# FEBS openbio

Volume 9 Supplement 1
July 2019
ISSN 2211-5463

THE 44th
FEBS
CONGRESS
6-11 JULY 2019
Krakow, Poland

FROM MOLECULES TO LIVING SYSTEMS

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Abstracts submitted to the 44th FEBS Congress, taking place in Krakow, Poland from 6th to 11th July 2019, and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this issue.

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### Indexing

Abstracts published in *FEBS Open Bio* Supplement for the 44th FEBS Congress will be included individually in the Conference Proceedings Citation Index published by Web of Science.

### How to cite these abstracts

AuthorOne, A., AuthorTwo, B. (2019). Abstract title. FEBS Open Bio, 9: Abstract number*. doi:10.1002/2211-5463.12675


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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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FEBS Open Bio **9** (Suppl.1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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# POSTERS

## Molecular mechanism of inflammation-related diseases

### P-01-001
### Mechanisms of accelerated ageing in gestational diabetic vasculature

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Gestational diabetes mellitus (GDM) is associated with epigenetic alterations in endothelial and vascular smooth muscle cells, causing morphological changes and dysfunction. Oxidative stress has been measured in human umbilical vein endothelial cells, which persists in cultured cells exposed to normoglycaemia for several passages. Our group are interested in identifying the mechanisms of endothelial and smooth muscle cell dysfunction. We have identified both multinucleation and increased senescence associated beta galactosidase staining in both endothelial and smooth muscle cells from umbilical cords of GDM pregnancies compared to healthy controls. The staining was particularly evident in multinucleated cells which is associated with ageing and senescence. The senescence associated secretory phenotype (SASP) is known to drive inflammation in aging. Measurement of relevant pro-inflammatory cytokine gene expression revealed increased interleukin 8 (IL-8) in GDM vascular smooth muscle cells and a tendency to increased IL-8, IL-6 and IL-17a in both endothelial cells and smooth muscle cells. We also demonstrated that insulin growth factor binding protein 5, a protein recently associated with senescence was upregulated. Other authors have identified increased senescence of umbilical vein endothelial cells from non diabetic volunteers after high passage numbers and our data demonstrates these characteristics are present in GDM cells cultured for minimal (less than 5) passages under normoglycaemic conditions. This may indicate that GDM causes accelerated aging of the vasculature which is likely to lead to increased risk for cardiovascular disease, a condition more usually associated with patients with advanced age. Taken together, our data suggests that GDM causes accelerated vascular ageing which may explain, at least in part, the increased risk of cardiovascular disease associated with the condition for mother and offspring. *The authors marked with an asterisk equally contributed to the work.

### P-01-002
### The role of the transcription factor Ets-2 in the pathogenesis of type 1 diabetes

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Autoreactive T helper (Th) cell populations and their signature cytokines are implicated in the pathogenesis of type 1 diabetes (T1D). Recent studies in our laboratory have shown a suppressive role of the transcription factor Ets-2 on the IL-2 gene expression, a major cytokine for the induction of naive Th cell differentiation. Moreover, it was reported that Ets-2 can induce Foxp3 expression in $CD4^+CD25^-$ Th effector cells cultured in specific conditions. Preliminary data of our laboratory have indicated that Ets-2 function may be affected by the acetyltransferase Tip60. In this study we investigated the expression of Ets-2, Foxp3, Tip60 and cytokine genes in Th subpopulations of T1D patients vs healthy controls, to delineate whether these factors are implicated in disease pathogenesis. Peripheral blood mononuclear cells (PBMCs) were isolated from T1D patients and controls. Determination of Th subpopulations was performed by flow cytometry. $CD3^+CD4^+$ lymphocytes were isolated from PBMCs, using immunomagnetic beads and were separated into $CD4^+CD25^-$ and $CD4^+CD25^+$ subpopulations. Ets-2, Foxp3, Tip60 and cytokine gene expression was determined by real-time PCR. Ets-2 and Tip60 protein determination was done by immunofluorescence. Our results showed increased levels of activated Th cells and increased Ets-2 and Tip60 mRNA and protein levels in $CD4^+CD25^-$ cells of T1D patients. Foxp3 gene expression was decreased in T1D $CD4^+CD25^+$ cells. Regarding cytokine gene expression IL2 and IL10 were increased and TGF-β1 was decreased in T1D $CD4^+CD25^-$ cells. Increased expression of Ets-2 and Tip60 indicates that are involved in the pathogenesis of T1D. Increased IL2 gene expression in T1D $CD4^+CD25^-$ cells suggests a reduced suppressive activity of Ets-2 on IL2 gene expression. The decreased Foxp3 gene expression possibly demonstrates Treg dysfunction in T1D patients.

### P-01-003
### Free fatty acid receptor type 4 ligands alleviate intestinal inflammation in mice

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Crohn's disease (CD) and ulcerative colitis (UC) are the main representatives of inflammatory bowel diseases (IBD), a group of chronic, immune system-mediated inflammatory diseases of the GI tract. High incidence of IBD was reported in western, developed countries which is likely related to the increasing environmental pollution, as well as "westernization" of the populations' diet and lifestyle (e.g. diet rich in animal fat and protein and low in fiber), all of which significantly affect individuals' immune response. Free fatty acid receptors (FFARs) are localized in the gastrointestinal tract and are possibly involved in the maintenance of gut function and implicated in IBD. The objective of this study was to test the anti-inflammatory activity of synthetic agonists of FFARs in mouse models of colitis. Therapeutic activity of GW9508 (FFAR1 agonist), 4-CMTB (FFAR2 agonist), AR420626 (FFAR3 agonist) and GSK137647 (FFAR4 agonist) was investigated in two models of semi-chronic colitis: induced by trinitrobenzenesulfonic acid (TNBS) mimicking CD as well as induced by DSS that recapitulates UC in humans. Body weight, macroscopic score, ulcer score, colon length, weight and thickness, as well as myeloperoxidase (MPO) activity were recorded. The systemic administration of GSK137647 (1 mg/kg, twice daily) attenuated both TNBS-induced and DSS-induced colitis in mice, as indicated by significantly reduced body weight loss as well as macroscopic parameters and MPO activity. This is the first investigation evaluating the anti-inflammatory activity of FFAR agonists and showing that pharmacological intervention targeting FFAR4 attenuates intestinal inflammation. Further experiments evaluating the signaling pathways triggered by FFAR4 stimulation are pending.


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## P-01-004
## Serum endocan and indoleamine 2,3 dioxygenase levels and diagnostic value in Acne rosacea

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In this study, we investigated the relationship between indoleamine 2,3 dioxygenase (IDO) and endocan in Acne rosacea (AR), which is implicated in changes in the immune system for the etiopathogenesis. 81 volunteers participated in the study. The AR group consisted of 52 subjects (36 females, 16 males) while the control group consisted of 29 healthy subjects (21 males, 8 females) without any disease. Serum IDO and endocan levels were determined by ELISA. AR is a chronic inflammatory skin disease in which dilated vascular structures and affecting the facial skin. Pathophysiology is not entirely clear, as there are many triggering mechanisms, including genetic and environmental factors, infectious causes, and the immune system. IDO is cytosolic enzyme composed of 407 amino acids in the first step in tryptophan catabolism. By proinflammatory cytokine release such as interferon-γ, it is expressed from antigen-presenting cells, and the main role is the immunosuppressive effect. Endocan is a cysteine-rich proteoglycan. It is excreted in the microvascular endothelial cells. Endocan plays a role in the pathogenesis of various malignancies and inflammatory diseases. Serum IDO levels were significantly higher in patients compared to healthy group ($P < 0.001$). Serum IDO levels of female AR patients were higher than healthy ones ($P < 0.001$). There was no difference between groups regarding serum endocan levels. There was no difference in serum endocan levels between sex groups. When the diagnostic value of the parameters was investigated, serum IDO level has high sensitivity (83.3%) and specificity (76.1%) with the cut-off value of 47.1 ng/mL for female AR patients. In conclusion, serum IDO, a parameter related to immune tolerance, was found to increase in AR patients in this study. However, serum IDO levels were found to be important in gender when assessed. Contrary to expectations, there was no correlation between serum endocan levels and AR with increased local vascularity.

## P-01-005
## Molecular and genetic markers of the disease severity in patients with autism spectrum disorders

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Autism has become the focus of attention for the modern medicine. As previously shown, the pathogenesis of autism is associated with oxidative stress. Aim: Revealing molecular and genetic markers of the disease severity in autistic children. Methods: A sample of children aged 4 to 10 (n = 133) with autism spectrum disorders (ASD) was divided into Group 1 (Gr1) with severe ASD (n = 71) and Group 2 (Gr2) with mild/moderate ASD (n = 62). The controls (n = 65) were healthy children aged 4 to 10. Cell-free DNA (cfDNA) was isolated from plasma using phenol extraction and quantified by Hoechst 33528 fluorescence. 8-oxodG frequency in DNA was determined on filters with antibodies. The degree of double-strand breaks (DSB) in G0-lymphocytes was estimated using gamma-H2AX detection. RNA was isolated with RNeasy Plus Mini Kit ('Qiagen'). Expression of *BCL2* and *BAX* was evaluated using real-time PCR. Findings: cfDNA content in Gr1 was 3 fold higher than in controls ($P < 0.01$) and Gr2 ($P < 0.01$). CfDNA in Gr1 had more oxidative modifications (OM) compared to controls ($P < 0.001$) and Gr2 ($P < 0.001$). The lymphocyte DNA in Gr1 had more OM and DSB than in controls ($P < 0.01$) and Gr2 ($P < 0.01$). The apoptosis rate was increased in Gr1 compared to controls ($P < 0.01$) and Gr2 ($P < 0.01$); the pro-/anti-apoptotic *BAX*/*BCL2* ratio was 4 to 5 fold higher in Gr1 than in controls ($P < 0.001$) and Gr2 ($P < 0.001$). Conclusion: An elevated content and a higher oxidation degree of circulating cfDNA, increased rates of OM and DSB of lymphocyte DNA, and activation of apoptosis in the blood cells were found in severe ASD. Supported by RFBR grant #17-04-01587A.

