two or more layers of polygonal cells that have well organized bands of actin microfilaments at the cell borders. The cells of the lower layer in the colony are attached to culture surface by stress fibers. The described structure of cellular interactions in hESC colony is necessary for preservation and maintenance of viability and unique properties of hESCs. We demonstrate that $PBS^{Ca\text{-}Mg\text{-}}$ acts like chelating agent and decrease in the extracellular calcium level leads to active disassembly of actin-containing microfilaments, loss of cell-cell and cell-surface contacts and complete dissociation of hESCs colonies to single cells. Disrupting cellular skeleton also lead to active bleb formation and cell lysis, presumably due to the difference in intracellular and extracellular osmolality. $PBS^{Ca\text{-}Mg}$ osmolality is slightly lower than osmolality of adapted to hESCs culture medium and can result in excessive water intake by cells and their swelling as a consequence. The described cell response to decrease levels of calcium must be considered in hESCs experiments setting.

The study was supported by the Russian Science Foundation grant No. 14-50-00068.

Thursday 14 September 13:00-14:00

Intrinsically Disordered Proteins

P.5.1-001 Proteomics study of *Danio rerio* otolith's proteins

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Otoliths are calcium carbonate ear stones of teleost fish. The major role of these biomineralized structures is sound transduction and sensation of linear acceleration. Similar structures – otoconia are located in mammalian inner ear. Otoliths consist predominantly of a mineral phase which is found within an organic matrix. This organic matrix consists of a network proteins and proteoglycans, responsible for mineral phase and crystal morphology. The highly acidic nature and structural properties (frequently IDPs) of these proteins promotes Ca^{2+} binding allowing them to act as a specific regulatory factors. The inner ear of a *Danio rerio* fish is divided into three semicircular canals and three otolithic organs: the sacculus, utricle and lagena, each contains an otolith known as the sagitta, asteriscus and lapillus, respectively. Otoliths differ in calcium carbonate polymorph which can be caused by differences in proteins components. There are only few proteins known to be important in biomineralization of *Danio rerio* otoliths. Proteomics (MS analysis) was applied to study a specific zebrafish otolith proteome, including information of protein content, their variations and modifications. The project was divided into several steps. The first part was focused on otolith extraction/purification and matrix protein extraction. A protocol for protein extraction included decalcification in EDTA and protein precipitation. After digestion in Trypsin/Lys-C mix peptides were analyzed with LC-MS/MS. Finally 63 proteins associated with the otolith matrix and collagen proteins were identified.

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P.5.1-002 Computational characterization of hybrid proteins containing ordered and intrinsically disordered regions

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Intrinsically disordered proteins (IDPs) characterized by polypeptide chains that fail to fold into stable and well defined tertiary structure in an isolated state have been under our interest. IDPs play key roles in processes such as molecular recognition, regulation of transcription and they are related to neurodegenerative diseases. Most of IDPs are in fact intrinsically disordered regions (IDRs) that are tethered to ordered domains (ODs). It is imperative that the biophysical properties of these regions be studied in their naturally occurring contexts, which is tethered to ODs. It is difficult to cope with such systems for the experimental techniques and for computational methods. Typical experimental methods for study of IDPs are nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS). The obtained data were used for verification of predicted values from the computational simulations. In our study, we generated structural ensembles of the δ-subunit of RNA polymerase and regulatory domain of human tyrosine hydroxylase using molecular dynamics simulations. The reliability of the obtained ensembles generated under different force field parameters (AMBER99SB-ILDN/CHARMM22* + TIP3P/TIP4P-D) was checked by the comparison of the corresponding calculated properties with their experimental values. Namely we monitored: NMR chemical shifts, residual dipolar couplings, paramagnetic relaxation enhancement, relaxation rates, and SAXS data. The best agreement was obtained for the AMBER99SB-ILDN/TIP4P-D force field parameters.

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P.5.1-003 NMR characterization of intrinsically disordered microtubule associated protein 2c

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Microtubule-associated protein 2c (MAP2c) is an intrinsically disordered 49 kDa protein expressed in developing nerve cells. MAP2c interacts with microtubules, affecting their dynamics, and plays an important role in neuronal morphogenesis. MAP2c function is regulated via phosphorylation by various kinases, including cAMP-dependent protein kinase (PKA). Multidimensional methods of nuclear magnetic resonance (NMR) spectroscopy with high resolution were used to obtain atomic-resolution description of structural features and dynamics of

unphosphorylated and PKA-phosphorylated MAP2c. Kinetics of phosphorylation by PKA was monitored using fast 2D NMR experiments. Propensities of individual regions of MAP2c to form transient secondary structures were revealed by analyzing chemical shifts of unphosphorylated and PKA-phosphorylated MAP2c. Long-range intramolecular interactions were described by paramagnetic relaxation enhancement, utilizing spin labels attached at different positions in the MAP2c molecule. NMR relaxation experiments provided a detailed picture of internal motions of the MAP2c backbone. Titration with a regulatory 14-3-3zeta protein revealed its binding sites in unphosphorylated and PKA-phosphorylated MAP2c. The results showed that properties of MAP2c significantly differ from those of the microtubule-associated protein tau, in spite of a high sequence homology of microtubule-binding regions of both proteins.

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P.5.1-004
The role of the intrinsically disordered C-terminus of the Tobacco mosaic virus coat protein in viral infection

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The coat protein (CP) of Tobacco mosaic virus (TMV) is the well-studied one both structurally and functionally among CPs of helical plant viruses with the positive RNA genome. Besides canonical functions including virion assembly and dissociation the TMV CP is involved in noncanonical (nonstructural) functions such as virus movement through the plant vascular system (long-distance transport) and modulation of the host responses to viral infection. In our work we tested the role of the intrinsically disordered C-terminus of the TMV CP exposed on the surface of viral particle for characteristics of viral infection. We obtained TMV U1 infectious cDNA clones with a set of deletions inside the gene *CP* encoding mutant CPs without 3, 6, 9, 15 and 20 amino acids residues from C-terminus respectively. Nicotiana benthamiana plants were infected using leave infiltration by Agrobacterium tumefaciens comprising wild type (wt) and mutant TMV U1 cDNA clones. Symptoms of viral infection and level of viral RNA in infected and systemic (non-infected) leaves were analyzed. This study demonstrates that the deletion of the CP C-terminal 3 a.a. only does not affect the infection characteristics (symptoms, cell-to-cell and long-distance movement, TMV RNA levels and virion appearance) in comparison with wt TMV infection. All other deletions inside the gene *CP* abolished infection in inoculated leaves: neither viral RNA nor virions were detected in these plants. However infected plants demonstrated similar mild symptoms such as curling of upper systemic leaves during two weeks post infiltration. We believe that the TMV CP C-terminus contain functional determinant/determinants which might interact with viral/cellular proteins involved in previously assumed participation of the TMV CP regulation of TMV RNA replication, production of viral movement protein or cell-to-cell transport.

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P.5.1-005
Analysis of LEA proteins evolution in closely related anhydrobiotic species of chironomid midges

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Larvae of the chironomid midge P. vanderplanki, can withstand complete desiccation, by accumulation a set of protective biomolecules, including intrinsically disordered late embryogenesis abundant (LEA) proteins in response to slow drying. Using complete genome data of the midge, we analyzed 27 PvLea-protein coding genes and 18 genes sharing similarities with 3'-part of Lea1 protein (LIL in Gusev et al, 2014) in several populations of P. vanderplanki in Nigeria as well as in closely related species *P. pembae* recently described in Malawi (Cornette, et al, 2017). We employed genome resequence using HiSeq 2500 and HiSeq200 Illumina platform. We found that LEA and LIL strongly diverge in the studied populations. The similarity ratio among primary sequences of LEA proteins was lower among midges from different populations, compared to the avergae value of genetic divergence. We further analyzed the distribution of some LEA proteins in the chironomid cells and found that in some cases, divergence of LEA and LIL-coding genes sequence correlate with mRNA expression pattern upon desiccation. We hypothesize that chromatin opening upon anhydrobiosis, and associated DNA damage can be one of the driving force for increased mutagenesis rate in the LEA and LIL genes. We anticipate that further deep investigation of DNA reparation mechanisms in the anhydrobiotic midge will provide additional details about relation of desiccation-induced DNA damage and gene evolution in this extra-ordinary organism.

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Medicinal Chemistry

P.5.2-001
Natural bispecific antibodies: new biochemical markers of autoimmune pathology

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Natural immunoglobulin G presented in biological fluids are considered as stable molecules with two identical antigen-binding sites. Exchange of Fab arms (HL-fragments) was first described for IgG4 subclass, later the reaction mechanism was described in details. We have shown that human milk IgG contains up to 54% of bispecific molecules comprising κ- and λ-light chains simultaneously, and up to 17% of human milk sIgA are bispecific. Interestingly, bispecific human milk κλ-IgG are presented mostly by IgG1 (74%) and lower amounts of IgG2–IgG4 (5–16%). Recently we have shown that similar to human milk, placenta antibodies undergo extensive Fab arms exchange. Since that placental IgG preparations in average consists up to 15% of the IgGs containing both κ- and λ-light chains. Chimeric placenta κλ-IgGs are consisted of: 43.5% IgG1, 41.0% IgG2, 5.6% IgG3, and 7.9% IgG4. Clearly, the relative content of chimeric IgGs in placenta is significantly lower than that of the milk. One can suppose that the observed phenomenon may appear due to a

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lower content of factor(s) stimulating the exchange in placenta comparing with milk. Here we show for the first time the content of chimeric κλ-IgGs in the blood of autoimmune patients with multiple sclerosis and systemic lupus erythematosus. Since the serum of autoimmune patients contains significantly higher concentrations of bispecific IgG molecules than in healthy donors, the presence of bispecific antibodies in the serum of systemic lupus erythematosus and multiple sclerosis is a new biochemical marker of autoimmune disorders.

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P.5.2-002
Structures of G protein-coupled purine receptors enable drug discovery

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We take synthetic chemical, pharmacological, and structural approaches to discover and characterize new compounds to modulate purinergic signaling, with the potential for treating chronic diseases. This encompasses 4 G protein–coupled receptors (GPCRs) for adenosine, 8 GPCRs activated by nucleotides (P2YRs), 7 ATP-gated P2X ion channels, and the associated catabolic and metabolic enzymes that regulate the levels of the native agonists. We use the high-resolution X-ray structures of the adenosine receptors (ARs) and P2YRs to rationally design ligands, either by modification of known agonists and antagonists or by virtual screening to discover novel chemotypes. In collaboration with Ray Stevens and colleagues, we determined $A_{2A}R$, $P2Y_1R$ and $P2Y_{12}R$ structures in complex with high-affinity ligands, which displayed surprising structural features that could not be predicted by modeling derived from previous GPCR templates. We introduced sterically constrained rings to mimic native ribose in nucleosides and nucleotides, to determine their preferred conformation when bound to protein targets. Novel A_3AR agonists for pain control were designed and screened using an *in vivo* phenotypic model, which reflected both pharmacokinetic and pharmacodynamic parameters. High specificity (>10,000 fold selective for the A_3AR) was achieved with the aid of receptor models based on related GPCR structures. Activation of the A_3AR in peripheral neurons, spinal cord, and brain was found to reduce chronic neuropathic pain *in vivo*. Chemical tools for the inflammation-related $P2Y_{14}R$, such as fluorescent probes, were designed with the aid of molecular modeling based on the $P2Y_{14}R$ X-ray structure and applied to discovery of novel antagonists. Thus, purine receptor structures and an interdisciplinary approach have enabled the elucidation of their biological role, the conceptualization of future therapeutics and novel ligand discovery.

P.5.2-003
The polyamine exchange modulating compounds as anticancer agents

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A deviation in polyamine (PA) metabolism is observed in various tumors in comparison with normal tissues. In this regard, the PA exchange is considered as a target for the drug design of potential anti-tumor agents. The compounds activating PA catabolism are supposed to cause production of cytotoxic PA-metabolites such as H_2O_2, acrolein, NH_3 and thus to be potential anticancer agents. We suggest 3 main criteria for the primarily selection of PA-exchange modulating compounds. They must inhibit or at least not activate PA biosynthesis, activate PA catabolism, and show good membrane permeability. We have tested 30 different compounds. The ODC and PAO activities were measured according to the standard PA protocols. The membrane permeability was evaluated through a standard PAMPA procedure. Cell growth inhibition was tested in a modified MTT-test in such cancer cell lines as PC3, MCF-7, MEL-7. Correlations between the cytotoxic activity and membrane permeability were shown in different groups of compounds for all cell lines with the highest one for MEL-7. A correlation between the cytotoxic activity and spermine oxidation was shown in the group of copper complexes for MCF-7. The most cytotoxic was copper complex with the fluoroaniline derivative. Its IC50 was found to be in the range of 10^{-4} M. It showed PA biosynthesis inhibition, PA oxidation activation and good permeability as well. As the antiproliferative effect is supposed to be mediated by PA themselves being the source of cytotoxic agents, the cell sensitivity is expected to be PA level-dependent. However PC3 cells were the most sensitive to these compounds, although their initial PA levels are lower than those in MCF-7 cells. Further investigations are necessary for this group of compounds to justify it as potential anticancer agents.

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P.5.2-004
Multiple sclerosis IgG effects the amidolytic activity of thrombin

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The pathogenesis of multiple sclerosis (MS) has long been imputed to T cells and B cells. But recent studies suggest factors of the coagulation cascade might also be involved in MS development. Moreover it's well known the consistently elevated levels of IgG during the MS development. This study aimed to determine effect of IgG, accumulated in the bloodstream after suffering MS, on the amidolytic activity of thrombin. Further to compare their effect with the analogical of healthy donor's IgG. IgG was separated by affinity chromatography on protein A Sepharose from the blood plasma samples obtained from 35 healthy donors and 20 patients with multiple sclerosis. To examine the IgG, in a concentrations 100 and 300 mkg/ml, influence on thrombin *in vitro* experiments were performed. The level of released of p-nitroaniline from the S_{2238} by thrombin as well as by the thrombin activated from the prothrombin in plasma under the effect of endogenous activation from the venom *Echis multisquamatus* (ecamylin) were measured. Control sample included vehicle instead of IgG. The elevation of amidolytic activity of thrombin under influence of both fractions of IgG in a concentration of 100 and 300 mkg/ml was observed. After applying of healthy donor's IgG to mixture the level of thrombin activity after 60 min of incubation was on the 45% higher (100 mkg/ml of IgG) and 35% higher (300 mkg/ml of IgG). MS IgG in a concentration of 100 mkg/ml elevated the tested activity on 30% and in a concentration of 300 mkg/ml on 58%. The effect of MS IgG on the thrombin activated from prothrombin in plasma by

ecamulin was analogical. Obtained results are the evident of the possible impact of IgG directly on the enzyme thrombin may therefore provide future targets for therapeutic strategies.

P.5.2-005
Lactoferrin-derived peptides with anti-hepatitis B virus properties

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Lactoferrin (Lf), an iron binding glycoprotein with host-protective effects against pathogenic microorganisms was reported to exhibit antiviral activity at an early phase of infection. It was suggested that Lf interaction with host cell surface molecules such as glycosaminoglycans (GAGs) could be part of the mechanism of action. Lf posses two GAGs binding sites located in the N-terminal (N-t) region that could be involved in the antiviral activity. In our work we have investigated seven human Lf (HLf)-derived peptides (HLPs), from the N-t region (1–47 amino acids sequence) for their capacity to prevent hepatitis B virus (HBV) infection and replication using HepaRG and HepG2.2.2.15 cell lines. Four HLPs inhibited HBV infection between 40 to 80%. HLP_{1-23}, a peptide containing the first GAGs binding site (GRRRR) was the most potent inhibitor, prevented HBV infection at 250 μM by neutralizing the viral particles. HLP_{20-45}, the peptide containing the second GAGs binding site (RKVR) led to only 50% inhibition. To improve the antiviral activity we further used computer modeling and a new mutant peptide with increased overall positive charge and aromaticity, supposedly displaying improved affinity through additional GAGs binding site and increased stability through supplementary aromatic stacking interactions was designed. The new HLP_{1-33} peptide had good solubility and was not toxic for concentrations up to 100 μM. The results showed that HLP_{1-33} is able to inhibit HBV infection by 50% at 100 μM, a concentration lower by 2.5 fold than HLP_{1-23}. HLPs may constitute a non-toxic approach for potential clinical therapy in inhibiting early steps of HBV infection or protection of regenerated hepatocytes from *de novo* infection.

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P.5.2-006
Quantitative relationship between the rate of enzyme cell lysis determined by microbiological and turbidimetric techniques

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Bacteriolytic enzymes cause the lysis of bacterial cells due to cleavage of cell wall components and are considered as potential alternative antibiotics. Not only enzymes can act as bacteriolytic agents. We showed recently, that such activity demonstrates interleukin-2 – one of the key cytokines, which is used for therapy of sepsis and oncology. Once a possible bacteriolytic agent has been identified, quantitative description of its specificity toward different types of microorganisms is essential to determine its potential as therapeutic agent. The simplest method for determination of lysis rate is turbidimetry, based on decreasing of cell suspension turbidity at 500–650 nm during cell destruction under the action of bacteriolytic agent. Usually, enzyme activity is expressed as reducing of optical density at initial time $(dD/dt)_o$. At the same time direct comparative analysis of enzyme specificity to different microorganisms measured by turbidimetry is very complicated. First, at the same D_o substrate concentration (CFU/ml) can varied 2–3 orders in dependence of cells size and form and microorganism type. Second, complete lysis according to microbiological method not always leads to the totally transparent suspension, i.e. $(dD/dt)_o$ value is not identical to the real rate of enzymatic cell lysis, $(dCFU/dt)_o$. Mathematical analysis allowed us to find necessary conditions for transfer turbidimetrically determined rate of cell lysis to the real one. They are cell concentration CFU_o at initial D_o and final density D_∞ when lysis is finished according microbiology – these two coefficients must be determined for the specific type of microorganism. Having a common methodological basis for action of bacteriolytic factors on cells we made a detailing comparison of lysozyme and interleukin-2 specificity toward different bacteria types.

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P.5.2-007
Discovery of potent isoflavone (Glabrescione B) as selective inhibitor of Hedgehog-dependent tumors

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Hedgehog (Hh) signaling has emerged in recent years as a druggable target for anticancer therapy. Its aberrant activation, occurring either by ligand-dependent or -independent mechanisms, has been observed in many tumors. Recently, particular interest has been highlighted by the identification of molecules able to hit glioma-associated oncogene (GLI) factors, the final effectors of the Hh pathway, which provide a valid tool to overcome drug resistance at the upstream level. Based on the knowledge of the

crystallographic structure of the zinc finger domain of Gli1 (Gli1ZF) in complex with DNA, together with NMR studies as well as computational and experimental mutagenesis, we clarified the structural requirements of Gli1/DNA interaction. We identified Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae), as a novel small molecule that proved to be able to bind Gli1ZF and interfere with its interaction with DNA. This small molecule turned out to be an efficient inhibitor of the growth of Hh/Gli-dependent tumors and cancer stem cells *in vitro* and *in vivo*. Moreover, since extraction methods developed allow to get very limited amounts of pure isoflavone, we provided the total synthesis of GlaB which foresees just three steps route with a overall yield 15%. This synthetic strategy allowed us the preparation of five derivatives with the aim to elucidate the structure-activity relationships and to clarify the molecular mechanism behind the Hh signaling modulation.

P.5.2-008
Rational design of FGFR1 inhibitors with antiproliferative activity

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Fibroblast growth factor receptor 1 (FGFR1) is a tyrosine protein kinase which plays an important role in the regulation of cell growth, proliferation and differentiation. Increased activity of FGFR1 is associated with pathogenesis of lung cancer, oral tongue squamous cell carcinoma, sinonasal cancer, gastric cancer, renal cell carcinoma, prostate cancer, acute myeloid leukemia, etc. Therefore, the inhibitors of FGFR1 can be interesting for anticancer drug development. To discover small-molecular FGFR1 inhibitors we have performed screening program, using both *in silico* and *in vitro* approaches. AutoDock software was used to conduct receptor-ligand flexible docking of OTAVA drug-like compounds collection. The best-scored compounds from different chemical classes were taken for the kinase assay analysis. *In vitro* observations revealed that compound 5-amino-4-(1H-bezoimidazol-2-yl)-1-(3-methoxy-phenyl)-1,2-dihydro-pyrrol-3-one inhibits FGFR1 with IC_{50} value of 3.5 μM. To find more active FGFR1 inhibitors 25 derivatives of this chemical class were synthesized and tested *in vitro*. Among them 17 compounds inhibited FGFR1 with IC_{50} values in the range from 0.32 to 28.2 μM. Structure-activity relationships were studied. Six the most active FGFR1 inhibitors have been investigated for anticancer activity toward acute myeloid leukemia cell line KG1. This cancer cell line is characterized by constitutive activity of FGFR1. Among studied inhibitors we have identified four compounds with antiproliferative activity toward cell line KG1 with IC_{50} values in the range from 2 to 9.3 μM which are non-cytotoxic against non-cancer cell line HEK293. Therefore, these FGFR1 inhibitors can be valuable candidates for further optimization and biological research.

P.5.2-009
PaMAP 1.9 and PaMAP 2: two antimicrobial peptides effective against a clinical isolated multiresistant bacteria

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Current century is bringing new challenges to the medical field, due to the new threats that are becoming responsible for high mortality ratios. Between them, multi-resistant pathogens have been increasing their number and inducing severe infections on patients that current available drugs cannot attend. This new paradigm lead to urgency in the development of new classes of drugs to deal with these pathogens, and antimicrobial peptides (AMPs) are pointed out as a future possibility. These small, hydrophobic and cationic peptides have been studied, mostly in terms of peptide-membrane interactions, but the urgency of new drugs have redirect the focus to pharmacodynamics, pharmacokinetics and effectiveness studies, principally with animal models. In this work, we focus in two AMPs (*Pa*MAP 1.9 and 2), synthetically designed using a natural AMP as template. After initial the promising results of tests against bacteria and cancer cells, as well as biocomputational, peptide-membrane interactions were extensively studied, using lipid vesicles as biomembrane models, as well as bacterial cells (*wt* and a clinical multi-resistant *Escherichia coli*, KPC). Different biophysical techniques, including fluorescent spectroscopy (using different probes to deduce about the membrane properties affected) and microscopy, flow cytometry, dynamic light scattering, zeta-potential, circular dichroism and atomic force microscopy allowed to infer about the efficiency in eradicating bacteria and promoting cell death. Data obtained were confirmed and further extend by *in vivo* studies. Both AMPs proved to be efficient in terms of activity toward bacteria, but by acting at different membrane levels, leading to distinct mechanisms of action. Importantly, both AMPs were also effective against the multiresistant strain. Bacterial metabolic pathways affected by these AMPs will be studied in the near future.

P.5.2-010
The interaction of antibiotics or antibiotics with nanomaterials with endothelial cells

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The interaction of nanomaterials with cells and lipid bilayers is critical in many applications such as phototherapy, imaging, and drug/gene delivery. These applications require a firm control over nanoparticles–cell interactions, which are mainly dictated by surface properties of nanoparticles. The aim of this study was to investigate the interaction of nanoparticles (Fe_3O_4) or nanoparticles fused with different antibiotics with cell membranes in order to reveal changes in the membrane organization. Endothelial cells were used to determine the effect of different antibiotics (gentamicin, kanamycin, amikacin, penicillin, polymyxin, vancomycin) on

the membrane organization. For recording the anisotropy of cell suspensions treated with antibiotics or nanoparticles fused with antibiotics we used 1-4-trimethyl-6-phenyl 1, 3, 5 hexatrien p-toluenesulfonate (TMA-DPH). We decided to use nanoparticles fused with antibiotics because they contain small amounts of antibiotics which makes them less toxic than simple antibiotics, which is very important in patients with genetic diseases such as cystic fibrosis, that should be treated with antibiotics for a long time. Our results showed that at temperatures between 32–36°C simple nanoparticles decreased the membrane fluidity. At physiological temperatures (37–39°C) nanoparticles fused with antibiotics (gentamicin, vancomicin) increase more the membrane rigidity compare with simple antibiotics or nanoparticles. The membrane rigidity was higher in the presence of amikacin and kanamicin compared with nanoparticles or nanoparticles fused with these antibiotics. Polymyxin and penicillin increase the membrane rigidity at 37°C, and at 39°C the same effect was obtained in the presence of nanoparticles fused with these antibiotics, suggesting that the nanoparticles are dependent to temperature for penetrating the membrane. In conclusion the membrane fluidity does not depend on antibiotics types. The presence of nanoparticles fused with antibiotics is very important for long term treatment, because in the presence of nanoparticles the concentration of antibiotic is much lover but the effect is similar.

P.5.2-011
Antibiotic modulatory effect of the methanolic extracts from Armenian herbs against several gram-negative and gram-positive bacteria

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Plant phytochemicals can act as antibiotic modulating agents. Currently, this strategy is considered one of prospective ways to combat antibiotic resistance. The aim of this study was to explore antibiotic modulatory effect of extracts from *Agrimonia eupatoria* L., *Hypericum alpestre* subsp. *polygonifolium* (Rupr.) Avet. & Takht., *Rumex obtusifolius* L., and *Sanguisorba officinalis* L. toward antibiotics: gentamicin, tetracycline, kanamycin, ampicillin, erythromycin, and ciprofloxacin against *Staphylococcus aureus* MDC 5233, *Escherichia coli* VKPM-M17, kanamycin-resistant *E. coli* PARS-25, and ampicillin-resistant *E. coli* dhpα-pUC18. The antibiotic modulatory activity was explored by determining MICs of antibiotics in the absence and presence of plant extracts at sub-inhibitory concentrations. The effect of heat treatment on antimicrobial activity of plant extracts was determined by testing their antimicrobial activity before and after heat treatment. The obtained data showed that tested plants extracts exhibited high modulatory activity toward all tested antibiotics. Particularly, high modulatory activity was observed with extracts of *H. alpestre* and *R. obtusifolius*. Many plant-antibiotic combinations induced decrease of MICs of antibiotics by up to 4 fold, indicating their synergy. Moreover, in most of the cases the similar change was observed at both MIC/2 and MIC/4 concentrations of the same plant extract. Thermostability tests revealed that methanol extract of *H. alpestre,* and methanol and acetone extracts of *S. officinalis* retained their antimicrobial activity even after 121°C heat exposure. In contrast, methanol extract of *A. eupatoria* and acetone extract of *H. alpestre* had lost their activity just after treatment with 60°C heat. Thus, the results highlighted high antibiotic modulatory effect of some Armenian herbs. Their combinations with commercial antibiotics could have potential in practical applications, however further more comprehensive studies will be needed.

P.5.2-012
Anti-angiogenic effect of *Macrovipera lebetina* obtusa snake crude venom and obtustatin

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Macrovipera lebetina obtusa (MLO) is a poisonous snake in Armenia. Obtustatin represents the shortest known monomeric disintegrin, isolated from the snake venom of MLO, and is known to specifically inhibit α1β1 integrin. Its oncostatic effect is due to the inhibition of angiogenesis, which likely arises from α1β1 integrin inhibition in the endothelial cells. To explore the therapeutic potential of the MLO snake venom and obtustatin, we studied activity of obtustatin and MLO venom *in vitro*, by testing their efficacy in human dermal microvascular endothelial cells (HMVEC-D) and *in vivo*, using chick embryo chorioallantoic membrane assay (CAM assay). Our *in vitro* results showed that pure obtustatin in comparison with MLO venom did not exibit cytotoxic activity in HMVEC-D cells in comparison to MLO venom. But *in vivo* results have shown that 4 μg/embryo (90 μM) of obtustatin inhibited angiogenesis induced by FGF2 by 17% while MLO snake venom induced 22% reduction of the angiogenic index. The concentration of obtustatin in the crude MLO venom was 0.3 nM, which is 300,000 times less than the concentration of the obtustatin itself. Given this enormous difference in concentration, it is likely that some components of the crude venom contribute to the observed anti-angiogenic effect. Hypotheses will be ascertained to justify this action: components in the MLO venom may increase obtustatin efficacy or we suggest that such component could be the effect of dimeric disintegrins which amount is 4 times more in the crude MLO snake venom in comparison to the obtustatin.

P.5.2-013
Investigating the effects of emulsion formulations of oleuropein isolated from olive leaf (*Olea europaea* L.) ethanol extract in diabetic rats

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This study was designed to investigate the effects of emulsion formulations of oleuropein isolated from olive leaf ethanol extract in streptozotocin (STZ)-induced diabetic rats. Experimental diabetes was induced in rats by intraperitoneal injection of STZ at a dose 50 mg/kg b.w. dissolved in freshly prepared cold citrate buffer (0.1 M; pH: 4.5). Rats with blood glucose levels above 300 mg/dL were considered to be diabetic. Diabetic rats were treated with oral administrations of emulsion formulations of oleuropein, at the different doses (30 and 45 mg/kg b.w.), in liquid form at a daily dose for 4 weeks. Serum glucose, AST and ALT levels in the blood samples of rats were analysed. In addition, SOD activities, GSH and MDA levels in the liver tissues of rats were analysed. GSH levels ($P < 0.05$) and SOD activities

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($P < 0.05$) significantly decreased, whereas MDA levels ($P < 0.05$) increased in diabetic rats compared with those of normal control rats. Also, a significant increase in their serum glucose, AST and ALT levels ($P < 0.05$) were observed. After the oral administrations of the emulsions containing oleuropein, at two different doses, a significant increase in GSH levels ($P < 0.05$) and SOD activities ($P < 0.05$) and a significant decrease in MDA levels ($P < 0.05$) of the treated diabetic rats were observed. Also, their serum AST and ALT levels ($P < 0.05$) significantly decreased. The oral administration of the emulsion containing oleuropein at the high dose was found to be more effective than that of the low dose ($P < 0.05$). In addition, a significant decrease in serum glucose levels ($P < 0.05$) of the rats receiving the emulsion containing oleuropein at high dose was observed. The results indicate that the antidiabetic effect of the emulsion formulation of oleuropein is due to its hypoglycemia effect and antioxidant effect restraining the oxidative stress which is widely associated with diabetes.

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P.5.2-014
Effect of retinoid derivates on cholinesterase activity

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Both retinoic acid treatment and butyrylcholinesterase-knockout attenuates β-amyloid in an Alzheimer's disease transgenic mouse model. In this study, we analyzed the interaction of retinoid derivates with purified human serum butyrylcholinesterase (BChE) and human erythrocyte acetylcholinesterase (AChE). BChE was purified from fresh plasma by Tris Acryl-M chromatography followed with Procainamide-Sepharose 4B affinity chromatography. Thirty-five derivates of retinoid were chemically synthesized. Cholinesterase (ChE) activities were determined by the modified Elman assay. Initial ChE activities were measured by 96-well plate assay at 37°C using butyrylthiocholine or acetylthiocholine as substrate at a final concentration of 1 mM in 50 mM MOPS pH 7.4, 0.25 mM DTNB at 412 nm. AChE and BChE activities were determined in the presence different concentration (0.1–1000 μM) of the retinoid derivate compounds. All assays were carried out in triplicates. We found that the IC50 value of retinoid derivates varied from 0.15 μM to 150 μM. Of the retinoids synthesized, the chemicals with highest affinity were of indole derivatives. The highest affinity was displayed by the indole derivative compound I4-g. This compound had 0.15 μM IC50 value. Our results show that ChE activity is inhibited by retinoid derivates. Functional group of the compounds which have lower IC50 value could be modified in the future research and might be of use as therapeutics.

P.5.2-015
Biocidal and anti-pathogenic acvity of *Juniperus communis* essential oil against multidrug resistant *Staphylococcus aureus* strains

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Multidrug resistant (MDR) and highly virulent *Staphylococcus aureus* strains urgently need innovative strategies for the development of new and efficient antimicrobial agents, targeting key virulence determinants. For this approach an important step is the critical evaluation of novel antimicrobial alternatives. We aimed to evaluate the biocidal and anti-virulence activity of *Juniperus communis* essential oil (JEO) on 11 MDR *S. aureus* strains isolated from different clinical specimens. The antimicrobial activity of JEO was assessed quantitatively by the minimum inhibitory concentration (MIC) and characterized by phenotypic and genotypic tools for their virulence and resistance profiles. JEO influence on bacterial adherence to acellular substratum was assessed by the microtiter plate assay. Ethidium bromide-agar cartwheel method and flow cytometry (FCM) were used to evaluate the influence of JEO on bacterial cell wall permeability and efflux pumps activity. JEO major compounds (GC-MS) were α-pinene, β-mircene and β-pinene. All strains exhibited resistance to penicillin and 10 strains exhibited methicillin resistance and the MLSB phenotype. 83% of strains produced proteases, pore-forming toxins and enzymes, and iron-chelating compounds. PCR assays identified mecA, CCR complex and ermA resistance genes, 5 adhesion genes (clfA, clfB, fib, ebpS, fnbA) and 2 synergohymenotropic toxins (hlg, luk-PV). All strains were susceptible to JEO (MIC = 1.04 – 8.33 μl/ml). JEO inhibited bacterial adherence to acellular substratum in 8 strains. EB-agar cartwheel method revealed no efflux activity in the tested strains. In exchange, the FMC has revealed that JEO induced cellular coatings permeabilization. The present study demonstrated that the tested JEO exhibited good anti-staphylococcal activity, proving a real potential for developing efficient anti-effective strategies.

P.5.2-016
Limonene functionalized magnetite nanoparticles for improved antimicrobial and immunomodulatory therapy

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Limonene is a widely encountered plant-derived compound with acknowledged antimicrobial properties. The aim of our study

was to obtain and characterize efficient magnetite-based nanoshuttles to better deliver, stabilize and improve the biological activity of limonene in order to develop more natural and efficient antimicrobial and immunomodulatory drugs. Co-precipitation synthesized Fe_3O_4 were functionalized with Limonene (Fe_3O_4@L) and characterized by IR, SEM and HR TEM. Antimicrobial activity was assessed by viable count, while attachment and biofilm formation were analyzed through an adapted microdilution method and microscopy on two main opportunistic pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). *In vitro* biocompatibility was tested using endothelial human cells by fluorescence microscopy and MTT assay. Production of inflammatory cytokines was assessed by ELISA. Our results revealed that the obtained nanosystem is highly efficient in bacterial biofilm inhibition in a dose and exposure time dependent manner; subinhibitory concentrations proved to inhibit initial microbial attachment to the substrata. Moreover, the magnetic nanosystem reduced the necessary active dose of L by approximately two fold (from 0.5% to 0.2%), by stabilizing the compound and ensuring a targeted and controlled release. The obtained nanosystem revealed a good biocompatibility *in vitro*. Fe_3O_4@L nanoparticles had a slight proinflamatory effect, stimulating the production of Il-10, Il-6 and Il-8, the stimulation being maximum after 48 h of exposure. Functional Fe_3O_4 nanoparticles are efficient drug delivery shuttles, able to stabilize pharmacological compounds, such as plant-derived bioactives, and their biocompatibility, immunomodulatory and antimicrobial effects make them competitive candidates for prophylaxis and treatment of severe infections.

P.5.2-017
Biomaterials based on functional graphene oxide and natural compounds with improved biocompatibility, biodistribution and low immunomodulatory effects

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This study aims to reveal the biocompatibility, biodistribution and impact on the production of inflammatory citokines of graphene oxide (GO) functionalized with natural compounds with proved antimicrobial and immunomodulatory effects. Prepared GO was functionalized with eucalyptol, carvone, limonene and β-pinene. Characterization was done by IR, SEM and HR-TEM, while *in vitro* biocompatibility was tested using endothelial human cells. *In vivo* biodistribution was tested in a balbC mouse model at 2 and 7 days. Production of inflammatory cytokines was assessed by ELISA. Results demonstrated that, at concentrations of 500 μg/ml, nanosystems have a good biocompatibility, allowing the development of cultured cells and also not affecting any visible behavior and organ morphology of the mice. Microscopy evaluation of the organs sections revealed that nanoparticles are not present in vital organs such as brain, heart, kidney and liver, but aggregates were visible in the lungs and spleen. At 7 days post-injection no visible aggregated were found in the lungs, few dark-brown nanoparticles clusters being visible in the red pulp of spleen. ELISA results revealed that functionalized GO significantly stimulated the production of IL-2, IL-10 and IL-6, while reducing the production of TNFα. Functional GO is efficient drug delivery shuttles, able to stabilize pharmacological compounds, such as plant-derived bioactives, and their biocompatibility, specific biodistribution and limited immunomodulatory effects recommend their use in pharmacological formulations.

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P.5.2-018
Functionalized graphene oxide nanomaterials: *in vitro* cytotoxicity and BRAF inhibition in melanoma cells

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Graphene nanomaterials have proven unique physicochemical properties, several applications being continuously explored. Among them, recent advances in nanomedicine revealed huge interests for fabricating graphene-based cell instructive environments for drug/gene delivery and cancer therapy, engineering stem cell responses, bacteria-killing and tissue engineering platforms, biosensing and cellular imaging. However, the full merging of graphene and graphene oxide (GO) with biotechnology is still in its infancy, many challenges remaining unexplored. In order to use GOs as drug release matrices for cancer cells targeting, it is mandatory to ensure that these molecules do not affect normal cells within tissues. Studies suggest that pristine and reduced GO with fewer surface functional groups tend to be more toxic than GO. In striking contrast, it has been reported that functionalized graphenes, can significantly reduce the cytotoxicity even at relatively high concentrations. In this study, we report on the fabrication of smart biointerfaces by Matrix-Assisted Pulsed Laser Evaporation (MAPLE) for targeting melanoma cells. We first addressed a comparison between GO and protein functionalized GO when submitted to *in vitro* cytotoxicity tests. BSA was used for the noncovalent GO surface conjugation. Safe concentration windows were identified by live/dead staining and MTS assays for different human melanoma cell lines, while melanocytes and human dermal fibroblasts were used as normality controls. Hybrid GO-BSA thin films incorporating Dabrafenib inhibitor for cells bearing $BRAF^{V600E}$ activating mutation were assembled on solid substrates by MAPLE. Our results evidence the successful laser immobilization of the drug within GO-BSA matrix and its efficient activity for BRAF inhibition, as resulted from decreased ERK phosphorilation in SK28 cells. Such bioplatforms present high potential for the cell-biomaterial interface engineering for biomedical applications.

P.5.2-019
Biotechnology for new targeted delivery strategies against malaria

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The concept of antimalarial therapy has been locked for over 100 years on the administration of drugs against which *Plasmodium* has evolved resistance shortly after their deployment. More often than not, economy-related issues have been hampering the

progress of nanotechnology-based medicines against malaria with the dubious argument that they are too expensive to be used in developing areas. Unfortunately, it is true that the application of nanoscience to infectious disease has been traditionally neglected, with most research resources overwhelmingly biased towards other pathologies more prominent in developed regions. Thus, extra ingenuity is demanded from us: malaria-oriented nanomedicines not only need to work spotless; they have to do so in a cost-efficient way because they will be deployed in low-income countries. In this regard, the use of molecular elements combining several antimalarial activities, whether drug, targeting, carrier, or booster of immune reactions, will contribute to reduce the cost of their development. The implementation of a new delivery method is usually cheaper than the process leading to the discovery of a new drug, and it has the additional advantage that, if well designed, these biotechnological strategies can be adapted to several drugs. Rather than focusing all efforts on identifying new drugs whose efficacy is rapidly diminished by the parasite's evolution of resistance, an important and often disregarded battlefront is the implementation of targeted delivery methods capable of increasing the doses reaching the pathogen up to local levels sufficiently high to minimize this resistance emergence. Regrettably, the search for this long sought-after *magic bullet* against malaria has not taken off in earnest yet. However, recent data outline the feasibility of some such potential novel approaches, among which we can count new types of combination therapies where one of the activities does not act on individual *Plasmodium* gene products.

P.5.2-020
The effects of two different antihipertansive drugs as beta blockers-adrenergic receptor antagonists on hca1 and hca2 isozymes in obstructive sleep apnea patients and control group

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The catalytic function of the enzyme carbonic anhydrase (CA) plays a fundamental role in carbon dioxide (CO_2), proton (H^+), and bicarbonate (HCO_3^-) homeostasis. A specific role of CA in respiration and ventilatory control is suggested by the presence of various CA isoforms in various tissues [1]. Carbonic anhydrase (CA) activity increased with apnea-hypopnea index and related nocturnal hypoxemia measures in patients with obstructive sleep apnea (OSA) [2]. In this study, patients referred to the Department of Pulmonary Diseases at the Balıkesir University Hospital with suspected OSA were randomly recruited. We investigated inhibition of carbonic anhydrase I and II from sleep apnea patients and healthy control gruop with two different antihypertansive drugs as a class of beta blockers (nebivolol and carvedilol). hCA-I and hCA-II were purified from human erythrocyte cells by affinity chromatography [3]. CA I and CA II enzyme activity was observed to be increased in obstructive sleep apnee patients. Antihypertansive ingredients have been shown to have a stronger inhibition in OSA patients than non OSA group for hCA I and hCA2 activity.

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P.5.2-021
Testing the prooxidant-antioxidant balance in patients with prediabetes and Type 2 diabetes mellitus

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Diabetes mellitus is associated with an imbalance between prooxidant mechanisms and the antioxidant defenses, contributing to oxidative-stress. Oxidative stress is associated with an insulin-resistance, impaired pancreatic β-cell function, increased susceptibility to endothelial dysfunction and atherosclerosis. In this work, the serum pro-oxidant/antioxidant balance (PAB) in patients with prediabetes and type 2 diabetes was tested. The study included 54 patients with prediabetes and 195 patients with type two diabetes mellitus (T2D) which were divided into two groups (95 newly diagnosed T2D patients and 100 T2D patients recieving therapy). All of the patients were recruited at Clinical Center University of Sarajevo. The main task of this study was to evaluate differences in PAB value between our patient groups. The values of uric acid, inflammatory markers (CRP, IL-6, fibrinogen), anthropometry parameters (BMI, waist circumference, hips circumference, WHR) and parameters of lipid profile were also tested. All subjects included in the study were free of evidence of chronic problem that can cause hyperglycemia (infections, surgery, thyroid disease, polycystic ovarian syndrome), active liver and kidney damage and were not using any hormonal or hypoglycemic therapy. Results of this study demonstrated statistically higher values of PAB in T2D patients receiving therapy when compared to newly diagnosed T2D patients and patients with prediabetes. Statistically higher values were also observed for CRP in T2D newly diagnosed patients and patients recieving therapy compared to prediabetics patients. Patients with T2D recieving therapy had highest VLDLc values and newly diagnosed T2D patients highest atherogenic index among three patients groups. Our study demonstrated imbalance between serum pro-oxidant and antioxidant activity. Furthermore, our findings indicate that this assay could be used along with other risk factors to estimate the oxidative stress in high-risk patients.

P.5.2-022
Effect of static magnetic field with antiproliferative compounds on MDA MB-231 cell line

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The recent studies show that the static magnetic field (SMF) can be used as an alternative treatment in cancer therapy. Especially

combination treatment through simultaneous delivery of antiproliferative compounds with SMF has been demonstrated to be an efficient approach for cancer therapy. The aim of this study is to investigate the effect of SMF combination treatments with antiproliferative flavonoids on apoptosis, proapoptotic and antiapoptotic protein expressions on MDA MB-231 (receptor-negative) breast cancer cell line. Quercetin (range 10–1000 μM) and hesperetin (range 10–1000 μM) were used as flavonoids. Cell proliferation was measured with MTT assay and IC50 values of flavonoids were calculated. Then, applications divided into six groups; I. Control (only cell); II. Hesperetin (110 μM); III. Quercetin (160 μM); IV. SMF (174 mT); V. SMF+hesperetin; VI. SMF+quercetin. Apoptosis was analyzed by flow cytometry and Bax/Bcl-2 expressions were analyzed by Western Blot. Statistical analyzes were evaluated by SPSS programme. Although SMF and flavonoid alone induced apoptosis of MDA MB-231 cell lines, this effect is observed more when co-administered ($P < 0.001$). Similarly, Bax expressions increased and Bcl-2 expressions decreased on cell line compared with control group ($P < 0.001$). Taken together, our results demonstrated that joint application of SMF with flavonoids promoted apoptosis of MDA MB-231 cell line. This *in vitro* study, which assesses the antitumoral activity of SMF on breast cancer cell, SMF can enhance the anticancer effect of antiproliferative compounds; this may provide a new strategy for cancer therapy.

P.5.2-023
Inhibition of dipeptidyl peptidase IV and adenosine deaminase activities as approach of diabetes treatment by natural compounds

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The enzymatic activities of dipeptidyl peptidase IV (DPPIV) and adenosine deaminase (ADA) rise in hyperglycemic condition. DPPIV inactivates GLP-1, the potent insulinotropic hormone and ADA decreases the level of the possessing insulin like activity adenosine, bringing to insulin resistance. Therefore, the inhibition of both enzymes considered as beneficial tools in type 2 diabetes treatment. The high cost and side effects of synthetic inhibitors forced the conducting of researches on the search and use of natural products. This work describes the *in vitro* inhibition of DPPIV and ADA by extracts and phenol glycoside fractions (PhGs) from medical plants. The IC_{50} values for some of the studied plant preparations (PP) in inhibition of the enzymes were evaluated as rather low. In ADA inhibition, the IC_{50} values for ethanol extracts from pistacia, grape, sorrel and blackberry leaves (PL, GL, SL, BL, respectively), walnut pellicles (WP), rose petals (RP) and melilot were in the range from 0.23 to 1.25 mg/ml. The IC_{50} values in DPPIV inhibition for the extracts from WP, BL and RP were between 0.13 and 0.63 mg/ml. Extracts from GL, SL and roots of bryonia alba did not inhibit the activity of DPPIV, the extract from melilot even activated the enzyme. The IC_{50} values for PhGs from RP and GL were close each other. In the case of DPPIV (0.029 mg/ml) they were by one order smaller than for ADA (0.34 mg/ml). It was shown, that the inhibition of DPPV by PhG fraction from GL is of the competitive nature, with Ki = 1.35 μg/ml. The anthracene derivatives and PhG from SL did not inhibit DPPIV. Interestingly, the absorption spectrum of PhG from SL, which did not inhibit DPPIV, differs sharply from the spectra of PhGs from RP and GL (similar one to other). The spectra of the last two fractions evidence the prevalence in them of flavin-3-ol(+)-catechines. The spectrum of PhG from SL evidences the presence of phenol oxides, flavonones and flavananes.