## P-01-006
## Expression levels of genes associated with Toll-Like Receptor signaling pathway in patients with carotid artery disease

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Atherosclerosis is known to be a fatally complicated and multifactorial cardiovascular disease. *Carotid artery disease* is typically caused by *atherosclerosis*, a *disease* in which plaque builds up in the *arteries*. Better knowledge of the pathophysiology of carotid atherosclerosis and its underlying genetic mechanisms is needed to improve diagnosis and therapy. Toll-Like Receptors ("TLRs") are the primary receptors of innate immunity. They play a key role in inflammation and expressed by a major number of cells in cardiovascular system. Therefore, TLRs are expected to play a part in the progress of carotid artery diseases. In this study, we aim at describing the expression of genes related to TLR pathway in carotid atherosclerotic plaques. The study included 15 endarterectomy specimens available from operated symptomatic carotid artery stenoses. The tissue specimens were separated according to anatomic location: internal carotid artery (ICA) and common carotid artery (CCA) as control region. Total RNA isolations and CDNA synthesis of the samples were conducted. Expression levels were investigated in Q-RT-PCR for IL-1 BETA, IL-6, IL-8, SPP1, TLR-7, and TNF-α genes located in TLR pathway using individual primers. GAPDH was used as a control to normalise mRNA levels. The fold change between carotid artery plaque tissue and control tissue was calculated using $2^{-\Delta\Delta CT}$ method. Q-RT-PCR data showed that IL-1 beta, IL-6, IL-8, TLR7, TNF-α gene expression levels were increased in ICA plaque regions when compared to CCA regions whereas SPP1 gene expression level was decreased. Determination of molecular mechanisms that indicate the presence of vulnerable plaques could be useful as diagnostic biomarkers for patients with stenosis of the ICA. This differential expression patterns needs further evaluation, with extended protein panels.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-01-007
## Analysis of MCPIP1 level in NAFLD patients

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) is an endonuclease that acts as a negative regulator of inflammation, through degradation of proinflammatory cytokines transcripts. MCPIP1 impacts also lipid metabolism – it inhibits adipogenesis by suppression of adipogenesis-related pre-miRNA maturation and degradation of CCAAT/enhancer-binding protein beta mRNA. Excessive accumulation of lipids in hepatocytes during nonalcoholic fatty liver disease (NAFLD) might be followed by development of fibrosis and inflammation. What is important, both processes might be regulated by MCPIP1. The aim of this study was to analyze MCPIP1 level in PBMC and liver biopsies of patients' who underwent bariatric surgery. For this study we enrolled 37 obese patients. We excluded subjects infected with hepatitis viruses or drinking alcohol (more than 20 g and 30 g of ethanol per day for women and men, respectively) and collected liver biopsies and blood of those qualified into the study. Liver histology analysis was performed to assess the level of steatosis, inflammation and fibrosis. We have also analyzed the level of plasma components. Moreover, expression of *ZC3H12A* gene (coding for MCPIP1) was evaluated in PBMC and liver samples. Body mass index (BMI) of 37 NAFLD patients enrolled to our study ranged from 38 to 58 and liver steatosis was from 5% to 80%. 15 out of 37 patients were diabetic with high postprandial glucose levels and moderate glucose control measured by percentage of HbA1c. CRP was above normal range in all subjects and 90% of them had elevated levels of ALT and AST. We detected a nine fold variation of *ZC3H12A* gene expression in PBMC isolated prior to the bariatric surgery. Additionally, there was a positive correlation between *ZC3H12A* expression in PBMC and patients' BMI. Previous studies showed that NAFLD is more prevalent in patients with high BMI. Thus, MCPIP1 can be considered as a new important player involved in the progression of NAFLD.

## P-01-008
## Biological role of MCPIP2 during viral infection of central nervous system

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The MCPIP (Monocyte chemoattractant protein-1 induced protein) family contains four members, MCPIP-1, 2, 3 and 4 encoded by the genes *ZC3H12A, ZC3H12B, ZC3H12C,* and *ZC3H12D* respectively. All of them are CCCH-type zinc finger proteins with highly conserved RNase NYN domain. Our and other studies indicate that proteins from the MCPIP family work as negative regulators of inflammatory response with a broad spectrum of substrates. According to Lin RJ et al. [1] overexpression of MCPIP1, but not other family members inhibits Japanese encephalitis virus (JEV) and dengue virus (DEN) replication through the degradation of their genetic material. The aim of this research was to investigate the expression of *ZC3H12A and ZC3H12B ZC3H12C* after viral infection of human neuroblastoma cell line – SH-SY5Y. In particular, we focused on ds-DNA-virus, herpes simplex type 1 (HSV-1). Our preliminary studies imply the induction of the expression of investigated genes after viral infection which suggest their involvement in the host innate defense. We also conducted our research on HSV-infected mice in which the presence of the virus has been detected in the central nervous system.

## P-01-009
## Oxysterols may contribute to hemolysis in sickle cell disease

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Sickle cell disease (SCD) causes severe anemia, oxidative stress and chronic inflammation in addition to various lipid abnormalities including reduced cholesterol levels. Oxysterols are oxidized derivatives of cholesterol. They are known to affect cholesterol metabolism and have been shown to increase eryptosis. Our aim was to determine whether the levels of 7-ketocholesterol (7-KC) and cholestane-3β,5α,6β-triol (C-triol) were associated with hemolysis and lipid profile in patients with SCD. A total of 32 steady-state pediatric patients with SCD (22 HbSS and 10 HbSß$^+$) and 25 healthy controls were included in the study. The hemoglobin, LDH, bilirubins, ferritin, serum iron, lipid profile, 7-KC and C-triol levels of all subjects were measured. Oxysterols were quantified with N,N-dimethylglycine derivatization via LC-MS/MS. Patients' 7-KC and C-triol levels were found to be significantly increased compared to controls ($45.39 \pm 3.89$ vs. $27.86 \pm 11.61$ and $20.31 \pm 1.78$ vs. $13.86 \pm 9.23$, respectively), while there was no difference between the HbSS and HbSβ$^+$ subgroups. 7-KC levels were found to be correlated negatively with hemoglobin ($r = -0.539$, $P = 0.007$) and positively with lactate dehydrogenase levels ($r = 0.518$, $P = 0.002$), while C-triol levels were correlated negatively with HDL cholesterol ($r = -0.439$, $P = 0.022$). However, there were no correlations between oxysterol levels and direct/total bilirubin levels. Additionally, while 7-KC and C-triol levels were highly correlated among controls ($r = 0.620$, $P = 0.001$), there was no correlation in patients. The findings of our study suggest that 7-KC and C-triol may have a role in SCD pathophysiology. Particularly 7-KC may contribute to hemolysis by affecting the erythrocyte membrane and activating eryptosis. The lack of correlation in patients' 7-KC and C-triol levels suggest alterations in the mechanism of non-enzymatic oxysterol production in patients with SCD.


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## P-01-010
## Integrative genomics reveal a role for MCPIP1 in adipocyte metabolism

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Metabolic diseases, including type 2 diabetes mellitus, nonalcoholic fatty liver disease, cardiovascular diseases correlated with obesity, result from adipocytes dysfunction. Excessive accumulation of lipids by adipocytes increases their pro-inflammatory activity, what leads to the generation of low-grade, chronic inflammation. In our studies we investigated the role of MCPIP1 as a factor controlling inflammatory processes and metabolic activity of adipose tissue. We performed global transcriptome and proteome analysis in 3T3-L1 adipocytes overexpressing wild-type ($_{WT}$MCPIP1) and the mutant form of MCPIP1 protein, ($_{D141N}$MCPIP1). Both RNA-Seq and proteomic analysis revealed that the elevated $_{WT}$MCPIP1 level downregulated factors involved in lipid and carbohydrates metabolism. Moreover, we found that $_{WT}$MCPIP1 overexpression in adipocytes impairs insulin signaling pathway, manifested by decreased level of insulin receptor, reduced insulin-induced Akt phosphorylation, as well as depleted Glut4 level and impaired glucose uptake. These results indicate that MCPIP1 affects glucose homeostasis by attenuated glucose uptake via regulating the insulin signaling pathway in adipocytes. To better understand the role of MCPIP1 in the adipose tissue biology, we also examined adipose tissue from patients with different BMI, including morbidly obese individuals. Interestingly, we found decreased level of MCPIP1 along with an increase BMI in SAT, whereas in VAT the difference was not significant. The presented data explain how the level of MCPIP1 protein in adipose tissue influence the tissue homeostasis and its lack or reduced level, as in the case of obese people is associated with the presence of chronic inflammation. Supported by the grant from the National Science Centre 2013/11/B/NZZ/00125 and grant for young scientists (BMN7/2016). FBBB of Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.

## P-01-011
## Development and validation of an in vitro model of colitis for the screening of potential therapeutic agents in Inflammatory Bowel Disease (IBD)

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IBD is a chronic disease with growing incidence, associated with significantly decreased quality of life and recurrent exacerbations requiring hospitalization. Conventional treatment is insufficient and frequently remission cannot be maintained, therefore the potential for new therapeutic options is widely studied. The research mostly employs animal models, which, in addition to the ethical dispute it evokes, is a costly, time-consuming and laborious solution. The aim of this project was to develop and validate an *in vitro* model of colitis. In the first stage, RAW264.7 macrophage cell line was stimulated with lipopolysaccharide (LPS) to induce inflammatory response. The cells treated with anti-inflammatory agents (budesonide and a novel gold(III) complex) were assessed for viability (XTT and NRU tests) and NO production (Griess assay). In this model, the gold complex showed better anti-inflammatory activity than budesonide (both tested at $10^{-6}$ M), which was in line with *in vivo* studies. The second stage involved coculture of RAW264.7 and Caco-2 human colorectal adenocarcinoma cells monolayer to mimic the change in permeability of intestinal barrier in inflammatory conditions, assessed by an alteration in trans-epithelial electrical resistance (TEER). To conclude, our study validates a novel approach in the in vitro studies on potential anti-inflammatory agents and encourages further research employing compounds of various chemical structure as well as targeting a wide range of intracellular pathways.

## P-01-012
## Fibroblasts-derived ROS as mitogens for keratinocytes in co-culture

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Our previous studies showed an elevated activity of NADPH oxidase in lesional skin fibroblasts obtained from psoriatic patients with respect healthy controls (CTR). In the present study we investigated in depth the redox imbalance in fibroblasts obtained from lesional skin of psoriatic patients (LES). A prominent redox imbalance in LES signed by increased levels (*vs.* CTR) of total ROS level, calcium, nitric oxide and mitochondrial superoxide ($O_2^{\bullet-}$) production was discovered using respective fluorescent probes and flow cytometry. We confirmed the data of other authors on elevated TBARS levels and decreased total antioxidant capacity in LES. Further, with the luminometric assay on intact cells treated with catalase and superoxide dismutase we defined H2O2 as the type of ROS that is over-produced by LES. Indeed, the expression of NADPH oxidase 4 (NOX4), the only NOX producing 90% H2O2 and only 10% $O_2^{\bullet-}$, but not NOX1 and NOX2, was augmented in LES. The silencing of NOX4 with the mix of three siNOX4 RNAs decreased significantly the extracellular ROS production by LES. Finally, performing co-culture studies with the primary keratinocytes obtained from healthy individuals we demonstrated that the co-culture with both CTR and LES fibroblasts brings to the increase in intracellular ROS


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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concentration and acceleration of keratinocytes' cell cycle. However, the co-culture with the LES fibroblasts had a significantly major impact on these two parameters with respect to CTR fibroblasts. Taking together, our findings demonstrate the direct impact of H2O2 derived from the LES fibroblasts on the proliferation and intracellular redox state of keratinocytes in co-culture. The use of primary cell cultures at their low passages prompt our results to be close as possible to the in situ conditions in the skin and indicate the dermal component as the trigger of psoriatic plaque development through the redox cross talk between fibroblasts and keratinocytes.

## P-01-013
## Identification, allergenicity, and effective mechanism of Der p 38 as a novel TLR4-binding allergen, homologous to the NlpC/P60 family protein in *Dermatophagoides pteronyssinus*

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The house dust mite is a common cause of allergic disease, and TLR4 acts as an important receptor of allergens and enhances allergic responses. We found a novel allergen that binds to TLR4 in *Dermatophagoides pteronyssinus* (DP). The allergen was submitted to WHO/IUIS allergen nomenclature sub-committee and was designated Der p 38 according to the allergen nomenclature. Der p 38 was purified and characterized by FPLC and LC-MS/MS-based peptide mapping analysis. The allergenicity of Der p 38 was confirmed by skin prick test, basophil activation, Western blotting, flow cytometry, and ELISA, and in a mouse model of asthma. Natural Der p 38 was identified as to bind to TLR4 (a protein present in the midgut of DP). The frequency of IgE reactivity to Der p 38 was 77.5% (31/40). Der p 38 increased CD203c expression in the basophils of Der p 38-positive allergic subjects, and induced clinical features of asthma via TLR4 in BALB/c mice and in C57BL/10SNJ and C57BL/10ScNJ TLR4 knockout mice. Der p 38 suppressed neutrophil and eosinophil apoptosis through a MyD88-dependent pathway and an independent pathway that included TLR4, Lyn, PI3K, Akt, ERK, and NF-κB. When treated with Der p 38, human bronchial epithelial cells produced IL-6, IL-8, and MCP-1, which inhibited the apoptosis of neutrophils and eosinophils. These findings regarding Der p 38 contribute to understanding of the pathogenic mechanism and diagnosis of allergic disease induced by DP.

## P-01-014
## Novel polypeptide biomarkers detected in TCA-soluble fraction of blood serum of multiple sclerosis patients

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Multiple sclerosis (MS) is an inflammatory autoimmune disease characterized by an impairing of the myelin sheaths around brain and spinal cord that leads to demyelination and scarring. A search for novel biomarkers convenient at diagnosing and treatment of the MS patients is an actual biomedical goal. Here we developed an original procedure for isolation of specific blood serum proteins and peptides in the MS patients. While in the traditional procedures, the 2,2,2-trichloroacetic acid (TCA)-pelleted material is mainly used for isolation of the bio-marker materials, our approach is based on 10% TCA-initiated precipitation of the ballast proteins, HPLC enrichment of the supernatant fraction, followed by the MALDI TOF/TOF identification of proteins and their immunochemical verification. We have shown that a TCA-extracted fraction obtained from blood serum of the MS patients contains 46-48 kDa short form of the unconventional myosin 1c (Myo 1C) and serum albumin identified by the MALDI TOF/TOF mass-spectrometry. Besides, such fraction contains IgGs and/or their heavy chains and Cys-Pro-Ser peptide. The TCA-extracted fraction isolated from blood serum of healthy human donors does not contain a significant amount of these proteins and mentioned peptide, while their content and frequency of appearance were increased in patients with MS, systemic lupus erythematosus, rheumatoid arthritis, Alzheimer's disease, and multiple myeloma. Potential role and mechanisms of appearance of above listed bio-markers have been considered. Our sincere thanks are to Prof. Tetyana Nehrych and Nazar Negrych (multiple sclerosis), Prof. Valentyna Chopyak (systemic lupus erythematosus, rheumatoid arthritis), Dr. Olga Shalay (multiple myeloma), Dr. Ludmila Stepanenko (Alzheimer's disease) for providing blood samples and histories of patients under observation, Prof. Serhiy Souchelnytskyi (mass-spectrometry), Dr. Volodymyr Tkachenko (HPLC), and Severyn Myronovskiy (electrophoretic studies). *The authors marked with an asterisk equally contributed to the work.

## P-01-015
## Aquaporins in immune cells: contribution for inflammasome priming and activation

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Aquaporins (AQPs) are protein channels that facilitate the transport of water and/or small solutes through membranes, essential for cell volume regulation. Variations in cell osmolarity and consequent cell volume regulation precede NLRP3 inflammasome activation and, interestingly, AQP blockage was shown to limit IL-1β release from NLRP3-activated macrophages. However, the role of AQPs in inflammation is still unclear. Human peripheral blood monocytes (HPBMs) and THP-1 macrophages cultured under stimuli for cell priming and inflammasome activation are valuable models to study inflammation. Here, we used HPBMs to evaluate AQPs expression in a healthy or pro-inflammatory phenotype. Then, we used THP-1 macrophages to uncover the mechanism where AQPs are players and potential new targets. Our results show that AQP9 and AQP3 are the most representative isoforms in primary monocytes and THP-1 cells, respectively, being both upregulated by LPS-priming. PMA-differentiated macrophages-like cells and LPS-primed macrophages-like cells showed similar basal water and glycerol permeability, being glycerol permeability reduced by Auphen, a selective AQP3 inhibitor. Therefore, using Auphen, we investigated the role of AQP3 during cell priming and inflammasome activation. AQP3 inhibition partially blocked LPS-priming, decreasing mRNA expression and protein release levels of IL-6 and IL-1β. This suggests an involvement of AQP3 in macrophage priming by Toll-like receptor 4 engagement. NLRP3 inflammasome priming and activation was also blocked when AQP3 was inhibited, decreasing mRNA expression and protein release of IL-1β after NLRP3 activation with nigericin and ATP. Moreover, challenging LPS-primed cells


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with hyperosmotic solutions of glycerol increased IL-1β release through Caspase-1 activation. Altogether these data evidence AQPs as candidate players in the setting of the inflammatory response.

## P-01-016
## Bacteriolytic activity of human blood plasma serotransferrin

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In the current work, bacteriolytic activity of serotransferrin was discovered. Although transferrins have been known for a long time, some of their functions are not completely clear. There is the information that transferrins can suppress the bacterial growth, however, bacteriolytic activity of these proteins has not been described before. In our experiments, human blood plasma proteins were separated to identify and study new bacteriolytic factors. It was found that the protein fraction with molecular weights between 60 and 80 kDa showed bacteriolytic activity on bacteria cells. After a few stages of chromatography the active protein was purified. Trypsinolysis of the isolated protein followed by mass spectrometry analysis allowed us to identify this protein as a serotransferrin. Purified serotransferrin demonstrates bacteriolytic activity against *Escherichia coli*, *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus megaterium* cells. pH profiles of activity are characterized at a maximum pH of 8.7–8.9. At an optimal pH value (8.8), the rates of lysis of various bacteria in the presence of serotransferrin and lysozyme were compared, considering the rate of lysis as a percentage of "lysozyme activity against *E. coli*". Lysozyme at a concentration of 0.3 μg/mL acts effectively against *E. coli* (100%) and *M. luteus* (68%), less efficiently against *B. subtilis* (18%) and practically does not act at all against *B. megaterium* (1%). Serotransferrin at a concentration of 3 μg/mL acts effectively against *E. coli* (61%), less efficiently against *M. luteus* and *B. subtilis* (13% and 12%), but relatively efficiently against *B. megaterium* (23%). The detailed investigation of serotransferrin bacteriolytic properties can be useful in drug-design to fight antibiotic-resistant pathogenic microorganisms. The authors thank Dr Marina Serebryakova, leading researcher of the Belozersky Institute of Physico-Chemical Biology, for conducting a mass spectrometric study.

## P-01-017
## Morin hydrate alleviate sepsis symptoms induced by *E. coli* ESBL (clinical isolate: KBN10P03347) by renovating autophagy in human hepatoma cells

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Flavonoid morin hydrate (MH) from *Moraceae* plant family is reported to have antioxidant and anti-inflammatory activity. This study was conducted to determine whether MH can modulate hepatic functional damage in a cell line model of sepsis with particular focus on the autophagy and lysosome signalling pathway. In silico analysis revealed MH followed the Lipinski rule of five and interacts with cytochrome family hence plays role in pharmacokinetics. The potential of MH treatment to reduce hepatic damage induced by *E. coli* ESBL (clinical isolate) in hepato cell line was measured by assessing levels of lactate dehydrogenase (LDH, $P < 0.001$), myeloperoxidase (MPO, $P < 0.001$), and lipid peroxidation. Pre-treatment with MH increased the cell viability after sepsis induction by clinical sample of *E. coli*. MH induced auto phagosome formation after ESBL treatment which was evident by the fluorescent microscopy and expression of ATG7 (1.004 ± 0.0), p62 and LC3 (4.795 ± 0.12). Furthermore cathepsin C (1 ± 0.00) expression helped in determining the increase in lysosome acidification due to the presence of ESBL (7.077 ± 0.15) which was attenuated by MH (4.676 ± 0.01). Loss of cell survival resulted in increased production of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and decreased mitochondrial membrane potential. Oxidative stress was controlled by MH as evident by SOD2 and HO-1 expression. Our findings suggest that MH protects against septic injury by restoring impaired autophagic flux. Therefore, MH might be a potential therapeutic agent for the treatment of sepsis.