P.5.2-024
A highly synergistic, broad spectrum, antibacterial activity of organic acids and transition metals

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Antibiotics are one of the keystones of modern medicine and are broadly used not only in clinical settings, but also in farm animal diets and crop protection. However, the widespread use of antibiotics is also the source of the emergence of drug-resistant strains of bacterial pathogens. Thus, it is generally agreed that there is a pressing need to replace the use of antibiotics with non-antibiotic alternatives whenever possible. For millennia, transition metals and organic acids have been exploited separately to inhibit bacterial growth. We report the potentiation of the anti-bacterial activity of transition metals by organic acids. A strong synergy between low, non-toxic concentrations of transition metals and organic acids was observed, with up to ~1000-fold higher inhibitory effect on bacterial growth when compared to their individual effects. We show that organic acids shuttle transition metals through the permeability barrier of the bacterial membrane, leading to increased influx of transition metals into bacterial cells. This increased influx can be counteracted by over-expression of metal efflux systems, or compensated for by chromosomal deletions of metal import systems. We demonstrate that this synergy can be effectively used to inhibit the growth of a broad range of plant and human bacterial pathogens, suggesting a revision of food preservation and crop protection strategies. These findings bear significant biomedical, agricultural, financial, and environmental opportunities.

P.5.2-025
Potential new antibacterial drug based on C115H mutant methionine γ-lyase and S-substituted L-cysteine sulfoxides

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Pyridoxal 5'-phosphate-dependent methionnine γ-lyase (MGL) catalyzes the γ-elimination reaction of L-methionine to give methylmercaptan, α-ketobutyric acid and ammonia. We have revealed that MGL effectively catalyzes the reaction of the β-elimination reaction of S-alk(en)yl-L-cysteine sulfoxides with the formation of thiosulfinates, which possess antimicrobial activity. To increase the efficiency of the enzyme in the β-elimination reaction of sulfoxides C115H mutant form of *Citrobacter freundii* MGL was prepared. The replacement has led to an increase of catalytic efficiency of the mutant form in the reaction with sulfoxides in comparison with the wild type MGL. Two-component systems composed of the mutant enzyme and S-substituted L-cysteine sulfoxides (alliin, S-methyl-L-cysteine sulfoxide, S-ethyl-L-cysteine sulfoxide and S-propyl-L-cysteine sulfoxide) were demonstrated to be effective against Gram-positive and Gram-negative bacteria. MICs of thiosulfinates were determined and ranged from 0.087 to 0.38 mg/L. The goal of our studies is the creation of an effective antimicrobial drug on the basis of such two-component systems. In order to prevent immunological reactions that might be produced by multiple dosing of C115H MGL and to prolong the serum half-life of the enzyme, the α-methoxy-ω(4-nitrophenoxycarbonyl)polyethylene glycol (ME NP-50H PEG 5000) was coupled to C115H MGL. Pharmacokinetic parameters of pegylated C115H MGL and unmodified enzyme

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were determined. The circulating half-life of pegylated MGL in mice has increased two-fold as compared to unmodified enzyme. Our findings can serve as a basis for further constructing of antibacterial drug composed of modified MGL and S-substituted L-cysteine sulfoxides for testing *in vivo*.

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P.5.2-026
In pursuit of cell-penetrating antimicrobial peptides: the transcriptome robust analysis

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The essential components of the innate immune system of living organisms are antimicrobial peptides acting in various manners. Cell-penetrating antimicrobial peptides (CPAMPs) translocate across the plasma membrane without its disruption and kill the pathogen affecting on the intracellular targets. The cell-penetrating peptides exhibiting antimicrobial activity are contained in the venoms of poisonous animals, such as spiders, snakes and scorpions. The guiding idea of the search for novel CPAMPs involves the identification of proteins with cell-penetrating ability and antimicrobial potential based on the analysis of the transcriptome data of the tentacles secretion of the sea anemone *Cnidopus japonicus* and the venom glands secretion of the spider *Poecilotheria fasciata*. Full-length transcripts reconstruction and identification of coding regions were performed by Trinotate annotation suite. The translated transcipts encoding the protein more than 10 aa were selected. According to the server SignalP proteins containing signal peptide were collected. The mature peptide was identified according to PQM: R(K)toR and EtoR/EafterR. Antimicrobial peptides with the cell-penetrating ability were identified by predictors CellPPD, CPPpred, AMPA, ADAM and CAMP and physicochemical properties corresponding to known CPAMPs. Additionally, the collected peptides of the sea anemone were verified against the proteome of *C. japonicus* secretion. For the moment, we have obtained lists of candidate peptides that likely penetrate the cell membrane without causing any damaging and exhibit antimicrobial activity against intracellular targets. Candidate CPAMPs will be chemically synthesised and antimicrobial activity of selected peptides will be evaluated against different bacterium *Bacillus subtilis*, *Escherichia coli* and intracellular pathogen *Chlamydia trachomatis*. The reported study was funded by the Russian Foundation for Basic Research according to the research project No. 16-34-00936 mol_a.

P.5.2-027
Modified methionine γ-lyase as potential anticancer drug

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The pyridoxal 5'-phosphate-dependent methionine γ-lyase (EC 4.4.1.11, MGL) is involved in the metabolism of sulfur-containing amino acids of bacteria, unicellular eukaryotes and fungus. It catalyzes the γ-elimination reaction of L-methionine the essential amino acid that is absolutely requirement for proliferation of cancer cells. This fact determines the perspective of the enzyme utilization in cancer treatment. In spite of the success obtained using MGL in several types of cancer *in vitro* studies, its usage *in vivo* revealed several problems typical of protein pharmaceuticals, including increased susceptibility to proteolysis, highly immunogenicity and a relatively short-lasting biological activity. MGL genes of pathogenic bacteria *Clostridium tetani*, *Clostridium sporogenes*, *Clostridium novyi* and *Citrobacter freundii* were cloned, and recombinant enzymes were purified and characterized. The cytotoxicity of MGLs was evaluated *in vitro* against several cancer cell lines. The enzymes exhibit a comparable cytotoxic activity with respect to well known antitumor drug asparaginase from *E. coli*. The pharmacokinetic properties and plasma methionine depletion were investigated after single administration of MGLs. The circulating $\tau_{1/2}$ of enzymes in mice varies from 73 to 123 min. To increase the half-life of the enzymes in the blood circulation two alternative methods (the covalent coupling with α-methoxy-ω-(4-nitrophenoxy carbonyl) polyethylene glycol and with polysialic acids) were used and the properties of modified enzymes were investigated. The MGL-polysialic acid conjugates fully retained the biological activity of the native enzyme whereas the activity of MGL-PEG conjugates reduced almost two times.
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P.5.2-028
Basic pharmacokinetic parameters of L-Lysine α-oxidase from trichoderma cf. aureoviride Rifai VKM F-4268D

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L-lysine α-oxidase (EC 1.4.3.14) (LO) deaminates L-lysine to form 2-keto-6-aminocaproate and hydrogen peroxide and exhibits antitumor, antiviral and antibacterial properties. The mechanism of these effects is associated with depletion of lysine and with accumulation of hydrogen peroxide. The rate of LO clearance is important for its further development as a medicine. The aim of the work was to determine the key pharmacokinetic parameters of LO in different schemes of i.v. administration to mice. Lyophilized LO (95 U/mg) was used; its specific activity was measured by the rate of hydrogen peroxide accumulation. LO content in blood was determined by the immunoassay. The dynamics of LO concentration in blood after i.v. administration determined by immunoassay practically coincided with the decrease of enzymatic activity, so it can be concluded that LO substance does not loose enzymatic activity in the bloodstream. Quantitative determination of LO content after i.v. administration at single doses of 1.0–3.0 mg/kg showed that the decrease of the enzyme concentration has a pronounced two-phase character, and the pharmacokinetic profile was nonlinear. LO concentrations were detectable in blood up to 24 h even upon administration of the minimal dose 1.0 mg/kg. The residual concentration of the enzyme ensured complete absence of L-lysine in serum one

day after administration. Pharmacokinetic parameters of the LO were: Cl – 0.05–0.1 ml/min; $T_{1/2}$ – 1.05–1.55 h, which is comparable to *E. coli* L-asparaginase. Repeated administration of LO can result in the L-lysine starvation and can lead to a significant weight loss. To avoid this complication, a short treatment regimen was proposed. Pharmacokinetic curves of LO after 5-fold administration with 48 h interval did not differ significantly from a single administration.

P.5.2-029
Effect of antioxidants from *Brassica napus* genotypes on catalase activities in *Galleria mellonella* (L.) (Lepidoptera: Pyralidae)

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Diet is effective on biochemical metabolism of insect. In this study, effect of antioxidants on enzyme activites of *Galleria mellonella* was studied. The insect was fed with different amount of two different antioxidants such as quercetin and cinnamic acids. In addition to these antioxidants, the methanolic extracts of two *Brassica napus* (Canola) genotypes were examined for the insect metabolism. The results showed that quercetin and cinnamic acid decreased catalase (CAT) activities. However, enzyme activities of the insect were increased by the extracts of two canola genotypes.

P.5.2-030
Relation between HOMA-IR, folat, vitamin B12 and MPV

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Introduction: Insulin resistance is a disease related to hypertension, high cholesterol and coronary heart disease. Insulin resistance can be defined as an fatal and metabolic disease which should certainly be recognized and treated.
Methods: All the patient registrations to Yozgat State Hospital in 2016 are retrospectively examined. Folic acid, vitamin B12 and MVP levels of a group of 60 people (30 women and 30 men who are between the ages of 30–60) with normal and high HOMA-IR levels (in equal numbers) are recorded. At last these results are evaluated in terms of statistics.
Results: Shapiro-Wilk test is used in order to determine how groups were distributed. Datas of normally distributed groups are analyzed as mean±standart deviation, the ones that are not distributed normally are analyzed as median (min-max) The relation between groups is researched by using Student's t-test and MannWhitney U tests in terms of distribution. According to this while there has been found a significant difference (P = 0.033) between the groups HOMA-High women and HOMA-Normal men in terms of Folic Acid Results, there has not been found any difference between other groups in terms of statistics.
Conclusions: When we are talking about such an important disease of which every single discovery is valuable, Folat levels of HOMA high women being higher than HOMA low men shows us how important our study is and why it should be repeated with a larger group.

P.5.2-031
The importance of reporting troponin I analysis results with measurement uncertainty estimation

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Objective: Troponin I is one of the most commonly used biochemical indicators in the diagnosis of acute myocardial infarction. But once troponin I analysis measured, is that result accurate and absolute? In principle, it is assumed that the real value of an analytical measurement is unknown. Uncertainty of measurement provides quantitative estimates of the level of confidence that a laboratory has in its analytical precision of test results and therefore represents the expected variability in a laboratory result if the test is repeated a second time. The aim of this study is to present the importance of reporting Troponin I analysis results with measurement uncertainty estimation.
Materials and Methods: We retrospectively reviewed the records of 16619 patients (8060 males and 8619 females) who were tested Troponin I from January 2016 to December 2016. Troponin I analysis results were re-evaluated by estimation of measurement uncertainty. The uncertainty of measurement on serum Troponin I level based on the top-down approach was estimated by this research, as a practical example.
Results: Measurement uncertainty (95% confidence interval) for Troponin I is estimated to ±13.35%. Troponin I reference values were determined as 0–34.2 pg/ml for male and 0–15.6 pg/ml for female in data sheets of commercial kits. In this study, 184 Troponin I analysis results (115 females-69 males) which are above cutoff value might be below cutoff value if they were assessed based on measurement uncertainty.
Conclusion: Medical laboratories must produce the necessary data and analytical results in order to achieve the correct interpretation and use of the results. A test result is not enough powerful without an assessment of its reliability. Finally, reporting Troponin I analysis results with estimation of measurement uncertainty is important to show measurements that contained within the true limits and the level of confidence.

P.5.2-032
Chemo-enzymatic synthesis and anti-herpesvirus activity of novel 6-substituted purine-based nucleosides

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In continuation of our research on chemo-enzymatic glycosylation of 6-substituted 2-aminopurines, we obtained a series of novel modified nucleosides using the transglycosylation reaction. For the first time, it has been demonstrated that (3*R*) and (3*S*)-4-[6-(2-aminopurin-6-yl)aminohexanoyl]-7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazines are good substrates for the genetically engineered recombinant *E. coli* purine nucleoside phosphorylase. Ribosides and 2-deoxyribosides were obtained in high (up to 95%) and moderate (up to 43%) yields, respectively. It has been found that yield in the transglycosylation reaction does not

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depend on the stereo configuration of the fragment of chiral N-heterocycle in 2-aminopurine derivative. It has been found that (3*R*) and (3*S*)-4-[6-(2-amino-9-(β-D-ribofuranosyl)-purin-6-yl) aminohexanoyl]-7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazines exhibit not high but sufficient antiviral activity against herpes simplex virus type 1 (HSV-1) in experiments *in vitro*. It should be especially emphasized that the compounds obtained are also active against acyclovir-resistant HSV-1 strain.

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P.5.2-033
Rational design of novel highly potent and selective phosphatidylinositol 4-kinase III-beta (PI4KB) inhibitors as broad-spectrum antiviral agents and tools for chemical biology

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Phosphatidylinositol 4-kinase III-beta (PI4KB) is an essential host factor for multiple +RNA viruses, which hijack this Golgi resident enzyme to remodel intracellular membranes of infected cells in order to establish replication organelles (ROs). Therefore, the inhibition of PI4KB leads to the arrest of viral replication. Here, we report on the synthesis of novel PI4KB inhibitors, which were rationally designed based on our previous structural analysis [1]. These "hybrids" excel in outstanding inhibitory activity and show high selectivity to PI4KB compared to other lipid and protein kinases. Their activity is in the single-digit nanomolar range and they also exert profound antiviral effect against hepatitis C virus, human rhinovirus, and coxsackievirus B3. We performed structural analysis to unveil the exact position of the side chains and explains their extensive contribution to the inhibitory activity. We also prepared their fluorescent derivatives that we show to be useful chemical biology tools especially in determination of dissociation constants and in lifetime imaging microscopy (FLIM) microscopy.

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P.5.2-034
Development of scaffolding molecule for improving function of biomolecules

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Biosensing based on the specific interactions of biomolecules (*e.g.*, antibody-antigen, receptor-ligand, enzyme-substrate, lectin-sugar chain, DNA aptamer-target) is a state-of-the-art sensing technique in the field of basic and applied biosciences. To improve the sensitivity and analyte-binding capacity of biosensing, the sensing molecules (*e.g.*, antibody, receptor, enzyme, lectin, DNA aptamer) on solid phase should be clustered and immobilized in an oriented immobilization manner. Here, we developed a novel ~30-nm scaffold ZZ-bio-nanocapsule (ZZ-BNC), consisting of transmembrane hepatitis B virus envelope L protein displaying the protein A-derived IgG Fc-binding Z domains outwardly (ZZ-L protein), that plays for both clustering and oriented immobilization of IgGs and Fc-fused receptors. In quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) using immobilized IgGs or Fc-fused receptors to detect targets in solution, ZZ-BNC could spontaneously and firmly adsorb onto the gold surface of sensor chips, and markedly enhance the sensitivity, target-binding capacity, and avidity of biosensors. High-speed atomic force microscopy (HS-AFM) observation revealed that the IgGs on ZZ-BNCs underwent rotational Brownian motion with the Fc region serving as the supporting point. Furthermore, we succeeded in the disassembly of ZZ-BNC into ZZ-L micelles by the detergent treatment. By mixing with liposomes on biosensor surface, they could transform into planar ZZ-L membrane spontaneously, allowing us to control the density of orientation-fixed ZZ-L proteins. The planar ZZ-L membrane could be more applicable to various biosensors than ZZ-BNC for enhancing the sensitivity and ligand-binding capacity. Taken together, both scaffolds ZZ-BNC and planar ZZ-L membrane can bring out the inherent function of biomolecules in biosensing techniques.

P.5.2-035
New 1-hydroxy-2-thiopyridines combat *Mycobacterium tuberculosis* violating copper homeostasis

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Tuberculosis is the most challenging threat to human health because of the increasing prevalence of primary resistance to the current drugs and wide distribution of latent TB infection that designate for a growing need for new compounds with a novel mode of action. New 1-hydroxy-2-thiopyridine derivatives were found to be highly effective *in vitro* against both actively growing and dormant non-culturable *M. tuberculosis*. To elucidate the mode of action of 1-hydroxy-2-thiopyridines we profiled transcriptomic response of *M. tuberculosis* to the leading compound and found transcriptional signs of copper stress. The most prominent primary transcriptome response was found to be induction of operons Rv1994c-Rv1992c and Rv2641-Rv2643 containing genes of ArsR/SmtR family involved in defense mechanisms from toxic concentration of divalent heavy metals. Transcriptome analysis also identified activation of copper responsive genes, which were known to be induced under toxic levels of copper, including metallothionein coordinating Cu^{1+} ions, multicopper oxidases and metal cation-transporting ATPases. What is more, copper was found to accumulate inside *M. tuberculosis* bacilli treated with 1-hydroxy-2-thiopyridines. 1-hydroxy-2-thiopyridines were found to form a stable charged complex with Cu^{2+} ion which evidently transports into mycobacterial cell. Being essential for the growth and development of almost all organisms, copper is known to be toxic at high concentrations. Thus, copper ions accumulate in phagosome infected with mycobacteria suggesting Cu provide an innate immune mechanism for combating the pathogen. 1-hydroxy-2-thiopyridine derivatives with strong bactericidal activity against both actively growing and dormant tubercle bacilli may help to elucidate the role of copper homeostasis in controlling *M. tuberculosis* infection.

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P.5.2-036
NFAT – calcineurin interaction: biophysical characterization and virtual screening of small molecule inhibitors

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T-cell activation switch is based on the interaction between the transcription factor Nuclear Factor of Activated T-cells (NFAT) and the phosphatase Calcineurin. In resting T-cells NFAT protein is heavily phosphorylated by various kinases, which keep NFAT inactive in the cytoplasm. Extracellular calcium influx activates Calcineurin to dephosphorylate NFAT and triggers NFAT's nuclear translocation and the activation of immune related genes. Under certain conditions (e.g. organ transplantation) it is necessary to suppress the immune system. Our research aims biophysical characterization of Calcineurin-NFAT interaction and finding a molecule to be further developed into immunosuppressant based on selective inhibition of this T-cell activation switch. Currently used drugs (CsA and FK506) block Calcineurin catalytic site and are toxic. Thus, finding a small molecule that can ispecifically nhibit Calcineurin-NFAT interaction without obscuring Calcineurin catalytic site is a promising lead towards the development of new immunosuppressant. This project combine experimental and computational techniques into an integrated approach of exploring the protein-protein interaction inhibitors chemical interaction space. The outcome will provide new patentable lead molecule for the development of a new immunosuppressant.

P.5.2-037
Charge influence of G2 and G4 PAMAM dendrimers associated with AFP receptor-binding domain on intracellular fate and endocytosis mechanisms in AFP-receptor positive cancer cells

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Polyamidoamine dendrimers are branched polymers, using as universal carriers in various drug delivery systems, containing a large number of primary amine groups. Amine-terminated dendrimers are characterized by high positive surface charge, leading to effective but nonspecific interactions with plasmatic membranes. We partially modified surface of dendrimers to reduce nonspecific internalization. As the vector molecule we used new recombinant alpha-fetoprotein receptor-binding domain (r3dAFPpG). In this work, the role of primary amine groups, which are localized on the surface of doxorubicin-conjugated (Dox) dendrimers (2nd and 4th generation), including r3dAFPpG-conjugated dendrimers, as well as Dox-labeled r3dAFPpG, were studied with regard to their endocytosis mechanism (using endocytosis inhibitors chlorpromazine, filipin, colchicine and ethyl isopropyl amiloride), intracellular distribution and internalization rates using SKOV3 human ovarian adenocarcinoma and MCF7 human mammary glands adenocarcinoma cells. As expected, all Dox-labeled dendrimer conjugates containing maximal numbers of amine groups and r3dAFPpG-Dox had high rates of cellular uptake. Incubation of these conjugates in presence of colchicine and ethyl isopropyl amiloride decreased levels of endocytosis dramatically. Endocytosis of r3dAFPpG-Dox was disrupted in presence of chlorpromazine and colchicine and, as had been shown, the conjugate recycled mainly. At the same time, all dendrimer-contained conjugates revealed colocalization with LAMP2, the marker of lysosomes and late endosomes. Interestingly, that endocytosis of neutrally charged conjugates contained r3dAFPpG was blocked by chlorpromazine as in case of unmodified protein, but conjugates were localized mostly in endolysosomal compartments of the cells. We conclude that surface charge of the conjugates synthesized influences dramatically on the mechanisms of endocytosis and intracellular fate despite the presence r3dAFPpG.

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P.5.2-038
Impact of quercetin on chemotherapy response in metastatic human breast cancer – a vibrational microspectroscopy study

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Regardless of all efforts, metastatic breast cancer disease still is the second most lethal type of cancer among women around the world. About 30% of cancer mortality is attributable to an inappropriate diet, with an insufficient amount of health-beneficial components (such as antioxidants). Indeed, there seems to be an inverse relationship between a decreased risk of cancer and a regular consumption of fruits and vegetables, which is ascribed to phytochemicals with potential chemopreventive properties. Cancer prevention may be achieved by pharmacologically safe compounds from natural sources that are capable of modulating multiple targets and cell signalling routes. Also, this type of non-toxic agents can be used alone to prevent cancer, or in combination with chemotherapy. The present study reports the combined effect of quercetin, an abundant dietary flavonol with a recognised antioxidant capacity, with cisplatin (*cis*-$Pt(NH_3)_2Cl_2$) and a new generation cisplatin-like polynuclear Pd(II) chelate (Pd_2Spm, Spm = spermine), aiming at an improved activity and selectivity towards the human metastatic breast cancer cell line MDA-MB-231. The influence of cell pre-sensitisation with the antioxidant agent on the pharmacodynamic behaviour of the tested drugs was assessed, with a view to understand the effect of the daily diet on chemotherapeutic intervention. Synchrotron-radiation infrared microspectroscopy (SR-IRMS) and microRaman (in formalin-fixed cells) were used, to analyse three distinct sets of cells: non-treated, treated with quercetin at 50 μM for 24 h, drug-treated (cisplatin or Pd_2Spm at 4 μM, 48 h) upon pre-sensitisation (24 h) with quercetin. Drug exposure after pre-sensitisation unveiled a greater impact on DNA (O-P-O stretching) and protein (NH_2 deformations) as compared to quercetin *per se*, which is in accordance with the known mechanism of action of this type of Pt-based drugs.

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P.5.2-039
Innovative, pharmaceutical nano-hybride materials for the specific functional cellular regeneration

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The research of our laboratory is focused on the current trend of micro-nano-structured materials used in pharmaceutical industry. For the know-how development in the field of Medicinal Chemistry, we study the hybrid support MCM-41-biopolymer, processed through innovative technologies for the loading of bioactive, standardized vegetal extracts. This hybrid material was obtained in a several steps process, as follow: MCM-41 synthesis using TEOS as precursor, at room pressure and temperature; the mesoporous material loading with bioactive compounds and depositing of a biopolymer nano-film on the surface of the encapsulated material in order to obtain a slow release of the active principle. All this complexes were characterized through DLS, FT-IR, SEM, TEM, X-RD, and BET. The design of the active biocomplex results from the inter-relationship of compounds classes (flavonoides, poliphenols, fatty acids, sugar derivatives, terpenes, etc), encapsulated in the nano-structured carrier, the adsorption/desorption capacity and molecular and cellular parameters modelation. We target regeneration mechanisms at dermal and epidermal level: cell proliferation, apoptotic pathways, keratinocyte differentiation (keratine K5/K14, transglutaminase, involucrine membrane expression). According to our results, the nano-hybrid complex acts as a regenerative agent, suggesting its use in skin chronic diseases therapies. This study was sustained by Romanian Research Authority, contract 49 PTE/2016.

P.5.2-040
A modular and generalizable route to potent, selective and pharmacologically compliant inhibitors of rhomboid proteases

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The rhomboids are a conserved family of intramembrane serine proteases. They are involved in the regulation of many biological processes, such as growth factor secretion, invasion of the malaria parasite, regulation of mitochondrial dynamics or membrane protein quality control[1]. These findings confirm the biological relevance of rhomboid proteases, but their therapeutic potential still needs to be validated. This is however hampered by the lack of potent, selective and pharmacologically compliant inhibitors[2]. Based on the current knowledge of rhomboid protease mechanism, taking together both enzymological studies and structural analyses[3,4], we discovered that peptidyl ketoamides substituted with a large hydrophobic tail at their ketoamide nitrogen are potent, covalent reversible inhibitors of rhomboids. Their activity is in low nanomolar range which surpasses all other rhomboid inhibitors by up to three orders of magnitude. Selectivity profiling against about a hundred human hydrolases shows that our rhomboid-targeting peptidyl ketoamides have minimal off-target effects. Structures of their complexes with rhomboid suggest a conceptual framework of how their design can be generalized. This prospect, together with the fact that some peptidyl ketoamides are clinically used pharmacophores[5], indicates that the inhibitors that we describe and their variants could be widely applicable in cell biology and drug discovery involving rhomboid proteases.

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P.5.2-041
Investigation of utility of homocystine as an internal standard for the determination of leukocyte cystine level by LC MS MS method

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Cystinosis is an autosomal recessive disorder characterized by the accumulation of cystine in lysosomes, causing irreversible damage to many organs, especially to the kidneys. Previously, for the determining leukocyte cystine levels cystine binding protein assay, ion exchange chromatography and HPLC methods have been used but nowadays the most commonly used method is LC MS MS. Generally, isotope of target analyte that is quite expensive is used as an internal standart for lc ms ms based methods. The aim of this study is to test the use of homocystine molecule as an internal standard, which is very rarely found at the intracellular level and resembles cystine. Using the homocystine as an internal standart instead of D_6-cystine will reduce the cost of test. In the developed method, ACQUITY UPLC BEH-C18, 2.7 µm-2.1 × 50 mm column was used as the stationary phase and 0.05% formic acid in acetonitrile/water 50/50% were used as the mobile phase. Injection volume was 2 µl while flow rate was 0.2 ml/min. Mass-spectrums were determined with Waters-Xevo-TQD MS/MS system. 12%trichloroacetic acid was used as the protein-precipitation agent for leukocyte proteins. Retention times for cystine/D_6-deuterated-cystine/homocystine were 0.75 min. The limit of detection was found as 0.0192 mM and the limit of quantification was found as 0.0582 mM. % CV values obtained from the intra-day and inter-day reproducibility studies are below 15% which is suitable for validation criteria. Recovery rate was found as 94–106%. The results obtained from the D_6-cystine and homocystine method are compared with each other, and the R^2 value of the obtained graph is 0.9838. As a result of all these findings we propose that homocystine may be an economic and easily available internal standart for LC MSMS analysis of leukocyte cystine leves in as alternative to D_6-cystine isotope.

P.5.2-042
Searching for new oligomycin A derivatives with improved selectivity against malignant cells

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Oligomycin A (1) inhibits F_0F_1-ATPase by binding to the F_0 subunit and blocking the H^+ translocation. Also, oligomycin could block P-gp activity, trigger apoptosis and inhibit K-Ras signaling pathway. With the aim of searching of selective antitumor agents we have prepared novel semi-synthetic derivatives of oligomycin A, evaluated their biological activity and performed modeling of binding with F_0F_1-ATPase by docking (Molsoft ICM-Pro version 3.8–3).Two novel semi-synthetic derivatives with a pointed modification of the C16, C17 and C33 positions – 16,33-*O,O*-diformyl-16,17-dihydro-16,17-dihydroxyoligomycin A (2) and 33-*O*-formyloligomycin A (3) were obtained by epoxidation and esterification of oligomycin A (1). Epoxidation of C16-C17 and HCOOH-provided ring opening as well as formylation of C33 hydroxyl group led to the decreasing of activity against *S. fradiae*, *Candida* spp. and filamentous fungi. Obtained results were in agreement with docking studies. A computer simulation of interaction of 1, 2 and 3 with the F_0-subunit of the ATP-synthase (PDB: 4f4s) revealed that these modifications led to a significant change in the solvation energy during the interaction of the derivatives 2, 3 with the target, and an increase in the conformational capacity of the ligands. This resulted in a decrease of the binding affinity for derivatives 2, 3. The main contributions to the binding energy for oligomycins 1–3 are from their hydrophobic components. However, 33-*O*-formyloligomycin A (3) showed high selective antiproliferative activity against tumor cell lines (HCT-116 colon carcinoma, K562 myeloid leukemia cell lines and MDR-subline K562/4) and was twice weaker against non-malignant human cells than 1. Thus, chemical modifications of oligomycin A could be useful for further optimization of the biological effect of this class antibiotics via modulation of affinity to ATPase and changing the mode of their action for mammalian cells.

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P.5.2-043
Study of the mechanisms of action of oligomycin A and its derivatives in the model system *Streptomyces fradiae* ATCC 19609

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Oligomycin A (olgA) inhibits FoF1-ATP synthase activity in eukaryotic cells by binding with its subunit C. Recent studies show that other olgA biotargets may exist within the cell. Actinobacteria including Streptomyces are the only representatives of prokaryotes sensitive to oligomycins. We synthesized more than 20 chemical derivatives in order to study the mechanisms of their action. The object of the study was Streptomyces fradiae ATCC19609 – a supersensitive to olgA, and two olgA derivatives – (33S) -33-deoxy-33-thiocyanatooligomycin (tcn-olg) and (33S) -33-azidoligomycin (azd-olg). The impossibility of obtaining *S. fradiae* ATCC19609 mutants resistant to olgA may indicate to the presence of several biotargets within the cell. The level of inhibition of the FoF1-ATP synthase activity in preparations of Streptomyces fradiae ATCC19609 inverted membrane vesicles was analyazed. It was found that the maximum degree of inhibition by the known inhibitor of ATP synthesis of N, N'-dicyclohexylcarbodiimide (DCCD) is 100%, oligoA is 31.3%., which in turn confirms the hypothesis of the presence of several olgA biotargets. Initiall, we obtained *S. fradiae* tcnR + mutants resistant to tcn-olg. Full-genomic sequencing of the mutant strains revealed a mutation in the conserved region of the DNA helicase IV gene (KDS85476.1). *S. fradiae* tcnR + was used to obtain mutants resistant to azd-olg – *S. fradiae* tcnR +/azdR +. Full-genomic sequencing of these mutant strains revealed a mutation in the FoF1-ATP synthase subunit A gene. The strain *S. fradiae* tcnR +/azdR + in its turn was used to obtain mutants resistant to olgA (*S. fradiae* tcnR +/azdR +/olgAR +). We plan to conduct full genomic sequencing and comparative genomic analysis of these strains. We also plan to determine the level of ATP synthesis inhibition by olgA, tcn-olg, azd-olg on the mutant of FoF1-ATP synthase subunit A.

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P.5.2-044
Some aspects of tissue specific JNK activity regulation under experimental insulin rasistance

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Since cJun-N-terminal kinases (JNK) has been indicated as one of the central mediators of insulin resistance (IR), a lot of researches try to find JNK inhibitors from natural sources as well to design synthetic ones. Taking into consideration that the biggest part of experiments was made *in vitro*, the aim of this research was to study *in vivo* tissue specificity of JNKs inhibition under diet-induced IR as possible correction for diabetic complications. Mature (180–200 g) male Wistar rats were randomized into groups according to the aim of the experiment. IR was induced by feeding animals high-calorie diet enriched with fructose (HCFD) during 90 days without and with JNK inhibitors administration since 75th day of experiment. It was used well-known JNK inhibitor SP600125 and G0007 (6,7-dimethoxy-4-N-(4 cyanophenyl) aminocinazoline) designed in National University of Pharmacy). One of the determined indices that evaluate JNK activation was determination of phosphorylated JNK (pJNK) in tissue homogenates of liver, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Our results demonstrated that feeding HCFD leads to increase of pJNK content for 24% in liver, 19% in VAT, at the same time in SAT was found no changes. SP600125 administration leads to decrease in pJNK levels. Thus, in liver pJNK level was lower for 20% compared with control pathology group, in VAT for 12%, in SAT also was found decrease of JNK level for 7% compared with intact group. G0007 administration resulted in lowering of JNK content in liver for 18% and for SAT for 7% compared to IR group, but there were no effect on VAT. So, HCFD caused JNK activation in investigated tissues independently to insulin sensitivity. Thus, SP600125 administration affected both subcutaneous and visceral adipose tissue as well as hepatocytes and decreased pJNK content. However, G0007 has tissue specificity towards insulin sensitive tissues that makes it more preferential for IR treatment.

P.5.2-045
Copper(I)-binding properties of de-coppering drugs for treatment of Wilson disease

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Wilson disease (WD) is an autosomal recessive genetic disorder caused by loss-of-function mutations in the P-type copper ATPase, ATP7B, which transports copper out of cells and is therefore crucial for biliary excretion of excess copper. WD is characterized by toxic accumulation of copper primarily in the liver and brain, leading to liver disorders and/or neuropsychiatric symptoms. Unlike many other genetic disorders, *WD* is treatable, primarily by copper chelation therapy, which mobilizes copper from the organism, enhancing its urinary excretion. Although a number of de-coppering drugs are currently available, their Cu (I)-binding affinities and interaction with essential cellular copper proteins have not been quantitatively characterized to date. By using an ESI MS-based approach we have determined the Cu(I)-binding affinities of five major de-coppering drugs – penicillamine, trientine, 2,3-dimercapto-1-propanol, 2,3-dimercaptosuccinate and tetrathiomolybdate – by exploring their ability to extract Cu(I) ions from two cellular Cu(I)-binding proteins, which have different Cu(I)-binding affinities. We report that Cu (I)-binding affinity of de-coppering drugs vary by four orders of magnitude and depends from the number of sulfur atoms in the drug molecule. Obtained structure-activity relationships are important for understanding the action of copper-chelating drugs and elaboration of new generation drugs that may provide better therapeutic outcomes.

P.5.2-046
Human genomic DNA and serum albumin binding of three novel zinc complexes with NSAID niflumic acid

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Fenamates, derivatives of N-anthranilic acid, belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs) which are widely used for their analgesic anti-inflamatory and antipyretic activity. Recently, transition metal complexes of NSAIDs are widely studied, since many of these compounds were found to be more effective than the original drug. As a part of our studies three novel Zn(II) complexes with NSAID niflumic acid (H*nif*) were synthesized and described, namely; Zn $(MeOH)_4(nif)_2$] (1), [Zn(*cyclam*)$(nif)_2$] (2) and [Zn$(nif)_2$(*tmen*)] (3), where *nif* is deprotonated niflumic acid, *cyclam* is 1,4,8,11-Tetraazacyclotetradecane and *tmen* is *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine. These complexes have been characterized by infrared spectroscopy, elemental and thermal analysis and single-crystal X-ray structure analysis. Fluorescence binding studies of the prepared compounds with human genomic DNA-EB (ethidium bromide) complex suggest that all complexes are able to bind to DNA via intercalation. In order to study binding to different types of human genomic DNA two samples of DNA were used for the experiment; control (cDNA) and DNA from the tissue with aortic aneurysm (aDNA). Obtained results indicate different binding properties of respective complexes; while 1 binds to both samples of DNA with comparable strength, 2 shows greater affinity towards aDNA and on the other hand 3 towards cDNA. Additionally, all complexes exhibit good binding affinity to human serum albumin (HSA) with high binding constant.

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P.5.2-047
Discovery and the mechanism of action of novel selective carbonic anhydrase IX inhibitors for anticancer therapy

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Carbonic anhydrase enzyme catalyzes a simple but highly important reaction of CO_2 hydration to acid and bicarbonate, every living cell contains this enzyme. Humans express 12 catalytically active highly homologous isozymes that participate in numerous pathological processes, glaucoma, high-altitude sickness and cancer. CA IX is nearly exclusively expressed in tumor tissue and its selective inhibition could potentially treat solid hypoxic cancers. We have synthesized and tested over 700 CA inhibitors and discovered ones that inhibit isoform IX with picomolar affinity and over 1000-fold selectivity over other vital isoforms. Rational design used over 50 high-resolution X-ray crystallographic structures of inhibitors bound to most CA isoforms. Furthermore, a detailed analysis of intrinsic thermodynamics of binding was important to understand the structure-activity correlations of these inhibitors. CA-compound interaction was measured by thermal shift assay, isothermal titration calorimetry and enzymatic stopped-flow CO_2 hydration assay. The Kd reached 50 pM for the strongest binders, among the tightest known protein-ligand binding reaction. There is abundant evidence that CA IX, essentially absent in healthy human body, is overexpressed on cell surface of hypoxic metastatic highly invading cancers. The action of CA IX caused the pH to decrease from 7.4 to 6.2. Our inhibitors fully stopped this acidification of the environment in hypoxic HeLa cell cultures, prevented hypoxic spheroid formation for numerous cancer cell lines and reduced the number of migrating highly metastatic cells. Heterologous human CA expression in *Xenopus* oocytes demonstrated nanomolar affinity and high selectivity towards CA IX over other isoforms. Compound toxicity was tested in zebrafish embryos and was found to be lower than CA inhibitors-drugs used in clinic. Thus this series of compounds are promising candidates for animal testing and development into anticancer drugs.

P.5.2-048
Comparable effects of nitrate ions on growth and survival of *Lactobacillus paracasei* subsp. *paracasei* and *Escherichia coli* BW 25113

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The main dietary sources of nitrate (NO_3^-) and nitrite (NO_2^-) ions are vegetables, foodstuffs (used as a food additive) or volatile nitrogen oxides (environmental pollutants), some of which are converted to nitrate or nitrite in the body. The nitrate ion itself has low toxicity, but there is possibility of conversion of nitrate to nitrite, a reaction that is catalysed by the bacterial enzymes. These ions are toxic not only for eukaryotic but also for prokaryotic cells. Today there is a grate interest to the effects of nitrate ions on human gut microflora. The aim of this study is to investigate the comparable effects of different concentrations

(2, 4 and 6%) of sodium nitrate ($NaNO_3$) on growth (during 24 h) and H^+ efflux throughout plasmatic membrane (PM) of human gut lactic acid bacteria (LAB) *Lactobacillus paracasei subsp. paracasei* and laboratory model parent strain *Escherichia coli* BW 25113. *L. paracasei* also used as probiotic strain. Bacteria were grown anaerobically in MRS (for *L. paracasei*) and peptone (for *E. coli*) broths or agars. Growth lag phase duration, colony forming units (CFU) number and H^+ efflux of bacteria was determined at different concentrations of $NaNO_3$. The H^+ efflux across PM were determined using ion selective electrode. It was shown, that in presence of 2% $NaNO_3$, bacterial growth and H^+ efflux were not suppressed, but 4% prolonged lag phase duration in ~2 fold for *E. coli* and ~1.8 fold for *L. paracasei*, H^+ efflux was increased by ~20 and 15% respectively. The CFU number was decreased by ~28 and 32% respectively, but 6% $NaNO_3$ was suppressed the CFU number of *E. coli* and *L. paracasei* by ~90 and 40% respectively, while H^+ efflux suppressed totally for both cases. According to obtained results *L. paracasei* subsp. *paracasei* is more resistant to nitrates than *E. coli* BW 25113. Resistance to different chemicals is one of the probiotic properties of LAB, therefore these results could be used in food industry and medicine.

P.5.2-049
Maternal serum hepcidin and IL-6 levels in preterm birth

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Termination of the pregnancy after 20th week and before the 37th week is defined as premature delivery. Approximately 10–11% of all deliveries end prematurely. Preterm birth is the most important cause of perinatal mortality and morbidity. Inflammation and infection have been shown to significantly increase the synthesis of hepcidin and IL-6 is responsible for this increase. In our study, the relationship between preterm delivery threat (EDT) and anemia and inflammation was planned. To investigate the concurrent effects of hepsin and IL6 in the pathogenesis of preterm labor with EDT patients and control grups. Our cases were 28th-35th gestatonal week in which the patients were followed up with EDT diagnosis (n = 54) and the control group (n = 26) and consisting of normal pregnancies at the same gestational weeks. In preterm, term and control groups, hepcidin and IL-6 was measured by ELISA method. In the preterm group, serum hepcidin and IL-6 were significantly increased compared to control and term groups, and serum iron was found to be significantly decreased ($P < 0.001$). In the term group, serum IL-6 was significantly increased according to the control group and serum iron was found to be significantly decreased ($P < 0.001$). There was a statistically significant negative correlation between hepcidin, IL-6 levels and birth week. Correlation coefficient (r) calculated as 57.8% ($P < 0.01$) for hepcidin and 43.5% ($P < 0.01$) for IL6. Negative correlation was found between cervical length and maternal serum hepcidin and IL-6 levels and the p values were $r = -0.490$ ($P < 0.0001$) and $r = -0.623$ ($P < 0.0001$). According to the results of our study, there was a negative correlation between the rising values of hepcidin and IL-6 and the birth week. These findings include preterm delivery; inflammation may play an important role. It has also been shown that hepcidin, and IL-6 levels in maternal serum can be important biomarkers for predicting preterm birth in EDT cases.

P.5.2-050
New approaches to the structural aspects of polyamine oxidase activation by heterocyclic analogues of polyamines as potential antitumor agents

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Di- and polyamine oxidases (DAO and PAO), which regulate the polyamine (PA) levels, may have direct or mediated effects on the enhanced cell proliferation and tumor growth. Our previous results showed that enzymatic activity of PAO can be down-regulated by high concentrations of PA and vitamin B_6 deficiency in early stages of hepatic malignization. In the late phase of neoplastic transformation the DAO function may be genetically regulated by arrest of apoenzyme synthesis. Elimination of DAO and PAO activity in tumor cells may be a kind of defense against toxic and proapoptogenic products of PA oxidation. Thus the activators of PA catabolic enzymes can have the cancerostatic potency. The goal of the study was to test the ability of some chemical compounds to activate PA oxidation. So, bisuracil PA analogues demonstrated activation of PA catabolism in acellular test-systems of both tumor and regenerating tissues. This is the probable reason for their cancer (CaOv) cell growth suppression in culture. The influence of acetophenone, azafluorene, benzimidazole, and dioxaboreninopyridine derivatives on the rate of PA oxidative deamination was evaluated in the acellular test-system of the rat regenerating liver. Only azafluorene compounds were activators of polyamine catabolism, especially: 1-amino-9-phenylamino-4-azafluorene and 1-amino-2-bromo-4-azafluorene-9. Docking with yeast PAO enzyme Fms1 using Molegro Virtual Docker software was performed with flexibility in torsion angles of ligands. There were identified few critical atoms in the sphere radius of 12Å from the PAO active center: His67, Tyr450, His191, Trp174, Gly487. These are the most important sites for the tested compounds. These results reveal the structural basis for the design and synthesis of novel activators of polyamine catabolism as potential antitumor agents.

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P.5.2-051
Antibacterial activity of *Trichoderma harzianum* Rifai – producers of enzyme L-lysine-α-oxidase

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The concentrate of culture liquid of *Trichoderma harzianum* Rifai being producers of the enzyme L-lysine-α-oxidase was gained after cultivation in the equipment of Experimental technological installation of the G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms (Pushchino, Russia). The concentrate has an activity 0.54–0.56 U/ml. The influence of this concentrate on the growth of mycoplasmas has been studied for the first time: two species of the family *Mycoplasmataceae*: *Mycoplasma hominis* (Mh) and *Mycoplasma fermentans* (Mf) and one species of the family Aholeplasmataceae: *Aholeplasma laidlawii*

(Al). For the growth of mycoplasmas was used broth (Difco PPLO Broth, Becton, Dickinson, USA) with adding of 20% horse serum, 2% fresh yeast extract, 1% arginine or 1% glucose (dependent on kind of mycoplasmas), and 0.005% phenol red indicator. 10-fold dilutions in physiological saline were used for serial titration. The material from all dilutions was seeded into 0.3% agar (BBL Mycoplasma Agar Base, Becton, Dickinson, USA) with the same additives as in the broth. After 3 days, the number of colonies in the last two samples from several dilutions was counted and the average colony count was calculated. The culture titer was expressed in the average number of the colonies, multiplied by cultivation dilution. For the experiment the culture with the previously known titer was taken. It was shown that the culture liquid of *Trichoderma harzianum* Rifai with the activity of L-lysine-α-oxidase 0.54–0.56 U/ml inhibits growth of *Mycoplasma hominis* after a preliminary contact. The degree of growth inhibition depends on the seed dose of mycoplasma and the content of *Trichoderma harzianum* culture fluid.