## P-01-018
## Morin hydrate induces hyper activation of protein kinase B in order to attenuate the *E. coli* induced systematic inflammation in RAW macrophages via ROS generation and caspase-1 mediated pyroptosis

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*Escherichia coli* ESBL causes blood infection, which leads to hyper activation of cytokines further leading to sepsis resulting in systematic inflammation and multiple organ dysfunction. Morin hydrate (MH) from *Maclura pomifera* contributes in controlling the hyper immune responses via antioxidant machinery. However, the exact mechanism of its protective actions during systematic inflammation needs to be elucidated. This study was conducted with RAW 264.7 macrophages to determine whether pretreatment with MH can modulate *E. coli* ESBL (clinical isolate KBN10P03347; MOI: 1:5) obtained from the blood of a 49 year old male sepsis patient. Cytotoxicity was determined using LDH (con: 100, ESBL: 238.7 ± 0.27, MH: 126.1 ± 0.65) which conferred MH potential in conquering ESBL infection. Differential expression was observed in the level of MDA (con: 1.06 ± 0.28, ESBL: 7.32 ± 0.70, MH: 2.85 ± 0.28) and MPO (con: 3.31 ± 0.02, ESBL: 9.603 ± 0.01, MH: 4.601 ± 0.04) which was significantly ($P < 0.001$) co-related with the increased ROS (con: 100 ± 6.63, ESBL: 464.89 ± 11.98, MH: 122.96 ± 13.63) generation as determined by fluorescence intensity. Dysregulation of redox cycle was confirmed by antioxidant enzyme SOD-1, SOD-2 and catalase. RNS levels were elevated in ESBL (9.82 ± 0.21) as compared to control (5 ± 0.84) which were significantly reduced after MH (5.65 ± 0.91). Analysis of conditioned media revealed significant induction of IL-1β in ESBL (44.42 ± 0.07) treatments as compared to control (11.34 ± 0.35). Results revealed, activation of IL-1β induces pyroptosis by caspase-1 as evident by both mRNA and protein expression. Hence, the results observed in this study reinforce the protective role of MH in controlling the systematic inflammatory response.


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## P-01-019

## Inhibition of BET bromodomain proteins suppresses inflammatory activation of gingival fibroblasts and epithelial cells from patients with periodontal disease

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Periodontal disease is a chronic inflammatory disease of the periodontium that leads to bone resorption and tooth loss. Activation of resident gingival cells significantly contributes to the pathogenesis of periodontitis. BET bromodomain proteins are important epigenetic regulators of gene expression that bind acetylated histone tails and regulate the formation of acetylation-dependent chromatin complexes. BET inhibitors suppress inflammatory responses in multiple cell types and protect against bone resorption in a mouse model of periodontitis. Here, we analyzed the role of BET proteins in inflammatory activation of gingival fibroblasts (GFs) and gingival epithelial cells (GECs). We show that the BET inhibitors I-BET151 and JQ1 significantly reduced expression and/or production of distinct, but overlapping, profiles of cytokine-inducible inflammatory mediators in GFs from healthy donors (IL6, IL8, IL1B, CCL2, CCL5, COX2 and MMP3) and the GEC line TIGK (IL6, IL8, IL1B, CXCL10, MMP9) without affecting cell viability. Activation of mitogen activated protein kinase and nuclear factor-κB pathways was unaffected by I-BET151, as was the histone acetylation status, and new protein synthesis was not required for the anti-inflammatory effects of BET inhibition. I-BET151 and JQ1 also suppressed expression of inflammatory cytokines, chemokines and other mediators in GFs and TIGKs infected with the key periodontal pathogen *Porphyromonas gingivalis*. Notably, *P. gingivalis* internalization and intracellular survival in GFs and TIGKs remained unaffected by BET inhibitors. Finally, I-BET151 and JQ1 reduced *P. gingivalis*-induced inflammatory mediator expression in GECs and GFs from patients with periodontitis. Our results demonstrate that BET inhibitors may block the excessive inflammatory mediator production by resident cells of the gingival tissue and identify the BET family of epigenetic reader proteins as a potential therapeutic target in the treatment of periodontal disease.

## P-01-020

## Impaired cellular energy metabolism contributes to joint pathogenesis in rheumatoid arthritis

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Objectives: To examine the role of cellular bioenergetics in promoting synovial tissue inflammation in patients with rheumatoid arthritis (RA). Methods: Patients with active RA underwent full clinical assessment, video arthroscopy to quantify macroscopic synovitis and *in vivo* synovial tissue oxygen ($tpO_2$) measurements. Synovial tissue microscopic markers of inflammation (CD3, CD68) and glycolysis/oxidative phosphorylation (GAPDH, PKM2, GLUT1, ATP) were quantified by immunohistology. Primary rheumatoid arthritis synovial fibroblast cell (RASFCs) were isolated from synovial tissue and cultured with hypoxia or glycolytic inhibitor. RASFCs energy metabolism was assessed by the Seahorse analyser, mitochondrial structural morphology by TEM, mitochondrial-DNA-mutations, cell invasion, cytokines, glucose and lactate were quantified using specific functional assays. Results: The median $tpO_2$ was 25.4 mmHg (range 3.2–63 mmHg) and correlated inversely with macroscopic synovitis ($P < 0.05$), synovial expression of inflammatory cells ($CD3^+$ / $CD68^+$; all $P < 0.01$) and glycolytic markers (GAPDH, PKM2, GLUT1; all $P < 0.05$). Functionally, hypoxia increased the rate of glycolytic activity of RASFC ($P < 0.001$) with concomitant attenuation of mitochondrial respiration demonstrated by a decrease in basal and maximal respiration and ATP turnover (all $P < 0.05$). Hypoxia significantly increased mtDNA-mutations, mitochondrial alterations and invasiveness in RASFCs (all $P < 0.05$). *In vitro* inhibition of glycolysis attenuated RASFCs invasion, migration and secretion of pro-inflammatory mediators (all $P < 0.05$). Conclusions: The RA joint is profoundly hypoxic as a result of impaired mitochondrial function and inflammation, which leads to a bioenergetic crisis. Regulating glycolysis *in vitro* can resolve inflammation in primary synovial cells.

## P-01-021

## Endocan predicts development of HCC in patients with compensated cirrhosis, portal hypertension and no varices

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Background/Aims. The role of endocan, a prognostic factor in compensated cirrhosis, has not been investigated as a predictor of the development of HCC. A total of 103 patients with compensated cirrhosis, portal hypertension and no varices were included in a prospective observational study. Predictors of the development of hepatocellular carcinoma (HCC), including parameters indicative of liver insufficiency and portal hypertension, and endocan were analyzed. Methods. Baseline laboratory tests, ultrasound and endocan measurements were performed. Patients were followed prospectively every three months until development of varices or variceal bleeding. The endpoint was HCC development according to standard diagnostic criteria. Univariate and multivariate Cox regression models were developed to identify predictors of HCC. Results. In a median follow-up of 52 months 15/103 (14.5%) patients developed HCC. Seventeen patients died without HCC. Twelve (82%) HCC developed in patients with HCV. On multivariate analysis endocan (HR 2.91; 95% CI 1.03 – 8.26), viral etiology (HR 3.28; 95% CI 1.35 – 7.97) and albumin (HR 1.07; 95% CI 1.02 – 1.12) were independent predictors of HCC development. ROC curves identified 5.19 ng/mL of endocan as the best cut-off; those who had an endocan above this value had a three-fold increase in the HCC incidence. Conclusions. Endocan is an independent predictor of HCC development. An endocan > 5.19 ng/mL is associated with a 3-fold increase of HCC risk.


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## P-01-022

## Synthesis and initial evaluation of efficacy of olean-12-en-28-ol, 3β-pentacosanoate for the symptomatic treatment of multiple sclerosis

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In this study, bioactivity of olean-12-en-28-ol, 3β-pentacosanoate (OPC) was evaluated, which was formerly isolated from *Capparis ovata* plant by our group. First of all, compound OPC was synthesized starting from oleanolic acid (OA) because both have same chemical skeleton. Initially, OA reduced to erythrodiol and its primary OH group at C28 position was selectively protected as silyl ether. 3β-hydroxyl group of corresponding silyl protected compound was converted into pentacosanoate ester derivative. Finally, compound OPC was synthesized deprotection of silyl group. Then OPC was applied at non-toxic doses to the human neuroblastoma cell line SH-SY5Y to study the regulation of the expression of MS-related genes. Total RNA from SH-SY5Y was isolated and reversely transcribed into cDNA. Tested and validated human MS primers along with the housekeeping genes were used for quantitative determination of differential gene expression profiles between different treatment groups. The results are the average of the experiments conducted in three independent set of experiments. The expressions of pro- and anti-inflammatory chemokines/cytokines such as CXCL9, CXC10, have been shown to be prominently reduced with OPC treatment. Also, expressions of IL6, NFKB, TNF and C1S were also down-regulated with OPC in these cells. Thus, these data strongly suggest that OA is a potent inhibitor of the T cell activation and differentiation. The expression of MMP9, which is known to be important in leucocyte infiltration, was also significantly decreased with OPC treatment. It was also found out that the transcripts levels of myelin-specific proteins such as MAG and PLP1 were upregulated by OPC. Collectively, these results indicate that OPC was associated with the suppression of molecules essential for disease development and induction of molecules important in healing. This work was supported by the Scientific and Technological Research Council of Turkey [TUBITAK-117S293].

## P-01-023

## The genotoxic effect of combined action of carbon tetrachloride and ionizing radiation on Wistar rat's liver cells

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To date, one of the methods of treatment of malignant tumors of the liver is radiation therapy. However, during irradiation, not only tumor cells are affected, but also cells of adjacent tissues that are not involved in the process of malignant transformation. These facts stimulate the development of technologies aimed at minimizing the negative effect of ionizing radiation on healthy tissues. The aim of the research was to determine the number of clustered damages DNA (OCDL) in liver cells of Wistar rat's during combined action of carbon tetrachloride and dose radiation of 0.5 Gy on the 30th day after irradiation. Methods. The experiments were performed on Wistar rats. The animals before irradiation were injected with CCl4 (50%, 2 ml/kg) and subjected to single-time total body irradiation using a closed source of γ-radiation, dose radiation 0.5 Gy samples selection was performed on the 30th day after irradiation. The DNA extraction was performed according to the method of High Pure PCR Template Kit (Roche). To determine the sites of DNA with a loss of oxidized base or modified nitrogenous base the endonuclease APE1 was used. Determination of number of clustered damages in DNA was performed according to the method of NALA. Results. On the 30th day number of clustered damages in DNA in Wistar rat's liver cells, which were injected with CCl4, has increased 6.49 (1657.45 APE1 clusters/Mbp) times compared to control and 18.33 (4680.77 APE1 clusters/Mbp) times in irradiation cells injected with CCl4 compared to control. Conclusion. During combined action of CCl4 and irradiation we observed increase a number of clustered damages in DNA compared to control. *The authors marked with an asterisk equally contributed to the work.

## P-01-024

## Roles of myricetin and dihydromyricetin on regulation inflammatory response in vitro

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Myricetin (MYR) and dihydromyricetin (DMY) are classified as natural flavonoids that have been shown as a compound with anti-inflammatory and antioxidant potential. MYR is especially found in fruits, vegetables, berries, tea and red wine. On the other hand, DMY is one of the most pharmaceutical active components that is isolated from the Chinese herb *Ampelopsis grossedentata*. The aim of this study was to evaluate the biological effect of MYR and DMY on the levels of inflammatory parameters, such as matrix metalloproteinases and the cytokines. The overexpression of selected cytokines may initiate production of MMP-1 and MMP-2 that often persist in non-healing wounds. Their elevated level may cause prolongation of the wound healing time and damaged of healthy tissue. Normal human dermal fibroblasts (NHDFs) and normal human epidermal keratinocytes (NHEKs) were scratched and exposed to lipopolysaccharide from *Pseudomonas aeruginosa*, and were used as *in vitro* models for testing of these flavonoids. The sub toxic concentrations of MYR and DMY (5–15 μM) were selected using MTT assay. The level of the inflammatory parameters were analysed in collected cell medium after the incubation period (24 hours) using the Enzyme-Linked ImmunoSorbent Assay (ELISA). According to the results the level of IL-6 was decreased with increasing concentration of MYR or DMY in LSP-stimulated NHDFs and NHEKs respectively. In addition, the same trend was observed at the level of MMP-1 in NHDFs. Contrarily to the results of NHDFs, the level of MMP-1 was increased with increasing concentration of MYR and DMY in NHEKs. In conclusion, MYR and DMY seem to possess the potential to regulate the inflammatory response. This work was supported by the grants IGA_LF_2019_015 and RVO:61989592.


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## P-01-025
## Serum amyloid A induces cytokine and growth factor production in pancreatic beta cells

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Decreased insulin secretion by pancreatic β-cells may be caused by amyloid deposition or lipotoxicity. Inflammation plays a key role in the development of β-cell dysfunction. Serum amyloid A (SAA), a classical acute phase protein, plays a role in the inflammatory process by increasing production of inflammatory mediators. Persistently elevated SAA levels characterise a number of metabolic disorders, such as obesity. We hypothesise that chronic pancreatic exposure to SAA may lead to alterations in insulin secretion by inflammatory mechanisms. The purpose of this study was to investigate the effects of SAA on the function of pancreatic β-cells. To define the toxic dose of SAA in pancreatic cells, we analysed the number of viable cells by flow cytometry. Cytokines and growth factors were quantified by ELISA in the supernatant of β-cells treated with SAA. After 24 h, the supernatants were collected, and cytokines were determined by ELISA. In the concentration range studied, SAA (0, 5, 10, 20 and 40 μg/mL) was not toxic to pancreatic beta cells. A dose-dependent increase in IL-1β ($r = 0.82$, $P < 0.05$), CINC ($r = 0.78$, $P < 0.05$) and VEGF-α ($r = 0.65$, $P < 0.05$) by β-cells incubated in the presence of SAA was observed. In the studied conditions, our results indicate that SAA is an important protein involved in signalling in the inflammatory environment. Knowing the mechanisms by which SAA interferes with signalling for β-cell dysfunction is essential for the development of new therapeutic agents because it has become clear that inflammation plays a key role in β-cell dysfunction. We suggest that beta cell amyloidosis may be linked to the genesis of diabetes because the exposure of beta cells to SAA increased the release of cytokines, which may cause dysfunction of these cells. Financial Support: FAPESP (18/17986-0) and CNPq 308700/2017-1.

## P-01-026
## Role of pentraxin-3 on dental pulp inflammation and regeneration

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The inflammation in dental pulp is a prerequisite for the regeneration of the dentin-pulp complex; otherwise, irreversible pulpitis or pulp necrosis occurs. Pentraxin-3 (PTX3) has been considered as an inflammatory biomarker in various inflammation-associated diseases. In this study, we investigated the role of PTX3 in the inflammation and regeneration of dental pulp. The treatment of recombinant PTX3 protein increased the expression of proinflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) in human dental pulp cells. PTX3 induces NF-KB activation through NF-KB p65 translocation into the nucleus of human dental pulp cells, which induces the expression of proinflammatory cytokines. Immunohistochemical study showed PTX3 is highly expressed with the inflamed pulp tissues. The expression of PTX3 was increased during the osteogenic differentiation in human dental pulp stem cells (HDPSCs). The inhibition of PTX3 decreased osteogenic differentiation of HDPSCs, which regulated the expression of osteogenic genes. Taken together, these results indicate that the PTX3 may play an important role in modulating the inflammation of dental pulp cells and osteogenic differentiation of HDPSCs.

## P-01-027
## Role of IDH2 in kidney fibrosis induced by ureteral obstruction

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Mitochondrial NADP + -dependent isocitrate dehydrogenase 2 (IDH2) produces NADPH, which is highly correlated with oxidative stress and dysfunction of mitochondria. Ureteral obstruction induces kidney inflammation and fibrosis via oxidative stress. Here, we investigated the role of IDH2 in unilateral ureteral obstruction (UUO)-induced kidney inflammation and fibrosis as well as underlying mechanisms using IDH2 gene deleted mice (IDH2-/-). IDH2 gene deletion did not affect other ROS scavengers, and UUO absolutely decreased IDH2 expression in the kidney. Although UUO increased oxidative stress, inflammation, and fibrosis in both wild type (IDH2 + /+) and IDH2-/- mice, IDH2 deficiency exacerbated UUO-induced oxidative stress, inflammation, and fibrosis. IDH2 gene silencing (KD) in raw264.7 cells increased lipopolysaccharide-induced F4/80 and α-SMA expression, and TGF-β-induced α-SMA and Collagen III expression suggesting that activation of macrophages plays a critical role. Taken together, our data demonstrate that IDH2 plays a protective role in UUO-induced inflammation and fibrosis through inhibition of oxidative stress and macrophage activation.

## P-01-028
## Examining the oxidative status in peripheral bloods of schizophrenia patients and first degree relatives

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The scope of this current work is to investigate oxidative status of peripheral bloods in both schizophrenia (scz) patients' and their unaffected relatives' and so try to assess if some oxidative markers can be identified and be used for both diagnostic and theranostic purposes. We think examining the patients' relatives' blood would also help to evaluate oxidative stress involvement in the disease pathology by referring to its genetical background. In this prospective case-control study, participants were categorized into three groups: 50 adult patients with schizophrenia diagnosis, 50 healthy controls and 50 patients' relatives. Venous blood samples were collected, and selected parameters measured from serum were: plasma myeloperoxidase (MPO), total oxidant status (TOS), total antioxidant status (TAS), total thiol (TT) and native thiol (NT). Oxidative stress index (OSI) and thiol disulfide level (TDS) were calculated. As a result, the plasma TOS and OSI levels were significantly higher and TAS levels were lower in the patient group compared to relatives and healthy controls. MPO enzyme activity also tended to be higher in scz patients. Plasma total and native thiol levels were highest in the control group, relatively lower in relatives' group but still higher than scz patients as that group has the lowest levels. In conclusion, data of patients' higher marker levels contribute and support the existing literature but the key finding that higher levels of oxidative markers in relatives' blood compared to control group, puts a question to the genetical background of disease pathophysiology. Being


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first to examine the relatives' oxidative status by measuring multiple parameters, our work fills the gap in the field and also suggests patients' relatives' involvement in further studies which would give essential hints to elicit the etiology of scz.

## P-01-029
## Nitro derivatives of purines (azoloazines) as potential inhibitors of phosphodiesterases

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Acute respiratory viral infections (ARVIs) are a group of clinically and morphologically similar acute inflammatory diseases of the respiratory system. One of the regulators of airway inflammation is phosphodiesterase 4 (PDE4). Inhibition of PDE4 leads to reduced inflammation in upper respiratory tract tissues, and antiviral therapies may benefit from strategies involving modulation of PDE4. In this study, we showed that the expression level of PDE4 increased 10-fold in the lungs of mice in response to influenza A infection. We hypothesize that triazavirin (sodium salt of 2-methylthio-6-nitro-1,2,4-triazolo [5,1-c]-1,2,4-triazin-7 (4H)-one) and its analogs, being synthetic analogs of the nitrogenous bases of adenine and guanine, may have inhibitory activity in relation to cAMP and cGMP specific phosphodiesterases. The ability of triazavirin to inhibit phosphodiesterase was tested on phosphodiesterase 1 (PDE1) using the PDE Activity Assay Kit (Abcam, ab139460). Several azoloazine derivatives showed the ability to inhibit PDE1. The concentration of azoloazines at which a 50% inhibition of PDE1 activity was observed was 100 μM. This concentration is comparable to the inhibitory features of a standard non-competitive inhibitor of phosphodiesterase, namely 3-isobutyl-1-methylxanthine (IBMX). We next considered that our compounds may also have inhibitory activity with PDE4, a regulator of upper respiratory tract inflammation. For confirmation, molecular modeling was performed by docking (MolSoft ICM); this showed that the most likely binding site for PDE4 is in the region of the active center. The interaction with PDE4 of both triazavirin itself and its derivatives according to the docking results is due to the formation of coordination bonds with ions located in the active center. In summary, inhibition of PDE1 by azoloazines has been shown. According to the results of molecular docking, we assume that azoloazines likely have inhibitory activity on PDE4.

## P-01-030
## Promoter acetylation of proinflammatory cytokines correlates with PARP-1 absence in immunotolerant cells

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Immunotolerance is induced in myeloid cells during their contact with a high concentration of bacterial endotoxin (Lipopolysaccharide – LPS). That phenomenon is associated with deficiency of cytokine (e.g. TNFα) production, what in consequence impairs the functioning of the immune system. Previous reports documented the role of PARP-1 protein in the regulation of gene expression during the proinflammatory response. Since our preliminary data suggested that the presence of PARP-1 on the chromatin inhibits the development of immunotolerance in macrophages we searched for the molecular mechanism that underlies this interdependence. High dose of LPS that induced macrophage tolerance was associated with strong acetylation of H3K27, EP300 recruitment to TNFα promoter and PARP-1 eviction. The latter event was responsible for bacterial endotoxin tolerance as evidenced by the role of PARP1 protein, as an important component of the immune response. Inhibition of histone acetylase kept PARP-1 associated with chromatin and prevented enzyme dissociation in response to LPS. Analysis of mRNA and protein level also confirmed that combination of PARP1 and EP300 inhibitors can protect cells from immunotolerance development after cell exposure to lipopolysaccharide. In conclusion, strong acetylation of TNFα promoter results in PARP-1 dissociation during LPS treatment, what can be one of the principal pathways of immunotolerance. For this reason, histone acetyltransferase and PARP-1 inhibition are essential to PARP-1 maintenance on chromatin in TNFα promoter, thereby causing lack of immunotolerance. Acknowledgments: A.R. is supported by the Polish National Science Centre grant no. DEC-2013/11/D/NZ2/00033. *The authors marked with an asterisk equally contributed to the work.