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P.5.2-052
Specificity of olivomycin and its derivatives to nucleotide sequence of the double helix

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The aureolic acid family antibiotics olivomycin, chromomycin and mithramycin are promising precursors for development of new anticancer drugs. Their antitumor properties are presumably attributed to the inhibitory effects on replication and transcription upon their binding to the minor groove of the DNA double helix. Although their preferences to GC rich sequences is known, the exact sites of binding to genomic DNA still need to be elucidated. In this work, we determine the specificity of olivomycin and its several derivatives to GC rich sequences of nucleic acid. Olivomycin exhibits strong preference to tetranucleotide sequences SGGS and SGCS (where S stands for G or C) over SCGS. We demonstrate that these recognition rules arise from kinetic aspects of the drug-DNA interaction and we present structural explanation for such behavior. The revealed mode of the antibiotic-DNA binding provides valuable basis for designing of new anticancer compounds.

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P.5.2-053
The medicinal potential of synthetic and naturally occurring anticancer pyran scaffolds

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Pyran is a therapeutically vital oxygen containing heterocyclic moiety which exhibits an array of credible pharmacological properties. Pyran is also one of the important structural units found widely in natural products, such as coumarins, benzopyrans, sugars, flavonoids, xanthones and other natural products. The diverse anticancer capabilities of pyrans have been additionally evidenced by a number of recent publications, which have demonstrated that this heterocycle has been a focal point for researchers worldwide. This review provides a summary of pyran-based anticancer compounds reported in past years and focuses on advancements in the field of naturally occurring pyrans as anticancer agents. The discussion will also include the structure-activity relationships, along with the structure of the most promising molecules, their biological activities against several human cancer cell lines, mechanistic insights discovered through the pharmacological evaluation, and molecular modeling of pyran-based molecules. Consequently, an overview of the state-of-the-art on pyrans and their analogs as anticancer candidates is presented. The promising activities revealed by these pyran-based scaffolds undoubtedly places them on the front stage for the discovery of prospect drug candidates and could also be of great interest to researchers working on the synthesis of antitumor drug candidates.

P.5.2-054
Alginate microencapsulated capsaicin reduces inflammation and stimulates extracellular matrix production by dermal fibroblasts cells

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Capsaicin, a major ingredient of hot pepper, exhibits anti-inflammatory properties and thus it has been traditionally used in topic formulations as adjuvant therapy in several chronic diseases. However, some side effects have been reported after its topic use. Consequently, the aim of our study was to develop a new formulation of capsaicin consisting in its encapsulation in alginate microparticles. In this view, the capsaicin alginate microcapsules were washed with PBS supplemented with 5% antibiotic-antimicotic and then immersed in complete culture medium (*Dulbecco's Modified Eagle's Medium* supplemented with 1% antibiotic-antimicotic and 10% Foetal Bovine Serum). Capsaicin free alginate microcapsules were treated identically and were used as reference in this experiment. In the end, the results were compared to those obtained after cell treatment with unencapsulated capsaicin. Briefly, during 24 h, 5 extracts were collected at specific time points and stored at −20°C until analysis. The anti-inflammatory potential of these extracts was tested on *lipopolysaccharide (LPS)* stimulated mouse macrophage cells (RAW 267.4 cell line) by evaluating the protein expression of IL-1β, IL-6, IL-10, IL-12p70, MCP1, MIP1α, RANTES and TNF α. Additionally, human dermal fibroblasts (CCD-1070Sk cell line) were exposed to the defrosted extracts for one week and fibronectin protein expression was evaluated at this time point by fluorescence microscopy. Our results show that the inflammatory status in LPS stimulated RAW 264.7 cells was lower after the treatment with capsaicin alginate microcapsules than capsaicin alone. Furthermore, the treatment with capsaicin alginate microcapsules determined a higher production of fibronectin by CCD-1070Sk cells than the treatment with capsaicin free alginate

microcapsules. Consequently, capsaicin alginate microcapsules might be further used for the development of pharmacological formulations for topic administration in diabetic neuropathy.

P.5.2-055
iBodies: modular synthetic antibody mimetics based on hydrophilic polymers decorated with functional moieties as tools for molecular recognition, imaging and specific drug delivery

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Monoclonal antibodies are widely used tools for research, diagnostics and anticancer therapy. However, their use is compromised by high production cost, limited stability, and complicated chemical modification. Recently, we reported the design and development of synthetic polymer conjugates capable of replacing antibodies in number of *in vitro* applications such as ELISA, flow cytometry, immunocytochemistry, and immunoprecipitation, and also useful for specific imaging of tumors and delivery of anticancer drugs to diseased tissues. The conjugates, named "iBodies", consist of a hydrophilic copolymer decorated with low molecular-weight compounds that function as targeting ligands, affinity anchors (typically biotin), and imaging probes (fluorophores or radiolabels). We prepared specific conjugates targeting several important proteins, such as prostate specific membrane antigen, fibroblast activating protein, carbonic anhydrase IX and number of other medicinally relevant enzymes and cancer antigens. We used these iBodies for enzyme inhibition, protein isolation, immobilization, quantitation, and live cell imaging. Specific iBodies could also be prepared for a non-enzyme proteins for which a suitable ligand is known, such as opioid receptors or proteins containing a polyhistidine tag (His-tag). Our data demonstrate that this highly modular and versatile polymer system can be used to produce inexpensive and stable antibody substitutes directed toward virtually any protein of interest with a known ligand, and could be leveraged for specific anticancer drug delivery.

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P.5.2-056
Novel PEG-containing polymeric nanocarrier enhances anticancer activity, provides circumvention of drug-resistance mechanisms, and protects of general toxicity

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We present the results of *in vitro* and *in vivo* application of a novel polymeric nanocarrier of 5-*tert*butylperoxy-5-methyl-1-hexene-3-yne and glycidyl methacrylate co-polymer containing PEG brushes (PNC). The anticancer drugs, such as Doxorubicin (Dox) and synthetic 4-thiasolidinone derivatives (antineoplastic activity towards 60 lines of human tumor cells was confirmed at NCI in USA) were immobilized on this PNC. Thus, universal binding characteristics of the developed PNC were demonstrated. Enhanced uptake of the immobilized anticancer drugs and their cytotoxic action (cell cycle arrest, DNA damage, as well as the apoptotic cell death (cleaved caspase-3, ERK1/2-kinase) in approximately 10 times lower doses for achieving the effect observed at the action of free drug were detected. PNC was also tested towards murine NK/Ly lymphoma *in vivo,* and in both experimental models the nanoformul ation of Dox resulted in 100% cured animals already at low concentrations (0.1 mg/kg), while free Dox solely extended survival time. Additionally, we present data that a PNC distinctly enhanced (2–18 times depending on cell line) the antineoplastic activity of Dox in 5 tested cancer cell lines including several cellular models. Complexation of the antineoplastic derivatives of 4-thiasolidinone with a PNC substantially lowered the activity of the biochemical indicators of the cardiotoxic, hepatotoxicity and nephrontoxic actions, namely creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, α-amylase and lactate dehydrogenase in rat blood serum. Thus, the application of PNC proved to be a promising strategy to enhance the efficacy and reduce general toxicity of the immobilized anticancer drugs used for treatment of drug-sensitive and drug-resistant cancer experimental models.

This study were approved by the Ethical Committee of Danylo Halytsky Lviv National Medical University (Ukraine), Protocol N4 from 18.04.2016.

P.5.2-057
Anti-adenoviral activities of 5-aminouracil and thiouracil derivatives

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Human adenoviruses (HAdVs) are a large group of viruses causing a broad variety of clinical manifestations. Most infections are self-limited in the setting of a normal immune response, although fatal disease can occur in immunocompromised individuals. Currently, specific therapy for adenovirus infection, other than supportive and symptomatic treatment, is not available. It has been suggested in our previous work that several 5-substituted pyrimidine nucleosides might reduce the replication of HAdVs. Therefore, we propose here to further explore therapeutic potential of

newly synthesized 5-aminouracil and thiouracil derivatives. The starting 5-aminouracils were alkylated with various benzyl bromides, which led to the formation of N^1-substituted 5-aminouracil derivatives. 2-Thiouracil derivatives were obtained by alkylation of the starting 2-thiouracil with different chloroacetanilides containing diverse modifications in the aromatic ring. To assess the inhibitory potency of these compounds, we infected HEK293 cells with HAdV 5 at an MOI of 1 FFU/cell. The compounds at concentrations of 0.5, 2.5, 5, 10, 15 and 25 μM were added 3 h post infection. Newly synthesized viral genomes were detected via quantitative real-time PCR after 24 h of incubation. All compounds were non-toxic at effective concentrations according to MTT test. IC50s of 0.5 and 8.9 μM were observed for the 5-aminouracil derivatives with the highest level of antiviral activity. Furthermore, six of tested thiouracil derivatives reduced viral genome replication by more than 90% in adenovirus-infected HEK293 cells. IC50 of 0.06 μM was observed for the most effective thiouracil derivative. 5-aminouracil and thiouracil derivatives inhibit adenoviral replication in the low micromolar range and thus can be considered for the development of antiviral agents.

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P.5.2-058
Synthesis of modified uracil derivatives and their potential use as prodrugs

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Fluorinated derivatives of uracil and uridine can act as prodrugs in the cancer therapy. The chemical synthesis of 5-fluoroisocytosine and the fluorinated 2′-O-methyluridine was performed. 5-Fluorouridine and 5-fluorodeoxyuridine were obtained by the enzymatic synthesis using thymidine phosphorylase (EC 2.4.2.4) from *Escherichia coli*. Thymidine phosphorylase mediates the nucleobase-exchange reaction to convert thymidine or uridine to modified nucleosides possessing a functional group at the 5th position and allows biosynthesis of modified nucleosides that are not possible to obtain by organic synthesis methods. The obtained compounds were converted into the 5-fluorouracil, a cancer drug, by action of newly discovered enzymes, such as isocytosine deaminase and nucleoside hydrolase. In conclusion, it was demonstrated that the synthesized fluorinated uracil derivatives could be used as prodrugs in the cancer therapy.

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P.5.2-059
Polyelectrolyte nanocapsules formed through layer by layer approach as an alternative camptothecin delivery system

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Camptothecin is natural anticancer compound isolated from the bark of *Camptothecca acuminata*. The synthetic camptothecin was firstly obtained by Stork in 1971 and, afterward, the route of synthesis had been modified and the optimized procedure for synthesis was demonstrated by Comins in 2001. Numerous studies involving different human as well as animal cancers confirmed excellent antitumor activity over a wide spectrum of human and animal cancers. However, the direct formulation is limited by insolubility in water and other biocompatible solvents, low stability in physiologic conditions, rapid plasma clearance and high systemic toxicity. An important approach to overcome limits in the clinical application of camptothecin can be brought by nanotechnology. Various type of nanocarriers have already been employed for camptothecin delivery and some of them have already reached clinical trials. In our study, pegylated polyelectrolyte nanocapsules were utilized to prepare camptothecin delivery system. Nanodroplets containing the camptothecin were encapsulated in polyelectrolyte shells formed by the layer by layer (LbL) technique. The size of synthesized nanocapsules was around 120 nm. The surface of the nanocapsules was pegylated through the adsorption of the pegylated polyelectrolyte (PGA-g-PEG). The biological effects of encapsulated camptothecin on two tumor cell lines: mouse colon carcinoma cell line CT26-CEA and the mouse mammary carcinoma cell line 4T1 were studied. Encapsulated camptothecin retained its strong cytotoxic/cytostatic activity. Observation suggests, that encapsulated drug, as well as free one, arrests the cell cycle progression at the G2/M stage. The obtained results indicate that pegylated polyelectrolyte nanocapsules are promising an alternative way of camptothecin delivery.

P.5.2-060
The effect of synthetic methoxy derivatives of resveratrol on the expression of enzymes involved in synthesis of estrogens in breast cancer cells

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Intratumoral synthesis and metabolism of estrogens is thought to be a major risk factor in development of breast carcinoma particularly in postmenopausal women. Key role in intratumoral estrogens synthesis plays aromatase converting androgens to estrogens along with 17 beta-hydroxysteroid dehydrogenases (17-beta HSD), sulfotransferases (SULT) and estrogen sulfatases (STS). The modulation of expression and activity of these enzymes is considered important strategy of breast cancer chemoprevention. Resveratrol was shown to be non-selective and moderate aromatase inhibitor. Substitution of hydroxyl groups with methoxy group may potentially improve its anti-estrogenic activity. In this study we assessed the modulation of aromatase, 17-beta HSD 1 and 2, STS, SULT1E1 by resveratrol and its three methoxy derivatives: 3,4,2′trimethoxy-*trans*-stilbene (3MS), 3,4,2′,4′-tetramethoxy-*trans*-stilbene (4MS) and 3,4,2′,4′,6′-pentamethoxy-*trans*-stilbene (5MS) in breast cancer cell lines: MCF7 and MDA-MB-231. Cells were treated with stilbenes for 72 h and mRNA transcripts and protein levels were evaluated. In both cell lines aromatase expression was affected the most. In MCF7 cells resveratrol and 5MS at the dose of 5 μM decreased both mRNA transcript and protein levels of aromatase by ~20%. In MDA-MB-231 the most efficient inhibitor of this enzyme expression was 3MS, which reduced aromatase protein level by ~30%. In MCF7 cells treatment with all analogs of resveratrol resulted in induction of SULT1E1, but had no effect on the expression of the other investigated enzymes. In MDA-MB-231 none of these enzymes was affected. These results indicate that methoxy derivatives might be slightly more efficient inhibitors of aromatase and

inducers of SULT1E1 than resveratrol. However, other modifications of stilbene ring should be search to improved resveratrol anti-estrogenic activity.

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P.5.2-061
Steroidal pyrazoles and pyrazolines as potent antfiproliferative agents

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Among the five-membered N-containing heterocycles, pyrazoles and 2-pyrazolines represent important scaffolds in heterocyclic chemistry research in view of the potentially high bioactivity profile. The introduction of an arylpyrazoline structural unit into a sterane core may also be beneficial from a pharmacological aspect by modifying the stereochemical properties and binding abilities of the original molecule. Several D-ring-fused analogs have been reported to display antiproliferative or antiandrogenic effect or to inhibit one of the key regulatory enzymes of steroid biosynthesis. Novel sex hormones containing a pyrazole or pyrazoline ring condensed to ring D of the sterane core were efficiently synthesized with different, readily available hydrazine derivatives. The reactions proceeded in a stereoselective manner to furnish 2-pyrazolines in good to excellent yields. The syntheses of pyrazoles were carried out *via* cyclocondensation reaction to afford a diverse set of novel derivatives, regioselectively, depending on the pH of the medium. The compounds were screened *in vitro* on four human breast cancer cell lines (MCF7, T47D, MDAMB-231 and MDA-MB-361) by the MTT colorimetric assay. Some pyrazoline derivatives exerted *in vitro* antiproliferative activities against all members of the utilized breast cancer panel which were higher than or comparable to those of the reference cisplatin. Among the pyrazoles, *p*-cyanophenyl derivative proved to be highly active against T47D cells. In order to obtain further information concerning its antiproliferative properties, cell flow cytometric cycle analyses were performed.

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P.5.2-062
Synthesis and modulation of NF-κB pathway by new conjugates of oleanolic acid oximes and aspirin

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Naturally occurring triterpenoids, such as oleanolic acid (OA), and their synthetic analogues possess the cytoprotective, anti-inflammatory and anti-tumorigenic activities and thus may be considered promising chemopreventive and/or chemotherapeutic agents. The chemopreventive activity of these compounds may results from the modulation of the nuclear factor κB (NF-κB), which controls the expression of inflammatory mediators including cyclooxygenase-2 (COX-2). The aim of the study was the synthesis of a series of novel oleanolic acid (OA) oximes and their conjugates with acetylsalicylic acid (aspirin) and the evaluation of their anti-inflammatory activity in hepatoma cell line HepG2. Our initial study showed cytotoxic activity of some oleanolic acid derivatives with modified A ring and the C-17 –COOH group. The series of oleanolic acid derivatives in which the C-3 hydroxyl function was transformed into =NOX group (X = proton or acetylsalicylic acid moiety) was synthesized. MTT assay was used to select the most active compounds and expression of NF-κB p50 and p65 active subunits and their binding to DNA along with COX-2 protein level was assessed. The oxime derivatives of oleanolic acid diminished the expression and protein level of NF-κB p50 and p65 active subunits and their DNA binding capacity (about 20–40%, respectively). The expression and protein level of *COX-2* was decreased after treatment with acetylsalicylic acid hybrids. Overall these results indicate that the new oxime derivatives of oleanolic acid and their conjugates with acetylsalicylic acid down-regulate *COX-2* expression in HepG2 cells by modulating the NF-κB signaling pathway and suggest that these compounds may have chemopreventive or therapeutic potential.

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P.5.2-063
Investigation of vitamin D and k2 status in patients with chronic obstructive pulmonary disease and smoking

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Vitamins D and K are the fat-soluble vitamins that have received great attention in recent years. Vitamin K and vitamin D work together to increase Matrix GLA Protein (or MGP), the protein responsible for protecting your blood vessels from calcification. The biological role of vitamin k2 is to help move calcium into the convenient region in your body, such as bones and teeth. It also helps remove calcium from areas where it shouldn't be, such as arteries and soft tissues. This effect may account for its beneficial role in reducing high blood pressure as well as the number of early cardiovascular disease. We investigate two groups that have already have risk factor for cardio vascular dissease. A total of 180 patients aged 29–80 years from Konya, presenting to Selçuk Faculty of Medicine hospital were included in the study. Diagnosis of Chronic Obstructive Pulmonary Disease (COPD) was confirmed based on airflow limitation defined as FEV1/FVC ratio < 70%. Serum 25-hydroxy-vitamin D and k2 level were measured in 60 COPD patients and 60 smoking people to compared with 60 age and gender matched controls. In each of the three groups, in the resume of the individual's rheumatologic disease, autoimmune disease, oncologic disease, and patients receiving multi-vitamin supplements were excluded from the study. COPD subjects had significantly lower 25-hydroxy-vitamin D concentrations control subjects ($P < 0.005$). There is no difference between plasma k2 level all groups. Mean± vitamin k2 levels were 1.43 ± 1.69 nM control, 1.19 ± 1.20 nM smoking and COPD 1.67 ± 1.96 nM respectively. These findings suggest that there is no correlation between vitamin K2 and 25-OH vitamin D level.

P.5.2-064
Novel structural motif for cytostatics – from synthesis to identification of biological target

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The limitations of many anticancer drugs have stimulated the search for new substances with enhanced pharmacological properties, such as low cytotoxicity to normal cells. Lately, we reported the synthesis of a novel pentamethinium salt with an incorporated quinoxaline unit and described its structure. Herein, we present the biological characteristics of this system before applying it *in vivo*. First, the subcellular distribution of this autofluorescent compound was studied in U-2 human sarcoma cells. When the cells were exposed to the methinium system, the drug rapidly accumulated in the mitochondria until cell death. Growth inhibition (IC50) was determined by monitoring cell viability. The tested substance showed high cytotoxicity towards the malignant cell lines, but was not cytotoxic to non-malignant BJ fibroblasts. Next, the system was tested *in vivo* on NuNu mice injected with malignant U-2 cells. The application of our system resulted in significant suppression of tumor growth. Our findings indicate that this compound could well improve cancer treatment while having low impact on normal cell lines.

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P.5.2-065
Discovery and substrate specificity of a novel nucleoside hydrolase

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Modified nucleotides are present in various RNA species and are critical for folding, stability, and interaction with cellular proteins. Biosynthesis of pyrimidine nucleotides and their modified derivatives in RNA is well studied, yet much less is known about the cellular degradation of these compounds and their salvage. Using methods of bacterial genetics, an *E. coli* strain, unable to convert 2′-*O*-methyluridine into uracil and therefore to grow on minimal medium, was created. The growth phenotype of such mutant was restored by introducing a genome fragment from the metagenomic libraries. Such fragment contains 2 ORFs, one encoding a bacterial nucleoside hydrolase, and the other – a ribose aldolase. The recombinant nucleoside hydrolase was expressed in *E. coli*, purified, and its substrate specificity was investigated.

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P.5.2-066
Ring A- or D-modified steroids with selective anticancer activity against androgen-independent prostate cancer cell lines

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Experimental findings have revealed that modification of the well-defined stereostructure and/or the substitution pattern of natural sex-hormones by different heterocycles may alter not only the physicochemical and pharmacokinetic properties but also the original bioactivity of the parent compound. The most reasonable and frequent alterations are introduced onto ring A or D of the sterane framework, which have essential roles in the development of hormonal activity. Over the past decades, several sex hormone-derived heterocycles have been found to possess antitumor rather than hormonal effect either by acting as an inhibitor of one of the enzymes of the steroid hormone biosynthesis or by affecting the cell cycle and inducing apoptosis in tumor cells. Pyrimidine moieties were efficiently incorporated into ring D- or A of androstane skeleton *via* multicomponent heterocyclization/oxidation sequences. The one-pot construction of six-membered heterocycles was carried out from steroidal β-ketoaldehydes or ketones, different arylaldehydes and ammonium acetate or urea as *N*- or *N*-*C*-*N* fragment sources. The synthesized compounds were tested *in vitro* on human cancer cell lines (PC-3, DU-145, MCF-7) as well as on non-cancerous fibroblast cells (MRC-5) by the MTT assay in order to assess their potential in pharmaceutical applications. As a result of the *in vitro* screen, a remarkable structure-activity relationship has been observed as the acetylated 1*H*-pyrimidin-2-one products exhibited higher toxicity compared to the deacetylated compounds. Furthermore, three 2′-arylpyrimidines were identified with prominent prostate cancer cell specificity. Regarding our findings, arylpyrimidines can be exploited as structural scaffolds of androstanes, motivating the rational design and pharmacological investigation of further derivatives. Financial support by the Hungarian Scientific Research Fund (OTKA K-109107) is gratefully acknowledged.

P.5.2-067
16,17-Seco-steroids as anticancer agents

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Antiproliferative and pro-apoptotic potential of selected steroidal 16,17-seco-16,17a-dinitrile derivatives against triple-negative breast cancer MDA-MB-231 cell line is presented in this research, as well as screening of their blood cells genotoxicity. Among tested seco-steroids, some compounds from a class of 16,17-seco-16,17a-dinitrile derivatives exerted strong antiproliferative effect against triple-negative breast cancer MDA-MB-231 cells, with no influence on the proliferation of the healthy cells (MRC-5). Anti-apoptotic potential of selected compounds was tested, comprising of flow-cytometric analysis, cell distribution or morphology changes and expression of pro-apoptotic proteins in the apoptotic signaling pathways. Genotoxicity tests were conducted in the aim to study pharmacological potential of these substances comprehensively. Almost all compounds affected the cell cycle distribution and induced apoptosis through increased expression of proapoptotic Bax protein and/or decreased expression of

antiapoptotic Bcl-2 protein. The completion of apoptotic process was confirmed by cleavage of PARP protein. In some cases inducing of apoptosis was in Caspase-dependent manner. Moderate genotoxicity of seco-steroids under study was noticed in a battery of genotoxicity tests, including chromosomal aberrations in peripheral blood lymphocytes, micronucleus and sister chromatid exchanges test and comet assay. Having in mind their great pro-apoptotic joined with low genotoxic potential and especially lack of their antiproliferative effect on the healthy cells, the results obtained indicate tested seco-steroids as potential candidates in anticancer drug development, with substantial biomedical potential.

P.5.2-068
Orally bioavailable prodrugs of a hydroxamate-based glutamate carboxypeptidase II inhibitor

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Glutamate Carboxy Peptidase II (GCPII, EC 3.4.17.21) also termed Prostate Specific Membrane Antigen (PSMA) is a zinc metallopeptidase that hydrolyzes N-acetylaspartylglutamate (NAAG) and produces thus free glutamate in the nervous system. Excess glutamate is highly pathogenic and has been shown to play a crucial role in many neurological disorders. Inhibition of GCPII reduces the extracellular glutamate levels and is therefore considered an attractive target for intervention. Over the last 20 years, great effort has been invested into discovery of orally bioavailable and brain/nerve tissue penetrable inhibitors of GCPII. Many of those compounds were found to be efficacious in several models of neuropathic pain, diabetic neuropathy peripheral neuropathy, stroke, amyotrophic lateral sclerosis (ALS), multiple sclerosis, schizophrenia, epilepsy and several others associated with pathological increase of glutamate concentration leading to excito-toxic effects and neuronal death. A major drawback that halted clinical development of GCPII inhibitors is the lack of their oral biovailability and low target tissue levels. 4-Carboxy-α-[3-(hydroxyamino)-3-oxopropyl]-benzenepropanoic acid (1) is a potent hydroxamate-based inhibitor of GCPII. Despite its high polarity, it exhibited good distribution to sciatic nerves, which is believed to be the site of action for GCPII inhibitors for attenuating neuropathic pain. 1, however, suffers from poor oral bioavailability, limiting its therapeutic utility as a treatment for chronic pain. Herein we report a series of prodrugs of 1, in which the hydroxamate and/or carboxylate group(s) was (were) masked to form more lipophilic species in an attempt to improve oral absorption. We present the design, synthesis, and pharmacological evaluation of prodrugs of 1 with novel promoieties attached to the hydroxamate group.

P.5.2-069
Design and synthesis of brain-targeted prodrugs of the glutamine antagonist 6-Diazo-5-oxo-L-norleucine

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6-Diazo-5-oxo-L-norleucine (DON) is a glutamine antagonist, which was isolated originally from Streptomyces bacteria found in Peruvian soil, and is one of a very few naturally occurring diazoketones. DON acts as an irreversible inhibitor of many glutamine utilizing enzymes critical for the synthesis of nucleic acids/proteins and the generation of alpha-ketoglutarate for energy metabolism. The anticancer and autoimmune activities of DON has been shown repeatedly in both preclinical and clinical studies. Although promising, clinical studies with DON were halted due to its marked dose-limiting toxicities, which were mainly gastrointestinal (GI)-related, as the GI system is highly dependent on glutamine utilization. Recently, we have demonstrated that DON inhibits glutamine metabolism and provides antitumor efficacy in a murine model of glioblastoma, although toxicity was observed. To enhance DON's therapeutic index, we utilized a prodrug strategy to increase its brain delivery and limit systemic exposure. Herein we describe the synthesis of several DON prodrugs designed to circulate inert in plasma and be taken up and biotransformed to DON in the brain. This strategy may provide a path to DON utilization in patients with brain tumors.

P.5.2-070
Collagen in production of novel medical and cosmetics drugs

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Unique molecular structure of collagen is a subject of close attention for scientists and specialists in different fields of Economy. Nature of physical and chemical and physiological and biochemical properties is caused by fibrillary form of spatial structure, specific aminoacid composition of primary structure and existence of multiple active functional groups on surface. Experiments *in vivo* and *in vitro* showed that collagen from fish and animal stuff is available to immobilization of antibiotics, metal ions and various aroma substances. Developed complexes from collagen are stable to externally acting factors, capable to structuring and have substantivity to heavy metal ions. Collagens also can be successfully used as biocorrector in role of matrix for biological active substances and elements. This mechanism of biological active substances immobilization opens availability of needed component delivery to aim organs. Approbation of such medical drugs as porous materials, gels and complex biological and active additives showed capabilities of application in cosmetology (rejuvenation and products purposeful aromatization), surgeries (while rehabilitation period for regenerative process, alimentary correction of reduction period after various diseases). Column chromatography, electrophorese, IR-spectroscopy, electronical microscopy, atomic force microscopy and other analysis allowed to study the structure of hydrates of fish-stuff collagens and animals collagens hydrolysates.

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P.5.2-071
Circulating endocan as disease-specific biomarker of diabetic vascular injury in patients with chronic hepatitis C

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Aim: To investigate the relationship between the endothelial biomarker endocan and diabetic vascular injury by comparing the selected biomarker for detecting vascular injury [vascular endothelial growth factor (VEGF)], and Nε-(carboxymethyl) lysine-advanced glycation end products (CML-AGEs) in patients with chronic HCV infection (CHC).
Methods and results: Eighty-five CHC patients with diabetes and 50 nondiabetic subjects were enrolled in the study. Circulating levels of endocan, CML-AGEs, VEGF, and tumor necrosis factor α (TNF-α) were assayed in serum samples by ELISA analyses. Compared with nondiabetic patients with CHC, the diabetic patients had higher levels of endocan, which were associated with increased AGEs-albumin (the ratio of CML-AGEs to albumin content). When the presence of liver cirrhosis was considered, the plasma levels of endocan were higher, as well as VEGF and TNF-α. Endocan positively correlated with AGEs-albumin level in all CHC patients with diabetes and this correlation was stronger in CHC patients who develop liver cirrhosis. In multivariate logistic regression analysis, the independent factors associated with the presence of compensated cirrhosis were high endocan levels and elevated levels of VEGF and AGEs-albumin.
Conclusion: The simultaneous monitoring of plasma endocan, AGEs-HSA and VEGF can be helpful for the alterations in vascular function control in diabetic patients with chronic HCV infection.

P.5.2-072
Isolation and analysis of phycobiliprotein from wild nostoc commune and its antioxidant activity evaluation

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Phycobiliprotein was isolated and purified from wild *Nostoc Commune* in Xuyi for the first time. The result of chromatographic analysis indicated that it was comprised of phycocyanin (PC) and allophycocyanin (APC), and the molecular weight of its two subunits detected by SDS-PAGE electrophoresis were15 kD and 23 kD, respectively. With common antioxidant butylated hydroxy toluene (BHT) or Vc as the positive control group, we measured the antioxidant activities of PC and APC through 5 antioxidant model systems. Results showed that in the 5 antioxidant systems, PC and APC exhibited relatively strong antioxidant activities in scavenging free radicals and anti-lipoperoxidation *in vitro*. On the aspect of total antioxidant activity, PC was the main antioxidant component of phycobiliprotein since it showed a stronger antioxidant activity than APC. On the aspect of free radical scavenging ability of PC and APC, the scavenging rate of DPPH free radical> the scavenging rate of ·OH radical> the scavenging rate of free radical was observed. On the aspect of antioxidant systems of rats *in vitro*, no significant difference was found between PC and APC, additionally, equivalent effects were shown between PC and APC and common antioxidant Vc.

P.5.2-073
Design and synthesis of brain-targeted prodrugs of the glutamine antagonist 6-Diazo-5-oxo-L-norleucine

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6-Diazo-5-oxo-L-norleucine (DON) is a glutamine antagonist, which was isolated originally from Streptomyces bacteria found in Peruvian soil, and is one of a very few naturally occurring diazoketones. DON acts as an irreversible inhibitor of many glutamine utilizing enzymes critical for the synthesis of nucleic acids/proteins and the generation of alpha-ketoglutarate for energy metabolism. The anticancer and autoimmune activities of DON has been shown repeatedly in both preclinical and clinical studies. Although promising, clinical studies with DON were halted due to its marked dose-limiting toxicities, which were mainly gastrointestinal (GI)-related, as the GI system is highly dependent on glutamine utilization. Recently, we have demonstrated that DON inhibits glutamine metabolism and provides antitumor efficacy in a murine model of glioblastoma, although toxicity was observed. To enhance DON's therapeutic index, we utilized a prodrug strategy to increase its brain delivery and limit systemic exposure. Herein we describe the synthesis of several DON prodrugs designed to circulate inert in plasma and be taken up and biotransformed to DON in the brain. This strategy may provide a path to DON utilization in patients with brain tumors.

The Human Microbiome

P.5.3-001
Proteomic analysis of biofilm on central venous catheter

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Biofilm formation on medical devices, particularly central venous catheters (CVCs), results in significant morbidity and mortality. Bacteria associated with the CVCs surfaces and embedded in an extracellular matrix are able to survive antimicrobial agents, and the host system. The goal of presented study was to elucidate the host and pathogen proteins of CVC biofilms *in vivo*. The set of CVCs (n = 150) was collected after 7 days of use from premature infants in the neonatal intensive care unit. To confirm the formation of biofilms on CVCs was used Scanning electron microscopy (SEM)(Quanta 200 3D (FEI Company, USA)). Further catheters were divided in half. One part of CVC was incubated in a tryptone soya broth (37°C, 24 h) to detect biofilm forming bacteria. Bacteria were cultivated on Columbia blood agar and identified by MALDI MS (AutoflexIII Bruker Daltonics, Germany). The biofilm of second part of CVC was degradated by lysostaphin (20 a.u.) and sonication (30 min, 37°C). The proteins of CVC biofilms and corresponding isolates were digested with trypsin in gel. Chromatography-mass spectrometric analysis of the peptides was performed by Q-Exactive HF(Thermo Scientific, Germany). Mascot v.2.5.1. software was used to protein identification. According to SEM data, the biofilms were observed on the inner

surface of CVCs. They were consisted of fibrin, formed elements of blood and bacteria embedded in a matrix. On the surfaces of CVCs 50 different isolates were identified, most of them were *S. epidermidis* (68%). The proteomic analysis revealed 735 proteins of *S. epidermidis* biofilm on the CVCs and 755 proteins of *S. epidermidis c*ulture. The ratio of bacterial proteins and human proteins in CVC biofilms was 1:6. The most interesting proteins of CVC biofilms related to Integral membrane components (GO:0016021). The organization of the cell wall (GO:0071555) and Catabolism of peptidoglycans (GO:0009253).

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P.5.3-002
The oral microbiome is predictive of child weight gain

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One in six children in the US are overweight or obese. Obesity is a complex disease with many environmental influences; in particular, the microbiome is emerging as important. Characteristic perturbations in the gut microbiome have been shown in obese adults and adolescents compared to their normal-weight peers. Less is known about the microbiome influence on weight gain in early childhood. This knowledge is critical because children with rapid weight gain have a greater risk for developing obesity later. Furthermore, the potential connection between *oral* microbiome and childhood obesity has not been studied. We studied the relationship between infant weight gain in the first two years and gut and oral microbiome at 2 years. For this, we recruited 215 mother-child dyads. Child weight & length were measured at 6 time points during these 2 years and child mouth swabs and stool were collected at 2. We computed conditional weight gain (CWG) scores- a metric used to determine rapid infant weight gain, and also modeled growth curves using novel Functional Data Analysis (FDA) techniques. The oral and gut microbiome was surveyed with 16S sequencing. Analysis of the oral microbiome revealed that it is predictive of weight gain. Children with rapid weight gain had a lower diversity and elevated Firmicutes-to-Bacteroidetes ratio in their oral microbiome community than children without rapid weight gain. Additionally, using multiple linear regression analyses, we discovered that gut microbiome is mostly determined by diet (vegetables & fruit and vegetables & meats are significant predictors of the gut microbiome diversity and Firmicutes-to-Bacteroidetes ratio, respectively). This study for the first time found significant associations between oral microbiome and child weight gain, and demonstrated the power of using growth curves as a predictor of microbiome composition.

P.5.3-004
The gut microbiota as potential modulator of gastrointestinal motility in patients with chronic constipation

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The intestinal microbiota may affect gut motility by altering host metabolism and production of metabolites, including gases, short chain fatty acids and bile acids, and therefore is considered to contribute to chronic constipation (CC). The aim of this study was to characterize mucosal microbiota and contractility of colonic muscle in CC patients. Colonic tissue samples were obtained from patients undergoing colectomy for CC and contractile activity was analyzed. Outcome was compared with the intestinal muscle contractions of patients undergoing colorectal surgery for gut diseases not associated with disorder of motor function. In CC patients, the spontaneous contractions were higher in both longitudinal (12.3 ± 4.4 vs 1.9 ± 0.9 g/s) and circular (13.47 ± 3.3 vs 5.8 ± 2.2 g/s) smooth muscle strips compared to controls. Moreover, the carbachol-induced response was also increased in CC in both longitudinal (EC50 0.50 ± 0.05 vs 0.65 ± 0.14 μM) and circular (EC50 0.76 ± 0.06 vs 2.01 ± 1.05 μM) muscle layers compared with those of the control group. The juxta-mucosal microbiota was studied with culture-based and 16S rRNA pyrosequencing techniques. The microbiota in constipated patients was dominated by bacteria belonging to the phyla *Bacteroidetes* (34–43%) and *Firmicutes* (31–52%), followed by *Proteobacteria* (4–26%) and *Actinobacteria* (1–4%). No definitive association between constipation and the abundance or lack of certain prokaryotic taxa in the gut microbiome was observed. Yet, we identified some microbes which may affect motility via production of methane, hydrogen sulfide, butyrate, acetate, and propionate. *Lactobacillus* species were isolated from colonic tissue samples from CC patients and their probiotic properties were compared with those of lactobacilli isolates from feces of healthy subjects, but no significant differences were detected. Our findings suggest a role for gut microbiota in constipation and promote new therapeutic approaches for treatment of patients with CC.

P.5.3-005
Genetic determinants of antimicrobial peptides produced by *Bacillus* species: identification and prospects for probiotics creation

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Bacteria of the genus *Bacillus* are characterized by the ability to synthesize a wide range of antimicrobial metabolites, including lipopeptides. Screening for strains with high antagonistic activity is necessary for the creation of probiotics with a potential to be used in medicine, veterinary medicine and poultry farming. From potato rhizosphere, we isolated 27 bacterial strains of the genus Bacillus with a high antagonistic activity. The isolates were identified by the homology of 16S rRNA gene as different strains of *B. subtilis*. Primers, based on bioinformatic analysis of the sequenced genomes of *B. subtilis*, were constructed to the genes of antimicrobial peptide synthesis: *ituC* (iturin A synthetase C), *fenD* (fengycin synthetase), *srfAA* (surfactin synthetase subunit

1), *bmyB* (bacillomycin L synthetase B), *bacA* (bacilysin biosynthesis protein BacA). It has been established that 96% of the strains have genes for surfactin synthesis, 92% – for bacillolysin, 78% – for fungicin, 59% – for iturin, 55% – for bacillomycin. Genes for the synthesis of 5 antimicrobial peptides were identified in 30% of the strains. Medium for the optimal synthesis of antimicrobial metabolites was selected. Liquid lipopeptide fractions were isolated from the bacterial culture and their ability to inhibit the growth of multidrug-resistant gram-negative bacterial strains of *S. enterica*, *E. coli*, *K. oxytoca* and *P. aeruginosa* was examined. The highest activity against the selected strains of pathogens was shown by the 3, 4, 8 and 23 isolates of *Bacillus*. The ability of bacilli to grow between the pH range of 4 to 10 and with a 1–10% NaCl concentration was investigated. On the basis of the data, it was concluded that isolated strains have a potential to be used as probiotics. This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University.

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P.5.3-006
Antimicrobial resistance of *Staphylococcus aeureus* to multi-heavy metal aqueous solutions

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Many bacteria species have specific genetic mechanisms of antimicrobial resistance to toxic heavy metals to the presence of virulence factors. The microorganism inhibition involves the entrance of heavy metal ions to the metabolic system and formation of secondary toxic metabolites containing heavy metal ions. As a consequent, growth inhibition or replication which lead to cell lysis will be observed. This study focused on the effect of multi-component heavy metal occurrence to the antimicrobial resistance of the *bacteria species*. *As model strain Staphylococcus aeureus ATCC 6538* which is a gram-positive commensal bacterium causing skin, soft tissue and wound infections, *septicaemia* and *endocarditis* was selected. The LB medium was prepared and sterilized at 121°C at 30 min. The study was conducted in a 96-well plate, in which each well contains broth, bacteria and heavy metal solutions at varying concentrations between 10–300 μM as a single, binary and ternary variations of Cu(II), Ni(II), and Cr (VI). At 37°C, plate was cultivated overnight and OD_{600} readings were performed as triplicate in VarioScan spectrophotometry. For single metal solutions, by using method of growth curves, minimal inhibition concentrations were determined. With respect to these concentrations the binary and ternary occurrence of the metals were investigated. IC_{50} values were determined at 6, 12, 18 and 24 h. With the statistical evaluation by viability of cells method, the results were supported. Also the assessment of anti-oxidative stress in cells was performed spectrophotometrically by ferric reducing ability power and Trolox equivalent antioxidant capacity tests. As a conclusion, the observations showed that the multi-component occurrence of the metals has a significant role in both growth inhibition and anti-oxidative potential of the strain. This can be explained with structural changes of the cell wall that determine the survival of the microorganisms depending on pollutant metal.

Metabolism and Signaling

P.5.4-001
Changes in metabolism of *Mycobacterium tuberculosis* Beijing B0/W148 cluster against the background of anti-tuberculosis therapy

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Beijing B0/W148, a "successful" clone of *Mycobacterium tuberculosis*, is widespread in the Russian Federation and some countries of the former Soviet Union and associated with drug resistance. The aim of the present study was to estimate changes in protein profiles of these endemic strains under the anti-tuberculosis (TB) treatment. Two clinical isogenic pairs of *M. tuberculosis* Beijing B0/W148 cluster strains were recovered from patients before and after anti-TB treatment. LC-MS/MS proteome analysis was performed on a Q-Exactive HF mass spectrometer in an IDA mode. The Mascot v 2.5.1 was applied for protein identification. Quantification was carried out using the MaxQuant v 1.3.0.5. According to proteomic data we identified 1329 proteins for both pairs. The comparative quantitative analysis revealed a low representation of ESX-3 and ESX-5 type VII secretory systems proteins in the strain obtained after treatment. We revealed an increased representation of proteins, encoded by mce 1 operon (Rv0169, Rv0170, Rv0172). Increase of representation of the corresponding proteins, considering the influence of anti-TB therapy, may testify in favor of the bacterial adaptation to the effects of anti-TB drugs. We detected an increased representation of proteins involved in lipid metabolism, in particular responsible for biosynthesis of long-chain fatty acids. Increased representation of the Rv0469 (UmaA) protein responsible for synthesis of mycolic acids, may testify in favor of the formation cell membrane, preventing the penetration of anti-TB drugs, lowering their intracellular concentration and thus creating favorable conditions for the survival of the cell and the development of drug resistance. Moreover, an increased representation of a large number of regulatory proteins was identified. These changes certainly indicate the regulation of bacterial pathways due to anti-tuberculosis therapy and effect of anti-TB drugs.

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P.5.4-002

Recategorized

P.5.4-003
Affect of exogenous creatine on blood lipid spectrum

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It is known that long-term social isolation and the disorder of natural circadian rhythm is considered an important stress factor, which cause a variety of metabolic and mental disorders. It is estimated that as a result of stress the metabolic processes change

in the organism, which is reflected in increased lipid peroxidation process, decreased of energy metabolism and activity of antioxidant system and the changes of activity other important systems, which affect the body's function adversely. In such circumstances, getting of some of the big amount of food adjunct in the organism may be caused changes, which is developed as a result of stress. Creatine is considered such a food adjunct, which is widely used by those individuals who are under physical activities. Despite the positive effect of creatine for energy metabolism, it is unknown the impact of creatine on such important processes as lipid spectrum changes in blood. The experiment was conducted on 50 adult male Wistar rats (150 ± 10 g) divided into two groups (control and stressed). The amount of total cholesterol, triglycerides, LDL, HDL and oxLDL was measured by cholorimetric assay kits (BioVision). The results showed that under stress condition and also intake of Creatine for a long time increases the numbers of total lipids, cholesterol, LDL-cholesteros, contrary to this HDL-cholesterol amount was decreased. It was not found any changes in measurement of oxLDL and ROS, it can be caused by melatonin antioxidant effect, which raised extremely under such stress condition. Thus, such condition of stress and creatine supplementation for a long time can cause development of diseases such as atherosclerosis, coronary artery disease and other cardiovascular system diseases.

P.5.4-004
Role of Mitofusin-2 in Sertoli cell phagocytosis

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Sertoli cells have multiple roles in germ cell development, ranging from physical support to supply of nutrients. The mechanisms that regulate Sertoli cell metabolism are central to the maintenance of spermatogenesis and male fertility. One of the functions of Sertoli cells corresponds to phagocytosis of residual bodies and apoptotic spermatogenic cells. It has been shown in phagocytes that there is a correlation between mitochondrial membrane potential and the engulfment capacity. Mitochondrial dynamics and quality control includes the exchange of mitochondrial components through mitochondrial fusion and fission. Mitofusin 2 (Mfn2) is a mitochondrial outer membrane protein involved in the rearrangement of these organelles through the regulation of the fusion process. In this work we evaluated the role of Mfn2 in the maintenance of mitochondrial and phagocytic function in Sertoli cells. We generated a Sertoli 42GPA9 Mitofusin 2 knockdown (KD) cell line and evaluated different parameters of mitochondrial function and observed an increase in ROS production, a decrease in mitochondrial membrane potential, higher calcium levels and a fragmented mitochondrial morphology, demonstrating an altered mitochondrial function. Phagocytosis was determined by incubating the cells with fluorescent beads and evaluated its internalization by microscopy and flow cytometry, which was reduced in a 30%. These results suggest that Mfn2 is involved in Sertoli cell phagocytosis. FONDECYT 1141033, KC: CONICYT and MECESUP AUS 1203 Fellowship.

P.5.4-005
The phosphorylation status of lymphocyte phoshatase-associated phosphoprotein (LPAP) changes dynamically upon lymphocyte activation

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Lymphocyte phosphatase-associated phosphoprotein (LPAP) was described in the 1990s, yet its function remains unknown. There is evidence that mice knock out for LPAP had decreased CD45 expression and abnormal B-cell development. Since CD45, which is tightly bound with LPAP, is an important player in lymphocyte signaling, LPAP may also be involved in this process, especially because its phosphorylation status changes after lymphocyte activation. We hypothesize that the function of LPAP can be understood by studying its phosphorylation. First, using targeted mass-spectrometry, generated phosphosite-specific antibodies, site-directed mutagenesis, and CRISPR/Cas9 knockout cells for stable transfection of mutants, we determined the LPAP phosphorylation sites, S99, S153, S163, and S172. Furthermore, we assigned the sites to various proteoforms and calculated their stoichiometry. Then, using Phos-tag-PAGE, we analyzed the LPAP phosphorylation status after Jurkat cell activation through T-cell receptor by CD3 cross-linking. We showed that shortly after 5 min of TCR stimulation, LPAP got partially dephosphorylated at S99 and S172 but became phosphorylated at S163, reaching the highest level by 30 min. The S153 site demonstrated constitutively high stoichiometry. These changes were abrogated in Lck KO cells confirming the involvement of TCR-triggered signaling. Stimulation of the cells with a PKC activator PMA to bypass the earliest signaling events restored the phosphorylation kinetics. By stimulating the cells in the presence of both broad spectrum and pathway-specific inhibitors, we assessed the contribution of different branches of the signaling cascade to LPAP phosphorylation changes. In conclusion, we showed individual site kinetics of LPAP phosphorylation changes after lymphocyte activation, and we hypothesize that LPAP may participate in T-cell signaling.

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P.5.4-006
Dephosphorylated AMPK facilitates HDAC4/HDAC5 myonuclear import and drives the slow muscle to the fast during 24-h Hindlimb unloading

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One of the key events that occurs during skeletal muscle inactivation is a change in myosin phenotype, i.e. an increased expression of fast isoforms and a decreased expression of the slow isoform of myosin heavy chain (MyHCI(β)). It is known that calcineurin/NFAT and AMP-activated protein kinase (AMPK) can regulate the expression of genes encoding slow isoform of MyHCI(β). Previously we showed that AMPK phosphorylation is significantly decreased in rat soleus after 24 h of hindlimb suspension (HS) [Mirzoev et al., 2016]. We hypothesized that a decrease in AMPK phosphorylation and subsequent histone deacetylases (HDAC) nuclear translocation can be one of the triggering events leading to a reduced expression of slow MyHCI(β). Wistar rats were treated with AMPK activator (AICAR) for 6 d before HS as well

as during 24-h HS. We found a significant 243% increase in the content of nuclear HDAC4 in the HS group, but in the HS+AICAR group the content of nuclear HDAC4 didn't differ from the control group. We observed a significant 41% decline in the content of nuclear HDAC5 in the HS group; in the HS+AICAR group a decrease in nuclear HDAC5 content was found. We also observed a decrease in pPKD content after 24-h HS. 24-h unloading resulted in a 0.3-fold decrease in MyHCI(β) pre-mRNA expression *vs*. the control group. MyHCI(β) pre-mRNA expression in HS+AICAR group didn't differ from the control. The results of the study indicate that AMPK dephosphorylation after 24-h HU had a significant impact on the MyHC I and MyHC IIa mRNA expression in the rat soleus. Through HDAC4 nuclear import we found a reciprocal relationship between AMPK and PKD in the rat skeletal muscle at the early stage of gravitational unloading.

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P.5.4-007
Control of energy balance by glucagon like peptide-1 may affect the nutritional support of spermatogenesis

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Obesity is a worldwide health issue and glucagon-like peptide-1 (GLP-1) related therapies appear as possible solution. GLP-1 stimulates glucose-dependent insulin release thus controlling energy status. Since glucose homeostasis, and metabolism by Sertoli cells is crucial to spermatogenesis we hypothesized that GLP-1 can have a role on male reproductive potential by acting on these cells metabolic function. Cultured human Sertoli cells (hSCs) attained from testicular biopsies of men with normal spermatogenesis (anejaculation) were exposed to different concentrations of GLP-1 (0, 0.01, 1 and 100 nM) during 6 h. We identified GLP-1 receptor and dipeptidyl peptidase 4 for the first time in hSCs. Glucose consumption in cells exposed to 0.01 and 1 nM of GLP-1 showed a decrease, though all GLP-1 concentrations increased lactate production. Notably, lactate dehydrogenase protein levels decreased in cells exposed to all GLP-1 concentrations. Lactate dehydrogenase activity increased in cells exposed to 100 nM when compared to 1 nM of GLP-1. Using JC-1 dye, we detected increased mitochondrial potential membrane in hSCs exposed to 1 nM of GLP-1 compared to 0.01 nM and decreased in 100 nM of GLP-1 compared to all conditions. The later condition also showed increased transcript levels of nuclear respiratory factor 1, as detected by real-time PCR when compared to cells exposed to 0 and 0.01 nM of GLP-1. Phosphorylated m-TOR increased in cells exposed to 100 nM when compared to 0.01 nM of GLP-1. Carbonyl groups decreased in cells exposed to 1 nM of GLP-1 when compared to cells exposed 0 nM and 0.01 nM and also in cells exposed to 100 nM of GLP-1 when compared with cells exposed to 0.01 nM. Our study reports for the first time the effects of GLP-1 in cultured hSCs and shows that GLP-1 modulates mitochondria functionality and improves the efficiency of glucose metabolism in hSCs. Further studies will be needed to clarify the role of GLP-1 in spermatogenesis.

P.5.4-008
Effect of GSK690693 and AZD2014, Akt/mTOR pathway inhibitors, on the development of the zebrafish (*Danio rerio*) embryos

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GSK690693 and AZD2014 are novel ATP-competitive inhibitors of the Akt and mTOR kinase, respectively, which are intracellular signal transducers in the Akt/mTOR pathway. This pathway controls protein synthesis, cell metabolism and proliferation, and therefore has a big impact on the embryonic development. We aimed to investigate the influence of GSK690693 and AZD2014 on the development of zebrafish embryos within 72 h post fertilization (hpf). Zebrafish, Tübingen wild type strain, were maintained and bred according to the Zebrafish Book. GSK690693 or AZD2014 treatments started from 2 hpf, 6 hpf or 24 hpf in a 24-well plate, with 12 embryos per well. The inhibitor concentrations were: 0.5 μM, 1 μM and 5 μM for GSK690693 and 0.1 μM, 0.5 μM and 1 μM for AZD2014. A combination of 1 μM GSK690693/1 μM AZD2014 was also tested. At least two replicates from three independent breeding were analyzed. The embryos were observed at 24 hpf, 48 hpf and 72 hpf. Mortality, hatching rate and malformations were recorded. Treatments with GSK690693 or AZD2014 showed decreased hatching rates at 48 hpf with increasing concentrations of the two inhibitors, independently of the starting time point. There were no significant changes in the mortality rate or developmental malformations compared to untreated control, which was dose and time independent. Embryos treated with 1 μM GSK690693/1 μM AZD2014 showed developmental malformations: all had smaller body length and 65% had yolk edema. No changes in mortality and hatching rate was observed compared to untreated control. Inhibition of Akt and mTOR kinases by GSK690693 and AZD2014 leads to disruption of the Akt/mTOR pathway in zebrafish. Akt/mTOR signal transduction pathway is essential for early stages of zebrafish embryonic development and its inhibition induces abnormalities in hatching rate and developmental malformations.

P.5.4-009
MiR-33a and its SREBF2 host gene as key orchestrators of the response of cardiac cells to palmitic acid and n-3 polyunsaturated fatty acids exposure

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Convincing evidence indicates that a high dietary content of saturated fatty acids, such as palmitic acid, increases the risk of

cardiovascular disease. Previously we have demonstrated that treatment with *n*-3 polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA) protects H9c2 rat cardiomyoblasts against palmitate-induced apoptosis. The aim of this study is to explore the involvement of sterol response element binding factors (SREBFs), the major transcription factors involved in the regulation of fatty acid and cholesterol biosynthesis, and of microRNAs (miRs) in the response of H9c2 cells to palmitate and its modulation by the *n*-3 PUFAs, docosahexaenoic acid (DHA) and EPA. We found out that palmitate increases apoptosis of H9c2 cells as well as mRNA levels of SREBFs, whereas both *n*-3 PUFAs exert a clear protective effect against palmitate-induced apoptosis and reduce palmitate-increased SREBFs mRNA levels. The intriguing presence of the miR-33 family within the intronic sequences of SREBF genes prompted us to speculate a possible mechanistic role of these genes in our model. Non-surprisingly, we observed that miR-33a, the only homologous isoform conserved in rats, appears to be co-expressed with the SREBF2. Preliminary data showed an opposite variation of miR-33a amount and protein level of carnitine palmitoyl transferase I (CPT1), direct target of this miR and key enzyme limiting the rate of long-chain fatty acids translocation to mitochondrial matrix to be β-oxidized. Our findings suggest that palmitate affects cardiac viability by reducing CPT1 levels and, in this way, accumulating in cytosol and converting to toxic products, e. g. ceramides. On the contrary, EPA and DHA may exert their protective effect through keeping low levels of miR-33a and its host gene SREBF2.

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P.5.4-010
MCPIP1 contributes to adipocyte differentiation

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Adipose tissue is a multifunctional organ, which maintains energy balance and homeostasis of the whole body. Process of fat tissue formation, known as adipogenesis, requires activity of transcription factors, growth factors and others molecules, including miRNAs. Monocyte chemoattractant protein-1-induced protein 1 (MCPIP1) has been shown to be an important regulator of adipocyte differentiation and its forced expression impairs adipogenesis *in vitro*. MCPIP1 exerts its antiadipogenic role by a direct degradation of the C/EBPβ transcript. Using Next-Generation Sequencing we identified altered expression of 58 miRNAs in MCPIP1-overexpressing adipocytes compared to control cells, which correspond to molecular pathways essential for adipocyte differentiation, including MAPK signaling pathway. Pivotal results were replicated on adipose-derived stem cells isolated from subcutaneous (SAT) and visceral adipose tissue (VAT) of obese patients (BMI >35), where we demonstrated important role of MCPIP1 in the regulation of adipogenesis *de novo*. Interestingly, we found higher expression of MCPIP1 in VAT compared to SAT which was correlated with decreased expression of pro-inflammatory cytokines in this tissue. Our data elucidate the role of MCPIP1 in adipocyte biology and may help to explain its negative action in adipocyte differentiation. MCPIP1 as a negative regulator of inflammatory response and adipogenesis *de novo*, might be an important target in therapy of obesity and metabolic syndrome.

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P.5.4-011
Reciprocal interaction of c-Met receptor tyrosine kinase and lncRNA HOTAIR in hepatocellular carcinoma

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c-Met pathway is particularly important in the development of hepatocellular carcinoma (HCC) and elevated c-Met expression is correlated with metastasis, poor prognosis, and drug resistance. c-Met activation in HCC is a critical phenomenon and became an important molecular target of HCC therapies. HOTAIR expression is defined to be up-regulated in various cancer types, linked to poor prognosis and aggressive cancer phenotype. Lower survival rates and increased metastasis were reported for HCC patients with upregulated lncRNA HOTAIR expression as well. Elevated expression of HOTAIR in HCC tissues, its relation with cancer stem cell phenotype, poor prognosis and metastasis lead us to investigate its possible link between HOTAIR and c-Met. In our studies, HOTAIR expression is found to be reversely correlated with c-Met expression and activation in HCC cell lines, for the first time. c-Met activation leads to down regulation of lncRNA HOTAIR expression and also, inhibition of c-Met activation recovers HOTAIR expression. c-Met pathway is defined to be activated in ligand dependent (HGF) and independent manner. Reciprocal interaction of c-Met and HOTAIR was conserved in ligand-independent c-Met activation, too. Bioinformatics analysis of genes that are epigenetically regulated by lncRNA HOTAIR revealed that some of the genes that are targeted by HOTAIR are known to contribute to c-Met pathway via enabling its activation. The possible molecules that are hypothesized to take part in reciprocal interaction of c-Met and lncRNA HOTAIR are analysed by RT-qPCR and western blot. To further understand the role of HOTAIR and c-Met interaction in metastasis; expression of HOTAIR, c-Met and related molecules were analysed in circulating HCC cells under shear stress in normal physiological and metabolic conditions. Our findings contribute to literature by mapping the interaction of lncRNA HOTAIR and c-Met which is particularly important for planning targeted HCC therapies.

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P.5.4-012
Development of fly resistance to consume high-protein diet requires physiological, metabolic and transcriptional changes

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Metabolic pathways known to differ between carnivores and those consuming high carbohydrate diets are implicated in the development of diabetes and insulin resistance in non-carnivores. However, hemochromatosis, fatty liver, obesity, and diabetes in carnivores have strong parallels with humans. In this study, we used *Drosophila melanogaster* to investigate how animals adapt metabolically to high protein diet. Five day-old flies w^{1118} were separated by sex and maintained on diets with different protein (P) to carbohydrate (C) ratios. The shortest lifespan was observed on high protein diet containing 5% Yeast (Y) and 0.25% Sucrose (S). On high P diet, 90% of flies died within first 10 days. Nevertheless, small minority of approximately 10% lived for over 30 days. We decided to artificially select these 'protein-resistant' flies for 5 generations. Each generation maintained for 24 days on high (selected) or optimal P:C (control) diet and transferred to 5Y-5S medium to get next generation. Fly mortality decreased every subsequent generation at high protein consumption. Behavior, physiological, biochemical tests and transcriptional profiles were made to characterize metabolic adaptation to high protein. Selected flies ate less high P food than controls. Under food choice conditions between high P and optimal P:C diets, selected flies ate less protein-enriched food. Selected females had higher fecundity and higher starvation resistance. Selected flies also had higher levels of both circulating and stored trehalose, glycogen, triacylglycerides and relatively higher level of total body lipids. Transcriptional profile depicts many metabolic, proteolysis, temperature and immune response genes with altered expression. Conclusively, animal adaptations to high-protein diet require changes in physiology, metabolome and gene expression levels.

P.5.4-013
Elevated acylcarnitine levels induce muscle insulin insensitivity through the interaction with Akt-related insulin signalling pathway

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The accumulation of long chain acylcarnitines has been linked to insulin resistance and prediabetes, but the molecular mechanisms causing these complications are still not completely characterized. Therefore, the aim of this study was to outline molecular effects of long chain acylcarnitine accumulation on protein kinase B (Akt), a central component in the insulin signalling pathway. Differentiated C2C12 mouse myoblasts were incubated overnight with palmitoylcarnitine (PC), after that cells were stimulated with insulin and Akt phosphorylation was determined by western blot analysis. During *in vivo* experiments, single intraperitoneal PC dose (50 mg/kg) was given to fed CD-1 mice, 30 min later insulin was administrated. After 1 h of initial PC administration, skeletal muscle samples were collected for western blot, qRT-PCR, and acylcarnitine content analysis. PC treatment decreased Akt Ser-473 phosphorylation in the C2C12 muscle cell line. Similar decrease in phosphorylation was observed after stimulation with low levels of insulin (10 nM). However, higher concentration (100 nM) of insulin overcame PC induced effect. Single *in vivo* administration of PC increased muscle acylcarnitine content by 3-fold, corresponding to acylcarnitine levels detected after short term fasting. PC administration caused an increase in insulin levels and disturbances in glucose tolerance. Similarly as *in vitro*, *in vivo* PC administration decreased Akt phosphorylation in muscles while the administration of insulin overcame the PC-induced effect. PC did not change the expression of genes involved in glucose transport and fatty acid metabolism. This demonstrates that PC can interact with Akt-related insulin signalling and accumulation of long chain acylcarnitines induces insulin insensitivity. Effects are not related to changes in gene expression, but to the inhibition of Akt phosphorylation at Ser-473.

P.5.4-014
Switching between planktonic and biofilm life-style: uncovering the roles of a new transcription regulator

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Microbes are capable of an extraordinary range of adaptations to challenging environments, making them extremely versatile organisms. In face of stress many bacteria commonly turn off flagella formation and switch to a sessile state of growth activating the production of fimbriae and extracellular polysaccharides. This process frequently leads to the formation of matrix-encapsulated communities of microorganisms named biofilms. BolA is a morphogen involved in several cellular processes, namely in the establishment of such microbial communities. Using High-throughput technologies we have shown that BolA protein is a new bacterial transcription factor, involved in the negative and positive regulation of several important genes. Interestingly, this protein plays a key role in the switch between motile and sessile lifestyles. BolA represses flagellar biosynthesis with severe consequences for bacterial swimming capacity, and enhances biofilm development, through fimbriae-like adhesins and curli production. Moreover, our results in *Escherichia coli* indicate that BolA modulates biofilm development through a complex network involving c-di-GMP, one of the most important players in the regulation of this pathway. We are also investigating the role of BolA in bacterial persistence and its role in virulence. We want to further decipher the role of BolA, this important transcription factor specifically involved in the transition between the planktonic and biofilm life-style.

P.5.4-015
Psycho-emotional stress induced Ca^{2+}-cytotoxicity in white rat hippocampus and creatine's possible neuroprotecting effect

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Disturbances in natural Circadian rhythm are well-known stress factors, affecting a range of metabolic pathways in the living body including the brain. Hence, discovery of natural compounds that could help to prevent and cure of adverse changes is very important. One of the recently discussed substances is creatine, that is actively taking part in high-energy phosphate transferring

and storage, and it is believed to have anti-stressor properties. Recent paper describes the impact of intraperitoneally injected creatine (140 mg/kg) into rats with a disturbed natural circadian rhythm for an extended period of time (30 days). Markedly, creatine-treated animals show positive changes in open-field behavioral parameters, and an increase in certain antioxidant enzymes' (SOD, catalase) activity in the hippocampus, whereas the concentration of nitric oxide, H_2O_2 and Ca^{2+} are approximated to the control value (non-stressed individuals). Similar findings were also observed in case of Na^+/K^+- and Ca^{2+}-ATPases. To sum up, the recent findings allow the conclusion that oxidative stress induced by long-term disturbances in natural circadian rhythm is accompanied and likely provoked by an increase in Ca^{2+}-cytotoxicity, which is supposedly normalized by the creatine's indirect action on the NMDA receptor. Therefore, its impact on energy mediating pathways has a positive effect on stabilization of antioxidant as well as different metabolic systems, thus protecting the hippocampal cells from further stress impact.

P.5.4-016
Human proteins SLURP-1 and SLURP-2 suppress proliferation of epithelial cells by interaction with nicotinic acetylcholine receptors

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Consumption of tobacco is associated with the development of lung, gastric, pancreatic, colon, breast, and kidney cancers. Proposed molecular mechanisms of tobacco-induced cancer development and progression imply a direct activation of α7 type nicotinic acetylcholine receptors (α7-nAChRs) by agonists like nicotine and its oncogenic nitrosoamine derivatives. Thus, α7-nAChRs are a promising target for development of new anticancer therapies. Here, we investigated the antiproliferative activity of human proteins SLURP-1 and SLURP-2 secreted by epithelial cells, antagonists of α7-nAChRs, against several human tumor cell lines (A431, SKBR3, MCF-7, A549) and glial cell lines (A172, U251 MG). SLURP-1 and SLURP-2 at concentrations above 1 nM significantly inhibited growth of A431, SKBR3, MCF-7 cells (to 50–80% of a number of viable cells in 24 h). On A549 cells antiproliferative effect was observed only for SLURP-1. Contrary, SLURPs didn't affect the growth of glial cells. It was demonstrated that antiproliferative activity of SLURPs on A431 cells (known to overexpress EGF receptors, which stimulate anti-apoptotic pathways leading to the tumor growth) was associated namely with α7-nAChRs, but not with β-adrenergic receptors and EGF receptors. Exposure to recombinant SLURP-1 down-regulated the expression of α7-nAChRs in A431 cells and diminished intracellular level of endogenous SLURP-1 causing its secretion to the extracellular milieu. This in turn could simulate the neighboring cells to release their own signaling proteins and further transmit the signal. Co-application of gefitinib, irreversible inhibitor of EGFRs presently used for therapy of tumors, and SLURPs additively inhibited the growth of A431 cells and fully blocked cell proliferation during 24 h. So, SLURPs can be considered as promising natural drugs with low immunogenicity and no systemic toxicity inhibiting the growth of epithelial cancer cells.

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P.5.4-017
The role of mitochondrial origin H2S in mitochondrial permeability transition pore opening and cardiac resistance to Ca^{2+} overload in rats

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Hydrogen sulfide (H_2S) is a gaseous mediator, which regulates a lot of functions in cardiovascular system and is produced by three enzymes: CBS, CSE, and 3-MST, which work in tandem with CAT. It was shown that 3-MST and CAT are located in mitochondria, thus it becomes clear that H_2S, produced in these organelles, takes a great role in the heart and vascular functions regulation. Calcium overload in mitochondria can induce opening of the mitochondrial pore (MP) – the key player in apoptosis development. So, the aim of our work was to investigate the role of H_2S, produced in mitochondria, in cardiac function under calcium overload and in Ca^{2+}-induced MP opening. We used 5–7 months Wistar rats. Cardiodynamic parameters were registered using Langendorf isolated rat heart. Calcium load was carried by adding of $CaCl_2$ in perfusion solution every 10 min to increase its concentration from 1.7 to 12.5 mM. Rat heart mitochondria were isolated using differential centrifugation method. MPTP opening was registered spectrophotometrically as mitochondrial swelling. It was shown that inhibition of mitochondrial H_2S-synthesis enzyme 3-MST by O-CMH had a negative influence on initial cardiodynamic parameters. In particular, the left ventricle pressure (LVP) and the rate of contraction and relaxation of myocardium decreased twice. Coronary blood flow (CF) decreased by 1.12 times, while the heart rate was tended to increase. We found that the hearts of experimental animals developed less powerful reaction under the calcium overload that manifested in reduced parameters of LVP, CF and heart work intensity. Also, the inhibition of 3-MST (*in vitro* and *in vivo*) causes significantly dose-dependent increase of Ca^{2+}-induced mitochondrial swelling in adult rat heart. The highest concentration of inhibitor 10–3 M increased this parameter by 2.3 times. Therefore, mitochondrial origin H2S, has the great impact on cardiac resistance to Ca^{2+} overload and calcium-induced MP opening in rat heart.

P.5.4-018
Molecular mechanism underlying the effect of Hsp72 on insulin sensitivity and lipid metabolism in skeletal muscle

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Insulin resistance, a condition in which cells fail to respond to normal action of insulin, is associated with many health related complications, including type 2 diabetes, which is one of the most common metabolic diseases in the world. Recently it was shown that heat shock protein 72 (Hsp72), chaperone catalysing refolding of unfolded substrates, plays an important role in regulation of insulin sensitivity. Muscle-specific overexpression of Hsp72 protects mice against diet-induced insulin resistance and type 2 diabetes, but the underlying molecular mechanism still remain far from clear. Thus, the aim of the study was to investigate the molecular mechanism involved in Hsp72-associated regulation of insulin sensitivity in skeletal muscle. Herein it was proved that overexpression of Hsp72 protects against palmitic acid-induced insulin resistance and lipid accumulation in C2C12 myotubes. Moreover, C2C12 cells overexpressing Hsp72 are characterized

by upregulation of 5'AMP-activated protein kinase (AMPK), one of the most important energy homeostasis regulators in cell. Using specific inhibitors it was shown that the effect of Hsp72 on insulin signalling is dependent on AMPK and its upstream kinase, calcium/calmodulin-dependent protein kinase kinase (CaMKK). It was also observed that Hsp72 overexpression in C2C12 myotubes increases β-oxidation rate and mitochondrial biogenesis, as well as protein level of slow isoform of myosine heavy chain and activity of enzymes involved in aerobic metabolism. Overall, the study showed that Hsp72 by shifting to slower contractile phenotype and more oxidative metabolism protects C2C12 myotubes against palmitic acid-induced insulin resistance in AMPK- and CaMKK-dependent manner.

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P.5.4-019
Analysis of chylomicron apoB48 secretion using everted intestinal sac system; effects of food ingredients

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Postprandial hyperlipidemia is a strong risk factor of atherosclerosis. In order to analyze chylomicron metabolism and effect of food, animal studies and cell studies have been employed but intestinal tissue studies have not been performed. Everted sac method has been applied to the studies on drug and glucose absorption and secretion. To study chylomicron metabolism, we raised antibody of apolipoprotein B48 (apoB48) and made quantitative assay possible. Using this assay, we analyzed the effects of several food ingredients on apoB48 secretion in intestinal cell line $Caco_2$. In this study we studied the absorption of lipid and secretion of lipoproteins by intestinal sac and applied the system to study the effect of curcumin, resveratrol and genistein. Intestines were excised out of New Zealand White Rabbits fed show with or without the food ingredients. 6 cm long pieces of intestines were everted to sacs absorptive side out. Sacs were filled with 1 ml DMEM and immersed in medium including lipid micelles, bubbled with oxygen. After 15 min to 60 min, the fluid inside the sac was collected to analyze chylomicron secreted inside, serosal side, of the sac. ApoB48 was assayed with ELISA and other apolipoproteins, triglyceride and cholesterol were also assayed with ELISA or enzymatic system. ApoB48 secretion was detected in the fluid in the sac. Chylomicron was accumulated in the sac and secretion continued over 60 min. Along the intestine, in sacs from upper oral-side quarter higher secretion of apoB48 was observed and it was decreased in sacs from lower anal-side quarters. ApoB48 secretion into the medium inside of sacs was significantly suppressed by addition of resveratrol by 47% and genistein by 56% compared to control. Triglyceride or cholesterol secretion was not suppressed. It is suggested that number of secreted chylomicron was decreased by these food ingredients, forming less chylomicron remnant particles which lead to atherosclerosis.

P.5.4-020
Root phosphatase activity of three *Arabidopsis thaliana* ecotypes in response to various sources of phosphorus in cultivation media

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In response to soil phosphorus limitation, plants induce and secrete various extracellular enzymes, including phosphatases. Using roots of *Arabidopsis thaliana* ecotypes Col-0, Wil-2 and Ler-0 we conducted a comprehensive survey of intracellular phytase, phosphomonoesterase and phosphodiesterase activities in response to phosphorus starvation (noP) and during plant growth on inorganic phosphorus (Pi), ATP and phytate (IHP) as the sole source of P. Phytase activity in crude root extracts of Wil-2 plants supplied with ATP was 3–4 times higher than in plants supplied with IHP. Similarly, phytase activity of Ler-0 plants grown on ATP was higher than on P-deficient media (noP or IHP). In contrast, there were no significant difference in phytase activities for ecotype Col-0 grown on P-deficient media (noP or IHP) and on P-rich media (Pi or ATP). In contrast to the situation with intracellular phytase activity, total root intracellular acid phosphomonoesterase activity showed no significant differences when Col-0, Wil-2 and Ler-0 plants were grown on various P substrates. Similarly, Col-0 and Wil-2 plants showed no significant differences in intracellular phosphodiesterase activity when grown on either P-deficient or P-rich media. However, roots of Ler-0 ecotype displayed a nearly two-fold induction of phosphodiesterase activity when grown on IHP as compared to noP. Overall, Col-0 ecotype, a reference ecotype often used to describe the effects of various environmental factors on *A. thaliana* physiology, showed no significant differences in baseline intracellular phosphatase activity on P-deficient and P-rich media. Taken together, our approach with several geographically distinct *A. thaliana* ecotypes has allowed us to document changes in plant intracellular phosphatase activity levels in response to a broad range of P sources. Our results confirm and extend previous data reporting substantial diversity between *A. thaliana* ecotypes under various environmental conditions.

P.5.4-021
Impact of nanoparticles on transcriptional response of HepG2 cells to tumor necrosis factor

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Tumor necrosis factor (TNF) is a pleiotropic proinflammatory cytokine involved in progression of many diseases. Activation of cellular signaling pathways by TNF can lead to different cellular responses: cell survival and proliferation or apoptotic/necrotic cell death. Recent evidence suggests that different types of nanoparticles (NPs) can interfere with cellular signaling pathways. The aim of the present study was to assess the possible interference of silver (AgNPs), gold (AuNPs) and iron oxide (SPION) nanoparticles with the cellular signaling activated by TNF in HepG2 cell line. HepG2 cells were incubated with TNF or/and NPs for various time periods. Absorption of NPs was confirmed cytometrically. Cell reproductive death was determined by clonogenicity test. Expression of genes related to apoptosis and NF-κB signaling was analyzed by qPCR. In the clonogenicity test, AgNPs significantly sensitized HepG2 cells to TNF.

Similar but not statistically significant tendency was observed for AuNPs. In contrast, no impact of SPIONs on the TNF action was observed. Analysis of transcriptional response to TNF in the presence or absence of AgNPs revealed that the expression of *TNFSF9* was enhanced in TNF and AgNPs treated cells compared to TNF alone after both 6 and 24 h. AgNPs augmented also the expression of cytokines *IL10*, *TNFSF15* and *TNFSF8* but only after longer treatment (24 h). On the contrary, the expression of *BAG3* was augmented only after short treatment (6 h). Presence of AuNPs and SPIONs had no significant impact on the transcriptional response to TNF. The presented results suggest that nanoparticles may affect cellular response to TNF. The knowledge about interactions between NPs and cellular signaling pathways will facilitate the purposeful design of nanomaterials and lay the foundation for the controlled manipulation of biological systems through nanotechnology.

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P.5.4-022
The effects of vitamin D onto the expression of caspase enzymes in osteoblastic cell line treated with sodium fluoride (NaF)

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Long-term high dose fluoride exposure causes chronic poisoning, called fluorosis, which affects many tissues and leads to serious health problems. This study was planned to investigate the effects of Vitamin D in the osteoblastic hFOB 1.19 cells treated with sodium fluoride (NaF). Cytotoxicity dose of all compounds were determined by MTT. Control, NaF, Vit D and NaF+Vit D groups were prepared for experiment. The cells treated with IC50 dose of NaF, proliferative dose of Vit D and the combination of these doses were used for enzyme analysis. In MTT results, it was observed that the Vit D showed inhibitory effect on the NaF-induced toxicity. To evaluate the role of Vit D on apoptotic pathway, caspase 3,8,9 levels were analyzed by ELISA and the expression of caspase genes by real time PCR. According to ELISA, although caspase-3 levels did not change for all groups, it decreased in the NaF+Vit D group. Caspase-8 levels were not changed, statistically. Caspase-9 levels were decreased in Vit D group, but in the NaF+Vit D did not change compared with NaF group. The gene expression of caspase 3 were higher 2–3 fold for all groups than control. Caspase-8 expression was not changed for Vit D, while increased 7.3 fold in the NaF and 2 fold in the NaF+Vit D group, respectively. Caspase-9 expression was increased approximately 1.2 and 1.3 in NaF and Vit D groups, while increased 2.5-fold in NaF+Vit D, interestingly. As a result, this study showed that Vit D has a protective effect on NaF-induced cell death. The inhibitory effect of Vit D on NaF cytotoxicity could be done via receptor mediated pathways in apoptosis. In order to exactly understand the inhibitory effects of Vit D on NaF-induced cell death, further studies using different doses, times and detailed methods should be performed.

P.5.4-023
Phenol oxidases from *Pectobacterium atrosepticum*: identification and cloning of genes, characteristics of the enzymes and assessment of the transcript levels

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Phenolic compounds, having antimicrobial effect, are involved in plant disease resistance. Detoxification of phenolic compounds is an important aspect of the interaction of phytopathogens with plants. No enzymes oxidizing phenolic compounds were found to date in *Pectobacterium* genus. The aim of our study was a search of genes encoding phenol oxidases in *Pectobacterium atrosepticum* SCRI1043 and characterization of the corresponding enzymes. The nucleotide sequences of ECA0347 and ECA3346 loci encoding proteins similar to phenol oxidases were revealed. ECA0347-encoded protein is annotated as cell division protein, whereas it has high sequence similarity to laccases. Besides, the signal peptide in this protein implies its transmembrane transport. ECA3346-encoded protein has a functional domain of Cu-oxidase 4 responsible for laccase activity and YfiH domain of novel-type laccases. Novel-type laccases lack conservative sequences, which act as metal ligands in classical laccases. Target enzymes were obtained by heterologous expression in *E. coli* cells. Both recombinant enzyme displayed laccase activity. The enzymes were characterized in terms of substrate specificity, optimal temperature and pH; an inhibitory analysis was performed as well. The conditions that induce the expression of the target genes in *P. atrosepticum* were elucidated. The expression of a gene of ECA0347 locus was induced under *in planta* conditions. The increased transcript level of a gene of ECA3346 locus was observed, when bacteria were incubated *in vitro* in the presence of sub-inhibitory concentration of copper sulfate of ferulic acid.

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P.5.4-024
Different roles for PAK1 and PAK2 in the interaction of leukemia cells with fibronectin

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P21-activated kinases (PAKs) are critical effectors of small GTPase binding proteins Cdc42 and Rac1 regulating cytoskeleton reorganization. IPA-3 inhibits class I PAK members (PAK1, 2 and 3). PAK1, the best explored member, regulates cell motility and morphology. PAK2 knock-out impedes engraftment of hematopoietic progenitors to the bone marrow but its role in adhesion signaling is unclear. We analyzed the effect of IPA-3 on adhesion of leukemia cells (cell lines and primary cells) to fibronectin (FN), a canonical component of the bone marrow matrix. Cell interaction with FN was monitored using real-time microimpedance measurement (ECIS system). The adherent cell fraction (ACF) was determined by fluorescence staining of cells which remained attached after a PBS/Ca^{2+}/Mg^{2+} wash. PAK inhibition was assessed from the phosphorylation status at the regulatory autophosphorylation site (Ser144/Ser141 at PAK1/PAK2). PAK3 was not detected in leukemia cells. The intracellular localization of GFP/RFP-tagged PAK1/2 was analyzed in HeLa cells. We found that IPA-3 inhibits PAK1 with lower EC50 (about 5 μM) than PAK2 (20 μM or more). In adherent cells and adherent-like leukemia cells (HEL cell line), IPA-3 induced a fast

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decrease of the ECIS signal, attributable to PAK1 inhibition, probably due to cytoskeleton contraction. Slower concentration-dependent changes were observed at higher IPA-3 doses in both adherent and leukemia cells, in correlation with progressive PAK2 inhibition. The fast drop also occurred upon inhibition of kinases of the Src family by dasatinib. Inhibition of PAK2 (but not of PAK1) prior to cell contact with FN decreased ACF. PAK2 (but not PAK1) localized to cell-FN contact areas. Our results point to substantial differences between PAK1 and PAK2. In agreement with previous reports, PAK1 regulates the tension of actinomyosin fibers. PAK2 does not compensate for PAK1 in this function, but is important for cell adhesion through integrin-associated complexes.

P.5.4-025
Comparison of silver ions and silver nanoparticles effect on *E. coli* and mice

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Silver (Ag) is known as an effective antimicrobial agent. Nowadays Ag nanoparticles (AgNP) are widely used. However, due to the electronic similarity of Ag^+ and Cu^+, Ag^+ can integrate into copper metabolism, so Ag is a potential ecotoxic agent. Aim of this work was to evaluate impact of Ag^+ and AgNP synthesized in our group on *E. coli* and mice. AgNP were synthetized by a chemical reduction of Ag^+ to Ag^0 with hydrazine in potassium oleate clathrates. AgNP were characterized by spectroscopy, refractometry and electron microscopy methods; they had spherical-like shape with the average diameter of 35 nm, crystalline structure and consisted of silver atoms. AgNP demonstrated antibacterial properties in time- and dose-dependent fashion. As it was shown by TEM, AgNP with strong adhesion cover bacterial surface and possibly penetrate into the cell, notably, that Ag^+ itself crystalize on or into the bacteria forming AgNP-like structure. Presence of recombinant N-terminus domain of hCTR1, chelator for Cu^+ and Ag^+, in *E. coli* increases its resistance to AgNP as it also was in case of Ag^+ treatment. Flow cytometry experiments with FITC-Annexin V and propidium iodide revealed that both AgNP and Ag^+ firstly lead to phosphatidylserine externalization and then to bacterial membrane permeability. All cytotoxic effects were more profound with Ag^+. In mice, AgNP and Ag^+ decreased ceruloplasmin oxidase activity (OA) to 1/10 of the initial value while its synthesis and secretion weren't changed. In both cases atomic Ag was unevenly distributed throughout the organs and was excreted with bile. Serum OA recovered almost to its initial level in few days after the last Ag dose. Ag^+ were excreted from body in 3 days, while Ag concentration in tissues did not decrease in 9 days after the last AgNP injection. The results show that both Ag^+ and AgNP disorganize mammalian copper metabolism, in addition AgNP accumulate in organs. It should be paid more attention because AgNP usage spreads fast.

P.5.4-026
Hemorphin-7 demonstrates blood glucose-lowering effect in streptozotocin-induced diabetes

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Hemorphins, an endogenous nonclassical opioid peptides derived from hemoglobin, were proposed to serve as homeostatic factors that switch on the compensatory systems in the organism during severe pathologies. Earlier we have revealed the antidiabetic effect of LVVYPW (LVV-hemorphin-3), in addition to its antitumor activity. In this study we have tested the blood glucose-lowering effect of hemorphin-7, another member of hemorphins family, which has been shown to demonstrate antitumor effect as well. Experiments were done on male Wistar rats (weight 200–220 g). Animals received single intraperitoneal (ip) injection of streptozotocin (STZ) at 60 mg/kg to induce diabetes. After ip injection of hemorphin-7 (1 mg/kg) into fasting STZ-induced diabetic rats, this peptide has been shown to decrease plasma glucose levels from 28.86 ± 1.6 mM to 20.3 ± 1.14 mM in 2 h. Naloxone and naloxonazine have been shown to abolish the plasma glucose-lowering effect of hemorphin-7 indicating that μ-opioid receptors (MORs) are involved in mentioned effect of peptide. Thus, we have revealed that hemorphin-7 exhibits blood glucose-lowering activity in pathophysiology of diabetes by the same way as LVVYPW. In the light of the finding that the levels of hemorphin-7 peptides are decreased in the serum of diabetic and obesety patients, data obtained in this study indicate that hemorphins are good candidates for creating the new, effective complex antidiabetic drugs without side effects.

P.5.4-027
Pyropia yezoensis peptide prevents dexamethasone-induced muscle atrophy by down-regulating the NF-kB signaling pathway in mouse C2C12 myotubes

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Muscle atrophy can develop as a result of different pathologies of aging, disuse, starvation, and a number of disease states, including diabetes, cancer, sepsis, and chronic kidney disease. Several complex mechanisms of proteolysis have been proposed to explain the induction of muscle atrophy. Studies have indicated that one of the mechanisms linking the activation of proteolytic systems (such as autophagy and the ubiquitin–proteasome system) to muscle atrophy is the accumulation of oxidative stress by increased production of reactive oxygen species (ROS). In the present study, we measured the intracellular ROS production by C2C12 myotubes using the redox-sensitive fluorescent dye 2′,7′-dichlorofluorescein diacetate (DCF-DA). The results showed that the level of ROS in dexamethasone-treated cells increased compared with that in control cells. By contrast, treatment with PYP1-5 led to a significant reduction of DCF-DA fluorescence intensity. Several studies have shown that ROS can induce the transcription of NF-kB. NF-kB is a transcription factor involved in muscle wasting as a mediator of TNF-α in the inflammatory response. To investigate the effect of PYP1-5 on muscle atrophy, C2C12 myotubes were treated with 100 μM dexamethasone and 500 ng/ml PYP1-5 for 24 h. We confirmed the expression of TNF-α, TNF-R1, IkBα, and NF-kB by western blotting and real-time polymerase chain reaction. The results revealed that PYP1-5 decreased the levels of protein phosphorylation and

mRNA expression of TNF-α, TNF-R1, and IkBα. It also significantly decreased the expression of NF-kB protein in the nuclear fraction. Taken together, these results indicate that PYP1-5 exerts beneficial health effects by inhibiting the NF-kB signaling pathway in skeletal muscle.

P.5.4-028
Novel targets of the flippase kinase Fpk1

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Target of Rapamycin (TOR) is a widely conserved serine/threonine protein kinase that assembles in two distinct multiprotein complexes: TORC1 and TORC2. TORCs independently regulate distinct aspects of eukaryote growth, but only TORC1 is inhibited by rapamycin. To dissect the pathways downstream of TORC2, we have engineered a yeast strain in which only TORC2 is sensitive to an ATP-competitive Tor inhibitor. The major target of TORC2 is the AGC kinase Ypk1. Amongst its many targets, Ypk1 phosphorylates and inactivates Fpk1, another AGC-family kinase. Fpk1, initially described as an amino-phospholipid flippase activator, is additionally involved in other cellular processes, including cell polarity and endocytosis. An Fpk1 consensus motif RxSLD/E was identified by comparing the residues phosphorylated in its known targets. In the proteome of *Saccharomyces Cerevisiae*, we identified 91 such Fpk1 consensus motifs and tested each in *in vitro* by kinase assays. Among the hits, we focused on Akl1, a serine/threonine protein kinase, member of the Ark family involved in endocytosis regulation. Through biochemical approaches and confocal microscopy, we could demonstrate that Akl1 regulates the uncoating and fusion of endocytic vesicles with endosomes in a TORC2-dependent manner.

P.5.4-029
ASK1/Trx system as a defense mechanism against oxidative stress

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis and thus it plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin (Trx) and the 14-3-3 protein that both function as inhibitors of ASK1. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear. The aim of this study was to investigate the role of individual cysteine residues from TRX1 as well as from ASK1-TBD in the interaction between these two proteins. Sedimentation velocity analysis together with the site-directed mutagenesis revealed that from five cysteine residues in human TRX1 molecule, the residue Cys32 is crucial for TRX1 binding to ASK1-TBD in reducing conditions. Formation of disulfide bond between Cys32 and Cys35 has a major effect on complex dissociation under oxidative stress. ASK1-TBD contains seven Cys residues from which Cys200, Cys206, Cys250 and one from the pair Cys225 or Cys226 seem to be well accessible at the surface of the ASK1-TBD molecule. Oxidation of ASK1-TBD leads to the formation of two intramolecular disulfide bonds, Cys200-Cys206 and Cys225-Cys226, which induce subtle but significant structural changes of ASK1-TBD. On the other hand, serine-scanning mutagenesis of ASK1-TBD showed that Cys250 is the only cysteine residue which is critical for the stability of ASK1-TBD: TRX1 complex[1,2].

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P.5.4-030
Coilin and fibrillarin are possible players in plant responses to abiotic stress factors

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Coilin is a major scaffold protein of the Cajal bodies, the subnuclear structures which are physically and functionally associated with the nucleolus. Coilin provides functions for the formation and operation of Cajal bodies which are involved in various biological processes including metabolism RNAs and RNPs. Fibrillarin is one of the major proteins of the nucleolus. It plays essential role in ribosome biogenesis including pre-rRNA processing and methylation of rRNA and snRNAs. In the present study we investigated the role of coilin and fibrillarin in abiotic stress responses. We used the wild-type *Nicotiana benthamiana* plants and transgenic plants triggering silencing (knock-down) of the coilin or fibrillarin genes. In the first experiments, leaf discs were vacuum-infiltrated with a hyperosmotic medium (250 mM NaCl) or medium containing H_2O_2 (0.1 и 0.01%). Both fibrillarin and coilin knock-down transgenic plants displayed markedly higher level of resistance to each type of these stresses. Indeed, the leaf discs of wild-type plants showed severe bleaching several days earlier than those of fibrillarin or coilin knock-down plants. Next, we studied the resistance of whole plants to hyperosmotic stress. The plants were watered with 50 ml of a solution containing 300 mM NaCl. The degree of turgor leaf loss and the number of leaves that lost the turgor, were considered as a criterion characterizing the sensitivity of plants to stress. The wild-type plants had more leaves with a lowered turgor (in average, three leaves, some reached five-six) than transgenic plants (usually two lower leaves). Based on the obtained results, we assume that coilin and fibrillarin are involved in the formation of the plant's response to abiotic stresses. Decreased expression of the coilin/fibrillarin genes led to an increase in plant resistance to these abiotic stress factors.

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P.5.4-031
Cyclic RGD-peptides as molecular triggers to induce stem cell self-aggregation

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Mesenchymal stem cells (MSCs) are of great interest for regenerative medicine due to their ability to differentiate into various cell

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types and to enhance tissue reparation. However, during *in vitro* culture on plastic surface, MSCs rapidly lose the expression of transcription factors associated with pluripotency and self-renewal which results in decrease of regeneration ability. To prevent this, cultivation of MSCs in three-dimensional aggregates (multicellular spheroids) has been proposed. Recently, we have synthesized cyclic peptides, containing Arg-Gly-Asp (RGD) motif, and have applied them to induce tumor cells aggregation. In the current research we proposed these cyclic RGD-peptides as molecular triggers to induce self-aggregation of human primary MSCs. For this purpose, MSCs were seeded in 96-well plate (10,000 cells per well) and cyclic RGD-peptides (1–100 μM) were added directly to the monolayer culture. It was shown that MSCs aggregation could be triggered by addition of 10–100 μM peptides after 2–3 days incubation. The mean size of the generated spheroids was 56 ± 13 μM, and the total viability of cells in the spheroids was confirmed by fluorescent-based live-dead assay. It shoud be noted, that the removal of the peptides by replacement the media led to the spheroid disaggregation and to cell reattachment within 24 h. Additionally, spheroids were transferred into a 95% collagen I gel derived from rat tails, which is relevant to extracellular matrix conditions *in vivo*. We have found that MSCs spheroids demonstrated an invasion into the gel after cultivation for 3–5 days. These findings mean the novel approach to stem cell management before the implantation. First, we can add cyclic RGD-peptides to stem cells to form spheroids which preserve MSCs regeneration ability, and then we remove the peptide to allow the cells spreading into the matrix. This RGD-triggered technique can be promising for tissue engineering applications.