## P-01-031
## Some aspects of the reaction of pro- and antioxidant components of blood of patients under abdominal surgery

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Impairment of antioxidant system under intra-abdominal emergency surgery is associated with risk of systemic inflammatory response syndrome (SIRS). Our study was aimed at evaluation of plasma MPO level (neutrophils activation biomarker) and antioxidant blood components (plasma thiols and erythrocyte glutathione peroxidase (GP1) activity) before and after abdominal surgery as potential predictors of SIRS. The patients were divided in 3 groups: (1) elective surgery for hernia or gallstone disease, n = 6; (2) surgery for uncomplicated acute abdominal pathology, n = 7; (3) emergency surgery for acute abdominal pathology complicated by generalized peritonitis, n = 4. Plasma and erythrocytes were collected before, 2 h and 48 h after surgery. MPO was analyzed by ELISA; thiols and GP1 activity by spectrophotometric methods and normalized by protein or hemoglobin concentration. Results. GP1 activity showed no difference between groups and no noticeable reaction to surgery. The groups did not differ in MPO level before surgery. After 2 hours it was lower in group 3 than in group 1 (96 ± 65 ng/ml vs 424 ± 189 ng/ml, $P<0.05$). In 2 h MPO varied from 40% to 200% respective to values before operation (groups 1 and 2) and in group 3 it changed 3-7-fold (18-780%). In each time-point for nearly half of patients MPO increased. In 48 h MPO level varied from 60 to 189% (group 1) and from 20 to 400% (groups 2 and 3). Before operation plasma thiols level in group 3 was lower ($2.0 \pm 0.5 \times 10^{-6}$ mole/g) than in groups 1 and 2 ($3.6 \pm 0.8 \times 10^{-6}$ mole/g). After operation only in group 3 thiol values increased by 10–50% except patient with severe multiorgan failure ($0.9 \times 10^{-6}$ mole/g). Conclusion. Low concentration of MPO in


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blood plasma accompanied by significant (3-fold and more) change in 2 hours after surgery and low plasma thiols level were associated with SIRS in patients under intra-abdominal surgery. This study was supported by the Russian Science Foundation grant No. 17-75-30064.

## P-01-032
## Role of ROCK1 kinase in postinflammatory fibrosis in experimental autoimmune myocarditis (EAM) mouse model

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Heart-specific inflammation (myocarditis) – is a common cause of pathological fibrogenesis which result in ventricles stiffening and heart failure and involves heart-infiltrating inflammatory monocyte-like cells constituting major cellular source of fibrosis in mouse model of experimental autoimmune myocarditis (EAM). TGF-β and its downstream effector – ROCK1 kinase contribute to cardiac fibrosis in various heart disorders but their role in inflammatory diseases remains elusive. The aim was to investigate role of ROCK1 kinase in development of myocarditis and postinflammatory fibrosis in EAM. EAM was induced in BALB/c mice by immunization with alpha myosin heavy chain (αMyHC) and Complete Freund's Adjuvant (CFA). We characterized inflammatory myeloid cells in reporter mice expressing GFP under collagen type I promoter (Coll-GFP) using flow cytometry. In acute phase of EAM, we observed an increased number of inflammatory cells in the heart and identified a subset of inflammatory CD45 + CD11b+ cells actively producing type I collagen. Isolated inflammatory cells were treated with TGF-β and analyzed by immunocytochemistry and quantitative RT-PCR. In response to TGF-β treatment these cells differentiated into myofibroblasts with high level of α-smooth muscle actin (αSMA). ROCK1 + /- cells showed impaired up-regulation of *Acta2, Postn* and *Nox4* genes in response to TGF-β. In the EAM model, ROCK1 + /- and wild-type mice showed, however, a comparable degree of heart inflammation at acute phase (d21) and fibrotic area at chronic stage (d40). Heart inflammatory CD11b+ cells seem to represent an important source of type I collagen in EAM model. ROCK1 plays an important role in TGF-β-mediated pathways, but it seems to be redundant in mouse model of EAM. Financed by the National Science Centre (Poland), grant 2014/14/E/NZ5/00175.

## P-01-033
## The role of miRNA in type I IFN signature in monocytes of patients with systemic sclerosis

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Systemic sclerosis (SSc) is an autoimmune rheumatic disease. One of the cytokines strongly contributing to pathogenesis of SSc is type I interferon (IFN), which is mostly produced by plasmacytoid dendritic cells and monocytes. It has been shown that abnormalities in miRNA expression are related to excessive IFN production; however, miRNAs have never been fully analysed in monocytes population in SSc. The aim of this study was to explore global miRNA expression and transcriptomic profiling of monocytes from SSc patients in order to predict which aberrantly expressed miRNA can negatively modulate IFN regulated genes (IRGs). Total RNAs from monocytes of 10 SSc and 10 healthy controls (HC) were isolated. Next generation sequencing (NGS) of RNA and miRNA samples were performed simultaneously on HC and SSc monocytes. Hierarchical clustering was implemented in order to select upregulated IRGs and downregulated miRNAs which are predicted to be the putative targets of IRGs. Following computational analysis, selected miRNAs-mRNA candidates were validated using qPCR and correlated with clinical parameters. Our results from NGS and qPCR analysis confirmed aberrant IFN score (based on significantly increased IFIT1, IFIT3, IRF7, Siglec1, IFI44, IFI44L) in SSc patients. Notable, localised SSc have 4.3-time higher ($P = 0.03$) IFN score than diffused SSc. Based on NGS analysis, we have selected 7 miRNA which were further validated using qPCR. Only the expression patters of miR-26a-2-3 and miR-485-3p were confirmed both by NGS and qPCR studies. The expression level of miR-26a-2-3 negatively correlated with modified Rodnan skin score ($P = 0.01$, $r = -0.78$) and miR-26a-2-3 will be further validated in functional assays inhibiting IRGs. In conclusion, our results identify new miR-26a-2-3 candidate which is predicated to negatively regulate IRGs. Thus, miR-26a-2-3 may be involved in altered IFN signature of SSc monocytes. Supported by 2015/16/S/NZ6/00041 from National Science Centre, Poland.

## P-01-034
## Temporal profile of lymphocytes in patients during sepsis and in the course of one year after hospital discharge

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Sepsis is caused by a dysregulated systemic immune response to infectious agent that can lead to death. However, there are also high mortality rates of post-septic patients in the years following the disease. The aim of this work is to evaluate the lymphocyte profile in patients with sepsis and in different periods after hospital discharge. Initially, 62 individuals from the University of São Paulo University Hospital who developed sepsis or septic shock were evaluated. Of these, there were 19 deaths prior to discharge from the Intensive Care Unit, 12 drop-outs and 10 patients were excluded from the study because they did not fit the prerequisites. In this way, 19 patients were followed up after hospital discharge (Phase A), after discharge from intensive care unit (Phase B), 3 months (Phase C) and 6 months (Phase D) after. The lymphocytes were isolated and the percentages of regulatory T cells (Treg), Th1, Th2 and Th17 were evaluated by flow cytometry. In the analysis of total leukocytes, we observed that there was a decrease in absolute number of cells in Phases B, C and D when compared to Phase A. However, for total lymphocytes, there was an increase in the number of cells in Phases C and D when compared to Phase A. When evaluating the different cell profiles, the results demonstrated that the percentages of Th1 and Th17 lymphocytes were higher in Phase A when compared to other Phases, but for Th2 cells there was a decrease in the percentage of cells only in Phase C compared to Phase A. For Treg, we observed a decrease in phase C compared to phases A and B. This study will can gain a better understanding of the impacts caused by sepsis to the body and particularly to the control of the immune system. In fact, our results indicate that a better prognosis depends of the increase of Treg cells, since the patients in the study that survived presented this increase, which then decreased in order to reach homeostasis again.


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## P-01-035
## C8J1298, thiol oxidoreductase of *Campylobacter jejuni*, affects Dsb (disulfide bond) network functioning

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The oxidation reaction between two cysteine thiol groups leads to the formation of a disulfide bond. The disulfide bond generation is a rate-limiting step in the folding process of a protein. Bacterial proteins of the Dsb (disulfide bond) system catalyze this process. Generally, these proteins operate in two pathways based on the oxidation (DsbA and DsbB) and the reduction/isomerization (DsbC and DsbD) reactions. *C. jejuni* 81116 oxidative Dsb pathway is composed of four enzymes, of which two (CjDsbA1 and/or CjDsbA2) are localized in the periplasm, and two others (CjDsbB and CjDsbI) are localized in the inner membrane. *In silico* analysis has led to identification the potential homologs of EcDsbC and EcDsbD, enzymes potentially operating in isomerization pathway responsible for the rearranging of nonnative disulfide bonds. The periplasmic dimeric thiol oxidoreductase C8J1298 (putative isomerase) was the object of presented research. Our research indicates that *c8j1298*, which expression is controlled by stress envelope CprRS system, plays a key role in the regulation of the *C. jejuni* network. The C8J1298 overproduction results in the lack of CjDsbA1 and CjDsbA2 (probably major oxidoreductases) in the cell proteome. As CjDsbA1 plays a role in the cell motility and CjDsbA2 is involved in oxidative folding of AstA, we examined these two features of analyzed strain. The analyzed strain displays wild type phenotype. Real-time PCR experiments should indicate the level of *cjdsbA1* and *cjdsbA2* genes transcription in strain harboring recombinant plasmid containing *c8j1298* in comparison to wt strain. Because C8J1298 is present in wt cells in both, reduced and oxidized forms, the obtained results imply that under certain conditions it may undertake the function of CjDsbA1 and CjDsbA2. The work was supported by the National Science Centre (grant no. 2015/17/B/NZ1/00230). *The authors marked with an asterisk equally contributed to the work.