P.5.4-032
Improving plant phosphorus uptake on phytate-rich medium through inducible expression of bacterial phytases PaPhyC and 168phyA in *Arabidopsis thaliana*

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The majority of the phosphorus pool in soils is present in the form of organic phosphorus compounds, including *myo*-inositol hexakisphosphate (phytate). Though phytate is very abundant in nature, plant roots are unable to absorb it, and consequently phytate can not be utilized for plant metabolism. Furthermore, phytate sequesters important ions, such as calcium, further negatively impacting plant mineral nutrition. Nevertheless, phytate is digested by specific enzymes, called phytases, which are abundant in microbes. Phytase-mediated dephosphorilation of soil phytate is a promising field for improving plant phosphorus nutrition. One of the proposed biotechnological approaches to improving plant phosphorus nutrition is the development of genetically modified plants able to secret bacterial phytases into the rhizosphere. Here we report the development of the system, where several lines of *A. thaliana* plants have been engineered to integrate genes of bacterial acid and alkaline phytases under the control of root-specific inducible promoter Pht1;2. The influence of recombinant phytase expression on growth and morphology of transgenic plants grown on phytate as the sole source of phosphorus was investigated. Phytase activity of recombinant enzymes was detected in cell wall protein extracts under phosphorus deficiency conditions, suggesting that both phytases are induced and localize predominantly to cell wall fractions of roots. Bacterial phytase expression in roots allowed plants to achieve larger rosette leaf area and diameter and increased dry weight. These data clearly demonstrate the ability of engineered plants to utilize phytate as the source of phosphorus for growth and development. In addition, our results with bacterial phytase expression in plant roots provide further evidence that biotechnological approaches carry great potential to improving plant phosphorus nutrition.

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P.5.4-033
Agonistic ligands targeting PRR-receptors exposed at outer cell membrane (TLR4), endosome (TLR9) or cytoplasm (NOD2) synergistically activate dendritic cells cytokine/chemokine response

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Dendritic cells (DC) sense infection using a broad range of pattern-recognition receptors (PRRs) which are exposed in different cellular compartments. We examined whether simultaneous activation of PRRs on plasma membrane (TLR4), in endosome (TLR9) and cytosol (NOD-2) can induce synergistic response of DC. The cells were *in vitro* differentiated from mouse bone-marrow using GM-CSF. For PRR-activation, known agonistic ligands were used such as bacterial lipopolysaccharide for TLR4, unmethylated CpG oligonucleotide for TLR9, and 6-O-stearoyl-N-Acetyl-muramyl-L-alanyl-D-isoglutamine for NOD2. Early DC responses were measured according to production of TNFα, IL-1α, IL-10, IL-12, MCP-1, MIP-α, RANTES, IFN-γ cytokines and chemokines. Wide range concentrations of TLR4-, TLR9- and NOD2-agonists were applied for the activation. Cellular response to each agonist alone was compared with the one to the TLR4+TLR9, or TLR4+NOD2, or TLR9+NOD2 pairs of agonists, or their three-component TLR4+TLR9+NOD2 composition. Synergistic production of cytokines was represented as a synergy factor (SF), calculated as the ratio of response induced by certain combination of agonists to a sum of response rates induced by each of these agonists used alone. According to production of IL-12 by DC, maximum SF were observed after activation with TLR4+NOD2 (SF = 12.8), and TLR4+TLR9 (SF = 7.1), and TLR9+NOD2 (SF = 5.8) agonists combinations. All studied combinations of agonists also gave considerable synergy in induction of MIP-1α, showing SF 4.5, 4.8 and 2.9 with TLR4+NOD2, or TLR4+TLR9, or TLR9+NOD2 combinations, respectively. A synergism was less prominent in production of TNF-α (SF = 1.3) and MCP-1 (SF = 2.8). Just additive, but not synergistic productions were observed as regards to RANTES and IL-1, when DC were activated using the pairs of TLR4, TLR9 and NOD2 agonists. The three component TLR4+TLR9+NOD2 combination was in some cases superior to the pairs of agonists.

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P.5.4-034
High cholesterol diet activates endoplasmic reticulum stress signaling pathways in non alcoholic fatty liver disease: role of fatty acids

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Non-alcoholic fatty liver disease (NAFLD) is a chronic metabolic disorder characterized by hepatic fat accumulation (steatosis)

which represents an increasing public health concerning westernized nations. Although it is associated with many diseases related obesity, the underlying pathogenesis of NAFLD is unclear. Endoplasmic reticulum (ER) is known to play a crucial role in protein folding, assembly, and secretion. Disruption of ER homeostasis may lead to accumulation of misfolded or unfolded proteins in the ER lumen, a condition referred to as ER stress. Recent studies suggests that excessive synthesis of saturated fatty acids inside the liver might enhance ER stress activation which involves in the progression of NAFLD. In this study, we analyzed the fatty acid profile by GC-MS in the liver tissue of the hypercholesterolemic rabbit model. Also we evaluated a number of well-identified ER stress and lipid metabolism markers including GRP78, GRP94, phospho IRE1, phospho PERK, Chop, LXRα, SREBP1, SREBP2, PPARγ and ABCA1 by western blotting. Briefly, our results demonstrate that high cholesterol diet might lead to a significant increase in intracellular lipid synthesis, especially saturated fatty acids, followed by the activation of ER stress mediated apoptosis in NAFLD progression.

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P.5.4-035
N-acyl-L-homoserine lactones are inter-kingdom signalling molecules controlling quorum sensing in the Gram-negative bacteria and priming plants increasing stress adaptation

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Quorum sensing (QS) is a mechanism used by bacteria to communicate within physically-restricted populations and with the outside world. N-acyl-L-homoserine lactones (AHLs) are the most studied QS molecules, and these coordinate bacterial gene expression in response to changing environmental conditions and opportunities. AHL-based QS is very important in Gram-negative bacteria such as the pseudomonads where QS regulatory pathways have been described for the opportunistic human *P. aeruginosa* and plant *P. syringae* pathogens, as well as for many other pseudomonads including soil and plant-associated *P. fluorescens* sp. However, QS has not been described for *P. fluorescens* SBW25 which nonetheless can form biofilms traditionally associated with QS regulation. In this work we show that SBW25 is able to produce C8-HSL to control biofilm formation and eDNA release, and have identified a number of genes in silico that may be involved in AHL synthesis and QS regulation. So, AHLs present in a phytosphere constantly. This raises the interesting possibility that plants may respond to bacterial AHLs directly, and that exogenous AHLs might be used to improve crop yields. We hypothesized that some AHLs can be used to prime wheat plants. We used a short-chain C6-HSL to prime wheat seeds as well as young plants to evaluate their effect on plant resistance under laboratory and opened-field conditions. Both direct and indirect priming effects been revealed. The productive tillering, number and mass of seeds in a spike as well as total seed mass found to be increased. The qualitative and quantitative changes in the composition of ecological groups of rhizosphere microflora been observed. Cytological analysis shown the increase in cell wall thickness in three days after priming and increase of chloroplasts stability under acid rain stress. Since the priming of wheat shown positive effect on wheat plants AHLs might be considered as a promising ecological phytostimulator and phytomodulator.

P.5.4-036
Revealing novel functions of glutamate carboxypeptidase II using knock-out mice

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Glutamate carboxypeptidase II (GCPII), also known as prostate-specific membrane antigen (PSMA), has been suggested as an important therapeutic and diagnostic target. Overexpression of GCPII has been implicated in prostate cancer while inhibition of GCPII activity has been associated with neuroprotection. GCPII is involved in several physiological processes as a transmembrane glycoprotein with large extracellular part possessing carboxypeptidase activity. Despite extensive research, the physiological function of GCPII is still not fully understood. Several different attempts to generate GCPII knock-out (KO) mice were reported with rather controversial results, ranging from embryonic lethality to generation of viable GCPII KO mice with no obvious phenotype. Interestingly, a remaining GCPII-like peptidase activity was detected in GCPII KO mice suggesting compensatory expression of a homolog of GCPII – glutamate carboxypeptidase III (GCPIII). Here, we present generation of GCPII KO mice on C57BL/6 background by targeting the active site of the enzyme using TALEN technology. We established reliable genotyping method based on nested PCR and show that no GCPII protein is expressed in our KO mice as determined by Western blot and carboxypeptidase activity analysis. We also explore possible compensatory expression of GCPIII. Our GCPII KO mice are viable, breed normally and do not show any obvious phenotype. This reliable mouse model could be further used for revealing yet unknown physiological functions of GCPII and its orthologs.

P.5.4-037
Soluble adenylyl cyclase controls AMPK activity, mitochondrial function and biogenesis and may play a role in estradiol-dependent protection against oxidative stress in cardiac cells

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AMPK, via cAMP signalling, modulates mitochondrial function and biogenesis. But the source of the cAMP remains unknown. There are two sources of cAMP: transmembrane adenylyl

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cyclases and intracellular soluble adenylyl cyclase (sAC). Since AMPK is also localised intracellularly, we hypothesised that sAC may regulate AMPK activity and, thereby, mitochondrial function and biogenesis. As estradiol (E2) has also been shown to affect cAMP signalling and AMPK activity, we supposed a potential role of E2 in regulating sAC–AMPK axis. AMPK activity was examined by western blotting and FRET-based live imaging. Cellular cAMP and ATP contents were analysed by ELISA- and luminescence-based assay, respectively. Mitochondrial ROS was estimated with MitoSox. Mitochondrial mass was investigated by MitoGreen staining and mitochondrial/nuclear DNA ratio. In H9C2 cells and neonatal rat cardiomyocytes, E2 treatment (24 h) increased sAC expression and protected against H_2O_2-induced cell death and ROS formation. In H9C2 cells, sAC knockdown (KD) led to reduced cellular cAMP, AMPK activity and cellular ATP level and increased mitochondrial ROS formation. Similarly, sAC KD reduced AMPK activity in adult rat cardiomyocytes and coronary endothelial cells. Though sAC KD increased mitochondrial mass in H9C2 cells, mitochondrial biogenesis was suppressed. These data suppose a potential disturbance of mitophagy. Consistently, sAC overexpression enhanced AMPK activity and cellular ATP content. Stimulation of sAC activity with 42 mM bicarbonate increased cellular ATP level and led to activation of AMPK in the cytosol, but not in other compartments. The effects of bicarbonate were attenuated by PKA inhibitor or by sAC KD suggesting the involvement of sAC–PKA signalling. In conclusion, sAC plays an important role in supporting basal AMPK activity, mitochondrial function and biogenesis. Since E2 enhances sAC expression, the role of sAC in E2-induced protection against oxidative stress is suggested.

P.5.4-038
Interrelation between age and platelet-rich plasma composition (in growth factors and exosomal miRNAs) for personalized regeneration therapies

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Platelet-rich plasma (PRP)-based therapy is currently used in regenerative medicine (RM) procedures, due to the beneficial effect of growth factors released by platelets on cell proliferation, fibroblast stimulation and wound healing process. However, the positive effect of PRP is dose-dependent and the concentration in growth factors is age-dependent. Neither the molecular mechanisms triggered by PRP, nor the miRNAs found in PRP with potential regulatory effect were sufficiently studied. The aim of this study was to evaluate age-dependent PRP composition in growth factors and its effect on cell proliferation, as well as to investigate miRNA profiles in extracellular vesicles (EVs) found in PRP. For this purpose, 80 PRP samples were collected from healthy individuals in 3 age groups: young (<30 years), adults (30–49 years) and seniors (>50 years) and analysed for EGF, FGF-2, VEGF, PDGF-AB, IGF1 and TGFβ1 levels via magnetic-based MilliPlex kits (Luminex). Cell proliferation studies (MTT, LiveDead assays) were performed. Exosomes from plasma samples were isolated and validated for EVs markers TG101 and Alix by antibody arrays. miRNAs expression was assessed by miRNA qPCR array. Multiplex assays revealed a higher concentration of growth factors in younger subjects, leading to the hypothesis that PRP therapy should be personalized in a dose and age dependent manner. Fibroblast proliferation rate registered a significant increase when using 10% activated PRP-supplemented culture media. Upon activation, platelets in PRP released important amounts of miRNAs, among which miR-16, miR-125b, let 7-a, miR-143, previously associated with wound healing, were found to be upregulated. Consequently, this study demonstrates the need for personalized PRP therapy in RM applications, based on the cumulative pro-regenerative effect of growth factors and regulatory miRNAs.

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P.5.4-039
The effect of low-dose polyphenols on two-dimensional (2D) and three-dimensional (3D) *in vitro* model of hepatic steatosis

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases, characterized by over-accumulation of lipids and low-grade inflammation in the liver tissue. Studies in mice have shown that polyphenolic compound resveratrol (RSV) protected the liver from fat accumulation induced by high fat diet. However, detailed mechanisms mediating RSV effects remain unclear. In our research we studied an effect of 2 polyphenols (PPLs): RSV and trans-piceid on both lipid metabolism and inflammatory process, utilizing 3 different *in vitro* model systems created by inducing lipid overload (using free fatty acids (FFA)) in: 2 monolayer 2D cultures: mouse hepatoma cells – Hepa1–6 and mouse macrophages – RAW264.7 and 3D hepatic spheroids created from Hepa1–6 cells. Evaluating the effect of PPLs on lipid metabolism we measured the level of lipids overload, as well as the surface expression of lipid-transporter – CD36. To assess the effect of PPLs on pro-inflammatory processes, we monitored the surface expression of TLR4 receptor, which stimulates pro-inflammatory pathways in both- macrophages and hepatocytes. According to our data, FFA-induced lipid accumulation provoked an increase in TLR4 surface expression, both in monolayer and spheroid culture of Hepa1_6 cells. Low concentrations of PPLs (≤10 μM) have induced a decrease in lipid load in monolayer and spheroid culture of hepatocytes, as well as in macrophages. PPLs-induced decrease in lipid overload was accompanied by certain modulation of TLR4 and CD36 surface expression. Further, we investigated whether the disruption of dynamin-dependent vesicular transport pathways will affect both: PPLs-induced decrease in lipid overload and PPLs-induced modulation of TLR4 and CD36 surface expression. According to the obtained results, while the modulation of surface receptors expression appeared to be affected by dynamin inhibitor, the effect of PPLs on lipid overload appeared to be dynamin-independent.

P.5.4-040
Profiling of phytoecdysteroids and gibberellins during early stages of ontogenesis of superfood quinoa

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Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal that is being cultivated in Andean region since 3000 BC. Increased interest has been recently shown in this plant species worldwide (and

especially in Europe) since it was found that quinoa seeds represent very interesting functional food and nutraceutical due to lack of gluten, high protein content with abundance of all essential aminoacids, and a wide range of vitamins and minerals. *Ch. quinoa* also belong to the limited number of plant species in human diet having capacity to produce phytoecdysteroids (PEs). These substances are considered as plant secondary metabolites, which protect plants against non-adapted insects and nematodes. Regarding mammals and humans, PEs attract great attention for their potent pharmacological and medicinal properties including antibacterial, antifungal, antidiabetic, antiosteoporetic, performance-enhancing and wound healing effects. The aim of this study was to investigate the dynamics of PEs content in the selected twelve varieties of quinoa originating from Bolivia (9 varieties) and Peru (3 varieties) during early stages of its ontogenesis – the first four days of quinoa vegetative development – germination. It is known that this developmental process of plant, as well as other physiological processes *in planta,* are mainly driven by some plant signalling molecules like plant hormones. Plants' intricate signalling networks are further complicated by links and interactions with synthesis and metabolic pathways of secondary metabolites. To study these interactions, we performed LC-MS based targeted profiling PEs as the representatives of secondary metabolites together with profiling gibberellins, a group of plant hormones playing indispensable role in seed germination. Presented work represents a pilot survey focused on crosstalk between these two groups of endogenous terpenoid compounds.

P.5.4-041
How to turn it off? Role of VapC toxin in mycobacteria dormant state formation

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Previously, we have shown that transition of *M. smegmatis* cells into dormant state is accompanied by activation of vapC expression; at the same time, overexpression of VapC toxin in exponentially growing cells leads to formation of morphologically altered ovoid forms. These ovoid *M. smegmatis* cells have a reduced metabolic rate, a decreased level of respiratory activity and keep the ability to resuscitate, i.e., possess all the properties of dormant cells. Thus, the toxin VapC is proved to be a factor of transition into resting state. Though the mechanism of such a transition is still unclear, it is known that under VapC overexpression, inhibition of growth and translation accompanied by accumulation of polysomes is observed. If VapC toxin provides a redirection of protein synthesis, it is essential to compare the protein profile of the wild type strain and the VapC overexpressing one. Protein extracts of both strains were used for LC-MS analysis. An increase in the abundance of ribosomal proteins, ABC-transporters, glutamine synthase, serine-threonine protein kinases, and aminobutyric shunt enzymes as well as a decrease of aminoacyl-tRNA synthases abundances were found in membrane fraction of the protein profile of VapC overexpressing strain. Apparently, the cells form a stress response in this way. In addition, a search for VapC toxin target in *M. smegmatis* was carried out, namely, the integrity of 23S rRNA transcripts was assessed. Using qRT-PCR, we found a decrease in abundance of amplicons corresponding to domain VI fragment of 23S rRNA in VapC overexpressing strain nearly by 70% compared to the wild type. 23S rRNA molecules were cleaved by ribonuclease VapC between A2929 and G2930 nucleotides. Thus, when making *M. smegmatis* cells dormant, VapC toxin inactivates ribosomes through 23S rRNA cleavage, associates them with the cell membrane somehow and redirects protein synthesis dramatically.

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P.5.4-042
Dendritic cell signaling phases triggered during the early stage of Francisella tularensis invasion

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Francisella tularensis is a highly infective bacterium responsible for tularemia. The disease is characterized by the delayed onset of the adaptive immunity which is followed by the excessive inflammation. The impaired host response is a consequence of *Francisella* ability to replicate inside primary phagocytes while avoiding their activation. *Francisella*-infected cells do not provide enough pro-inflammatory stimuli and serve rather as bacterial reservoirs. From this point, dendritic cells (DCs) represent important hosts due to their migratory nature and their ability to present antigens. In this work, we applied quantitative phosphoproteomic approach for the analysis of DC signaling in response to *Francisella* in hope that results will help to understand the behavior of infected cells. In particular, we wanted to describe the very early moments of the invasion because these events represent the instructive signals for DC activation and maturation. Primary bone marrow-derived DCs (BMDCs) were SILAC-labeled and infected either by virulent *Francisella* FSC200 strain or by an attenuated mutant lacking *dsbA* gene. Following the infection, BMDCs were lysed and processed for LC-MS-based phosphoproteome analysis. The majority of sites differentially regulated during the first 10 min of infection were connected to the activation of PAKs, Akt-mTOR-p70S6K pathway and ERK signaling. While there were no quantitative differences between BMDC response to virulent and mutant strain during the bacterial internalization, only virulent *Francisella* induced the second wave of protein phosphorylation in infected cells at 60 min p.i. The most prominent feature of the latter signaling phase was the activation of ERKs and p38 kinases and their downstream transcription factors. We conclude that this second signaling wave represents the initial event of DC activation where virulent and attenuated *Fracnisella* strains differ.

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P.5.4-043
Translation initiation factor 6 (eIF6) regulates apoptosis in *D. melanogaster* eye development

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Eukaryotic initiation factor 6 (eIF6) regulates translation initiation by binding the 60S subunit and it is upregulated in some cancers. We previously demonstrated that eIF6 haploinsufficient

mice show a reduction in lymphomagenesis. These data indicate that cells need to tightly regulate eIF6 gene dosage. The eif6 gene is highly conserved from yeast to human. The well characterized development of *D. melanogaster* and the easy manipulation of its genetics led us to the fly model to study the effects of eIF6 altered gene dosage. Ubiquitous overexpression of eIF6 is lethal. We then focused on the eye, which is dispensable for life. We found that eIF6 overexpression in all eye's cells results in a rough eye phenotype. In addition, eIF6 overexpression only in cone or pigment cells is sufficient to alter the eye morphology. It is well established that the crosstalk between these two cell types during the pupal stage is responsible for the programmed cell death (PCD) of extra-numerary pigment cells to determine the correct structure of the adult eye. We indeed found that eIF6 is critical at this stage, because its overexpression is associated to a delayed and increased PCD, resulting in an aberrant adult eye. Moreover, we found an alteration of translation efficiency with a two-fold increase of general translation upon DeIF6 overexpression. We then analyzed the gene expression by RNASeq analysis, which revealed alterations in genes specific for eye development and, surprisingly, a tremendous decrease in ecdysone pathway related genes. In addition, administration of 20-HydroxyEcdysone (20HE) partially rescued the rough eye phenotype. This is the first evidence of PCD regulation by a translation initiation factor such as eIF6 via the ecdysone metabolism.

P.5.4-044
Role of ceramide synthase 5 as transcription factor identified by ChIP and LC-MS/MS

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Over the last few decades, sphingolipids and their metabolizing enzymes, like ceramide synthases (CerS), have been realized to be important in diseases for example cancer development and progression. In women, breast cancer is the most frequently diagnosed form of cancer with over 1.5 million new cases worldwide diagnosed each year. It is the second leading cause of cancer death in women. Therefore, identifying the exact signaling pathway is important for an efficient and individual breast cancer therapy. CerS-2, -4, -5 and -6 contain a homeobox (Hox)-like domain. The function of this domain in CerS isoforms is mostly unclear but its presence suggests a potential role as transcription factor. First results received by Chromatin-immunoprecipitation (ChIP) support this hypothesis. We established a screening method for proteins and their role as transcription factors based on CerS-5. Formaldehyd was used to cross-link the proteins to DNA, crosslinked lysate was sonicated and CerS-5 was precipitated with an anti-CerS-5 antibody. CerS-5 protein and anti-CerS-5 antibody were captured with beads and the crosslink was reversed. The DNA was degraded to nucleosides and was quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The nucleosides were separated with reversed phase chromatography on an Atlantis T3 column. The target DNA sequence was determined. This is a new innovative procedure, which can be assigned to any other protein and provides new insights into the role of CerS-5 in the regulation of cellular mechanism and enhance our understanding of CerS signaling.

P.5.4-045
Distinguishing of pea aminoaldehyde dehydrogenase isoenzymes in plant extracts using a synthetic coenzyme analog

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Aldehyde dehydrogenases (ALDHs) are widespread in living nature. Their role resides in $NAD(P)^+$-dependent oxidation of aldehyde substrates to carboxylic acids, which provides important metabolites or represents a way to remove toxic compounds. Members of the ALDH10 family in plants are aminoaldehyde dehydrogenases (AMADHs) as they readily oxidize omega-aminoaldehydes. Due to their broad substrate specificity, the enzymes used to be named 4-aminobutyraldehyde dehydrogenases (EC 1.2.1.19), 4-guanidinobutyraldehyde dehydrogenases (EC 1.2.1.54) or betaine aldehyde dehydrogenases (EC 1.2.1.8). Plant AMADHs exist as homodimers with a typical ALDH subunit fold comprising a catalytic domain, a coenzyme-binding domain and an oligomerization domain. The active site contains three strictly conserved and essential residues (Cys, Glu and Asn). Usually, there are two isoforms, which differ in their molecular properties and substrate specificity, but the biological significance of this fact has not satisfactorily been addressed. In this work, pea isoenzymes PsAMADH1 and PsAMADH2 were assayed in roots and shoots of germinating seedlings by spectrophotometry and native gel electrophoresis or isoelectric focusing followed by activity-based staining. The assays exploited a different activity of the isoenzymes with NAD^+ and its synthetic analog thio-NAD^+. The latter coenzyme form is accepted efficiently by PsAMADH2 but not PsAMADH1. We demonstrated that the specific activity of AMADH with thio-NAD^+ increased during the germination period in contrast to that with NAD^+ and since the day 5 it was even higher in absolute numbers. This observation indicates a gradual increase in the representation of PsAMADH2 towards PsAMADH1 during germination in both roots and shoots. The same trend was observed by activity staining of gels. The isoenzymes 1 and 2 were identified and distinguished in neighbor bands displaying a different staining intensity using nanoLC-ESI-MS/MS after a tryptic digestion.

P.5.4-046
Siderophores produced by *Serratia marcescens* SM6

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Iron is an essential micronutrient for nearly all forms of life. Its availability is tightly controlled by the mammalian host during infection. To overcome iron deficiency bacteria produce small molecules – siderophores which exhibit high binding affinity to Fe (III) ions and make iron available for bacterial cells. In addition to well-known role in iron acquisition siderophores also contribute to cell to cell signaling and oxidative stress defense. Here we report identification of siderophores in opportunistic pathogen *Serratia marcescens*. Genome of *S. marcescens* SM6 encodes two gene clusters involved in siderophore production. The gene that encodes the key enzyme for siderophore synthesis in cluster 1 shares 63% identity with gene *cbsF* of *Dickeya chrysanthemi* EC16. In *D. chrysanthemi cbsF* is involved in production of siderophore chrysobactin. LC-MS analysis of secondary metabolites

present in the conditioned media used for growth of *S. marcescens* SM6 under iron-limiting conditions showed the presence of the compound with mass-to-charge ratio (m/z) 370.16 and chemical formula $C_{16}H_{23}N_3O_7$ that matches the expected size of chrysobactin along with three additional compounds with m/z 721.32, 1072.45 and 1054.44 (dichrysobactin, trichrysobactin and cyclic trichrysobactin, respectively). Genomic organization of siderophore cluster 2 *S. marcescens* SM6 is similar to serratiochelin biosynthetic cluster of *Serratia plymuthica* V4. LC-MS analysis of metabolites in the siderophore extract of *S. marcescens* SM6 identified the compound with m/z 430.16 and a chemical formula $C_{21}H_{24}N_3O_7$. ^{1}H-NMR analysis confirmed that this compound is Serratiochelin A. We are currently working on characterization of phenotypes associated with the loss of individual genes in both siderophore biosynthetic clusters.

P.5.4-047
Loss of ELOVL3 reduces lipogenesis in skeletal muscle

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The fatty acid elongase ELOVL3 is involved in the synthesis of C20–C24 very long chain fatty acids. It was shown that ablation of ELOVL3 leads to reduced levels of adiponectin and leptin, constrained expansion of adipose tissue, and resistance against diet-induced obesity. Moreover, in cold-acclimated ELOVL3-/- mice, there is an increased heat loss due to impaired skin barrier, and lack of hyperrecruitment of brown adipose tissue. Instead, the muscle shivering in order to maintain body temperature was observed. This fact suggests a possible involvement of ELOVL3 in the regulation of skeletal muscle metabolism. Therefore, the aim of the present study was to investigate the effect of ELOVL3 ablation on the lipid metabolism in skeletal muscle. First, we measured level of sterol regulatory element-binding protein 1 (SREBP1), a transcription factor involved in regulation of lipogenesis. Our study showed decreased protein levels of both premature SREBP1 and mature SREBP1 in white gastrocnemius (WG) muscle of ELOVL3-/- mice. Also the protein levels of targets of SREBP1, i.e. fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase 1 were decreased in WG of ELOVL3-/- mice. These results suggest decreased fatty acid *de novo* synthesis and reduced lipogenesis in WG of ELOVL3-/- mice. Next, we analyzed protein level of adipose triglyceride lipase (ATGL), the rate-limiting enzyme in TG hydrolysis. ATGL protein level was decreased in muscle of ELOVL3-/- mice indicating reduction of lipolysis. Summarizing, presented results show important role of ELOVL3 in the regulation of skeletal muscle energy metabolism.

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P.5.4-048
The role of urokinase-type plasminogen activator system in regeneration properties of resident stem cells

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Mesenchymal stem cells (MSC) supposed to take a part in regeneration especially when it became activated by injury and inflammation. Restoration of tissue function is accompanied by angiogenesis, progenitor cell activation/migration and extracellular matrix reorganization. These effects are associated with two major fibrinolytic factors: urokinase plasminogen activator (uPA; urokinase) and uPA receptor (uPAR). It was shown that uPAR is expressed on the surface of MSC and in addition that the proteolytic activity of urokinase may regulate MSC behavior during regeneration. The aim of this work was to investigate the effects on MSC developed in our laboratory recombinant uPA and its forms (proteolytically inactive uPA with His204Gln mutation (uPA-H/Q), uPA lacking the growth factor-like domain (ΔGFD), kringle domain (KD), and aminoterminal fragment (ATF)). We studied human adipose tissue-derived mesenchymal stromal cell (ADSC) migration, proliferation and matrix metalloproteinases secretion induced by uPA. We found that uPA and its recombinant forms had no effect on ADSC proliferation assessed by MTT test. Directed ADSC migration was slightly induced by uPA, with no effect of other uPA forms. Full urokinase and kringle domain enhanced spontaneous migration of ADSC. Chemotaxis induced by platelet-derived growth factor (PDGF) was attenuated by proteolytically inactive uPA, KD and by blocking antibody to urokinase receptor. Urokinase and all of its recombinant forms induced secretion of matrix metalloproteinase-9 by ADSC, this effect was absent in PDGF. These findings suggest that uPA system is involved in the regulation of migration and secretion of MMP9 by MSCs. Modulation of uPA-system activity may be considered as a possible tool for induction of regeneration potential of MSC.

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P.5.4-049
Metabolic changes in the *Opisthorchis felineus* infected liver

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Opisthorchiasis caused by *Opisthorchis felineus* is a fish-borne parasitic worm infection spread in Russia and some European countries. This fluke inhabits the bile duct of the host liver, causing local tissue damage and chronic inflammation. The aim of the study was to investigate the local metabolic changes provoked by *O. felineus* in infected liver an experimental animal model. 5-week-old hamster *Mesocricetus auratus* were infected intragastrically with 50 metacercariae per hamster and divided in 3 groups; age-matched intact hamsters were used as controls for each group. After 5 weeks of infection hamsters were euthanized; blood and liver samples were collected for examination. Serum and blood analyses were performed using routine procedures. Liver samples were analyzed by histological (to determine the fibrosis stage) and biochemical analyses (to estimate cholesterol, triacylglycerols (TAG), phospholipids, proteins, glycogen and ATP concentration in tissue). Western blot analysis was applied

to evaluate AMPK phosphorylation level in the liver. For the gluconeogenesis rate estimating pyruvate tolerance test was performed using hamsters of 2nd group (both infected and intact); blood glucose concentration was monitored during 2 h. To analyze the liver TAG production rate the hamsters of 3th group (both infected and intact) were injected with triton WR1339 and concentration of TAG in blood was measured during 8 h. In conclusion, opisthorchiasis provoked metabolic change in the host liver, increased gluconeogenesis and accompanied by cholesterol accumulation in hepatic tissue. The knowledge about the mechanism that leads to the observed changes in host metabolism may be applied for seeking a target for anti-helminth therapy and serve as the basis for developing new approaches to human metabolic disease regulation.

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P.5.4-050
Characterization of several aldehyde dehydrogenases in moss *Physcomitrella patens*

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Aldehyde dehydrogenases (ALDHs) comprise a protein superfamily of $NAD(P)^+$-dependent enzymes (EC 1.2.1.-) and catalyze irreversible oxidation of aldehydes to carboxylic acids. ALDHs play a crucial role in detoxifying aldehydes produced by various metabolic pathways and during various stress conditions such as salinity, heat, cold and drought. The superfamily of plant ALDHs currently contains 13 distinct families and ALDHs sharing more than 40% sequence identity constitute the family. In this work, we studied ALDH2, ALDH10 and ALDH21 family members from the moss *Physcomitrella patens*, which belong to bryophytes, a group of non-vascular plants comprising mosses, liverworts and hornworts. Bryophytes represent an important transition step in the conquest of land and they retain a significant drought tolerance. Plant ALDH2 family comprises mitochondrial and cytosolic isoforms while ALDH10 family comprises peroxisomal and chloroplastic isoforms, respectively. The genome of *P. patens* contains two *ALDH2* genes and only one *ALDH10* gene. This number is low compared with six *ALDH2* and three *ALDH10* genes in maize. The *ALDH21* gene is unique to lower plants. It is well known that plant ALDH10 isoforms display aminoaldehyde activity, mitochondrial ALDH2 isoforms display acetaldehyde activity while cytosolic ALDH2 isoforms catalyze the oxidation of aliphatic and aromatic aldehydes such as coniferaldehyde and sinapaldehyde. We analyzed the enzyme kinetics of four moss isoforms and we further performed a structural study on PpALDH2A and PpALDH21 followed by the site-directed mutagenesis in order to identify key residues involved in catalysis and the substrate specificity.

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P.5.4-051
Morphological and metabolic alterations in human Sertoli cells induced by inhibition of carbonic anhydrase

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Carbonic anhydrases (CAs) are metalloenzymes present in all prokaryotes and eukaryotes. These enzymes catalyse the conversion of CO_2 to HCO_3^- and H^+. This reaction is involved in multiple physiological processes. CAs have been identified in the reproductive tract of various species, but their precise physiological roles in the male reproductive tract is still unclear. Herein, we determined the impact of CAs inhibition with acetazolamide, a general inhibitor of CAs, on the metabolism (by means of nuclear magnetic resonance spectroscopy), and on the mitochondrial morphology (by means of electron microscopy), biogenesis (using quantitative reverse transcriptase polymerase chain reaction) and bioenergetics (using the fluorescent probe JC1) of primary cultures of human Sertoli cells (hSCs), obtained from biopsies from men with conserved spermatogenesis. When CAs were inhibited in hSCs (by exposure to acetazolamide during 24 h), we observed a decrease in the number of mitochondria and an alteration on their morphology, though the average mitochondrial membrane potential was not affected. Still, cells treated with acetazolamide exhibited an accumulation of cytoplasmic lipid droplets, suggesting a diminished mitochondrial function. In fact, when assessing the expression of mitochondrial biogenesis markers, we observed a striking decrease in the abundance of HIF-1α, Sirt1, PGC-1α and NRF-1α mRNAs, when CAs were inhibited. All these mitochondria-associated alterations were accompanied by an increased production of lactate and alanine (although the consumption of glucose was maintained) indicating a reduced conversion of pyruvate to acetyl-coA, possibly due to the diminished mitochondrial function. Our results suggest that, in hSc, CAs are essential for mitochondrial biogenesis, morphology and physiology. Since Sertoli cells are essential for the occurrence of spermatogenesis, CAs should play an important role in the establishment of male reproductive potential.

P.5.4-052
Notch signaling is involved in regulation of cardiac stem cells behavior in scaffold-free cell sheet after epicardial implantation

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Cardiac progenitor cells (CPC) based cell sheet transplantation is emerging as a promising method to repair heart injuries. CPC contributes recovery of heart injuries by multilineage

differentiation, paracrine secretion and anti-inflammatory effects. However, signaling pathways which regulates cell suvirval and function after transplantation are not investigated in detail. Here we investigated the role of Notch pathway in the regulation of CPC function *in vitro* and after epicardial implantation in scaffold free cell sheet. After coronary artery ligation in rats syngeneic c-kit+Lin CPC marked with CM-DIL were grafted by epicardial placement of cell sheet generated using temperature-responsive dishes. Cell sheets integration, neovascularization, Notch signalling activation state, proliferation and differentiation were assessed by immunofluorescence analysis of myocardial frozen sections harvested 14 days after transplantation. For Notch signalling activation NICD overexpression and cultivation of CPC on Jagged 1-coated dishes were used. Histological analyses revealed that CPC sheet grafts produced thick, well vascularized tissues on the epicardial surface of the heart. Part of transplanted CPC migrated into myocardium, showed signs of Notch signaling activation (NICD in nucleus) and differentiation to cardiomyocytes and endothelial cells. Cultivation of CPC *in vitro* on dishes coated with Jagged 1 released NICD and activated expression of Notch target genes (Hes and Hey). Activation of Notch signaling upregulated expression of vascular cell transcription factors in CPCs and γ-secretase inhibitor prevented Notch signaling activation and CPCs commitment to endothelial lineage. Notch activation in CPC increased their ability for tube formation in Matrigel angiogenesis assay. These findings suggest that targeted modulation of Notch1 signalling may be useful for upregulation of cardiac progenitor cell sheets functionality and vacularization.

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P.5.4-053
Protective effect of betaine against paraquat-induced oxidative stress and pulmonary fibrosis in rats

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Betaine is a methyl donor utilized in remethylation of homocysteine to methionine, which is mediated by betaine-homocysteine methyltransferase (BHMT) mostly localized in the liver. However, our recent study revealed that the metabolism of sulfur-containing substances in extrahepatic tissues was also influenced significantly by betaine intake, probably due to a change in supply of sulfur-containing amino acids in blood. In this study we determined the effect of betaine against paraquat (PQ)-induced pulmonary injury in rats. Rats received betaine in drinking water (1%) for 2 wk prior to PQ challenge (0.3 mg/kg, it). In 2 wk after PQ instillation, 4-hydroxyproline levels in the lung and oxidative DNA damage measured by the Comet assay were increased significantly. Similar results were shown in histopathological assessment of lung tissues. Betaine supplementation effectively inhibited the fibrogenic changes in the rats treated with PQ. PQ instillation decreased methionine levels in the lung, but betaine supplementation elevated methionine and *S*-adenosylmethione (SAM) levels significantly. Putrescine and spermidine levels were also elevated by betaine supplementation. On day 4 after PQ instillation, the metabolomics of sulfur-containing substances was disturbed markedly, but glutathione (GSH) and its metabolic substrates, including methionine, *S*-adenosylhomocysteine (SAH), homocysteine and cysteine, were all elevated in the lung by betaine supplementation. Elevation of proinflammatory cytokines was also inhibited. Taken together, the results suggest that betaine may protect the lung from PQ-induced oxidative stress and pulmonary fibrosis most probably via enhancement of antioxidant capacity and polyamine synthesis.

P.5.4-054
Serotonergic signaling system in granulosa cells of the developing ovarian follicle

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Granulosa are the somatic component of the ovarian follicle, through which the interaction of the maturing egg with the mother's organism occurs. Serotonin is one of the important factors regulating both functional activity of granulosa cells and processes of oogenesis. The expression and functional activity of the components of the serotonergic system in postovulatory cumulus cells were shown earlier, but little is known on the composition of the serotonergic system in granulosa cells at the initial stages of folliculogenesis. We performed a RT-PCR screening of mRNA expression of all known components of the serotonergic system in granulosa cells obtained from mouse primary, secondary (antral) and preovulational ovarian follicles. Although tryptophan hydroxylase *tph1* is expressed at all stages, the aromatic L-amino acid decarboxylase *ddc* is not, hence the synthesis of serotonin in granulosa cells is impossible. Both the serotonin membrane transporter *sert* and the vesicular monomamine transporter *vmat2* are expressed in granulosa cells, suggesting the possibility of serotonin uptake from the intercellular medium and its accumulation into vesicles. mRNA of several serotonin receptors are expressed in granulosa cells – *htr1b*, *htr1d*, *htr2a* and *htr7*. In addition, the main serotonin degradation enzyme monoamine oxidase A *maoa* is expressed at all stages of folliculogenesis. Thus, all major components of the serotoninergic system that are necessary for the implementation of all the steps of the signaling process, with the exception of serotonin synthesis, are expressed in granulosa cells during all stages of folliculogenesis.

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P.5.4-055
Genetically encoded fluorescent probe to visualize phosphatidylinositol

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Phosphatidylinositol (PI) is an important lipid that serves as a membrane constituent and also participates in cell signaling as a precursor for phosphoinositides made by phosphorylation of one or more hydroxyl groups in positions 3, 4 or 5 of the inositol ring. However, the temporal and spatial distribution of PI remains unclear owing to the lack of a specific biosensor to visualize PI. Here we derive a new PI optical probe using phosphatidylinositol-dependent phospholipase C, an enzyme secreted by *Bacillus cereus* specifically cleaving PI into diacylglycerol and inositol 1-phosphate. Unlike eukaryotic isoforms bacterial inositol-dependent phospholipase C (PI_PLC) cannot use either PtdIns4P or PtdIns(4,5)P2 as a substrate, what makes it an ideal candidate for construction of genetically encodable fluorescent PI-specific biosensor. To potentially increase the affinity and specifity towards PI, we designed several mutant forms of PI_PLC. Further, we structurally characterized these mutants using protein crystallography. We performed lipid binding assay using phospholipid vesicles with and without PI, which showed that PI_PLC binding to membranes is PI-dependent. To asses *in vitro* PI-binding properties of each PI-PLC mutant GUVs recruitement assay using fluorescently labeled PI_PLCs was carried out. Our results revealed no binding to other phospholipids tested. Overall our findings indicate that our PI_PLC probe

displays high *in vitro* specifity for PI and is suitable candidate for biosensor design.

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P.5.4-056
Mechanisms of protective effect of sodium nitrite on the isolated rat heart at ischemia-reperfusion

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One of the main directions in the search for effective cardioprotective mechanisms against ischemia/reperfusion (I/R) injury is the use of various methods of preconditioning. The goal of this research was to examine the effect of pharmacological preconditioning using sodium nitrite on the functioning of an isolated working heart of male Wistar rats (180–300 g) with I/R. Preconditioning was induced with a single subcutanic injection of sodium nitrite at 3 mg per 100 g of body weight, control – saline. In the first series of experiments 30 min after the injection rats were decapitated, hearts were excised for biochemical analyses. In the second series of experiments 10 min after preconditioning animals were anesthetized, the heart rate (HR) was measured. 20 min after the anesthetic administration, the hearts were immediately excised using thoracotomy and perfused in a working heart system and subjected to 30 min global ischemia, followed by 30 min reperfusion. HR and coronary flow (CF) were measured in isolated heart experiments. After perfusion protocol the tissues of heart were used to the determination of TBA-active products and catalase activity. *In vivo* experiments we found that 30 min after preconditioning there were a significant negative chronotropic effect of sodium nitrite on the contractile function of the heart. HR decreased by 43% compared to the control. Simultaneously, in the tissues of the heart there was a significant decrease in the concentration of TBA-active products by 50% compared to the control. In ex vivo experiments HR and CF were significantly lower compared to the controls by 12.6% and 16.3%, respectively. A similar trend was observed after I/R. Additionally, we observed significant increase of catalase activity in the tissues of the isolated rat heart after I/R. Thus, preconditioning using sodium nitrite has a protective effect on the functioning of an isolated rat heart.

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P.5.4-057
Thermogenic food molecule RALIG prevents diet induced obesity by activating brown adipose tissue

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Thermogenesis is the process through which warm blooded animals generate heat. Brown adipose tissue (BAT) is the site of non-shivering thermogenesis in mammals, wherein energy is dissipated as heat rather than being stored as fat. The uncoupled respiration is the key phenomena accounting for anti-diabetic and anti-obesity property of BAT. Thus, we screened several foods traditionally known to induce thermogenesis *in vitro* for mitochondrial uncoupling. One of the food sources (coded as *LIPIC-60*) triggered an increase in the expression of Uncoupling Protein 1 (UCP1) and other brown fat specific markers. The active component from the extract was purified using activity guided fractionation (ability to induce UCP1) and positive fractions were further purified by HPLC and size exclusion chromatography. Intriguingly, the pure molecule (coded as *RALIG*) increased expression of brown fat specific genes including Pparα, Cidea, and Ucp1 in primary brown and white adipocytes *in vitro*. Also, *RALIG* treatment (5 mg/kg BW, i.p, 10 days) reduced serum levels of glucose, LDL cholesterol and leptin levels significantly in C57BL/6J mice. Strikingly, *RALIG* treatment substantially increased adiponectin level and protected mice against high fat diet induced obesity. The expression levels of UCP1 and the respective cellular events during non-shivering thermogenesis are highly dependent on the activation of ß3-adrenergic receptors (ß3-AR) of brown adipocytes. Understanding the molecular interactions of *RALIG* towards the mouse ß3-AR *in silico* revealed that it embedded in the same active site pocket as the known agonists. Furthermore, *RALIG* failed to induce thermogenesis in primary brown adipocyte culture in the presence of ß-AR antagonist, propranolol. Together, our results suggest that *RALIG* induces BAT activation through ß3-AR and therefore, thermogenic food molecules represent a potential complementary approach to combat diet induced obesity and associated metabolic disease.

P.5.4-058
Disorders of microelements profile in serum of overweight/obese adult females with past and repeated adenoviral infection

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Increased number of well documented evidences indicate that adenoviruses ((ADV) with adipogenic activity could be linked with human obesity but significance of some trace elements on the immunomodulatory function and susceptibility of the host on ADV infection are not fully recognized. Thus 18 microelements influencing antioxidant status were analyzed by IPC-MS in the nitric acid diluted serum samples collected from ADV-positive adult females (n = 59, av. age 46.5 years) from urbanized region of Poland. Past and repeated ADV infection were confirmed by IgA, IgG and IgM immunoenzymatic and real-time PCR serum assays. All females were classified under body-mass index (BMI) values to normal-weight (NW), over-weight (OW) and obese group (OB). Age-matched ADV-negative adult females (NDV, n = 23) comprising the control group. Prevalence of past ADV infection (IgG+) increased with age, BMI and body fat content of studied females and was significantly higher among OW/OB subjects in comparison with NW females (71.2 vs. 28.8%; ($P < 0.03$). Elevated V, Co, Zn, Cd, Sb, Ba, Hg, Pb conc. and declined B, Al, Mg, Mn, Fe, Ni, Cu, Se conc. were observed in serum of NW, OW and OB females indicating past ADV infection. In each IgG+ subgroup the serum conc. ratio Cu/Zn and Cu/Fe significantly decreased after ADV infection. However, as compared to NDV, strongest decline in Cu/Zn and Cu/Fe ratio (1.6 and 1.3 fold, respectively) and vit. A and E content (ca. 20%) was observed in NW-ADV group. Highest decline in serum Mn and Fe conc. was

noted in females NW-ADV (36.1 vs 15.9%) followed by OW-ADV (16.2 vs. 11.0%) and OB-ADV (30.5 vs. 11.2%). Similarly, after repeated ADV infection decline of serum Cu was observed in order: NW-ADV (33.7%), OW-ADV (0.5%), OB-ADV (16.6%). In addition, for all ADV infected groups no significant change in serum Cr and As was observed, thus suggesting that epigenetic events linked to these microelements could trigger ADV related systemic inflammation in obese females.

P.5.4-059
Nucleoside N-ribohydrolases in maize (*Zea mays*)

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Nucleosides and nucleobases are recycled to nucleoside monophosphates in a salvage pathway. This pathway preserves an energy, which would otherwise be needed for *de novo* synthesis. In the purine salvage, an important role has been shown for adenosine kinases (ADK, E.C. 2.7.1.20) and adenine phosphoribosyltransferases. Purine (as well as pyrimidine) nucleosides can be hydrolyzed by nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-) to corresponding nitrogenous bases and ribose. NRHs impose a strict specificity for the ribose moiety while residues interacting with the nucleobase highly vary. Plant NRHs can be divided in two subclasses with one preferentially targeting purine ribosides and the other one acting especially on uridine and xanthosine. Both subclasses hydrolyze also plant hormones cytokinins (N^6-substituted adenine derivatives). In this work we analyzed spatial and temporal expression of all *NRH* and *ADK* genes present in maize (*Zea mays*). A transient expression of ZmNRH-GFP fusion proteins was further studied in maize protoplasts. Further on, dexamethasone-inducible *ZmNRH* overexpressor lines in *Arabidopsis thaliana* were prepared to analyze the enzyme function *in planta*. Our experiments proved that NRHs metabolize also cytotoxic metabolites like 5-fluorouridine and 2-chloroadenosine. We further combined a site-directed mutagenesis with kinetic and structural analyses to study nucleoside binding sites in two maize NRHs. Crystal structures in complex with several ligands were solved up to 1.75 Å resolution. Twelve mutant variants were studied in detail and certain mutations were found to increase the hydrolysis of cytokinin ribosides.

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P.5.4-060
Suppressed mitochondrial oxidation of the carbon derived from glucose due to impaired mitochondrial pyruvate transport in renal cell cancer

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Glucose-dependent oxidation is disturbed in many cancer cells through HIF–dependent induction of lactate dehydrogenase A (LDHA), which favorably converts pyruvate to lactate, and pyruvate dehydrogenase kinase 1 (PDK1), which inhibits pyruvate dehydrogenase (PDH) activity, thus resulting in the suppression of mitochondrial pyruvate utilization. However, recent reports demonstrated that mitochondrial pyruvate carrier (MPC), which is composed of MPC1 and MPC2, is required to import pyruvate into mitochondria. In this study, we asked whether MPC could be essential for mitochondrial pyruvate usage in RCC since glucose- and pyruvate-derived OCRs were severely compromised in RCC cell lines. The mRNA level of MPC1 showed marked decrease in RCC tissues compared with those in matched normal kidney tissues although MPC2 mRNA expression decreased to a lesser extent. Protein levels of MPC1 and MPC2 were also severely decreased in RCC tissues and RCC cell lines when compared with HEK293 cells and HCC cell lines and the mRNA level of MPC1 was also markedly reduced in RCC cell lines without comparable changes in MPC2 mRNA levels. We investigated whether the reduced expression of MPC contributes to impaired pyruvate oxidation in Caki2 cells. Caki2 cells overexpressing MPC1 and MPC2 showed enhanced pyruvate oxidation in both basal and maximal respiration. Furthermore, MPC inhibitor, UK5099 treatment completely suppressed the elevating effects of MPC on the OCR. These results suggest that a defect in MPC may block mitochondrial pyruvate utilization in RCC.

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P.5.4-061
The relationships among an activity of the alternative pathway respiratory flux, a content of carbohydrates and a frost-resistance of winter wheat

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A content of carbohydrates, respiratory rates and coupling degree of oxidative phosphorylation in the isolated mitochondria, activities of the alternative pathway (AP) flux and the cytochrome pathway (CP) flux after different conditions of cold hardening (light, light/dark, dark, dark+sucrose) and relationships among these parameters and a frost-resistance have been investigated. The mitochondria have been isolated from leaves of winter wheat (*Triticum aestivum* L.) and purified in Percoll. It has been shown that the frost-resistance of winter wheat is generally associated with the carbohydrates content and the increased AP activity. The most resistance developed after exposure under continuous light or dark conditions with sucrose when the more significant increase of carbohydrates content (8.8-fold and 10.5-fold, respectively) and AP activity in respiration of mitochondria under malate (increase of AP/CP was a 1.6-fold and 2.1-fold, respectively), succinate (increase of AP/CP was a 1.1-fold and 1.2-fold, respectively) and exogenous NADH (increase of AP/CP was a 1.7 –fold and 2.0-fold, respectively) oxidation have been observed. In addition, after exposure continuous light or dark with sucrose was marked increase of the AOX protein content in mitochondria. Thus the positive relationships between the AP activity of mitochondria and plant frost-resistance have been shown. We have also observed the increase in AP activity at the oxidation of glycine by mitochondria (increase of AP/CP was a 2.0-fold) after cold hardening under continuous light, which indicates the important role of the AP in maintaining processes photorespiration in the conditions of low temperature. It is supposed that the AP activity in leaves under cold hardening protects

photosynthetic apparatus and prevents the oxidative stress, promotes to accumulate of carbohydrates and increase the winter wheat frost-resistance.

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P.5.4-062
A specific oligopeptide transporter mediates quorum-sensing regulation of the extracellular xylanase gene in *Geobacillus stearothermophilus*

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Geobacillus stearothermophilus T-6 possesses a single extracellular xylanase (Xyn10A), capable of producing short, decorated xylo-oligosaccharides from the naturally branched polysaccharide, xylan. The specific activity of the extracellular xylanase increases over 10-fold during early exponential growth, suggesting cell density regulation (quorum sensing). Addition of conditioned medium to low cell density cultures resulted in high expression of *xynA*, indicating that a diffusible extracellular *xynA* density factor (XDF) is present in the medium. XDF is heat-stable, sensitive to proteases and was partially purified using reverse phase liquid chromatography. Based on these results, it is likely that XDF is a small hydrophobic peptide or peptides. Secreted extracellular signaling peptides can be imported to the cell *via* specific oligopeptide (Opp) transport systems. Based on its genome sequence, *G. stearothermophilus* T-6 possesses a single Opp transport system composed of five genes (*opp*ABCDF). Bialaphos is a toxic tri-peptide that is known to enter bacteria *via* oligopeptide permeases. We have isolated a bialaphos-resistant mutant of *G. stearothermophilus*, and found that the *oppB* gene is interrupted by an insertion element and presumably lacks a functional Opp transport system. In this bialaphos-resistant mutant the *xynA* gene does not appear to be regulated by cell density, suggesting that an intact oligopeptide transport system is required for quorum-sensing regulation of the *xynA* gene.

P.5.4-063
Signaling events at the early stage of muscle disuse

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Postural muscle disuse is followed by the progressive atrophy development and slow-to-fast myosin phenotypical shift. It is believed that these events are triggered at the very early stage of unloading. Using the rat hindlimb unloading model we found 49% decrease of AMP-activated protein kinase phosphorylation and unexpected 56% increase of p70S6K (mTORc1 effector) phosphorylation in soleus muscle after 24 hrs unloading. Also HDAC4 accumulation was found in the nuclear fraction. The pretreatment of animals with AICAR (AMPK selective stimulator) prevented AMPK dephosphorylation and HDAC4 nuclear accumulation as well as the decline of type I myosin heavy chain mRNA content. Thus HDAC4 nuclear translocation and slow MyHCI(β) mRNA decline depend on AMPK dephosphorylation. AMPK dephosphorylation may influence also on the p70S6K phosphorylation level, since AMPK is known to suppress the mTORc1 activity [Bolster et al, 2002]. We demonstrated that AICAR pretreatment also prevented the p70S6K hyperphosphorylation. We may assume that it is the AMPK dephosphorylation to facilitate the p70S6K hyperphosphorylation. We speculate that the p70S6K hyperphosphorylation could inactivate IRS1 via inhibitory Ser 636 phosphorylation [Hsieh et al., 2014] and facilitate reduction of Akt-1 and FOXO3 phosphorylation. Indeed after 24 hrs of unloading Akt (Ser 473) and FOXO3a (S253) phosphorylation levels significantly decreased (60% and 45% respectively) while the content of MuRF-1 and MAFbx mRNA significantly increased (1.4- and 1.9 fold). Thus the proteolytic signaling pathway was found to be activated as early as after 24 hrs of unloading. It is concluded that it is the first day of unloading when the signaling background for main remodeling events (atrophy and MyHC phenotypical shift) in soleus muscle are triggered on.

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P.5.4-064
Alterations in the function of the mitochondrial respiratory chain during short-term exposure of BEAS-2B cells to total particulate matter from cigarettes or a candidate modified-risk tobacco product

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In our work we investigated the effect of 7-day treatment with total particulate matter (TPM) from the reference cigarettes 3R4F and TPM from aerosol of a candidate modified-risk tobacco product, the Tobacco Heating System 2.2 (THS2.2), on the function of the mitochondrial respiratory chain and intracellular ROS levels in human bronchial epithelial BEAS-2B cells. We found that 3R4F TPM has the strongest inhibitory effect on both basal and maximal oxygen consumption rate measured in intact BEAS-2B cells compared to TPM from THS2.2. Moreover, measurement of the oxygen consumption rate in the permeabilized BEAS-2B cells using a combination of respiratory complex-specific substrates and inhibitors showed that 3R4F TPM has the strongest inhibitory effect on complex I coupled oxygen consumption. These results together with calculated respiration "portion" used to drive ATP production under basal conditions can explain decreased level of intracellular ATP in the BEAS-2B cells treated with 3R4F TPM for 7 days. We have found that alterations in the oxidative phosphorylation (OXPHOS) are accompanied by increased mitochondrial superoxide levels in cells treated with 3R4F TPM or a 20-fold higher concentration of THS2.2 TPM, while cytosolic ROS levels estimated with the use of CM-H_2DCFDA revealed no differences between the two treatments. Interestingly, oxidative protein damage measured as levels of protein carbonyl groups was significantly increased only in cells treated with 3R4F TPM. Taken together, these results indicate that 3R4F TPM has a stronger effect on OXPHOS and manifestation of oxidative stress in comparison with TPM from the candidate modified-risk tobacco product THS2.2.

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P.5.4-065
Schisandrae Fructus suppresses RANKL-induced osteoclast differentiation by scavenging ROS generation

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Osteoclasts are capable of resorbing mineralized bone and excessive bone resorption by osteoclasts causes bone loss-related diseases. During osteoclast differentiation, the reactive oxygen species (ROS) acts as a secondary messenger on intercellular signal pathways. Schisandrae Fructus (SF), the dried fruit of *Schisandra chinensis* (Turcz.) Baill. (Magnoliaceae), is widely used in traditional medicine for the treatment of a number of chronic inflammatory diseases. In this study, we investigated whether SF can suppress RANKL-induced osteoclastogenesis through suppression of the subsequently formed ROS and inhibit H_2O_2-induced oxidative signaling pathways during osteoclast differentiation. We found that SF markedly inhibited the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells in RANKL-stimulated RAW 264.7 cells and scavenged intracellular ROS generation. SF also acted to significantly suppress the gene expression of nuclear factor of activated T cells c1 (NFATc1), TRAP, and osteoclast-associated immunoglobulin-like receptor (OSCAR), which are genetic markers of osteoclast differentiation in a dose-dependent manner. In addition, SF significantly blocked nuclear factor κB (NF-κB), phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs) signaling pathways activated by H_2O_2. Together, the present results indicate that SF acts as an osteoclastogenesis inhibitor through suppression of ROS-mediated signaling pathways and SF has potential usefulness for osteoporosis and pathological bone resorption.

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P.5.4-066
Modulation of the Nrf2 signaling pathway by the new oleanolic acid derivatives in human hepatocellular carcinoma cells

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Hepatocellular carcinoma is one of the most frequent malignancies in the world, usually with poor prognosis. Food components play a crucial role in both the etiology and prevention of this disease. Naturally occurring triterpenoids such as oleanolic acid (OA), have been shown to possess anti-inflammatory and antitumor properties. Modification of OA structure is expected to enhance its chemopreventive/therapeutic activity. One of the chemopreventive strategies is the modulation of Nrf2-ARE signaling pathway. The aim of this study was to evaluate the effect of OA oximes and their conjugates with aspirin on the activation and expression of *Nrf2* and *GST, NQO1, SOD* which transcription is controlled by Nrf2 in human HepG2 hepatoma cells. HepG2 cells were treated with the selected doses (2–20 μM) of the tested compounds for 24 h. Appropriate mRNA transcript and protein level was assessed by RT-PCR and Western blot respectively. ELISA plates with immobilized oligonucleotides containing the Nrf2 consensus site were used for the measurement of the activation of Nrf2 in terms of the amount of Nrf2 contained in the DNA binding complex. Treatment of the cells with OA derivatives and their conjugates resulted in the translocation of Nrf2 from cytosol to the cell nucleus and increased binding to Nrf2 consensus site. The most significant effect was observed at the dose of 20 μM. All OA derivatives showed higher activity than parent compounds. Activation of Nrf2 was accompanied by the reduced expression of *Keap1* gene. Activation of Nrf2 by OA derivatives resulted in the up-regulation of expression of *GSTP, NQO1* and *SOD* genes both on mRNA and protein levels. These results indicate that OA oxime derivatives and their conjugates with aspirin are potent inducers of Nrf2-ARE pathway in HepG2 cells. Further studies are necessary in order to explain the mechanism of their modulation of this signaling pathway.

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P.5.4-067
The essential role of DHPs in promoting tumor progression

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Tumor-dependent metabolic remodeling is mainly associated with production of building blocks needed to meet the replicating cells' demand for macromolecule biosynthesis. Our interest is in unique metabolic processes that do not fall under this category, but instead are more likely to present a regulatory role in cell fate determination. Epithelial-mesenchymal transition (EMT) is a cellular program during which cancers such as breast cancer gain mesenchymal-like properties, resulting in increased malignant aggressiveness. Despite the extensive study of cancer-related EMT, the role of cellular metabolism in this program is poorly understood. To systematically identify the metabolic genes that play a role in tumor progression we created Metabolic gEne RApid Visualizer (MERAV, http://merav.wi.mit.edu), a web-based tool for the analysis of human gene expression in normal tissues, primary tumors, and cancer cell lines. Analysis of this bioinformatic framework, resulted in the identification of 44 metabolic genes that are upregulated in high-grade tumors with mesenchymal markers and thus designated by us as "mesenchymal metabolic signature" (MMS). Among them is dihydropyrimidine dehydrogenase (DPYD), the rate-limiting enzyme for pyrimidine degradation pathway, which is the only member of this pathway to be expressed upon EMT induction. We found DPYD expression to be necessary for cells to acquire mesenchymal characteristics in *in vitro* experimental setting and to be required for tumorigenic cells to extravasate into the mouse lung. Interestingly, this role of DPYD is mediated through its enzymatic products, dihydropyrimidines (DHPs), that function as oncometabolites which affect cell fate mainly through the induction of mesenchymal markers. Thus, in this study we identified metabolites that play as signaling molecules that regulate the progression of tumors to a more aggressive state.

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P.5.4-068
Impact of extracellular vesicles from genetically modified human iPS cells on cardiac cells differentiation *in vitro*

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Extracellular vesicles (EVs) are population of small (100–1000 nm) circular membrane vesicles secreted by most cell types including stem cells (SCs). It has been recently reported that EVs may carry bioactive cargo including proteins, microRNAs and mRNAs. They also play a crucial role in cell-to-cell communication in both physiological and pathological conditions. Recent studies conducted by our group revealed that native human induced pluripotent SC (iPS)-derived extracellular vesicles (hiPS-EVs) may enhance several cardiac cells (CCs) function *in vitro* including their survival in cytotoxic environment. The aim of this study was to verify if treatment with EVs derived from hiPS cells overexpressing procardiomyogenic miR1 and miR199a, might have impact on various properties of human CCs including proliferation, migration, differentiation and survival. EVs derived from wild type (WT) and copGFP overexpressing hiPS were used as a control. EVs were isolated from conditioned hiPS culture media using differential centrifugation followed by ultracentifugation. NHCF-V cells (Lonza) were used as a model of target CCs. In each experimental setup cells were treated with 1 ug of EVs per 1000 cells. Our preliminary data indicate that hiPS-EVs may protect cardiac cells from apoptosis and inhibit the progress of this process. Antiapoptotic effect was stronger after miR1-hiPS-EVs or miR199a-hiPS-EVs treatment compared to control (EVs from unmodified hiPS cells). hiPS-EVs had also impact on NHCF-V cells proliferation, migration and differentiation toward cardiomyocytes. These results may suggest positive impact of EVs from hiPS cells overexpressing miR1 and miR199a on cardiac cell properties in myocardium after ischemia, which needs to be tested in further experiments *in vivo*.

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P.5.4-069
Orlistat is a fatty acid synthase (FASN) inhibitor caused the modulation of AMPK and lipogenesis signaling axis in PC3 prostate cancer cells but not in PNT1A prostate epithelial cells

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Prostate cancer is one the leading cancer type after lung cancer among male. Obesity is one of the known risk factor for developing highly aggressive and metastatic prostate cancer, which is an obstacle in cancer therapy. For these particular reasons, drugs are used in the therapy of obesity is suggested with their anti-tumoral effect, whether blocking adipogenesis and lipogenesis pathways or not. Orlistat inhibits fatty acid synthase (FASN), a large multifunctional enzyme, is responsible for de novo synthesis of long chain fatty acids. FASN activity is confirmed in the most of the xenograft models of prostate cancer cells. In this study we utilized PC3 cells, medium FASN expression profile compared to DU145 and LNCaP cells, and PNT1A prostate epithelial cells to evaluate the molecular targets of orlistat in adenosin mono phosphate kinase (AMPKa) and lipogenesis signaling axis. We demonstrated that orlistat inhibited cell proliferation and led to cell viability decrease in PC3 prostate cancer cells but not in PNT1A prostate epithelial cells. The activation of AMPKa was observed in both cell lines, which led to inhibition of protein translation through dephosphorylating mTOR at ser2448 and phosphorylating mTOR at ser2481 after treatment with Orlistat at 15 μM and 20 μM concentrations for 24 h. Concomitantly, orlistat induced autophagy via phosphorylation of ULK-1 at ser555. The modulation of Acetyl-CoA carboxylase (ACC), which regulates biosynthesis and oxidation of fatty acids was observed following orlistat treatment. However, AMPK phosphorylates ACC at ser79 and inhibits its activity. These findings suggest that orlistat altered AMPK-related a number of critical pathways to exert its anti-tumor activity in prostate cancer cells. However, AMPK is a metabolic stress regulator protects epithelial normal cells against orlistat treatment. Thereby, it is crucial to have deep understanding about the role of AMPK in prostate cancer cells.

P.5.4-070
Effect of palmitate on mitochondria in endothelial EA.hy926 cells

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A dyslipidaemia-related increase of the concentration of long-chain fatty acids in the plasma is an important risk factor leading to insulin resistance of muscle and liver cells and eventually to type 2 diabetes. Particularly saturated fatty acids impair insulin-induced glucose uptake by skeletal muscle and diminish insulin-dependent inhibition of gluconeogenesis in hepatocytes. A reduction of the sensitivity of endothelial cells to insulin is considered to be a very early step preceding the development of insulin resistance of the skeletal muscle. Palmitate at excessive concentrations has been shown to have a harmful effect on endothelial cells impairing NO generation, stimulating reactive oxygen species formation and affecting their viability. Here we found that palmitate applied for 48 h at a subliminal concentration sufficient to induce inflammatory response, stimulate reactive oxygen species (ROS) generation and reduce insulin sensitivity of EA.hy926 cells unexpectedly also stimulates NO synthesis and mitochondrial biogenesis. The latter is accompanied by increased oxygen consumption with succinate added to permeabilized cell suspension while the respiration rate of intact cells with respiratory complex I substrates was unaffected. This is in line with previously suggested inhibitory effect of palmitate on complex I [1]. This finding unveils a potential protective mechanism allowing cells to survive under conditions of a moderate deregulation of lipid homeostasis.

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P.5.4-071
A novel retinoic acid biosynthesis center required for normal head development

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Retinoic acid (RA) is essential for normal vertebrate embryogenesis. Reduced RA signaling causes numerous embryonic defects including head and brain malformations. In humans, several syndromes attributed to reduced RA levels exhibit overlapping malformations including head malformations. Microcephaly is a common abnormality observed in individuals with Fetal Alcohol Syndrome. In agreement, *Xenopus* embryos exposed to ethanol or treated with RA biosynthesis inhibitors exhibited reduced or absent head structures. During early frog embryogenesis (gastrula), several RA biosynthetic enzymes are expressed together in Spemann's organizer, the dorsal lip of blastopore. RDH10 that oxidizes vitamin A (retinol) to retinaldehyde is expressed in the organizer. Together with RDH10, are RALDH2 and RALDH3 that further oxidize retinaldehyde to RA. In the organizer are also two regulatory enzymes, DHRS3 and ADHFe1. As development proceeds, RALDH3 expression moves rostrally with the cells, that will induce the forebrain, the prechordal mesoderm (PCM), while RALDH2 remains in the more posterior, trunk region. This separation of the two RALDH domains establishes two RA biosynthetic centers. We can show that knock-down of the RALDH3 activity induces head malformations, further supporting the requirement for RA in the head induction/formation process. No additional RA biosynthetic enzymes are known to be expressed in the forebrain inducing prechordal cells with RALDH3. For this reason, we have devised a dissection scheme to separate both RA biosynthetic centers and to determine their respective transcriptomes. The accuracy of the dissection was studied using markers of the PCM and the trunk by qPCR. Expression of candidate RA biosynthetic genes was studied as well as transcriptome analysis of these RNA samples. This analysis allows us to characterize the head-inducing function of RA further, and the enzymatic network affected in the syndromes with reduced RA in humans.

P.5.4-072
Extracellular vesicles released by hiPS cells enhance functional properties of human hematopoietic stem cells isolated from cord blood

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Human cord blood (CB) represents a rich source of several stem cell (SCs) types, particularly hematopoietic SCs (HSCs). Thus, clinical application of CB cells became an alternative for bone marrow (BM) transplantation. However, successful application of CB-SCs requires the development of effective strategies improving their therapeutic potential. Our group proposed the enhancement of SCs functionality by their treatment with extracellular vesicles (EVs), which were shown to transfer bioactive components into target cells. Thus, in presented study, we evaluated an influence of human induced pluripotent SCs (hiPS)-derived EVs (hiPS-EVs) on selected functions of CB-HSCs, important for their hematopoietic potential. hiPS-EVs were harvested from media collected from feeder-, serum- and xeno-free cultures of hiPS cells by ultracentrifugation. Next, two HSCs-enriched fractions: CD34+ and cells negative for hematopoietic lineages (Lin-), were isolated from CB and further expanded in dedicated media. Subsequently, we evaluated the impact of hiPS-EVs on several biological and functional properties of CB-derived CD34+ and Lin-cells. Our results revealed that iPS-EVs treatment increases hematopoietic differentiation of CB-SCs. Subsequently, we have shown, that clonogenic potential of CB-HSCs was significantly higher in cells stimulated with hiPS-EVs. Moreover, we found that short incubation with iPS-EVs elevates chemotactic activity of CB-SCs into SDF-1. Finally, we also observed differential changes in the activity of several kinases involved in important signaling pathways in CB-HSCs shortly incubated with hiPS-EVs. In conclusion, our findings suggest that transfer of iPS-EVs may improve several functions of CB-derived HSCs important for their homing and hematopoietic activity following transplantation. Further studies are required to understand the mechanisms of observed phenomenon and its potential relevance for applications in regenerative medicine.

P.5.4-073
Mechanism of nicotinamide riboside and nicotinic acid riboside import into human cells

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Nicotinamide adenine dinucleotide (NAD) is known as a coenzyme of redox reactions in essential metabolic pathways. Moreover, NAD has a key role in various signaling pathways. To ensure proper control of vital reactions, NAD must be permanently resynthesized. The major extracellular precursors for NAD biosynthesis in humans are nicotinamide and nicotinic acid as well as nicotinamide riboside (NR) and nicotinic acid riboside (NAR). So far, little is known about the import mechanisms for the nucleosides NR and NAR into human cells. In this work we have tested whether members of the ENT (equilibrative nucleoside transporters) and/or CNT (concentrative nucleoside transporters) family can mediate the transport of NR and NAR across the plasma membrane. HEK293 or HeLa cells were grown in standard culture medium, containing Nam as the sole precursor of NAD. NAD synthesis from Nam was inhibited by adding to the medium the potent Nam phosphoribosyltransferase inhibitor FK866, which led to cell death. Cell survival was restored by supplementation of the culture medium with NR or NAR. Under these conditions, the cells were also treated with various nucleoside transport (ENT or CNT) inhibitors. Then we analyzed the cell viability and the utilization (disappearance from the medium) of NR and NAR using an NMR-based method. We found that treatment with NBTI and dipyridamole (inhibitors of ENT) significantly reduced the utilization of NR and, to a lesser extent, NAR and consequently inhibited the ability of these nucleosides to restore cell survival in the presence of FK866. On the other hand, when human cells were treated with phloridzin (inhibitor of CNT), NAR but not NR uptake was prevented. Thus, our

results indicate that both CNT and ENT activities can mediate import of NR and NAR into human cells.

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P.5.4-074
Cytokinins in poplar

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Cytokinins (CK) are phytohormones with fundamental role in the regulation of plant growth and development. They are derived from adenine and occur naturally either with isoprenoid or aromatic side chain. We have utilized UHPLC-MS/MS to screen content of aromatic CKs in 13 *populus* species and selected *Populus* × *canadensis* (cv. *Robusta*) as a model for the presented work. Mass spectrometric data show transient increase in the endogenous levels of poplar aromatic CKs after daybreak while levels of tRNA derived *o*-topolin remain unchanged. To examine CK metabolism further, all nine isopentenyl transferase genes (*IPTs*) found in the poplar genome were cloned, sequenced and their expression in different tissues analyzed by qPCR. We have also managed to express eight out of nine *IPTs* in *Escherichia coli*. However, they aggregate in the form of inclusion bodies and it was not possible to obtain pure proteins for kinetic measurements. Majority of the *IPT* genes were further subcloned into pMDC7 vector under estradiol inducible promoter for transformation of *Arabidopsis thaliana*. The clear difference in *Arabidopsis* CK levels before and after induction indicates whether the corresponding proteins are functional *in vivo*.

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P.5.4-075
The potential of various extracellular NAD precursors to maintain the synthesis of intracellular NAD in cultivated human cells

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Nicotinamide adenine dinucleotide (NAD) is an essential electron carrier in redox reactions. Moreover, NAD also serves as substrate of several families of regulatory proteins such as protein deacetylases (Sirtuins), ADP-ribosyltransferases and Poly-ADP-ribosyl polymerases and thereby plays a key role in a variety of central signaling pathways. Dysregulation of NAD-dependent signaling pathways is associated with metabolic diseases such as diabetes and metabolic syndrome. Alterations of NAD are also associated with neurodegeneration, cardiovascular disease and cancer. Generally, human cells regulate their NAD supply through the biosynthesis using various precursors delivered with the diet. In this work we characterized the efficiency of NAD biosynthesis from its major precursors: nicotinamide (Nam), nicotinic acid (NA), nicotinamide riboside (NR) and nicotinic acid riboside (NAR) as well as from nicotinamide mononucleotide (NMN) in cultures of various human cell lines. Cells were grown in standard medium, containing Nam as the sole precursor of NAD. NAD synthesis from Nam was suppressed by addition of FK866, a potent inhibitor of the phosphoribosyltransferase NamPRT, which triggers a rapid decline of the NAD levels and cell death. In the presence of the inhibitor, cells were also treated with alternative precursors each of which was the only source for generation of intracellular NAD. Then we assessed the cell viability and intracellular NAD levels. We found that NA does not maintain intracellular NAD level and viability of HepG2, A549 and IMR-32 cells, while A431, HEK293 and HeLa cells as well as primary human dermal fibroblasts efficiently utilized NA for NAD biosynthesis. NAR and NMN were the most and the least efficient NAD precursors, respectively, in the majority of human cell lines used in this study.

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P.5.4-076
Stable isotope 18O-assisted dynamic phosphometabolomic of adenylate kinase phosphotransfer and nucleotide triphosphate metabolism

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Adenylate kinase is ubiquitous enzyme critical in cell nucleotide metabolism and metabolic signaling. The energetic role of adenylate kinase is related to its ability to facilitate transfer and distribute energy from both γ- and β-phosphoryls of the ATP molecule between different cellular compartments thus increasing energetic efficiency. Beside ATP, intracellular energy distribution depends on the ability to transfer phosphoryls to GTP, UTP and CTP reflecting activity of protein, carbohydrate and lipid biosynthesis and cell growth. Here, using stable isotope ^{18}O-based dynamic phosphometabolomic approach we followed adenylate kinase metabolic flux by measuring ATP β-phosphoryl turnover and ^{18}O-labeling of nucleotide triphosphate γ-phosphoryls reflecting high-energy phosphoryl distribution in cell lines. Labeling experiments indicate that in cells such as HepG2 (liver) and 293T (kidney), the rate of label incorporation into ATP β-phosphoryls, which is a measure of adenylate kinase velocity, closely approximates that of γ-ATP reflecting cellular ATP turnover rate. This indicates that cells efficiently using both γ- and β-ATP high-energy phosphoryls and that majority of phosphoryls utilized in cells are processed through the adenylate kinase-catalyzed phosphotransfer system. In other type of cells, MEF (mouse embryonic fibroblasts) and primary astrocytes, β-ATP[^{18}O] labeling was by 20–30% lower compared by γ-ATP[^{18}O] reflecting diminished adenylate kinase flux. In breast cancer cells MCF7 γ- and β-ATP turnover was similar while highly aggressive cell line MDAMB231 had lower β-ATP labeling compared to γ-ATP. Relative amount of label incorporation into γ-ATP[^{18}O], γ-GTP [^{18}O], γ-UTP[^{18}O] and γCTP[^{18}O], reflecting energy distribution to biosynthetic processes, was 100%, 12%, 9% and 3%

respectively. Thus stable isotope phosphometabolomics permits assessment of changes in dynamics of cellular energetic and metabolic signaling circuits.

P.5.4-077
Cutting down tRNA modification care doomed scopolin induction

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Cytokinins (CK) act in numerous plant processes encompassing regulations of plethora metabolites in response to diverse exogenous and endogenous cues. Phenylpropanoids, namely coumarin scopoletin and its glucoside scopolin, are suspected to be targets of CKs modulation. Aglycone is proven phytoalexin with medicinal properties, antifungal and antibacterial, and recent studies imply its function in combating nutrient deficiency. Almost three decades ago, initial evidences of CK-induced elicitation of these compounds have been reported, however any molecular background for observed phenomenon has not been proposed yet. We tested whether functional tRNA isopentenyltransferase (IPT), CK biosynthetic and presumably tRNA modifying enzyme, might be important for the positive response. Analyses of scopolin content in roots of *Arabidopsis* T-DNA insertional mutant *atipt9* supplied with exogenous benzyladenine suggest vitality of the functional gene for CK-mediated scopolin accumulation. Elucidation whether the stimulus utilized downstream cascade executed by CK signaling pathway components is under progress.

P.5.4-078
Loss of calreticulin uncovers a critical role for calcium in regulating cellular lipid homeostasis

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Deficiency in calreticulin, an endoplasmic reticulum (ER) Ca^{2+} buffering protein, leads to abnormal cardiogenesis and embryonic lethality in mice (Mesaeli et al., 1999). Cardiac specific expression of a constitutively active form of calcineurin can rescue the embryonic lethality and defect in cardiac development, however these calreticulin deficient rescue mice display an abnormality in energy metabolism (Guo et al., 2002). In this study, we investigated the relationship between calreticulin, ER luminal Ca^{2+} and the sterol regulatory element binding protein (SREBP) pathway. SREBPs are members of the basic helix-loop-helix leucine zipper family of ER associated transcription factors that control the expression of genes involved in lipid metabolism. The processing and activation of SREBPs is controlled by a sensitive system dependent upon the changes in ER membrane unesterified cholesterol. SREBP forms a complex with the SREBP cleavage activating protein (SCAP) and insulin induced gene-1 (INSIG-1). When the content of ER membrane unesterified cholesterol is low, the SREBP-SCAP complex dissociates from INISIG-1, and translocates to the Golgi where SREBP is processed to yield nSREBP the active form of the transcription factor that is responsible for the expression of genes involved in lipid metabolism. We discovered that the decrease in ER Ca^{2+} stores by deleting the gene of calreticulin (calreticulin-deficient cells) or by depleting extracellular Ca^{2+} concentration, increased levels of neutral lipids and nSREBP activity. When ER luminal Ca^{2+} was reduced, there was a change in the intracellular distribution of unesterified cholesterol away from the SCAP-SREBP complex. We concluded that reduction of ER luminal Ca^{2+} increased nSREBP activity by a redistribution of ER cholesterol, establishing a role of ER Ca^{2+} status in the cholesterol sensing mechanism controlling lipid metabolism.

P.5.4-079
Nanopipette biosensors as a new tool for metabolism and signaling investigation

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Recently we have developed the new system for nanopipette navigation with feedback control. The ability to precisely move the nanopipette to target specific regions such as neuronal processes allows an unprecedented level of control of drug application. The speed of data acquisition positions this as a technology which may be suited to relatively high-throughput application in human neuronal preparations which would greatly facilitate drug discovery. This nanopipette navigation system can be used in combination with other techniques such as microinjection, electrochemical measurement, and patch-clamp recording. This has the potential to open new horizons in medicine and biology and could be of particular value to the pharmaceutical industry. We have demonstrated unique application of nanopipette as a sensor for local electrochemichal measurements. Nanopipette can be filled with a carbon using butan decomposition in argon atmospher as a result we get a disk-shaped nanoelectrode. We have developed the method of platinum deposition at the tip of nanoelectrode. Such probe with platinum can detect various oxidgen consuptions. As a demonstration we have shown the detection of oxygen photosynthesized by plant cell on light. Using nanopipette navigation system we can do electrochemical mapping of a living cell surface with nanoscale resolution.

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P.5.4-080
Role of hypoxanthine in adverse effects of red blood cells transfusion

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Red Blood Cells (RBCs) stored in blood bank conditions, undergo biological/biochemical changes collectively referred as "storage lesions". In our metabolomic studies, we observed a significant increase of hypoxanthine (HX) concentration on aged RBCs units. This intermediate of purine pathway is a pro-oxidant molecule since it's metabolism by xanthine oxidase (XO) produces reactive oxygen species (ROS: $O_2^{\cdot-}$ and H_2O_2) as catalytic by-products. XO is abundant in the intestine, liver and endothelial cells of the microvasculature. Many pathological conditions such as acute respiratory distress syndrome, ischemia reperfusion injury, atherosclerosis, diabetes, and autoimmune disorders seem to be related to XO-released ROS. Moreover, HX production has been implicated in the oxidative damages observed after hypoxia-ischemia and reperfusion. After transfusion of three old RBCs units (42 days of storage), in a

normovolemic patient, HX is expected to reach a concentration that is cytotoxic in cultured human cells. ROS play a central role in inflammation, since they can mediate neutrophil activation, as well as being involved in their downstream inflammatory response. Activated neutrophils secrete pro-inflammatory mediators such as chemokines and cytokines. We evaluated the effect of 42 day old RBCs supernatants and HX (in a concentration similar to that reached during multiple transfusions) on isolated neutrophils, by means of flow cytometry and ELISA assay. An increase of intracellular TNF-α and a release of IL-8 were observed. These effects were reverted by allopurinol-mediated XO inhibition, demonstrating the role played by HX. Some, *in vitro*, studies on neutrophil's priming/activation, induced by RBCs storage medium, attributed this effect to the presence of bioactive substances, such as lipids (arachidonic acid and derivatives). The search of other mediators responsible for neutrophils activation remains an open question: an additional candidate is HX.

Miscellaneous

P.Mis-001
Different impact of physical activity on plasma myokines content in athletes and untrained volunteers

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This research is devoted to the study of the physical activity effect on the myokine production by skeletal muscle in athletes and untrained volunteers. Exercise increases the IL-6, IL-8, IL-15, leukemia inhibitory factor (LIF) plasma content. Myokines production in skeletal muscle cells is carried out via their augmented transcription. The literature describes the effect of cyclic exercise on myokines production, but the effect of static loads remains understood. In addition, the effect of fitness level on the myokines production is unknown. In this study, we compared the effects of dynamic and static load on cytokine plasma content in elite strength- and endurance-trained athletes versus healthy untrained volunteers. The enzyme-linked immunosorbent assay was used for the measured cytokine content in plasma. We observed that cyclic exercise increased IL-6 and IL-8 content in the plasma of trained athletes by about 4- and 2-fold, respectively. The static load had negligible impact on these parameters in strength trained athletes. Also pedaling on a bicycle ergometer had no impact on IL-15 and LIF. In contrast, the static load increased the content of IL-15 and LIF by ~50%. We also observed the differences in the IL-8 and IL-15 production in athletes and untrained volunteers. We assume that differences revealed in athletes and untrained volunteers are caused by differential impact of HIF-1a-, $[Ca^{2+}]_i$-, and $[Na^+]_i/[K^+]_i$-mediated excitation-transcription coupling in overall myokine production by skeletal muscles.

This study was supported by a grant from the Russian Science Foundation (16-15-10026).

P.Mis-002
Distribution of the EDNRA H323H polymorphic genetic marker of cardiovascular diseases among populations of Syria, and Central Russia

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Endotheline-1 is a potent vasoconstrictor that exerts this effect through ETA receptors; much evidence suggests that genetic variations in the *EDNRA* gene encoding ETA receptors may play a role in the pathogenesis of many cardiovascular diseases. The ethnic characteristics of the distribution of the *EDNRA* gene polymorphism may influence the development of these disorders in different populations; therefore we investigated for the first time the distribution of the synonymous polymorphism H323H (T>C) of the *EDNRA* gene (rs5333) among 50 Arab Syrians with the mean age of 26.19 ± 8.03 years and 59 Russians from Central Russia (19.67 ± 1.96). The genotypes were determined with the use of PCR-RFLP (restriction fragment length polymorphism). The C allele frequency of the H323H polymorphism in Syrians and Russians was 27% and 8%, respectively. The genotype frequencies were in Hardy-Weinberg equilibrium in both populations ($P > 0.05$). The *TT, TC* and *CC* genotype frequencies were 58%, 30% and 12% among Syrians and 84.75%, 15.25%, 0% in the Russian population, respectively. A significant difference was found in the H323H genotype distribution between Russians and Syrians ($P = 0.011$), as well as in allelic distribution ($X^2 = 11.22$; $P = 0.0008$). These results show that the distribution of the H323H *EDNRA* polymorphism among Syrians and Russians should be investigated in larger samples, and considered in the further study of genes associated with cardiovascular diseases.

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P.Mis-003
Actin remodeling as coordinator of mast cell communication with the outside; mDia1 is a novel regulator of mast cell chemotaxis and exocytosis

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Mast cells (MCs) are secretory cells implicated as key effectors in diverse immune responses including allergic inflammation and host defense. To perform their physiological tasks, MCs first migrate to sites of pathogen invasion or inflammation in response to chemoatrractant gradients. This migration is followed by secretion by exocytosis of immune and inflammatory mediators that are prestored in the MC secretory granules (SGs). Migration and exocytosis are tightly regulated in a spatiotemporal manner, whereby migrating MCs do not secrete, and degranulating MCs stiffen. This regulation is actin dependent and is manifested in different actin dynamics that are respectively stimulated by chemoatrractants or secretagogues. We have characterized the actin infrastructure underlying migration and secretion of MCs. Furthermore, we show that Diaphanous-related formin 1 (mDia1), a member of the formin family of actin nucleating proteins, is endogenously expressed in MCs and that knocking-down mDia1 expression interferes with MC responses. Moreover, a constitutively active mutant of mDia1 (CA mDia1) displays opposite effects, inhibiting MC secretion but enhancing cell

migration. Our results show that this mutant eliminates cell protrusions and markedly increases the density of the actin meshwork in triggered cells, resulting in dense bundles of actin filaments that cluster the SGs at a perinuclear location. Collectively, our results assign a labyrinth pattern of actin a critical role in regulating MC exocytosis and migration and identify mDia1 as a novel regulator that controls MC responses by shaping the intracellular actin micropattern.

P.Mis-004
The influence of sodium dodecyl sulfate and sodium penta decyl sulfate on the electrical breakdown of bilayer lipid membranes

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In the cell, there are some molecules which have similar structures like sodium dodecyl sulfate (SDS) and sodium penta decyl sulfate (SPDS). SDS consists of 12 and SPDS consists of 15 carbon tail attached to the sulfate groups. SDS is a commonly used detergent to solubilize membranes and to isolate and purify membrane proteins. It is known to cause harmful effects on humans and animals. Because of its intensive use in households and in industry, and release into the environment, the volume of water pollution by surfactants comes with great speed. Therefore, it is important to investigate the influence of this detergents on the cell membrane electrical breakdown. But it seems more expedient to do a detailed study on the bilayer lipid membranes (BLM).The membranes were formed according to the Muller–Rudin method in the solution of electrolyte in a special teflon cell. For measurements of parameters of the BLM, two silver-chloride electrodes were located in two compartments of the experimental cell, which were separated by a membrane. The electrodes were connected to the ADC and controlled by a computer. It is shown that the presence of SDS leads to the loss of stability of the BLM, which is associated with the decrease in the value of the linear tension of pore edge in BLM. The presence of SPDS also leads to the loss of the BLM stability. But in the presence of SPDS the BLM lifetime decreases dramatically faster, than in the presence of SDS. And, with adding of SPDS in the solution, the value of the linear tension of pore edge in BLM decreases more than in the presence of SDS in the same concentration. The number of lipid pores on the BLM in the presence of SPDS increases more than in the presence of SDS. The reason is, that the presence of SPDS, with 15 alkyl chain length carbon tail, increases the probability of the pore formation more than it is happening in the presence of SDS, wich has 12 alkyl chain length carbon tail.

P.Mis-005
The immobilization of ficin, a nonspecific plant protease, for the biomedical applications as a wound-healing agent

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The enzymatic treatment of wounds by various proteases like trypsin, chymotrypsin, collagenase, papain etc. is widely used in medicine as a wounds-cleaning therapy to speed up wounds healing. Recently we have shown that Ficin, a nonspecific plant protease from the Ficus tree, efficiently eradicates staphylococcal biofilms increasing thereby the efficacy of antibiotics, and exhibits attractive wound healing activity. We aimed to produce a series of Ficin preparations immobilized on a various carriers for enzyme stabilization during storage and application. A virtual screening of high-affinity carriers for immobilization has been performed using computer modelling. Based on the comparative analysis of the total energy and the localization of the ligand binding sites, some assumptions about the enzyme interactions with suggested carriers were made. A number of heterogeneous enzyme preparations have been offered and the structural features of these complexes were predicted. The Ficin adsorption on chitosan, cation (VION KN-1) and anion (VION AN-1) exchange fibers allowed preserving up to 70% of the catalytic activity. Being immobilized on chitosan, the enzyme stability was increased 10-fold in compare with soluble protein. While the biofilm matrix hydrolysis by immobilized enzyme was less in compare with the soluble Ficin, an overall the antibiofilm activity of the chitosan-immobilized Ficin was higher because of mechanical removal of the biofilm matrix form the surfaces *in vitro*. Neither soluble nor immobilized Ficin and carriers did not exhibit any mutagenic, DNA-damaging, or cytotoxic activity, suggesting them as perspective start points for development of the staphylococcal biofilms treatment therapies.

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P.Mis-006
Survival and reactivation of dormant "non-culturable" mycobacteria depends on trehalose content and trehalase activity.

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Mycobacterium tuberculosis (MTB) viable cells transition to the dormant state causes latent TB – the asymptotic disease spread through the third of the human population. The mechanisms and metabolic processes responsible for dormant cells surviving for long periods and their resuscitation to a viable, multiplying state, are not fully elucidated. Under gradual acidification of growth medium resulting in the formation of dormant *Mycobacterium smegmatis* up to 64% of total organic substances was represented by trehalose. Varying the concentration of trehalase in dormant cells by expression of MSMEG_4535 coding for trehalase, we found that cell viability depends on trehalase activity: cells with low trehalase activity accumulates a high amount of trehalose and survive much better in contrast to wild type dormant cells. Upon resuscitation of dormant *M. smegmatis*, a decrease of free trehalose and an increase in glucose concentration occurred in the early period of resuscitation. The breakdown of trehalose by trehalase was observed in the first hours of resuscitation, and trehalase activity was increasing. Activation of trehalase was not due to *de novo* biosynthesis but because of self-activation of the enzyme from the inactive state in dormant cells. The activity of trehalase from dormant cells is not measurable in the presence of 2 mM ATP. In contrast, trehalase of active cells was tolerant to this concentration of ATP and was inhibited only in the presence of more than 20 mM ATP. The negative influence of the trehalase inhibitor validamycin A on the resuscitation of dormant cells proves the importance of trehalase for resuscitation. These experiments for the first time demonstrate the significance of free trehalose accumulation for the maintenance of dormant mycobacterial viability and the involvement of trehalose breakdown in early events leading to cell reactivation similar to yeast and fungal spores.