## P-01-036
## Study of the effect of succulent extracts on hypoxia-induced inflammation, proliferation, and energy metabolism

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Hypoxia, a condition in which cells are deprived of adequate oxygen supply, is known to cause apoptosis, inflammation, and altered metabolism, in turn leading to tissue damage. Upon hypoxia, cells induce prolyl-hydroxylase (PHD) and hypoxia-inducible factor (HIF) signaling pathway, consequently inhibiting cell proliferation and aerobic energy metabolisms such as aerobic glycolysis and fatty acid oxidation (FAO), and PHD works by hydroxylating the HIF. Moreover, PHD which hydroxylates HIF has IκB inhibitory activity, and in turn induces the activation of NFκB, consequently resulting in inflammation. Succulent plants are used to reduce or eliminate inflammation. In this study, we therefore investigated the effect of succulent plant extracts on hypoxia-induced inflammation and cell death, particularly focusing on the HIF-PHD signaling pathway. We induced the hypoxia in vitro by using cobalt chloride, a chemical inhibitor of PHD and confirmed up regulation of HIF expression, decreased expression of genes involved in glycolysis and FAO, and reduced cell proliferation. Upon treatment with succulent extract, HIF-PHD signaling pathway was found to be significantly stabilized to a normal level, and furthermore, glycolysis and FAO were also elevated compared with vehicle. In parallel, succulent extract increased the expression of anti-inflammatory cytokine IL-10 and inhibited proinflammatory cytokines such as TNF and IL-6. In conclusion, succulent extract can be used for anti-inflammatory therapeutic products via controlling the HIF-PHD molecular pathway. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01323005)" Rural Development Administration, Republic of Korea.

## P-01-037
## Effect of recreational dance practice on lymphocyte profile and function in diabetic women

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During the development of type 2 diabetes mellitus (T2DM), there is a systemic inflammatory state with elevation of Th1 and Th17 and a decline in T-regulatory (Treg) cell function. Although it has been observed that physical exercise promotes immune response reestablishment, there is currently no available data about the effects of physical exercise on the lymphocyte response profile in women with T2DM. Thus, the aim of this study was to investigate the effects of a recreational dance program on the lymphocyte differentiation process in women with T2DM. In the end of the protocol 11 women with T2DM (67.2 ± 7.7 years) and 12 healthy patients were evaluated (63.2 ± 7.2 years). All volunteers participated in a recreational dancing protocol for 16 weeks. Anthropometric and body composition measurements were taken and blood samples were collected. The levels of C-reactive protein (PCR), lipids, and glucose were evaluated in the participant's plasma. Expression of CD25 and CD28, percentage of Treg cells, proliferative capacity, and concentration of cytokines in the culture supernatant were assessed by flow cytometry of peripheral lymphocytes. The T2DM group had a lower percentage of CD28 + lymphocytes, a higher percentage of Treg cells, and a higher lymphocyte proliferative capacity at baseline as compared with the control group. However, after the dance program, there was no longer any difference between the groups. We also did not find any differences between the groups at any time point in regard to the secretion of CD25. The practice of recreational dancing promoted an increase of IL-10 in both groups; a decrease of IL-4, IL-2, and IL-6 secretion in only the control group. IL-17 and IL-10/IL-17 ratio increased only in the T2DM group. In conclusion, the practice of recreational dancing promoted an increase in important markers of lymphocyte regulation in the T2DM group. However, these effects were more pronounced in the healthy control group. *The authors marked with an asterisk equally contributed to the work.


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## P-01-038
## Effects of *Momordica charantia* extract on proliferation, oxidative stress, and apoptosis of C6 glioma cells

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Inflammation is a key part of the immune response to injury and infection caused by various pathogens in terms of a defense mechanism. However, if the inflammatory response lasts for too long, it can cause pathological legions. *Momordica charantia* is an annual or perennial climber that has been used for the treating of inflammation and cancers, and thus has been paid attention to utilize as a variety of pharmaceutical auxiliary substances. In this study, we therefore examined the effect of *Momordica charantia* on C6 glioma cells particularly focusing on anti-tumor activity. *Momordica charantia* extract was prepared by using a high-pressure and high-temperature method. Cells were treated with serially diluted extract for 12 h, followed by measurement of cell counts, cell cycle, and gene expression. As a result, *Momordica charantia* increased the activity of antioxidant enzymes along with decreased reactive oxygen species in C6 glioma cells compared with vehicle. In addition, treatment of *Momordica charantia* extracts inhibited cell proliferation via enhanced apoptosis, and cell cycle arrest. In parallel, the expression of p53, p21, Bax, matrix metalloproteinases were found to be inhibited by the treating of C6 glioma cells with *Momordica charantia* extract. In conclusion, *Momordica charantia* extract has a potential anti-cancer therapeutic agent by regulating cell death and cell cycle, although further studies should be conducted to identify a single compound derived from *Momordica charantia*. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01323005)" Rural Development Administration, Republic of Korea.

## P-01-039
## Endocan and cardiac biomarkers predict prognosis in cirrhotic patients with chronic HCV infection

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Background/Aims: Hepatitis C virus (HCV) infection is frequently associated with myocarditis and cardiomyopathy. The functional and structural cardiac abnormalities are present in the majority of HCV patients with cirrhosis. Cardiac biomarkers such as endocan (novel cardiac marker), high-sensitivity troponin T (hs-TnT), and pro-brain natriuretic peptide (NT-proBNP), and pro-inflammatory biomarkers including tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and high-sensitive C-reactive protein (hs-CRP) are related to the cardiac complications. We aimed to investigate if endocan and pro-inflammatory biomarkers are related to cardiac function and long-term survival in cirrhotic patients. Methods: A total of 84 stable cirrhotic patients (Child class: A = 28; B = 26; C = 30) were included in the study. Baseline laboratory tests, ultrasound and endocan, vascular endothelial growth factor (VEGF), TNF-α, IL-6, and hs-CRP, NT-proBNP, and hs-TnT measurements were performed. Results: Endocan, VEGF, hs-CRP, NT-proBNP, and hs-TnT were significantly different throughout the Child classes ($P < 0.01$; $P < 0.001$; $P < 0.05$; $P < 0.001$). All biomarkers except IL-6 correlated with indicators of disease severity in cirrhosis; endocan, VEGF, TNF-α correlated with hs-TnT ($r = 0.32$ $P < 0.01$; $r = 0.28$ $P < 0.01$, $r = 0.42$ $P < 0.001$, respectively) and NT-proBNP ($r = 0.30$, $r = 0.32$ and $r = 0.43$; $P < 0.01$). Endocan ($P < 0.01$), cardiac (NT-proBNP, hs-TnT; $P < 0.01$) and pro-inflammatory (TNF-α, hs-CRP; $P < 0.05$) markers were associated with mortality in a univariate Cox analysis, however, the strongest predictors of mortality in a multivariate Cox analysis were hs-TnT and ascites. Conclusion: The simultaneous monitoring of serum endocan and hs-TnT, and/or NT-proBNP can be helpful for the alterations in myocardial function control in cirrhotic patients. Endocan is a promising prognostic marker in cirrhotic patients with chronic HCV infection. *The authors marked with an asterisk equally contributed to the work.

## P-01-040
## UVB promotes the initiation of uveitic inflammatory and changes in the hydration of the cornea in vivo

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Recently, active research has been conducted in the field of terahertz (THz) scanning of human tissues for non-invasive determination of their hydration level, which have shown high diagnostic efficiency of this technology in various pathological conditions. Recently, we have developed a laboratory model of the facility for monitoring the state of the water balance of the cornea using THz scanning in vivo, which opens up the possibility of applying this approach in ophthalmology. The aim of the work was to compare the results of the THz scan of the cornea with its clinical changes using the example of an experimental model of the UV-induced keratouveitis. An experimental study, which included a comprehensive assessment of clinical changes in the cornea of rabbits during keratouveitis induction, revealed a decrease in the stability of the tear film, pathological changes in the corneal epithelium and stroma, as well as its anatomical and optical parameters. Comparison of data obtained in the THz scan of the cornea with tears production, optical coherence tomography and confocal microscopy showed their consistency in all observation periods, which allows us to conclude that the developed laboratory setup works and the feasibility of further research to promote the corneal hydration evaluation technology in clinical practice. Acknowledgements: Research was funded by the RSF, grant number 16-15-00255. *The authors marked with an asterisk equally contributed to the work.


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## P-01-041
## Inefficient repair of oxidative DNA damage in rheumatoid arthritis – a correlation with the key BER genes expression

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Rheumatoid arthritis (RA) is a systemic, inflammatory disease of the joints and surrounding tissues. RA manifests itself with severe joint pain, articular inflammation and oxidative stress. RA is associated with certain types of cancer. We have assumed that increased susceptibility to cancer of RA patients may be linked with genomic instability induced by disturbed DNA repair and sensitivity to oxidative DNA damaging agents. One of the main pathways involved in repairing oxidative DNA damage is base excision repair (BER). We analyzed the sensitivity and repair efficiency of peripheral blood mononuclear cells (PBMC) isolated from RA patients and healthy individuals to DNA damaging agent tert-butyl hydroperoxide (TBH) by using the alkaline version of comet assay. The data from DNA damage and repair study was correlated with profiles of the expression of 19 genes related with BER pathway. The genes expression was determined by Prime Time qPCR Assay. The levels of oxidative DNA damage induced by tert-butyl hydroperoxide (RA-21.6 vs 10.9 in control) was statistically higher in RA patients than in healthy subjects. We observed inefficient DNA repair (ROC area curve 0.7872; $P < 0.001$) in RA patients as compare to control. In RA PBMC cells we also notice impaired production of sufficient transcripts of key protein involved in the BER pathway. Therefore our results may suggest that disturbed expression of DNA repair proteins is responsible for inefficient DNA repair in RA. Acknowledgement: This work was supported by the National Science Center (Poland) – UMO-2017/25/B/NZ6/01358.