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P.Mis-007
Development of monoclonal antibodies for the detection of immunreactive tyrpsin for use in diagnosis of cystic fibrosis

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Cystic fibrosis (CF) is an autosomal recessive genetic disease affecting multiple systems and organs simultaneously; including lungs, pancreas, sweat glands and external glands in respiratory and digestive system. It has an incidence rate of one in 3,000 infants worldwide. Early detection of the disease is very important to start an effective treatment strategy to increase the life quality of the patients. Therefore, in recent years, CF was included in the neonatal screening programs in many countries such as USA, New Zealand, most European countries and Turkey. Immune reactive trypsinogen (IRT) levels are relatively high in newborns with CF and therefore, it is used as a neonatal screening marker for the disease. It is important to develop a sensitive, cost effective and easy method for efficient monitoring and early diagnosis of the disease. ELISA tests are commonly used in the screening programs and the development of high affinity antibodies is the key for the development of efficient diagnostic tests. The aim of this study was to develop novel monoclonal antibodies for IRT to be used in a direct sandwich immunoassay. In this study, 50 ng IRT2 was used for immunization of 6–8 weeks old BALB/c mice. By using hybridoma technology, lymphocytes obtained from the spleen of antibody producing mice and myeloma cells were fused in the presence of polyethylene glycol. Antibody producing hybrid clones were screened by indirect ELISA against IRT2 and their cross reactivities were tested against human blood proteins. As a result of 2 fusions, 4 highly specific anti-IRT monoclonal antibodies were developed. The monoclonal antibodies were purified and labeled with biotin in order to be utilized in sandwich immunoassay Developed monoclonal antibodies were shown to be efficient in use as capture and/or detection antibody for the detection of IRT2 in Sandwich ELISA system.

P.Mis-008
Expression, immunogenicity and protective activity of chimeric protein based on E protein domain III of tick-borne encephalitis virus and OmpF porin of *Yersinia pseudotuberculosis*

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Tick-borne encephalitis (TBE) poses a serious public health threat in the endemic regions. Therefore, great expectations are in the development of the prophylactic and therapeutic vaccines. The domain III of E protein of TBE virus (TBEV) is the main antigenic domain which includes virus-specific epitopes recognized by neutralizing antibodies. However, it is low immunogenic. We have expressed and isolated chimeric protein based on the fusion of domain III of E protein of TBEV and porin OmpF from *Yersinia pseudotuberculosis* to assess its immunogenicity and protective activity in the content of novel nanoparticulate adjuvanted delivery system – tubular immunostimulating complex (TI-complexes). Membrane protein OmpF was necessary to incorporate antigen in TI-complexes comprising glycolipid monogalactosyldiacylglycerol (MGDG) from marine macrophytes, triterpene glycoside cucumarioside A2-2 from holothuria *Cucumaria japonica* and cholesterol. Chimeric protein was successfully expressed by the *Escherichia coli* cells and purified using a metal-affine chromatography. The protein was recognized with immunoblots by anti-E protein monoclonal antibodies. Individual chimeric protein induced the 2.5-fold increase in the level of anti-EIII antibodies in the blood serum of immunized mice compared with control, whereas the same antigen incorporated in TI-complex caused the 4–7 fold increase of anti-EIII antibodies compared with animals immunized with individual antigen. 70% and 85% of mice immunized with chimeric protein in individual form and incorporated in TI-complexes, respectively, survived at the14th day after infection with TBEV against 30% in control. The newly obtained chimeric protein possesses high immunogenicity and protective activity which remarkably increase in result of antigen incorporation in TI-complex. Therefore, it could be valuable for the development of the preventing TBE subunit vaccines.

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P.Mis-009
Protein microarray biosensor based on total internal reflection imaging ellipsometry

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Biosensor based on imaging ellipsometry (BIE) has been developed for decades. Working under the external reflection condition, BIE has been used to visualize biomolecule interaction on a patterned surfaces for it enjoys high spatial resolution and fast data acquisition. Although BIE can work in aqueous environment to perform real-time detection, it suffers from the disturbance of solutions and the sensitivity limit of BIE. Hoping to visualize the biomolecule interaction in real-time with high sensitivity and no solution disturbance, total internal reflection mode of imaging ellipsometry (TIRIE) has been introduced into the biosensor approach. Its high sensitivity results from the evanescent field under the total internal reflection condition. However, the image contrast is insufficient for practical applications. To increase the image contrast, a gold film has been introduced into the sensing surface of TIRIE chip to enhance the amplitude of the evanescent wave. By optimize the polarization of the light beam and the angle of incidence, the image contrast is further improved. By our estimation, the sensitivity of TIRIE biosensor is 30 times larger than that of conventional surface plasmon resonance system which is agreed with others' work. After the optimization, we use TIRIE biosensor to detect cluster of differentiation 146, a cell adhesion molecule used as a marker for endothelial cell lineage, quantitatively and the weak affinity interaction between tris and lysozeme.

P.Mis-010
Growth medium optimization of *Cryptococcus albidus* D24 lipase by statistical design of experiments

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Lipases are constitute an important class of enzymes used in several industries such as detergent, food, pulp, and paper industries which catalyze a large number of reactions such as hydrolyzation, esterification, interesterification, and transesterification. *Cryptococcus albidus D24* is a newly identified lipase-producing microorganism isolated from petroleum-contaminated refinery basin soil. In order to produce an efficient amount of microbial metabolites, manipulation of nutritional requirements and physical parameters are required. Selection of appropriate carbon and nitrogen sources and other nutrients plays a substantial role to reduce production cost and to enhance enzyme production. In our current work, we aim to optimize lipase production by *Cryptococcus albidus* D24 using a statistical approach. In order to screen the most effective nutrients for D24 lipase production, different carbon and nitrogen sources, minerals, and a surfactant (Tween 80) were tested. The significance of the twenty components was analyzed to determine the most effective medium components to achieve the highest lipase production.

P.Mis-011
Cell state transitions during early differentiation of mouse embryonic stem cells

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Cell state transitions during development or *in vitro* differentiation result from the cell's current transcriptional and epigenetic state, as well as the processing of external signals, both mechanical and biochemical. Discerning the relative effect of internal state and external signals in early differentiation is essential for understanding differentiation dynamics during development and for future control of in-vitro tissue composition. Cell lineage, correlated with the epigenetic state memory, and external signal, correlated with cell location, may play different roles in fate decisions. We combine live imaging, spatial analysis and statistical approaches to quantify these effects on cell state transitions. We developed a high throughput method to characterize the decision point of mouse embryonic stem cells in colonies (2D) and in Embryoid bodies (EB, 3D) in mesoderm differentiation. Comparing these systems provides insights on the signals affecting the decision as they differ in cell to cell contact and cells exposure to medium. We look for tissue scaling effects on signal and fate patterns by decoupling the differentiation time from the tissue size. Our preliminary results show that mesoderm formation in EBs is largely depends on a mechanical signal regardless the starting EB size. Differentiation in an ECM-like environment abolishes this effect and changes the timing and dynamics of the mesoderm formation. Using a localized BMP4 signaling inside an EB precedes the mesoderm onset and affects its localization. Enhancing Wnt pathway at specific time during differentiation causes varied expression of mesodermal marker, suggesting a differential tendency to mesodermal differentiation prior to the timing of the intervention. We expect the results of this project will enhance our understanding on how internal state and external signals are integrated to result in early differentiation decisions. It will also provide a methodology to dissect other cell fate changes.

P.Mis-012
Structural and functional properties of *Bauhinia forficata* lectin (BfL)

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BfL is a legume lectin isolated from seeds of the tree *Bauhinia forficata*. Naturally occurring BfL is N-glycosylated on two asparagine residues. We have characterized the structural and functional properties of recombinant BfL expressed in *E. coli*. Carbohydrate specificity of BfL was studied using a glycan array, "in-printed" with 500+ different carbohydrate moieties. These experiments showed a strong preference of BfL for carbohydrates with a terminal α-GalNAc group, which includes blood group A antigens, as well as for Ser– and Thr-linked Tn-peptides. The affinity of BfL for the α-GalNAc-containing moieties presented on the surface of array and presumably on the cellular surfaces is in the picomolar range, rendering the recombinant BfL as one of the most potent α-GalNAc-binding lectins. Nine different crystal structures of the recombinant BfL were determined and refined at medium-to-high resolution, including the apo-form and complexes with a series of biologically-relevant ligands, including blood group A antigen and several Tn-peptides. Combined, the crystal structures reveal details of the architecture of a BfL molecule, structural basis of ligand specificity, and represent one of the most complete structural descriptions of a single plant lectin. To assess the potential of BfL to inhibit proliferation of cancer cells, the lectin was subjected to the one-dose SRB assay against the NCI-60 panel. While only modest overall activity of BfL was recorded, quite a significant effect was observed against specific cancer cell lines. In particular, BfL at the concentration of ~2 μg/ml (70 nM) showed ~97% inhibition of the growth of the melanoma LOX IMVI cells line. Slightly lesser, yet significant effect was observed for several lung, breast, or ovarian cancer cell lines. The molecular/mechanistic basis of this activity are under investigation.

P.Mis-013
An old scientific challenge: identification of the genes, catabolic enzymes and regulators involved in the catabolism of histamine

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Histamine is a biogenic amine widely distributed in nature including many foods of vegetable or animal origin. Because of its complex physiological functions, histamine could cause quite serious damages or important syndromes when its pool in the body increases abnormally. Thus, in order to avoid its harmful effects, the quantity of histamine in foods should be strictly controlled. By this reason we have approached the study of the genes and enzymes involved in the degradation of this amine using a paradigmatic bacterium (*P. putida* U) which is able to assimilate efficiently other biogenic amines (tyramine, dopamine, 2-phenyletylamine). In this report, we describe, a novel route which transforms histamine into fumaric acid. The catabolic pathway required for the degradation of the biogenic amine histamine (Hin) involves the participation of eleven proteins (HinABCDGHFLIJK) encoded by the genes located in three unrelated clusters (*hin1*, *hin2* and *hin3*) and by *hinK*. The enzymes HinABCD (encoded in *hin1*) catalyzes the transport and oxidative deamination of histamine to 4-midazoleacetic acid (ImAA).

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This reaction is tightly coupled to other enzymatic systems (DadXDR and CoxAB-C) ensuring: (i) the recovery of the pyruvate required for the deamination of histamine, and (ii) the generation of the energy needed for its uptake. Other proteins (HinGHFLKIJ) catalyze the sequential transformation of ImAA into fumaric acid, *via* aspartic acid. Knowledge of this pathway is important because it is the first description of all the genes and proteins (transporters, energy generating systems, catabolic enzymes and regulators) necessary for the degradation of histamine in an organism. Furthermore, some of these genes (*hin1*/*hin2*+*hinK*) can be transferred to other microbes giving them the ability to remove histamine or ImAA from different sources. We also reported the collection of mutants which accumulate certain metabolites enlarging the interest of this research for biotecnologists.

P.Mis-014
Silk fibroin scaffold in reconstruction of intestinal wall

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The high mortality from the lack of donor tissues and organs has stimulated the development of tissue engineering. Silk fibroin is increasingly being recognized as a promising material for implant fabrication in the area of tissue engineering. We examined the regenerative properties of an implant made of silk fibroin fibers in the model of rat small intestine wall damage. The jejunum of Wistar rat was wounded in the form of an oval opening (0.8 × 0.6 m). Oval opening was closed with a fibroin scaffold. The implant was fixed from the side of the intestinal serous membrane with a nondegradable polyester thread to ensure the possibility of finding the surgical site later. In the rats of the control group, the small intestine defects were sutured with the polyester thread. In the control group, most of the animals died within the first 5 days as a result of peritonitis and intestinal obstruction. In the experimental group after 3 weeks post the surgery, the implant material practically could not be detected, except for the area contacting with the polyester thread. Microscopic study of the preparation of the jejunum wall on the site of the injury showed the restoration of the jejunum wall. The luminal surface of the implant area was covered with the epithelial layer with crypts and villi. Restored intestinal wall had serous membrane and signs of smooth muscle. Mucosa and submucosa contained blood vessels. The presence of goblet cells, fibroblasts, smooth muscle cells, Paneth cells and dividing cells in the villi indicated the restoration of villus structure. Thus we demonstrated that silk fibroin implants allow effective close the defect of the intestinal wall with recovery of the anatomic integrity and can be successfully applied in the repair of the parts of gastrointestinal tract.

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P.Mis-015
Obtaining polarized functional retinal pigment epithelium from IPSCs on substrates mimicking the Bruch's membrane

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Macular degeneration affects tens of millions of people worldwide. Finding reliable sources for transplantation retinal components is an extremely important problem. Human pluripotent stem cells (embryonic stem cells, ESCs, and induced pluripotent stem cells, iPSCs) are able to self-renew, and to differentiate into all types of somatic cells. This make them a valuable source of differentiated cells for replacement therapy. Clinical trials of the retinal pigment epithelium (RPE) differentiated from the pluripotent stem cells recently began in the United States and Japan. Pioneering clinical trials highlighted the need to optimize the technique of RPE differentiation to standardize material for future cell therapy. For effective treatment, iPSC-RPE must recapitulate the physiology of native human RPE. The Bruch's membrane is an important eye structure that provides the support for intact and functional RPE sheet formation. We developed a robust protocol of differentiation and expansion of RPE cells from integration-free iPSCs from healthy donors. Resulting RPE cells accumulated pigment, expressed markers of pigmented epithelium and had the ability of phagocytosis. In order to obtain highly polarized mature RPE we prepared by electrospinning a set of porous nanofibrous scaffolds from various materials (hydrophilic polyurethane, PLGA, PLA, and their modifications, supplemented with silk fibroin, gelatin, collagen) to mimic Bruch's membrane. We tested the ability of scaffolds to support the maturation and polarization of IPSC-derived RPE cells. RPE cells cultured on the modified polyurethane showed good adhesion, formed hexagonal honeycomb morphology, tight junctions and developed apical microvilli. Collectively, our data report the establishment of original tissue equivalent mimicking RPE layer on Bruch's membrane.

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P.Mis-016
Biosafety study of vaccine complexes based on structurally modified plant virus

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Plant viruses are promising platforms for design of new generation vaccines. Previously we described the unique spherical particles (SPs) which formed by thermal structural remodeling of rod-like tobacco mosaic (TMV) virions. It is possible to obtain SPs of predetermined size (from 50 to 2000 nm). We have demonstrated that SPs are very stable to external factors, RNA-free, have unique adsorption properties (could form complexes with different proteins) and can be an effective adjuvant. Here the biosafety studies of SPs in complex with protein containing influenza virus M2e epitope (model antigen) were performed. SPs complexes with M2e epitope (a highly conserved antigenic determinant of human influenza A virus) is a prototype of universal

candidate vaccine against influenza virus A. Acute toxicity studies indicated that SPs-M2e antigen complexes were unable to achieve any lethality in mice, rats and rabbits by intramuscular or intravenous routes. The maximum feasible dose was accepted according to recommendations of Laboratory Animal Science Association. Rats and rabbits in chronic toxicity studies have not demonstrated any weight gain lag or pathological changes on complete blood count. Thus, experiments in acute and chronic toxicity demonstrated the safety of the candidate vaccine in mice, rats and rabbits. Our results suggest that SPs can act as a safe platform (depot) for antigen and can be a promising tool in development of plant virus-based vaccines.

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P.Mis-017
Gentle staining of halophilic microorganisms for fluorescence microscopy

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Halophiles are microorganisms habitate environments with extremely high salt concentrations. Being evolved in extremely hostile environment halophiles developed unique biomechanisms which make them one of the most exciting scientific objects. The family of retinal photoactivatable membrane proteins often serving as model for all other membrane proteins is a bright example. The ability to survive under extreme conditions makes halophiles also the promising targets for extraterrestrial life search. Thus sensitive and gentle detection of living halophiles in hypersaline medium is a question of high concern. Fluorescent staining under growth conditions could provide sensitive methods for detection of halophilic species (i.e. via flow cytometry) or become an essential tool for their analysis via fluorescence microscopy. The main challenges for fluorescent staining of halophiles are high ionic strength, hostile pH and low permeability of halobacterial S-layer. Notable, for none of the dyes previously used for halophiles staining availability of long-term staining without cytotoxic side-effects was shown, contrary for DNA-staining dyes dominating in the field cytotoxicity was shown to be the intrinsic limitation. Here we show that dyes designed to stain mitochondria (MitoTracker's) easily permeate S-layer of Halobacterium salinarum and cause bright stable staining. To test our labelling procedure we observe the process of Halobacterium salinarum cells conversion into spheroplasts. MitoTracker Orange CMTMRos has no effect on cell' growth rate. The dye remains bright during long observations (hours to days) and is inherited in cell division. Therefore we show that mitotracker dyes can be used as effective and non-harmful dyes for halophile labelling.

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P.Mis-018
Transcription from ribosomal genes rrn modulates DNA initiation and cell cycle parameters in *Escherichia coli*

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Links between chromosome structure and replication initiation have been made in a number of works, strongly suggesting that favorable chromosome structure (i. e. supercoiling) is required for initiation in all cells. Transcription from strong promoters of the *rrn* ribosomal genes has been demonstrated to alter chromosome supercoiling. Additionally, 5 of 7 rRNA genes are positioned near the *oriC* sequence being candidate to promoting a significant elevated negative supercoiling in the origin region. In this work we have studied cell cycle parameters in the strain SQZ5 *thyA* derivative deleted for 6 of the 7 chromosomal *rrn* genes but carrying pTRNA67 plasmid to provide the required tRNAs genes included in a plasmid. By flow cytometry, radioactive DNA-labeling and microscopy analysis we show that the deletion of *rrn* genes into the chromosome: increases generation time, slightly decreases the number of replication rounds per chromosome and reduces the DNA replication velocity, as longer C period is observed. Furthermore, cells are smaller and contain higher DNA content than wild type strain. DnaA protein is a key element for initiation of DNA replication in *E. coli*. By combining *D6rrn* background with several mutations altering DnaA protein levels, such a: *dnaA174*, *DDARS2*, *DdatA* and low copy number plasmid-containing *datA* extra copies; we show a different requirement for DnaA protein in the absence of *rrn* operons. These results suggest an important role of the transcription of ribosomal genes on chromosomal DNA initiation and cell cycle parameters, supporting the importance of surrounding transcription events to create a favorable origin scenario. +Note: Patricia Gil, Encarna Ferrera and Antonio Arribas contributed equally to this work.

P.Mis-019
The role of FOF1-ATPase in biological hydrogen production by *Rhodobacter sphaeroides* during mixed carbon fermentation

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Biological hydrogen (H_2) production is considered as one of the promising ways to generate efficient, ecologically clean and renewable energy from various organic substrates, including industrial waste products, and it can have a significant impactin the development of energy technology. Nowadays, the interest of H_2 production by various bacteria is mixed carbon fermentation, mainly because of the cheap and effective carbon sources like glycerol or industrial wastes. Batch photofermentation experiments were carried out to reveal the input of the F_oF_1-ATPase on H_2 production by *Rhodobacter sphaeroides* MDC6521 from Armenian mineral springs during mixed carbon (succinate, glucose, glycerol and acetate) fermentation. Initial pH, temperature and light intensity were optimized for maximal H_2 production based on our previous research: pH 7.0, 28°C and 2000 Lx, respectively. F_oF_1-ATPase activity was determined by the amount of liberated P_{in}. The results show that membrane vesicles obtained from bacteria grown in a medium containing glucose, glycerol or acetate (15 mM) in addition to succinate (15 mM), exhibit an increased F_oF_1-ATPase activity. Significant ~1.7 fold increase of DCCD sensitive ATPase activity was obtained with glycerol addition compared with control (30 mM succinate), and since it had a positive effect both on bacterial growth and H_2 production, further experiments will be performed to obtain the optimal succinate/glycerol ratio to get the maximum H_2 yield. The role of F_oF_1-ATPase in coupling mechanisms and bioenergetics of *R. sphaeroides* during mixed carbon fermentation might be applicable in biotechnology as a tool to interfere the hydrogen metabolism of these bacteria.

P.Mis-020
Antimicrobial effect of edible coating produced from *Pistacia vera* resin to prevent growth of Listeria monocytogenes and Salmonella Typhimurium

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Outbreaks of Listeriosis and Salmonellosis has serious effects on human health and economy of countries in Europe. European Centre for Disease Prevention and Control (ECDC) reported 2194 Listeriosis and 89883 Salmonellosis confirmed cases in 2014. *Pistacia vera* L. tree (Pistachio nut tree) resin (PVR) is known with its anti-ulcer effects and is abundantly present in Gaziantep province. In this study, PVR and its essential oils used to produce antimicrobial edible coatings. Antimicrobial effects of PVR based coatings on chicken breast fillets initially contaminated with *Salmonella enterica subsp. enterica ser.* Typhimurium ATCC 14028 and *Listeria monocytogenes* ATCC 35152 were examined during storage of chicken breast fillets stored at refrigerator temperature (10°C) for seven days. Edible coating dispersions were prepared with PVR and different concentrations of additional PVR essential oils as; 0%, 0.5%, 1%, 1.5% and 2%. Chicken breast fillets were coated with dispersions and kept at refrigerator. During storage, total aerobic mesophilic bacteria, salmonella and listeria counts (in colony forming units, cfu) recorded. The increase of total aerobic mesophilic, salmonella and listeria counts of Uncoated control samples at the end of storage time were 2 log cfu/g, 1.8 log cfu/g and 1.5 log cfu/g, respectively. Samples coated with PVR showed 2 log cfu decrease for both total aerobic mesophilic count and salmonella count but 1.1 log cfu decrease for listeria count. Highest decrease was seen at 2% PVR essential oil added coatings as 2.1 log cfu/g. Increasing PVR essential oil concentration increased the antimicrobial effect of coating significantly ($P < 0.05$). As a conclusion, *Pistacia vera* L. tree resin is a promising natural antimicrobial additive for edible coatings to prevent growth of *Listeria monocytogenes* and *Salmonella* Typhimurium at chicken breast fillets.

P.Mis-021
StkP-mediated phosphorylation of EloR (Spr1851) regulates cell elongation in *Streptococcus pneumoniae*

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The Gram-positive ellipsoidal shaped bacterium *Streptococcus pneumoniae* maintains its shape by having two cell wall synthesis machineries – the divisome and the elongasome. The divisome is responsible for synthesizing new cell wall material at the division site, while the elongasome performs lateral cell wall synthesis responsible for cell elongation. Proteins known to play important roles in the elongasome includes PBP2b, RodA, DivIVA, CozE, RodZ, MreC, and MreD. During studies of the essential penicillin binding protein PBP2b, we discovered suppressor mutations that allowed the bacterial cells to survive despite the lack of PBP2b. 3 out of these six suppressor mutants produced a truncated version of the predicted protein Spr1851. As the requirement for PBP2b is lost in a *spr1851* knockout, it is likely that Spr1851 has a regulatory function in the elongasome. To verify this, we used the image analysis program MicrobeJ to compare cell length distributions in wild type cells and *spr1851* knockout cells. The knockout cells were significantly less elongated compared to the wild type cells. This indicates that the loss of Spr1851 results in reduced elongasome activity, and we named the protein elongasome regulating protein (EloR). EloR is phosphorylated on T89 by the kinase StkP. A phosphoablative form of EloR (T89A) resulted in the same phenotype as *ΔeloR* cells, indicating that EloR is activated through phosphorylation. Surprisingly, a phosphomimetic form of EloR (T89E) displayed even less elongated cells. Further investigations of the phosphomimetic mutant revealed a frameshift mutation leading to premature termination of *mreC* mRNA translation. It seems that the phosphomimetic form of EloR is not tolerated, and the cells respond by inactivating the elongasome. These findings demonstrate that pneumococci can survive without a functional elongasome in the absence of EloR. This implies that EloR and the elongasome are part of the same functional network.

P.Mis-022
The role of heme as an important mediator of biochemical processes

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The heme, an important cofactor of proteins, takes part in many physiological processes. It can act as an oxygen binding site (hemoglobin), an electron transfer site (cytochromes) and an activation site (cytochromes P450). In these cases the heme iron complex functions as an active center itself. Recently it was found that heme iron can serve as a binding site for gas molecule and effect the protein functioning indirectly in proteins called gas sensors. These proteins were found in bacteria, in which they usually serve as transcriptional factors or enzymes. They are involved in bacterial functions such as antibiotic resistance, sporulation, biofilm formation or virulence. Some of these bacterial properties can be harmful for human's health. That is why gas sensor proteins have become an important target for studying. The gas sensor proteins are composed by two parts: sensor domain which contains heme molecule and binds signal molecule (O_2, CO, NO, H_2S) and functional domain which is responsible for enzyme or transcription activity of the protein. According to heme iron ligand and/or redox state in the sensor domain the functional domain is either active or inactive. The aim of our research is to explore the molecular mechanism of the signal transduction between the sensor and functional domain. Two model proteins were selected: a globin-coupled histidine kinase from soil bacteria *Anaeromyxobacter* (*Af*GcHK) and a globin-coupled diguanylate cyclase from *E. coli* (YddV). Since both proteins are enzymes we performed detailed kinetic analysis. We determined apparent kinetic parameters (K_m, V_{max}, k_{cat}) of the autophosphorylation reaction (*Af*GcHK) and the diguanylate cyclase reaction (YddV) under various heme iron redox and ligand states [Fe(III), Fe(III)CN^-, Fe(II), Fe(II)O_2,]. It will be discussed how the kinetic analysis can be utilized for explanation of the molecular mechanism of gas sensor proteins action and signal transduction.

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P.Mis-023
Carbon nanotubes, graphene oxide and platelet-rich plasma enhance the versatility of biomaterials between adipose and bone tissue engineering

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The current trend in regenerative medicine is to improve patient health by using innovative and versatile scaffolds that allow cells to populate the implantation site and to develop *de novo* and functional tissue. Natural compounds such as cellulose and its derivatives (eg. Cellulose acetate, CA) are more often preferred over synthetic non-biodegradable materials. Graphene oxide (GO) was shown to have positive effect on bone regeneration while carbon nanotubes (CNT) positively influence adipogenic differentiation. Platelet-rich plasma (PRP) is used in tissue engineering procedures to enhance cell proliferation and fibroblast activation. The aim of this study was to investigate the impact of GO, CNT and PRP on human adipose-derived stem cells (hASCs) differentiation. CA/CNT/GO 0.25–1wt.% were put in contact with hASCs and tested for cytocompatibility by MTT and LiveDead assays. Cells adhesion to the substrates was tested by confocal microscopy. hASCs were exposed to adipogenic and osteogenic conditions for 21 days. The evolution of differentiation markers was monitored both at gene level by qPCR and protein level by confocal microscopy. CA composition improved with 1% CNT and GO proved to be the most cytocompatible film with hASCs. The addition of >5% PRP further improved cell adhesion and hASCs differentiation. Perilipin late adipogenic marker revealed an increasing gene expression profile up to 21 days, also confirmed by lipid vesicle accumulation in confocal microscopy, probably due to CNT contribution. Osteogenic marker osteopontin (OPN) showed an increase over 21 days of *in vitro* differentiation, corresponding to the influence of GO. CNT and GO added to a CA scaffold could have a contribution to directing hASCs differentiation towards osteogenic or adipogenic lineages. Therefore, CA/CNT/GO 1 wt.% is a suitable biomaterial for regenerative medicine applications and autologous PRP could improve its effect.

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P.Mis-024
Genomic profile of Bacillus pumilus 3–19 strain extracellular membrane vesicles

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Extracellular membrane vesicle (EV) secretion is a widespread intercellular communication process in prokaryotes. However, there is not a lot of information about the DNA content and functions of vesicles. So we performed genomic characterization of vesicles of *Bacillus pumilus* 3–19, chemical mutant overproducing extracellular ribonuclease. EVs were isolated from an exponential-phase culture with ultracentrifugation and ultrafiltration method and were visualized by Hitachi HT7700 Exalens transmission electron microscope. The fragment library obtained after multiple displacement amplification (MDA) of DNA extracted after DNase treatment of purified vesicles was sequenced on the Miseq (Illumina) platform. Reads mapped on the *B. pumilus* 3–19 genome assembly with Bowtie2 aligner were summarized with featureCount program. In this study, we found that vesicular DNA fragment library covered over 20% of the entire *B. pumilus* 3–19 assembly sequence. An overabundance of reads was revealed within the region containing 34 genes (about 40 kb). It is interesting that 50% of this region represented genes of hypothetical proteins with unknown function. The remaining genes were phage related ones, N-acetylmuramoyl-L-alanine amidase and genes annotated as transcription regulators. This overabundant region also was found in vesicles by sequencing the fragment library prepared with commercial kit NEBNext Ultra II for small amount of DNA without applying MDA technique. Although functional role of the revealed overabundant region is unclear to date, the obtained data can be useful for further investigations of the principles of DNA fragment generation and the mechanism of packaging into vesicles.

P.Mis-025
Gastrointestinal microbiota of the Japanese quail: sex-based differences

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Gastrointestinal tract (GIT) microbiota of farm animals is considered to be a perspective modulator of animal health and productivity. Avian GIT microbiota is poorly studied, except chicken, whose microbial community differs from the mammalian one and could be associated with fast metabolic rate of birds. Japanese quail (*Coturnix japonica)* is a popular and economically important natural food resource all around the world. This study presents the bacterial profiles of domestic quail GIT in 4 sections: gizzard, cecum, ileum and colon. The 16S rRNA gene sequencing was performed on the MiSeq platform for 5 mature male and 5 female quails. Sequences were analyzed using QIIME pipeline. The microbial composition was found to be different between GIT sections, as well as between sexes. Female birds had higher diversity levels (Shannon index 5.2 $\pm$ 1.4) than males (3.9 $\pm$ 2.3). Quail gizzard was mostly inhabited by representatives of phyla *Firmicutes* and *Proteobacteria*. Female samples had significantly increased abundance of unclassified *Enterobacteriaceae, Acinetobacter* and *Comamonas* genera than the male birds. The cecal microbial community was represented by *Firmicutes* and *Bacteroides* phyla with increased amount of *Bacteroides*, *Dialister* and *Megamonas* genera in female quails and *Lactobacillus* in males. In the ileum section of GIT the most dominant phyla were found to be *Firmicutes, Bacteroides, Actinobacteria* and *Proteobacteria* in both sexes. Differentially abundant taxa were unknown genera of *Helicobacteriaceae* and *Enterobacteriaceae* families in females and *Ruminococcus*, *Bifidobacterium* and *Peptococcus* genera in male samples. *Firmicutes* and *Proteobacteria* representatives were showed to be the main bacteria in the large intestine. Female birds had significantly increased proportion of *Sporosarcina* genus and decreased percent of *Collinsella*. Such gender-related differences could be explained by hormones level and should be taken into account when balancing poultry feed rations.

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P.Mis-026
Muscle-specific gene expression and calpain and proteasome activities in parr of Atlantic salmon *Salmo Salar* L. of different age groups

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The study was designed to evaluate the features of muscle growth regulation in parr of Atlantic salmon *Salmo salar* of different ages. The expression of myogenic regulatory factors (MRFs: *MyoD1, Myf5*, *myogenin*), myostatin (*MSTN-1a*) and a most abandoned myofibrillar protein myosin heavy chain (*MyHC*) along with protein-degrading activities of proteasome and calpain were studied in the white muscles in salmon parr of different year-classes (0+, under-yearling; 1+, yearling; and 2+, two year old). The maximal mRNA levels of *MyoD1a*, *Myod1b*, *myogenin*, and *MyHC* were revealed in parr 1+. The study on protein degradation systems in salmon parr revealed gradual decrease in proteasome and calpain activities from 0+ through 2+. Apparently, age and size-dependent decrease in muscle protein degradation would benefit muscle accretion and weight gain since muscle growth in fish becomes more efficient. Elevated levels of *MyHC* and MRFs expression in salmon yearlings (1+) reflect the intensive body and particularly muscle growth processes. The high levels of *MyHC* and MRFs expression was associated with high mRNA level of *MSTN-1a*, a negative regulator of muscle growth. Presumably, MSTN is expressed in response to high MRFs content as an inherent regulation mechanism attenuating hyperplasia and hypertrophy and governing muscle growth.

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P.Mis-027
RNA-seq analysis of *Bacillus altitudinis* with an emphasis on ribonucleases

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Bacillus altitudinis is a producer of numerous extracellular hydrolytic enzymes. In this study we performed the whole transcriptome shotgun sequencing of *B. altitudinis* B-388 and analyzed a gene expression profile of RNA-hydrolyzing enzymes. *B. altitudinis* B-388 was grown in Luria-Bertani medium until the early stationary stage. Total RNA from bacteria was collected using Silica beads and Trizol Reagent via FastPrep Homogenization System. mRNA isolation and library preparation was performed using SOLiD Total RNA-Seq Kit with a Duplex-specific nuclease. The sequencing was performed on SOLiD 5500xl Wildfire next-generation sequencer. The reads were mapped to the *B. altitudinis* B-388 genome (GenBank Acc. No JOVS00000000.2) with Bowtie as the aligner. Read summarization was performed using the featureCounts program. As a results, we found 3002 (mean normalized read counts > 5) protein coding transcripts of *B. altitudinis*, among them 530 had unknown function. The 20 ribonuclease-encoding genes were expressed in the cells at the indicated time which corresponded to 1.83% of the total expression of all genes. The most expressed ones were intracellular ribonucleases Y, R and J (1.24%, 0.25% and 0.16%, respectively). Together with ribonucleases PH, III, H, HII, HIII, YbeY, M, M5, P, and Z, these enzymes are responsible for exo- or endoribonucleolytic decay and maturation of single- or double-stranded RNAs. Two ribonucleases, HQ51_RS08215 (guanyl-preferring balnase catalyzing the cleavage of single-stranded RNA via a two-step mechanism) and HQ51_RS07265 (wide-spread among bacteria, eukaryotes and viruses HNH nuclease domain-containing RNase), are extracellular ones with 29 and 28 a. a. signal peptides, respectively. The expression rates of extracellular ribonucleases were nearly the same and notably less than that of intracellular ones – 0.035% for balnase and 0.031% for HQ51_RS07265, respectively.

P.Mis-028
Genetic engineering of human mesenchymal stem cells leading to VLA 4 overexpression; the *in vitro* assessment of modified cell functionality

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Transplantation of mesenchymal stem cells (MSCs) is widely used in regenerative therapy for tissues damaged by various pathological conditions. The way of systemic infusion is the optimal approach for exogenous cell grafting, however it demands the efficient homing to the area of injury. The goal of the study was focused to reveal whether genetic cell engineering leading to overexpression the molecules responsible for homing capacity increase transplanted cell migration to desired tissues. In our project we have chosen to induce the overexpression of α4 integrin in MSCs which is known as a key factor for leukocyte migration to the inflammation site. Overexpression of α4 subunit of VLA4 integrin in human bone marrow mesenchymal cells (hBM-MSCs) (PT2501, Lonza) was obtained by mRNA-ITGA4 based transfection. With flow cytometry we confirmed the presence of α4 protein in 70% of hBM-MSCs after mRNA transfection. Then the functional analysis of genetically modified hBM-MSCs was applied *in vitro* by using microfluid assay to estimate the movement pattern of flowing cells and transwell technique to assess migration ability of VLA-4 overexpressing cells. By using microfluidic assay (an advanced *in vitro* model of cell flow in blood vessels) hBM-MSCs were observed to roll, crawl, capture and arrest in the chamber channel. Quantitative analysis showed that most VLA-4 overexpressing hBM-MSCs moved faster than naïve cells and revealed improved interaction with the artificial blood vessel wall. Surprisingly, the migration of genetically modified hBM-MSC in transwell insert was reduced probably as a result of their stronger and prolonged interaction with proteins covering microporous membrane. Our results show that mRNA-ITGA4 transfected hBM-MSCs revealed enhanced adhesive properties *in vitro,* the *in vivo* properties need to be proved after transplantation.

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P.Mis-029
Microcarriers based on silk fibroin for cultivation and directional osteogenic differentiation of mesenchymal stem cells

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Tissue engineering constructs containing osteogenic cells can be used to repair defects in bone tissue and to treat some pathologies. In this work we studied the possibility of creating such constructs with silk fibroin based microcarriers (MCs). 100–250 μm MCs were obtained from porous fibroin-based scaffolds. The MCs were also mineralized with calcium phosphate. Both types of MCs were used for culturing mesenchymal stem cells (MSCs) isolated from bone marrow of C57BL/6N mice. The stem nature of the cells was confirmed by the analysis of CD73, CD90, CD105, CD45 gene expression. Adhesion and proliferation of the cells cultured on the surface of the MCs was evaluated by MTT test and compared with commercially available MC Cytodex-3. The cells were placed in an osteoinductive medium after a week of cultivation on the MCs. The activity of alkaline phosphatase (Alp) was measured to assess the osteogenic differentiation of MSCs. The adhesion of MSCs was higher to the surface of the non-mineralized fibroin-based MCs than to Cytodex-3. Increased activity of Alp was registered on all types of the MCs on the 7th day after induction, and it continued to rise up to the 15th day of cultivation. It is worth noting that during cultivation of the cells on the mineralized MCs, the activity of Alp was significantly higher compared to unmodified ones, while the proliferation rate was lower. The arears of mineralization were found on the 25th day of cell cultivation on the unmodified MCs that indicates osteogenic activity of the cells on their surface. Thus, the fibroin based MCs are suitable for cultivation and directed differentiation of MSCs.

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P.Mis-030
Cellular and molecular effects of a small sea fish extract on hyaluronan homeostasis

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Hyaluronan is the main component of the synovial joint, acting as a lubricant and as a shock absorber. In degenerative processes, hyaluronan is strongly depolymerized by hyaluronidase, the matrix of connective and cartilaginous tissues being destroyed. One of the main goals in osteoarticular treatments is to maintain the dynamic balance between synthesis and local degradation of proteins in the extracellular matrix. The purpose of our studies was to discover interdependent mechanisms for extracellular homeostasis of hyaluronan, namely cellular synthesis and enzymatic degradation and their possible modulation by a bioactive extract of sea fish. The experimental project focused on the acellular interaction of hyaluronidase and the marine innovative complex compared to its known inhibitors, glycyrrhizin acid, chondroitin sulfate. These studies were completed at the cellular level by the response of stimulated proinflammatory chondrocytes (extracellular hyaluronidase and hyaluronan synthase). Correlations will be made with results obtained on synovial fluid samples. The techniques used were zimography, colorimetry and Taq MAN q-PCR. All of the biological models relevant to the degenerative joint pathology used show that small fish of the sea stimulate HAS1 biosynthesis and inhibit hyaluronidase in a dose-dependent manner, suggesting improvement of cellular response in the catabolic processes of hyaluronan.

P.Mis-031
Effects of the superparamagnetic iron oxide nanoparticles on the synthesis of the extracellular matrix proteins and their reorganization by the mesenchymal stem cells

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Therapy by mesenchymal stem cells (MSCs) has been reported to provide beneficial effects at different disorders treatment. Visualization of cells and indication of their role at tissue repair the superparamagnetic iron oxide nanoparticles (SPIONs) can be used. SPIONs can effectively and quickly label most cells *in vitro*, and date suggests that such labeling does not compromise the proliferation or differentiation of cells. However it is still unknown how the inclusion of SPIONs can influence the functional activity of cells. In this study, we analyzed the possibility of any SPIONs negative influence on cell function, in particular on synthesis of extracellular matrix (ECM) proteins and ECM reorganization by matrix metalloproteinases (MMPs) at 3D cell culture conditions.

Materials and methods: Human MSCs were incubated with SPIONs (Fe concentration of 150 μg/ml) for one day in a CO_2-incubator. 3D-scaffold based on poly(ε-caprolactone) was used for cells cultivation. The cultivation of MSCs without SPIONs under 2D conditions was used as control. Cells viability and proliferation were assessed by Trypan blue and MTT assays. The evaluation of ECM proteins synthesis and ECM reorganization by MMPs was performed at the various cultivation terms by of protein electrophoresis, immunoblotting and zymography methods. The samples of mediums and scaffolds were analyzed separately.

Results and conclusion: Essential distinctions of ECM proteins and MMPs synthesis for cells, which were containing and not containing SPIONs have been shown. The cells with SPIONs synthesized much more ECM proteins (collagen I and III types, fibronectin etc) and demonstrated higher MMPs activity then control cells. Thus, there was not any negative influence of SPIONs on cell functions. Opposite the SPIONs increased the ECM proteins synthesis and ECM reorganization by MMPs.

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P.Mis-032
Assessment of genetic diversity of three Kazakh Edilbay sheep populations using microsatellite markers

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The genetic variability of 139 Edilbay sheep breed growing in three different flocks (Bayserke-Agro, Birlik and Azhar) was analyzed by using 10 microsatellite markers. The analyzed loci were: BM1824, ILSTS5, ILSTS28, INRAO63, MAF33, MCM140, OARVH72, SRCRCP5, SRCRSP1 and MAF70 all markers recommended by FAO and ISAG. Blood samples were collected from Bayserke-Agro (30), Birlik (67) and Azhar (42) sheep. A total of 321 alleles were found from the 10 microsatellite loci in three populations. The greatest genetic diversity was found in the loci of MAF70 – 18 alleles in all populations of the Edilbay sheep breed. The mean number of alleles and effective alleles number per locus were 10.70 ± 0.73 and 7.33 ± 0.58. The higest and the lowest observed heterozygosity in Bayserke-Agro, Birlik and Azhar ranged from 0.154 (SRCRCP5) to 0.692 (MAF70), from 0.254 (MCM140) to 0.51 (SRCRSP1) and from 0.167 (MCM140) to 0.676 (MAF70), whereas expected heterozygosity varied from 0.704 (BM1824) to 0.922 (SRCRSP1), from 0.692 (BM1824) to 0.922 (MAF70) and from 0.717 (BM1824) to 0.927 (MAF70) respectively. The inbreeding coefficients were F_{is} 0.620 ± 0.075 and F_{it} 0.640 ± 0.075. F_{is} and F_{it} values showed same results, all examined locus were positive, which indicates deficiency of heterozygosity. F_{st} values of genetic differentiation varied between 0.016 (MAF33) and 0.060 (BM1824), the average were 0.032, in that case, between in three populations F_{st} values indicated low differentiation. *Nei* genetic distances of Edilbay sheep populations ranged from 0.157 to 0.665 and the smallest genetic distance (0.157) was between Bayserke-Agro and Birlik. In conclusion, all tested markers were polymorphic and high informative for analysis genetic diversity of Edilbay sheep breeds. Investigation of results demonstrated deficiency of heterozygosity and low genetic differentiation within in three populations. All these results will give approach for breeding three farms of Edilbay sheep breeds.

P.Mis-033
Comparative study of the biocompatibility of polydioxanone and polyglycolide absorbable meshes for hernia and pelvic defects repair

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The recurrence frequency of tension-free hernia repair with synthetic unresorbable and resorbable mesh-implants compared to plastic surgery by own tissues decreased from 54% to 16%, but the frequency of mesh-related complications achieved 32%. By now there is no ideal prosthesis, so the development of a fully absorbable synthetic mesh implant is an effective way to solve this problem. The aim of the study was to assess the biocompatibility of polydioxanone (PDO) and polyglycolide (PGA) mesh implants. *In vitro* evaluation of cytotoxicity of mesh materials was performed by MTT assay. Evaluation of *in vivo* biocompatibility of meshes was carried out on rat full-layer abdominal wall defect model. On day 3, 10, 30 and 60 after surgery tissues were collected for macroscopic, tensiometric, morphometric, immunohistochemical and western blot studies. MTT assay showed that both meshes did not possess cytotoxic properties. *In vivo* experiment showed significant difference in adhesion intensity between the groups: PDO caused less adhesion score on day 3, 30, and 60. The resorption rate also differed: PGA filament area reduced by 36.5 ± 4.7% vs 17.4 ± 2.3% for PDO filament area. Maximal capsule thickness was observed in PGA on day 10 and 30 after the surgery, while PDO and Permacol capsules were thinner and almost didn't change throughout the whole experiment. PDO, PGA and Permacol meshes induced complex differences in the type of FBR over the time course of implantation. PDO evoked a milder early inflammatory response than other meshes. Significantly higher number of FBGC and Langhans cells were apparent throughout the time course in PGA group. PDO led to better tissue integration and new collagen deposition compare to PGA. PDO provided better vascularization compared to Permacol. PDO mesh has higher biocompatibility than PGA mesh or even Permacol.

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P.Mis-034
Effects of Miro1 on mitochondrial transfer and efficiency of cell bioenergetics recovery after stem cells and neural cells interaction

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Intercellular transport has recently been actively studied as a fundamental phenomenon of interaction of various cell types, as well as the basis for cell therapy. We showed that MSC co-cultured *in vitro* with neurons, demonstrated a more pronounced neuroprotective effect when administered to rats with a stroke. We found that when coculturing of MSC with neurons or astrocytes, mitochondria from MSC were transferred into neurons/astrocytes although the reverse transfer was not observed. This was accompanied by elevated levels of Miro1 protein responsible for intercellular transfer of mitochondria. Studying the conditions that stimulate intercellular transfer of mitochondria, the efficiency of the intercellular transport of mitochondria was analyzed both under normal conditions and during the induction of mitochondrial damage, as well as with the increase of the *Miro1* gene expression. It has been shown that the transfer of mitochondria into astrocytes is enhanced when their own mitochondria are damaged by oxygen-glucose deprivation. Similar, mitochondrial transfer was observed when mtDNA replication in PC12 cells was impaired by ethidium bromide treatment. PC12 cells with the damaged mitochondrial DNA are known to provide energy mostly through glycolysis due to impaired respiration, which results in a rapid increase of lactate levels in the medium. After coculturing of these cells with MSC lactate levels dropped almost to baseline which means that the oxidative phosphorylation processes were restored. We have produced MSC that overexpressed Miro1, such cells transferred their mitochondria to astrocytes and PC12 cells more efficiently. When grafting such cells to rats after stroke, we observed a more pronounced and rapid recovery of neurological functions than in case of native MSC. Thus, we can conclude that mitochondrial transfer is the most important component of the neuroprotective effect exerted by MSC after stroke.

P.Mis-035
Structural and functional insights in the evolutionary relationships between LonA proteases and ClpB chaperones

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Homooligomeric LonA proteases are key components of the protein quality control (PQC) system in the cells of bacteria and eukaryotes. LonA proteases form a unique class of AAA^+ proteins due to the presence of a two-domain N-terminal region that has no analogues with other members of the AAA^+ proteins of PQC system. A fragment, which is located just before a single AAA^+ module of LonA proteases, is an α-helical domain that includes an extended coiled-coil fragment (HI(CC) domain), containing a long helix. An arrangement of the helices in this fragment is reminiscent of a helical arrangement in the structure of H1 domain of the first AAA^+ module of ClpB chaperone. That resemblance led to a hypothesis that LonA proteases with a single classical AAA^+ module include a part of another AAA^+ module, which is present in full in the ClpB enzymes. The remaining part of this hypothetical AAA^+ module in LonA proteases contains only an α (H) domain, which is embedded between the N domain and the single AAA^+ module, but does not include an α/β domain. Since no structural data on any LonA protease that includes an intact coiled-coil fragment are available to date, we have used known crystal structures of various fragments of LonA proteases and ClpB chaperones to test and establish a structural basis for this hypothesis. Similarities and differences between the HI(CC) domains of LonA proteases and α-helical H1(M) domains of chaperone-disagregases ClpB are discussed, and α/β domains of their AAA^+ modules are compared.

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P.Mis-036
Ultrahigh-throughput single-cell screening of biocatalytic and antibiotic activities enable to select unique functionalities from combinatorial libraries and natural biodiversity

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Modern screening techniques facilitate selection of new drug candidates and accelerate the creation and the development of novel pharmaceuticals. The most promising approaches for drug selection are associated with ultrahigh-throughput screening (uHTS) techniques that enable to select specific functionality from a population of more than 10^7 different variants. However, most of biotechnological uHTS techniques like mRNA, phage, bacteria, yeast and mammalian display are designed to isolate specific binders. Alternatively, we developed simple and versatile approach that enable uHTS of a broad range of activities including different enzymatic and antimicrobial activities that are not based on binding. Individual living cells were encapsulated in droplets of biocompatible microfluidic double emulsion (MDE) together with fluorogenic substances or reporter indicator strain and the most active variants were selected using FACS based on the fluorescence of droplet compartments. The efficiency of this technique was demonstrated by isolation of novel butyrylcholinesterase variants displaying artificial paraoxonase activity from the combinatorial enzyme library. Biocompatibility of MDE enabled us to cultivate different bacterial species in droplets and expand this approach to investigate bacterial cell-cell interactions. Culturable oral microbiota species demonstrating efficient growth inhibition of a common pathogen *Staphylococcus aureus* were isolated using MDE-based uHTS. Moreover, we predicted slow-growing species that were associated with this inhibitory activity using 16S and whole-genome sequencing of the selected droplets.

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P.Mis-037
A novel recombinant β-glucuronidase enzyme for improved drug analysis

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β-Glucuronidase plays an important role in the analysis of biological fluids for the presence of drug metabolites. This enzyme hydrolyzes glucuronide metabolites back to the native parent drug. The use of β-glucuronidase is often necessary when extensive drug metabolism complicates drug detection. The b-glucuronidase derived from limpets (*Patella vulgata*) has been used for many years as the enzyme of choice for the hydrolysis of drug-glucuronides in urine tests. Although the native enzyme from limpets is robust and has broad specificity its use is limited due to its low purity, and the limited availability of high quality raw material for production. For the development of a next-generation recombinant enzyme, we have cloned the β-glucuronidase gene from *Patella vulgata*, expressed it in a proprietary expression system to obtain a highly pure and defined enzyme. The recombinant enzyme is highly purified to minimize protein content that can interfere with the analytical methods used. It is devoid of monoacetylmorphine (MAM) esterase activity (6-monoacetylmorphine → morphine) that interferes with discrimination between morphine and heroin, and is highly active against the traditionally difficult opioid substrate, Codeine-6-β-D-glucuronide. In addition, the enzyme is highly stable and active in a wide temperature range.

P.Mis-038
Molecular cloning of phytase gene from Pantoea sp. 3.5.1 into non-conventional yeast Yarrowia lipolytica

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Yarrowia lipolytica is a non-conventional and GRAS yeast which is very useful as a protein expression system that has high secretion capacity and product yield, less hyperglycosylation of products and simple scaling-up production. For expression of the phytase gene from *Pantoea* sp. 3.5.1. in *Y. lipolytica*, a modified sequence of phytase gene *agpP* (AN KJ783401.1) was used – C-terminal His-tag was added to the structural region of the gene, which allows purification of the enzyme from the yeast culture media. For the correct expression, the codon-optimization of the nucleotide sequence of the gene was carried out. The codon-optimized phytase gene was synthesized by Genscript (USA). Two variants of the phytase *agpP* gene were used for cloning into

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yeast – a sequence with its own signal peptide (SP) and without SP. For this study we chose the yeast vector pINA1296, which contains a strong hybrid promoter hp4d and a secretion signal (XPR2 pre region). To clone the phytase gene, restriction sites KpnI and SfiI were used. Restricted vector and gene sequences were ligated. The resulting ligation constructs pINA1296/agpP (with SP) and pINA1296/agpP(without SP) were transformed into *E. coli* DH5α cells. Transformants were examined for the presence of the phytase gene by PCR and restriction analysis which was confirmed by sequencing. Resulting plasmids were isolated and linearized by *Not*I restriction enzyme prior to transformation. *Y. lipolytica* strain Po1 g was used for transformation by electroporation. Transformants were selected on the medium containing no leucine. Integration of the bacterial phytase gene into the *Y. lipolytica* genome was confirmed by PCR analyses. Expression of AgpP phytase in yeast is now being studied.

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P.Mis-039
Diversity of microbial siderophores excreted by electrogenic bacteria in microbial fuel cells treating swine wastewater

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Bio-electrochemical systems such as microbial fuel cells (MFCs) are promising new technologies for efficient removal of organic compounds from industrial wastewaters, including that generated from swine farming. Inside the confined anaerobic chamber of an MFC a consortium of bacteria catalyze oxidation reactions, depositing electrons on the anode by a variety of means, including directly via outer membrane proteins or conductive pili or indirectly via secretion and recycling of redox-active molecules. We inoculated two pairs of laboratory-scale MFCs with sludge granules from a beer wastewater treating anaerobic digester (IGBS) and from sludge taken from the bottom of a tank receiving swine wastewater (SS). Using a metagenomic approach we describe the microbial diversity of the MFC planktonic and anodic communities derived from the different inocula. Among the class *Deltaproteobacteria*, *Geobacter,* which produce electron-transferring pili, was identified as the most highly abundant genus on the anodes of both MFCs. The most abundant genera of *Archaea* were *Methanosarcina* on the anode of the SS-MFCs and *Methanothermobacter* on the anode of the IGBS-MFCs. We further carried out functional analysis to identify genes encoding for the production of a diversity of potential low weight redox active mediators, such as siderophores. We found that the most abundant types of siderophore producing genes were fluorescent siderophores, such as pyoverdin and pyochelin, in anodic and planktonic communities of both MFCs. Additionally, genes encoding for production of catechol-type siderophores like enterobactin and bacillibactin were identified. Genes for hybrid NRPS-PKS siderophores were represented by yersiniabactin in the anodic and planktonic communities of both MFCs. Thus, despite the fact that dominant bacterial genus was *Geobacter,* it is likely that the variety of redox active mediators excreted by other abundant species contribute to electricity generation in the MFCs.

P.Mis-040
Mortality and developmental delay of marsh frog (*Rana ridibunda*) embryos exposed to oil

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The decrease of biodiversity and population of aquatic animals can be connected with contamination of the environment with oil and oil products. Therefore, the study of impact of water-soluble fraction of oil (WSFO) on the early developmental stages of *R. ridibunda* was carried out. To obtain eggs, 5 sexually mature specimens of *R. ridibunda* were used: 2 females with body length of 110 ± 2.5 mm and body weight of 150 ± 6.2 g and 3 males with body length of 102 ± 2.7 mm and body weight of 115 ± 5.4 g. The animals were intraperitoneally injected with hormonal preparation consisting of des-Gly10, D-Ala6, Pro-NHEt9-GnRH (GnRH-A) and metoclopramide HCL (MET) at a dose of 5 μl/g of body weight. 4800 fertilized eggs were placed into experimental 20 L aquaria containing dechlorinated water. The water temperature was maintained at 23 ± 0.5°C. Exposure to the WSFO (Dunga oil field, Mangystau region, Kazakhstan) was started when all eggs reached gastrulation stage, which corresponds with Gosner stage (GS) 10. WSFO was obtained by mixing 100 ml of oil with 900 ml of distilled water for 48 h with following filtration. In total there were 3 replicates for each experimental group containing 400 eggs which were exposed to: I – control (pure water), II – 0.05% of WSFO, III – 0.5% of WSFO, IV – 1% of WSFO. The development of eggs was observed using stereoscopic microscope Motic (China). In 24, 48, 72 and 96 h mortality was checked, and photographs of embryos were also taken to measure morphometric parameters. Mortality in control groups was 6% ($P \geq 0.05$), and 17% ($P \geq 0.05$) among embryos of group II, mortality of embryos at higher concentrations in groups III and IV was 46% ($P \geq 0.01$) and 80% ($P \geq 0.01$), respectively. Also among the surviving embryos in groups III and IV, their smaller size and developmental delay and abnormalities were noted compared to the control group. Thus, oil pollution can cause high mortality, morphological disruptions and supress development rate of amphibians.

P.Mis-041
Self-assembling triton-based micellar clusters: formation features and modification strategies for new functional materials creation

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Low aqueous solubility of many compounds is usually a major obstacle in the development of therapeutic agents, drug delivery, sensing or during investigation of properties of materials. There many approaches commonly used to enhance the solubility of poorly soluble drugs exist. Micellar solubilization is a widely used alternative for the dissolution of many hydrophobic compounds. However, not always using of pure micellar aqueous solutions is suitable for solubilization. The concept of micellar conjugation followed by clusters formation showed a great potential in the aspect of hydrophobic compounds solubilization beyond their solubilization limit. With the aim to extend the application fields of micellar clusters, we developed new schemes for micellar

clusterization, which based on sequential self-assembly of non-ionic detergent micelles followed by encapsulation of various objects. Proposed strategies allowed us to sufficiently increase solubility of not only hydrophobic endogenous toxic compounds (e.g. bilirubin) but several fluorescent dyes (coumarins; BODIPY family compounds) in aqueous media, which even may serve as micellar clusterization agents themselves. Hydrophobic fluorescent compounds-loaded micellar complexes demonstrate good optical response in aqueous media without crystallization beyond their solubilization limit in aqueous phase. We also showed that clusters are also very tunable and can be modified by various nanoparticles of noble metals or magnet nanoparticles. Therefore, the conception of micellar clusters modifying allowed to obtain various nanosystems, such as fluoromagnetic clusters or drug-loaded composites that open a new horizons for new advanced functional materials creation.

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P.Mis-042
TolC is involved in protection of *Serratia marcescens* against oxidative stress

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Emergence of bacterial drug resistance is one of the most important problems of public health worldwide. Bacterial drug efflux systems are membrane protein complexes that function to expulse drugs from the cell. They play a crucial role in the rising rates of antibiotic therapy failures. Multiple efflux pumps frequently present in the genome simultaneously. TolC is the major outer membrane channel in many bacterial species involved in excretion of wide range of molecules including antibiotics, antimicrobial peptides, bile and enterobactin. In *Salmonella* Typhimurium TolC is required for function of seven out of 11 drug efflux pump including RND-type efflux pump AcrAB and an ABC-type efflux pump MacAB. In agreement, TolC homolog in *Serratia marcescens* (HasF) was also shown to be required for function of RND-type drug efflux pump SdeAB. Here we used lambda red technology to knockout *tolC* gene in *Serratia marcescens* TT392. Resulting mutant was confirmed by PCR genotyping. As expected, inactivation of TolC resulted in the elevated sensitivity to sodium dodecyl sulfate (SDS). Because TolC forms a complex with an ABC-type efflux pump MacAB in *E. coli* and *S.* Typhimurium to efflux macrolide antibiotics we next tested whether inactivation of *tolC* will affect sensitivity of *S. marcescens* to hydrogen peroxide, a phenotype associated with the loss of MacAB efflux pump in *S.* Typhimurium. We found that in the contrast to wild type *S. marcescens*, Δ*tolC* mutant cells completely lost viability within the first hour of growth in media with 5 mM or 10 mM hydrogen peroxide. Co-culture of Δ*tolC* with wild type *S. marcescens* cells in H_2O_2-containing media protected the mutant cells from peroxide-mediated killing. Our data suggest that TolC is involved in protection of *S. marcescens* against oxidative stress through a secretion of anti-H_2O_2 molecules.

P.Mis-043
Secreted low molecular weight metabolites protect *Serratia marcescens* against hydrogen peroxide

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The emergence of bacterial multi-drug resistance is a growing problem of public health worldwide. Bacterial drug efflux systems are membrane protein complexes that function to expulse drugs from the cell. They play a crucial role in the rising rates of antibiotic therapy failures. In addition to well established role of multidrug efflux pumps in antibiotic resistance, efflux pumps also play important additional roles in natural physiology of microorganism that are independent of their role in drug efflux. Macrolide-specific ABC-type drug efflux pump MacAB first identified in *E. coli* has been linked to virulence of Salmonella enterica serotype Typhimurium in mice. We have recently showed that MacAB efflux pump is required for protection of Salmonella Typhimurium against oxidative stress both *in vitro* and *in vivo*. Moreover, the function of MacAB is conserved across other members of Enerobacteriaceae family. Here we show that MacAB is essential for survival of Serratia marcescens SM6 in the presence of hydrogen peroxide. We further show that growth of *S. marcescens* ΔmacAB mutant cells in the peroxide-containing media could be restored by co-culture with wild type cells. This protection is mediated by heat- and proteinase K-sensitive metabolites present in the media used for growth of wild type *S. marcescens* SM6 cells but not in the media used for growth of its isogenic ΔmacAB mutant cells. Fractionation of the conditioned media showed that protective antioxidant molecule is present in the fraction containing low molecular weight metabolites (under 10 kDa). We are currently working on identification of MacAB substrates with anti-H_2O_2 properties.

P.Mis-044
Cellular structural biology of nucleic acids

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The nucleic acid structure, particularly that of DNA, is sensitively modulated by non-specific physical and chemical environmental factors such as nucleic acids concentration, water activity, molecular crowding, viscosity, temperature, pH and/or concentration and nature of counter ions. Existence of environmentally promoted conformational polymorphism thus indicates that characterization of DNA structure, dynamics, and DNA-ligand/protein interactions should be ideally performed under native conditions in complex environment of living cells. We elaborated approach, based on that originally developed for proteins, to introduce DNA oligonucleotides into complex environment of nucleus of living mammalian cells in quantities required for their structural characterization using in-cell NMR. We show that this approach allows monitoring of structural properties of DNA in living mammalian cells at atomic resolution. The procedure does

not compromise viability of cells. We demonstrate the method can be directly employed for semi-quantitative assessment of stability of DNA-ligand complexes under native conditions. While concept of in-cell NMR of nucleic acids in eukaryotic cells was introduced almost a decade ago, the concept/method has remained limited to the Xenopus laevis oocytes serving as a model of the eukaryotic cell. In contrast to this established method, our approach allows performing in-cell NMR investigations of nucleic acids in (any) mammalian cells. The realization of this project was allowed due to financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601), and by the grant from Czech Science Foundation (16-10504S).

P.Mis-045
Cloning and expression of phytase agpP gene from Pantoea sp. 3.5.1 and phyC gene from *Bacillus ginsengihumi* in *Pichia pastoris*

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The significant amount of feeds' phosphorus is presented by phytate and is unavailable for animal nutrition. Microbial phytases hydrolyze phytates and are used as feed additives. However, there is a search for new phytases and phytase production systems. Yeast *Pichia pastoris* is a handy tool for efficient production of heterologous proteins. The aim was to clone and express bacterial phytases in *P. pastoris*. The gene sequences of histidine acid phytase of *Pantoea* sp.3.5.1 – *agpP* and beta-propeller phytase of *Bacillus ginsengihumi* M2.11- *phyC* were used. We altered the genes by excluding the sequences of signal peptides, adding a C-terminal His-tag and performing the codon-optimization. Cloning of bacterial genes into integrative yeast vectors pPINK-HC and pPINK-LC was carried out by three-way ligation of a vector, a bacterial gene and a signal peptide. The signal sequence of *Aspergillus niger* α-amylase gene was used. Vectors were digested with *Eco*RI and *Kpn*I restriction enzymes to create compatible ends to the phosphorylated 5′*Eco*RI end of the signal sequence and the phosphorylated 3′ end of bacterial phytases genes. After the transformation of *E. coli* DH5α with the ligation mixtures, transformants were screened for the presence of phytases genes, which was confirmed by sequencing. The transforming DNA for integration into the genome of protease knockout *P. pastoris* strain PichiaPink4 was prepared: vectors were linearized with *Spe*I restriction enzyme. Transformation was carried out by electroporation and transformants were selected on the adenine dropout media. Integration of the bacterial phytases genes into the *P. pastoris* genome was confirmed by PCR analyses. The expression of bacterial phytases in yeast culture media was detected by immunoblotting. This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University.

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P.Mis-046
Chasing the tail of human IgA through rational design of peptide mimics of a *Staphylococcus aureus* protein

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Staphylococcus aureus produce multiple proteins that interact with the host to establish infection and modulate the immune system. Staphylococcal superantigen-like proteins (SSLs) represent an important class of secreted proteins with roles in binding components of both the innate and adaptive immune systems. In particular, SSL7 binds IgA in the Fc region with high affinity and is a potent inhibitor of complement component C5. Crystal structures of complexes of SSL7 with IgA-Fc and C5 have defined recognition to be mediated by separate domains, the OB-fold and the β-grasp domain, respectively. Binding of IgA occurs through an extended interface, but most interactions involve two loops (L1 and L4) in the OB-fold of SSL7. In attempts to rationally design peptide mimics of the IgA binding site of SSL7, we identified some challenges and interesting properties of the IgA-binding peptides. While both linear (L1 and L4) and disulfide-constrained (L1ds and L4ds) peptides bound IgA, only L1ds showed some inhibition of SSL7. A computationally-designed peptide mimic containing both L1 and L4 and two disulfides (L1L4ds) showed stronger binding to IgA but no capacity to inhibit SSL7 binding to IgA. Circular dichroism (CD) spectroscopy revealed random coils for L1, L4 and L4ds and only a small proportion of β-structure in L1L4ds. In contrast, L1ds showed a CD signal indicative of β-structure suggesting the presence of larger multimeric assemblies. Unexpectedly, upon cold storage, L1ds formed a stable hydrogel after 1 week in physiological saline. This gel was found to be composed of a fibrillar network by atomic force microscopy and transmission electron microscopy. Thus, the observed inhibitory activity of L1ds for the SSL7:IgA interaction may be a result of formation of fibrils prior to gelation. Overall our findings suggest that IgA-binding peptides may require a stabilized structure or multivalent binding to effectively block bacterial immune evasion proteins such as SSL7.

P.Mis-047
MyoD1 paralogs gene expression in Atlantic salmon *Salmo salar* L and brown trout *Salmo trutta* L at different ages

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The transcription factor MyoD belongs to specific myogenic regulatory factors (MRF), which are important in myogenesis regulation. MyoD play a key role in specification and proliferation of myoblasts. In result of tetraploidization salmonids expressed three MyoD1 paralogs – MyoD1a, MyoD1b, and MyoD1c. The MyoD paralogs have sub-functionalized and exhibit distinct expression patterns during development and in different fiber types. The study was conducted to evaluate the age-dependent features of MyoD1 paralogs genes expression levels in parr of salmon *Salmo salar* L and brown tout *Salmo trutta* L. The gene

expression levels of *MyoD1a*, *MyoD1b*, and *MyoD1c* were evaluated in white muscles of parr at ages 0+ (under-yearling), 1+ (yearling) and 2+ (two year old). The *MyoD1c* expression was the highest in under-yearlings (0+) and then decreased both in salmon and brown tout. The *MyoD1a* and *MyoD1b* gene expression peaked in salmon yearlings (1+) and did not differ between age groups in brown trout. It was sugested that paralogs differently expressed in the ontogenesis of fish studied and MyoD1c is of great importance at the earlier stages (in under-yearlings 0+). It is possible that character of *MyoD1* paralogs changes between age groups is species-specific.

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P.Mis-048
The biological activity of the silver nanoparticles to MDA-MB-436 cells

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Literature studies have shown that various nanoparticles can modulate epithelial-mesenchymal transition (EMT) in cancer cells. EMT is one of the mechanisms of cancer metastasis which is manifested for instance by actin cytoskeleton disruption. The aim of this study was to evaluate the cytotoxicity of silver nanoparticles (AgNPs) and their impact on actin cytoskeleton organisation. For this purpose MDA-MB-436 cells were exposed to 20 and 200 nm AgNPs, as well as silver nitrate. Human breast cancer MDA-MB-436 cells are well recognized and widely used cellular model of EMT studies. Cytotoxicity was estamished by NR and MTT assays, 24 and 48 h after exposure. NR allows to investigate the cytoplasmatic membranes integrity, while MTT examine cells metabolic activity. Actin cytoskeleton organization was determinated by TRICT-labeled phalloidin staining. Studies have shown that both AgNPs dimensions induced concentration and incubation time dependent reduction in the survival of MDA-MB-436 cells, demonstrated in NR and MTT assays. Silver nitrate induced marked higher toxicity, which rather excludes the hypothesis that nanoparticles toxicity comes from the release of silver ions. In MDA-MB-436 cells incubated with toxic AgNPs concentrations actin network was destroyed, which indicates apoptosis and confirm cytotoxicity assays results. There were no major changes in actin cytoskeleton organization of cells incubated with non-toxic AgNPs concentrations, however inconsiderable formation of actin clusters, stress fibers and lamellipodia was observed. Obtained results revealed that AgNPs affect membrane integrity and MDA-MB-436 metabolism and may influence actin cytoskeleton organization. While the results of *in vitro* studies cannot be directly related to exposure *in vivo*, remarks of this work encurages further, more advance studies in this field.

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P.Mis-049
Experimental evolution of RNA coliphage MS2

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Experimental evolution in laboratory populations facilitates the detailed investigation of adaptation in real time. RNA viruses provide several advantages for studying evolution in action due to their large populations, short generation times, small genomes and high mutation rates. In this study, we used MS2, an RNA bacteriophage with a tremendous burst size and a tiny genome. We characterized the phage's response to a higher than usual temperature, comparing the identity and fitness effects of mutations at 41°C, vs. the optimal growth temperature of 37°C, using high-fidelity deep sequencing. Our experiment began with a nearly homogenous population derived from a single clone, which was serially passaged and sequenced at high and low temperatures. We first inferred the phage mutation rate (according to our experimental conditions) based on the change in frequency of neutral mutations. Our results revealed a mutation rate that was substantially lower than what has been previously reported. We next set out to infer the fitness of all mutations detected, based on the change in their frequency over time and our calculated mutation rate. Here we focus on the "one-step" fitness landscape, i.e., all mutations one step away from the original clone we began with. While multiple deleterious mutations appeared and then became extinct throughout the course of the experiment, our data also revealed several interesting adaptive mutations that occurred in coding regions at 37°C and/or 41°C, both synonymous and non-synonymous. To directly test the impact of these mutations, we conducted competition experiments between some of these mutants and the w.t. phage. We also used the fitness landscape that we obtained to test the mutational robustness of specific loci within the four open reading frames of MS2, revealing sites that could be manipulated in future biotechnological applications for this phage.

P.Mis-050
Construction of the super productive endopeptidase in *B. pumilus* strain

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In the culture liquid of the non-pathogenic soil bacterium *B. pumilus*, a minor zinc-dependent metalloendopeptidase MprBp was detected. The enzyme is classified as the adamalysin-like protein of the metzincin clan. To date, MprBp is the only known prokaryotic adamalysine. We established the primary structure of the protein, as well as the physicochemical and catalytic properties of the enzyme. However, its function in bacilli cells remains unclear. For a more detailed study of the enzyme, it was necessary to obtain it in a homogeneous form in an amount sufficient for the study. The task of this study was to create a recombinant strain-producer, actively expressing MprBp in a culture liquid. It was also important to ensure the subsequent rapid and simple purification of the protein from the culture liquid. To solve this problem, the *mprBp* gene was extract from the genomic DNA of *B. pumilus* and cloned into the pGP382 vector. The vector contains a strong constitutive promoter capable of overexpressing the target protein, as well as a Strep-tagg sequence, for subsequent purification of the protein by affinity chromatography. The assembly of the modified vector was carried out in *E. coli* strain DH5α cells. The constructed plasmid was transformed into two protease-deficient strains of *B. subtilis* BRB08 and *B. subtilis* BRB14 with deleted extracellular proteinases. As a result, two recombinant strains of *B. subtilis* MRB68 and MRB69 were obtained, actively expressing MprBp metalloproteinase to the nutrient medium. The active enzyme is expressed and contains a Strep-tag label at the C-terminus of the protein.

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P.Mis-051
Development of high affinity antibodies for enrichment and quantification of IL-6 by mass spectrometry (MS)

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Antibody-based technologies have been the major well-established method for quantification of proteins. In recent years, Mass Spectrometry (MS) has been used extensively in protein research as a tool enable to detect, characterize and quantify biomarkers in various biological samples. One of the major limitations of the MS technique is its ability to quantify medium to low abundant proteins in complex biological samples. A combined approach of Immunoaffinity Enrichment (IAE) and MS can be performed to achieve selective and accurate quantification and characterization of low abundant proteins with a high degree of specificity. This can be carried across a wide range of analyte concentrations in different matrices including tissues, cell extracts and bio-fluids. We aim to develop high-affinity antibodies that can be used in the MS workflow for specific enrichment of biomarkers in biological samples. Herein we describe the development of SILu™Rich IL6, a biotinylated monoclonal antibody against Interluking-6 (IL6) cytokine. This antibody showed high affinity to both IL6 from activated MG-63 human Osteosarcoma cells and recombinant stable isotope labeled IL6 protein standard with a Kd of 0.04 nM as measured by Biacore. Moreover, quantification of IL6 in MG-63 cells by IAE-MS showed comparable results to ELISA, with over 80% recovery of the recombinant IL6 protein standard. These results serve as proof-of-concept for the quantification of low-abundant proteins by MS using SILu™-Rich Antibodies. This scientific approach might be valuable for both research proteomics and clinical diagnostics.

P.Mis-052
Immobilization of serine subtilisin-like proteinase of bacilli with thrombolytic activity on the surface of the catheter

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Immobilized microbial enzymes can be used in a wide range of processes. In recent years, a variety of new approaches have emerged for the immobilization of enzymes that have greater efficiency and wider usage in science and medicine. There is an urgent problem in medical practice the clogging of the lumen of the catheter by thrombus. It remains important to search for new solutions of this problem. The main purpose of the work was to immobilize thrombolytic bacterial proteinase on a metal plate, which can act as a prototype of the surface of the catheter. We have shown that the subtilisin-like proteinase of the recombinant *B. subtilis* MRS50 strain had thrombolytic properties. The immobilization of subtilisin-like proteinase was carried out using the layerwise application of oppositely charged polyelectrolytes – polyallylamine hydrochloride (polycation) and polystyrene sulfonate (polyanion). It was shown that the proteolytic activity of the immobilized proteinase was 75% compared to the non-immobilized enzyme. Thus, the immobilized proteinase of *B. subtilis* MRS50 recombinant strain, which had thrombolytic activity on a metal plate, was obtained. The results of the work can be used in creating catheters with immobilized enzymes on them to destroy the blood clots that clog the lumen of the catheter.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P.Mis-053
The effect of cell surface N-linked glycosylation on mating efficiency in the halophilic archaeon *Haloferax volcanii*

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Halophilic archaea use a fusion-based mating system for lateral gene transfer across cells, that is thought to be unique to archaea among the prokaryotes. Long believed to be restricted only to Eukarya, it is now known that cells of all three domains of life perform N-glycosylation, the covalent attachment of glycans to select target asparagine residues in proteins. Previous work has indicated that cell fusion relies on some cell-cell recognition aspects, since fusion occurs more efficiently between cells from the same species. We are investigating how differences in the glycoproteins decorating the *Haloferax volcanii* surface layer may affect cell to cell contact and recognition and therefore also the cell fusion process.

P.Mis-054
Chlorpyrifos impact on preadipocytes proliferation and differentiation

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Organophosphorus pesticides (OPs) are an important environmental factor, which people are exposed. There have been reports in the literature suggesting that exposure to pesticides increase adipose tissue level and/or body mass. The aim of this study was to determine chlorpyrifos (CPF; being a most often used in Poland representative of OPs) impact on preadipocyte proliferation and differentiation. Experiments were performed on murine 3T3-L1 cells, which are one of the best characterized and reliable *in vitro* models for studying adipogenesis. 3T3-L1 treatment with differentiation medium containing corticosteroids and high doses of insulin led to differentiation of preadipocytes into mature adipocytes. Cytotoxicity of CPF was assessed by MTT and NR assays. 3T3-L1 cells were treated with medium containing CPF at different concentrations (5–250 μM). CPF induced concentration dependent reduction in 3T3-L1 cells survival, however the relationship was not linear. To assess the effect of CPF on proliferation of murine 3T3-L1 cells a MTT, NR and BrdU assay were conducted. 3T3-L1 cells were treated with indicated concentrations of CPF (5–250 μM) for 24, 48 and 72 h. Cells proliferation was dependent on CPF dose and time of exposure. Although CPF rather did not induce cells proliferation, some concentrations stimulated proliferation of cells when exposure was prolonged. CPF effect on differentiation of 3T3-L1 preadipocytes was determined by an Oil Red O staining. 3T3-L1 cells were treated with differentiation medium containing CPF at different concentrations. Lipid droplets were observed on a microscope. Moreover, spectrophotometric measurement was determined to access amount of the accumulated dye. CPF did not induce differentiation when measured spectrophotometrically, however

observation under microscope suggested that CPF might induce hypertrophy. This project was financed by the National Science Centre founds, based on the decision No. DEC-2014/15/N/NZ7/03065.

P.Mis-055
Does chitotriosidase activity increase in elderly obese people?

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In this study, we aimed to determine the activity of serum chitotriosidase (ChT), an enzyme secreted by activated macrophages in chronic inflammatory states, and the relationship between ChT activity, obesity and aging. Meanwhile, we investigated the relationship between ChT activity and lipid parameters (Apo A1, Apo B, Lp(a), total cholesterol, triglyceride, HDL-cholesterol), anthropometric measurements, fasting insulin, fasting glucose and hsCRP concentrations. 30 newly diagnosed obese, 30 overweight and 30 healthy individuals within different ages (25 to 71) were enrolled in this study. Serum ChT activity was measured with fluorometric method. In addition, anthropometric measurements, insulin sensitivity (HOMA-IR) were calculated and hs-CRP, lipid parameters, fasting insuline, fasting glucose concentrations were measured. Serum ChT activity was found significantly higher in obese and overweight patients than healthy individuals ($P < 0.001$). In addition, ChT activity was significantly higher in obese elderly patients than young obese patients ($P < 0.001$). When the all groups were considered, ChT activity was positively correlated with age, body mass index (BMI), Lp(a), triglyceride, fasting glucose; but negatively correlated with Apo A1 and HDL-cholesterol concentrations. Although hsCRP concentrations were higher in obese patients, there was no correlation between ChT activity. The association of ChT activity with obese elderly people indicated that macrophages were activated due to the chronic inflammatory states. Similarly, former studies have suggested that lipofuscin and lipid components accumulate with aging and activate macrophages chronically. Recently, gene analyses studying the relationship between aging, obesity and ChT activity may be hopefull.

P.Mis-056
Acetylcholinesterase and novel oxime reactivators in counteracting organophosphates exposure

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The high toxicity of organophosphorus compounds (OPs; tabun, sarin, cyclosarin VX and soman) originates from the irreversible inhibition of acetylcholinesterase (AChE), an essential enzyme in cholinergic neurotransmission. The poisonings lead to life-threatening toxic manifestations and call for immediate treatment, which usually consists of a combined administration of anticholinergic drugs and an aldoxime as the reactivator of AChE. Our previous research showed that AChE mutagenesis can enable oximes to substantially accelerate the reactivation of OP-enzyme conjugates, while dramatically slowing down rates of OP-conjugate dealkylation (aging). Here we analyzed the reactivation potency of nearly 130 novel oximes synthesized using [3+2] cycloaddition between alkyne and azide building blocks. We identified several oximes with significantly improved human AChE-reactivating efficacy against OPs and antidotal efficacy in mice. We furthermore screened the same library for the reactivation activity of two AChE mutants inhibited by OPs, and identified more effective reactivators of the tested phosphylated enzymes than standard reactivators. Further ex vivo testing of selected oximes and AChE mutants confirmed efficient oxime-assisted catalytic bioscavenging and neutralizing of OP exposure in whole blood. Our findings offer a platform for further antidote and scavenger development for exposure to organophosphates.

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P.Mis-057
Truly human – recombinant human proteins expressed in human cells

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Though human recombinant proteins can be expressed in prokaryotic and lower eukaryotic expression systems, expression in human cells yields native human post-translational modifications, such as disulfide bonds, glycosylation and other human-specific modifications that can result in better bioactivity. A human cells-expressed protein is an authentic protein because human cells have all the necessary components to properly process, modify, and fold the protein into a mature, genuinely human molecule capable of carrying out its biological function. We show that the expression of human proteins in human expression systems helps generate more potent mature proteins with correct posttranslational modifications and oligomerization, and with ultra high purity.

P.Mis-058
Diversity of antiviral defense strategies of bacteria at the population level

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Prokaryotes are faced with a constant threat of predation, and have developed a broad range of antiviral defense mechanisms that act at every stage of the phage life cycle. While previous studies of bacterial defense systems were focused on the mechanistic basis for their activity, little is known about what determines the choice of anti-phage strategies at the population level. Using bacteria-phage co-evolution approach one can not only study known defense strategies, but also detect novel ones, as the memory of host-predator interactions could be recorded in the genomes of bacteria as rearrangements at specific loci, and could be monitored during real-time evolutionary experiments. In this work we performed over 70 separate coevolution experiments with dozens of strains of *Bacillus* and *Thermus* and their phages. Each bacterial strain was infected with one of its phages in 500 μl of liquid media, allowing coevolution for seven days with serial daily transfers. Whole genome sequencing of bacterial and phage genomes was performed, followed by analysis of genome changes suspected as responsible for phage and bacterial survival during the multiple coevolution passages. Different bacteria-

phage growths dynamics were observed during co-incubation, indicating different defense strategies of bacteria and phages over a period of time. Mutations of cell wall receptors and flagellum, lysogenisation, and rearrangements in CRISPR arrays were among most common arm-race tactics of bacteria, while mutations in tale fiber proteins were mostly utilized by phages. Apparently, flagellum was used as a receptor by a much broader range of phages than previously thought. The details of defense strategies of bacteria of two different phyla against viral infection during a period of time will be further discussed.

P.Mis-059
Cytogenetic evaluation of the effect of ultra-small gold nanoparticles on spermatogenic cells of 129/JY mice

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Gold nanoparticles (GNPs) hold great promise for future applications in consumer products, medical and biomedical research. Their small size uncovers new properties that are very important to study; especially the effect these particles have on germ cells because any changes in the hereditary information will be passed on to the next generations. In this study we use meiotic micronucleus test to investigate the cytogenetic effects of single and fourfold exposure to ultra-small gold nanoparticles (2–3 nm) on spermatogenic cells of 129/JY mice. It is well known, that these mice have a mutation in the gene encoding DNA polymerase iota, which results in the absence of this enzyme in the organism. Moreover, combined effects of GNPs and alkylating mutagen dipin were evaluated. In all cases, with exception of the experiment with fourfold GNPs injection, we observe slight, statistically insignificant increase in frequency of occurrence of round spermatids with micronuclei compared with the control (saline). Previously in similar experiments on CBA×C57Bl/6 hybrid mice we have shown that depending on the chosen experimental conditions GNPs demonstrated mutagenic, antimutagenic or comutagenic properties. The difference in GNPs effect on mutability of spermatogenic cells of 129/JY mice and CBA×C57Bl/6 mice can be explained either by some specific peculiarities in meiotic chromosomes organization in 129 mice or that these mice possess their own molecular repair mechanisms linked to the lack of DNA polymerase iota.

P.Mis-060
Effect of laparascopic sleeve gastrectomy in early and late term to hemogram parameters

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The rising prevalence of obesity in today populations has led obese individuals to seek medical interventions. A side from special diets, routine exercise and medical treatment in most of morbid obese patients, favoring those with morbid obesity can benefit from bariatric surgery to lose weight. Laparoscopic sleeve gastrectomy (LSG) is relatively new method to limit the compliance of stomach. Most of these patients are lost due to postoperative embolism. We want to follow postoperative patients with hematogical parameters for this purpose. In our study, 23 morbid obese patients (average age and BMI are 43.3 ± 12.83; 49.27 ± 7.46 respectively) including 5 male and 18 female were operated with sleeve gastrectomy method, we compared the preoperative and postoperative 1 st, 6 th months leukocyte (WBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), erythrocyte (RBC), neutrophil (NE), lymphocyte (LY), monocyte (MO), eosinophil (EO) and basophil (BA) values. Whole blood samples were collected and measured with Abbott Cell-Dyn 3700 hematology analyzer (Germany). Paired sample t-test test was used to examine the differences between the terms. $P < 0.05$ was taken to be statistically significant. The results between preoperative and postoperative 1st, 6th months terms were evaluated, ıt was observed WBC_0-WBC_1 ($P = 0.001$), WBC_0-WBC_6 ($P = 0.002$), NE_0-NE_1 ($P = 0.001$), NE_0-NE_6 ($P = 0.001$), LY_0-LY_1 ($P = 0.031$), HCT_0-HCT_6 ($P = 0.001$), RBC_0-RBC_6 ($P = 0.001$) levels were decreased respectively, but there was not significant differences in other parameters. Sleeve gastrectomy has been shown to provide significant weight loss in morbid obese patients of surgical treatment. The obtained laboratory results indicate that the applied surgical method effects hematopoietic and immunity systems in patients.

P.Mis-061
The effect of obesity surgery on ghrelin levels

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Ghrelin is a appetite regulating orexigenic hormone and has a multi-pronged role in the regulation of energy homeostasis, including glucose metabolism. Bariatric surgery is the most effective treatment that provides effective weight loss in morbidly obese patients and positively affects obesity-related comorbidities. We investigated the effect of obesity surgery on ghrelin levels. In our study, 23 morbidly obese subjects (4 men and 19 women), [mean age and initial body mass index (BMI) are 43 ± 12.83 years; 49.27 ± 7.46 kg/m² respectively] were operated with Laparoscopic Sleeve Gastrectomy (LSG) ($n = 16$) or Roux-en-Y Gastric Bypass (RYGBP) ($n = 7$). Preoperative and postoperative 1st, 3rd, 6th months plasma ghrelin levels, glucose, insulin, C-peptide levels and insulin resistance (HOMA-IR) were analyzed. For biochemical and hormonal analyses, preoperative and postoperative 1st, 3rd, 6th months blood samples was obtained from 23 morbidly obese subjects. Ghrelin levels were studied by ELISA method. Paired Samples T-Test was used to examine the differences between the periods. $p < 0.05$ was taken to be statistically significant. The difference between the periods in the ghrelin levels at preoperative and postoperative 1st, 3rd, 6th months was not statistically significant ($P = 0.840$; $P = 0.314$; $P = 0.101$). HOMA, insulin and BMI values between the groups according to the time were statistically significant diminished ($P = 0.014$; $P = 0.005$; $P = 0.001$); ($P = 0.002$; $P = 0.001$; $P = 0.000$); ($P = 0.000$; $P = 0.000$; $P = 0.000$) respectively. Bariatric surgery didn't produce statistically significant changes in ghrelin levels over the long run. It has been shown that bariatric surgery provide significant weight loss, blood glucose regulation and decreased insulin resistance in morbidly obese subjects.

P.Mis-062
Development of an efficient expression system in *Pichia pastoris* for bacillary proteases production

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Proteases are enzymes that are highly used in biotechnology, medicine and as detergents. *Bacillus pumilus* is well-known producer of proteases. However, its native activity is not large for using these enzymes in biotechnology. Therefore, the development of high-efficiency expression systems is an important task of biotechnology. *Pichia pastoris* are yeasts, which are able to metabolize methanol as its sole carbon source. Oxidation of the methanol is controlled by the genes *AOX1* and *AOX2*. The *AOX1* gene induced by methanol synthesizes over 30% of total proteins in the cell. Therefore, the promoter of this gene is often used to produce highly effective expression systems. In this study we developed the expression system in a recombinant PichiaPink strain based on two shuttle vectors – pPink-HC and pPink-LC. Codon-optimized proteinase gene from *B. pumilus* were used as gene for expression in new system. The chemically synthesized gene of subtilisin-like proteinase (*aprBp*) of *B. pumilus* was cloned into the vector pUC57 and transformed into *Escherichia coli* DH5α. The vectors pPink-HC and pPink-LC with the *ADE2* gene were ligated with optimized aprBp gene. Furthermore, three different signal peptides were used for optimization of proteases secretion. All constructs were cloned into *E. coli* DH5α strain and confirmed by sequencing. To obtain the expression system, the recombinant shuttle vectors were transformed by electroporation into PichiaPink cells. The selection of the transformants was carried out on a PAD medium without adenine. White colonies were tested for the presence of vector by PCR. In conclusion, we obtained recombinant strains of PichiaPink, carrying the synthetic protein of *B. pumilus*, under the influence of three signal peptides. This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University.

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P.Mis-063
25 hydroxy vitamin D3 levels in patients admitted to our hospital

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Aim: 25-hydroxy vitamin D3 is associated with bone metabolism and blood calcium and phosphorus levels. Vitamin D deficiency can occur in people who live without sun exposure or whose dietary intake is low. The aim of this study was to determine VitaminD3 levels in patients admitted to our hospital and to examine the difference between according to gender and age groups.
Material and Method: Vitamin D levels of patients admitted to our hospital in 1.04.2015–31.03.2016 were analyzed retrospectively. Subjects were classified according to age groups; <17, 18–25, 26–30, 31–35, 36–40, 41–45, 46–50, 51–55, 56–60, 61–66 over age.
Results: Mean Vitamin D3 levels were 19.8 ± 15.6 in < 17 age group, 15.5 ± 16.8; 18–25 in age group, 15.7 ± 15.9; 26–30 in age group, 17.1 ± 16.6; 31–35 in age group, 18.3 ± 17.8; 36–40 in age group, 18–17.4; 41–45 in age group, 18.5 ± 17; 46–50 in age group, 18.6 ± 16; 51–55 in age group, 18.8 ± 16; 56–60 in age group, 18.9 ± 18.6; 61–66 in age group, 18.7 ± 17.9 in 66 over age group respectively. Vitamin D3 levels were significantly higher in <17 age group compared with other group. Mean Vitamin D3 levels were 17.9 ± 17.6 in woman group (n: 10991) and 19.0 ± 14.5 ng/ml in man group (n: 3666) There was a statistically significant difference between groups in terms of gender
Conclusion: These data suggest that the prevalence of vitamin D deficiency is high in the Konya population.

P.Mis-064

Withdrawn

P.Mis-065
Total analytic error in laboratory of biochemistry

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Introduction: Effective quality system should be composed for accuracy of test results in Medical Laboratories. The aim of the quality control processes is to achieve the specified analytical quality goals. Specified quality goals are expressed as Allowable Total Error (ATE). The total analytic error (TAE) should not exceed the allowed total error limit.
Material and Methods: TAE is the sum of random error and systematic error reflected in a test result. Random Error was expressed as variation of coefficient (CV%). Systematic error was expressed as bias%.The calculation was performed for Glucose, Urea, Creatinin, Albumin, HDL, LDH, ALT, AST, ALP, Total Protein, Sodium, Potassium, Chlor, Triglyserid, Cholesterol which were analysed on biochemistry analysers (Beckman Coulter AU 2700 (two), AU 680(one)) in our Laboratory of Biochemistry. Between-day CV% was calculated from 20 different days and two different level quality control results. Bias% was calculated using six consecutive external quality control results, according to the peer group value. TAE was calculated for each parameters. TAE% = Bias%+1.65+CV%.
Results: Results of TAE%: AU2700-1: Glucose: 3, Urea: 7.5, Creatinine: 5.8, Albumin: 6, HDL: 7.5, LDH: 13.6, ALT: 8.6, AST: 6.4, ALP: 1.6, Totalprotein: 4.3, Sodium: 4.4, Potassium: 4.7, Chlor: 6, Triglyserid: 11.1, Cholesterol: 7.9; AU2700-2: Glucose: 4.3, Urea: 13.2, Creatinine: 7.6, Albumin: 4.7, HDL: 5.6, LDH: 8.3, ALT: 7.4, AST: 9.3, ALP: 13.4, Total protein: 4, Sodium: 5, Potassium: 6.1, Chlor: 6, Triglyserid: 11.9, Cholesterol: 5.6; AU680: Glucose: 7.2, Urea: 10.6, Creatinine: 16.7, Albumin: 9.1, LDH: 11, ALT: 6.1, AST: 10, ALP: 17.8, Total protein: 9, Sodium: 5.2, Potassium: 5.4, Chlor: 5.8. ATE: Glucose: 11, Urea: 15, Creatinine: 20, Albumin: 15, HDL: 30, LDH: 21, ALT: 20, AST: 20, ALP: 30, Total protein: 15, Sodium: 9, Potassium: 9, Chlor: 9, Triglyserid: 15, Cholesterol: 11. ATE was defined by the Ministry of Health.
Conclusions: We observed that our laboratory is suitable determined quality targets for patient safety and health in medical laboratory.

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