## P-01-042
## Impaired function of transcription factor Ets-2 results in the creation of pathological T helper (Th) cells in multiple sclerosis (MS)

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In MS, pathogenic Th cells, mainly Th1/Th17, recognize myelin antigens and contribute to the damage to the central nervous system. An important unresolved issue of MS pathogenesis is at which stage of Th cell differentiation errors occur, at the molecular level, which result in the development of autoreactive Th cells. We previously showed that in healthy individuals the IL-2 gene is repressed in naive Th cells by the transcription factor Ets-2 that binds to the ARRE-2 element of the proximal IL2 promoter, pointing to Ets-2 as a crucial factor influencing early events of Th cell differentiation. Our results from Th cells derived from 10 patients with remitting-relapsing MS and 10 age/sex-matched healthy controls, showed significantly reduced mRNA and protein synthesis of Ets-2 in naive Th cells from MS patients, lack of Ets-2 binding to the ARRE-2 of the IL2 promoter in vitro and in vivo and significantly higher constitutive expression levels of cytokines in patients' Th cells (IL2 and IL17A naive and IFN-γ and TNFα memory Th cells). Also, in patients' Tregs Foxp3 mRNA/protein levels were significantly reduced whereas the expressions of Ets-2, IL17 and IFN-γ were significantly increased. Ets-2 expression levels were also assayed in in vitro polarized Th cells isolated from lymph nodes and spleens of naive C57BL/6 mice, in Th cells from wild-type C57BL/6 mice susceptible to experimental autoimmune encephalomyelitis (EAE) and from EAE-resistant Spp-1-/-C57BL/6 mice. Our results showed increased Ets-2 relative expression in non-differentiated Th cells compared to in vitro differentiated Th cells. In addition, Th cells isolated from draining lymph nodes of EAE-resistant mice showed a 3-fold elevated expression of Ets-2 compared to EAE-susceptible mice. We suggest that in MS low-level synthesis and dysfunction of Ets-2 in Th cells are responsible for downstream aberrant Th cell differentiation resulting in the creation of pathological Th cells.

## P-01-043
## GC-rich cell-free DNA regulates TLR9-signaling cascade in rheumatoid arthritis patients

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Cell-free DNA (cfDNA) can accumulate in circulation during inflammatory processes in the human body. In chronic pathology, both concentration and composition of cfDNA may change. We have shown that an exacerbation of rheumatoid arthritis is associated with 6-7-fold increase in the number of GC-rich sequences (mainly ribosomal DNA repeats (rDNA)) in cfDNA compared with healthy controls. Computer analysis showed the presence of TLR9 binding sites in GC-rich sequences. The aim of the study was to investigate the effects of model rDNA fragments on the transcriptional activity of the *TLR9* and *MYD88* genes in the peripheral blood lymphocytes of healthy donors and patients with rheumatoid arthritis. Lymphocytes were isolated from peripheral blood by gradient centrifugation. The level of gene expression was determined by real-time PCR. Flow cytometry analysis showed 2-2.5-fold increase in expression of TLR9 in lymphocytes of patients with rheumatoid arthritis compared with healthy controls. Accordingly, an analysis of mRNA expression of the *TLR9* and *MYD88* genes (the latter coding for the adapter protein in TLR9-signaling cascade) in lymphocytes from patients with rheumatoid arthritis revealed an increase in transcriptional activity of the studied genes by 3–4 times ($P < 0.001$) compared with controls. A 3-hour incubation of healthy donor's lymphocytes with model rDNA fragments at a concentration of 50–100 ng/ml caused an increase in the expression level of the *TLR9* and *MYD88* genes by a factor of 3–4 ($P < 0.001$) compared with intact lymphocytes. In lymphocytes of the patients, model rDNA fragments at a concentration of 50–100 ng/ml caused a decrease in the expression level of the *TLR9* and *MYD88* genes by 2–2.5 times ($P < 0.01$) compared with intact cells. In conclusion, the results suggest that the modulating effect of rDNA fragments on the TLR9 pathway may depend on the initial level of the *TLR9* and *MYD88* genes expression in target cells. *The authors marked with an asterisk equally contributed to the work.


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## P-01-044

## Determination diagnostic and prognostic value of soluble urokinase type plasminogen activator receptor (SuPAR) and serum amyloid-A Levels in familial Mediterranean fever with MEFV gene mutation

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Familial Mediterranean Fever (FMF); non-infectious fever, characterized by predominantly neutrophil inflammation attacks and can cause the formation of amyloidosis. Soluble urokinase plasminogen activator receptor (SuPAR) has been identified as a biological marker of inflammation and immunological activation. The increase of SuPAR concentrations was associated with disease severity and mortality in various patient populations. Although the levels of SuPAR have been investigated in many rheumatological diseases, no studies on SuPAR levels have been found in the FMF disease. It is planned to investigate SuPAR's and other inflammatory markers' [Erythrocyte Sedimentation Rate (ESR), C-reactive peptide (CRP), Serum Amyloid A (SAA)] predictive value of diagnosis, activity, and prognosis in FMF patients who have homozygous or heterozygous mutations in different exons of the MEFV genome. This study was performed in İzmir Ataturk Education and Research Hospital in between 22.12.2016–22.06.2017 with 53 FMF patients and 27 healthy volunteers. SuPAR molecule was measured by enzyme linked immunoassay (ELISA) method. SAA levels in serum samples were measured by nephelometric method, CRP levels were measured by turbidimetric / immunoturbidimetric method, ESR was measured by Westergren method. SuPAR levels of patients were higher than control group, but this was not significant ($P = 0.178$). SAA, CRP, ESR levels were significantly higher in FMF patients than healthy subjects ($P < 0.001$). There was no significant correlation between SuPAR levels and ESR, CRP, SAA in study group ($P > 0.05$). However, there was a significant positive correlation between SAA and CRP and ESR in study group (rho = 0.823, $P < 0.001$, rho = 0.568, $P < 0.001$, respectively). There is insufficient correlation between SuPAR and SAA which is considered to be the best indicator of subclinical inflammation. According to our data, SuPAR could not be a good marker for subclinical inflammation.

## P-01-045

## The molecular basis for specific and cross-reactive T cell responses in celiac disease

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Celiac disease (CeD) is considered an autoimmune-like HLA-DQ2.5/8-associated T cell-mediated inflammatory disorder stimulated by ingestion of gluten derived from wheat, rye, barley and oats. We investigated T cell responses towards highly homologous and immunodominant gluten epitopes, DQ2.5-glia-a1a (PFPQPELPY) and DQ2.5-glia-w1 (PFPQPEQPF) presented by HLA-DQ2.5 molecules by determining a representative nbsp; T cell antigen receptor (TCR) repertoire from CD4 + T cells derived from CeD patients. The majority of patient-derived T cell clones (TCCs) were specific for either of the two epitopes with limited cross-reactivity. We determined the binary crystal structures of HLA-DQ2.5-glia-a1a and HLA-DQ2.5-glia-w1. Alanine scanning mutagenesis and cell stimulation assays on isolated TCCs highlight the p7 residue as critical for antigen recognition by epitope specific T cells and perturb the surrounding HLA-DQ2 substructure significantly enough to enable discriminatory recognition. The binary structures displayed highly similar pMHC landscape with slight differences in electrostatic potential surrounding the p7 residue. To investigate the basis for cross-reactivity, hypothesised to amplify the CeD inflammatory response, we determined the DQ2.5-glia-a1a/DQ2.5-glia-w1 cross-reactive L6 TCR structure, conducted surface plasmon resonance (SPR) and cell stimulation assays. The L6 TCR was cross-reactive shown by SPR and cell stimulation assays of L6 TCR-transduced SKW3 T cells towards HLA-DQ2-glia-a1a and DQ2-glia-w1. The CDR3 loops of L6 are highly mobile as flexible structural components cannot be resolved clearly in refined structures. This study highlights subtle differences between highly homologous gluten epitopes largely govern the non-cross-reactive TCR repertoire. These remarkable small variations in peptide-MHCII architecture were shown to be tolerated by cross-reactive T cells. Discriminatory and cross-reactive T cell responses may contribute to overall CeD pathogenesis. *The authors marked with an asterisk equally contributed to the work.

## P-01-046

## Monoacetyldiacylglycerol attenuates STZ-induced DAMP release and chemokine expression

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Streptozotocin (STZ) specifically acts on pancreatic beta cells and induces cell destruction. It is known that STZ increases the intracellular reactive oxygen species (ROS) generation to induce oxidative stress, and consequently causes cell apoptosis. In this study, we found the necroptotic cell death caused by STZ treatment in rat pancreatic beta cell line, INS-1. One of the damage-associated molecular pattern (DAMP) molecules, high mobility group box 1 (HMGB1) was released by STZ. HMGB1 is an intracellular DNA-binding protein which can be released and initiate inflammatory response. Phosphorylation of receptor-interacting protein kinase 3 (RIPK3) mediating the necroptosis was also observed in STZ-treated cells. In addition, the expression of cytokine-induced neutrophil chemoattractant 1 and 3 (CINC1 and CINC3) was increased after STZ treatment. CINCs are major neutrophil chemotactic factors in rat tissues, which induce the neutrophil recruitment into inflammatory sites. We investigated the biological activity of monoacetyldiacylglycerol (MADG) on STZ-induced cell damage. Our data showed that MADG reduced HMGB1 release in STZ-treated cells. Results from western blot analysis revealed that RIPK3 phosphorylation was decreased in MADG treatment as compared with in STZ alone. MADG also significantly alleviates the increase of CINCs expression. These findings suggest that MADG may attenuate the STZ-induced necroptotic pancreatic cell death.


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## P-01-047

## Anti-inflammatory effects of new compounds derived from *Portulaca oleracea* on inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the colon and rectum. Even though development of therapeutic agent for IBD, many patients experience side effects such as nausea, vomiting, abdominal discomfort, diarrhea, headache, pancreatitis, agranulocytosis, and pulmonary toxicity. This being so, the development of herbal medicines that fewer side effects is an important goal. *Portulaca oleracea L.* (PL) has been widely used as a traditional medicinal herb for the treatment of various diseases, such as diarrhea, skin inflammation and bloody stools. Although a variety of studies have been reported on PL, few studies have been conducted to investigate compounds derived from PL role in IBD. This study investigated the protective effects of compounds on epithelial barrier integrity by TJ protein expression in Caco-2 cells. Compounds inhibited decreased expression of the TJ proteins such as Claudin-1, zonula occludens-1 and occludin. These results suggest that compounds protect the epithelial barrier of Caco-2 cells. In addition, compounds inhibited the production of inflammatory mediators such as NO, iNOS, COX-2, MCP-1 and pro-inflammatory cytokine in LPS-induced J774A.1 cells. And study investigated the therapeutic effects of IBD in DSS-induced mouse. Treatment with compounds significantly ameliorated symptoms of DSS-induced IBD, including body weight loss, colon length shortening, disease activity index (DAI) increase, and histological changes. In addition, compounds inhibited the production of inflammatory cytokines as interleukin (IL)-6, IL-1β, IL-17 and tumor necrosis factor (TNF)-α in the colon of DSS-treated mouse. These results suggest that compounds can efficiently ameliorate the clinical signs and inflammatory mediators of IBD. Compounds are potentially of using natural medicine for treating IBD. *The authors marked with an asterisk equally contributed to the work.

## P-01-048

## Thrombin stimulates C/EBPβ activation and IL-8/CXCL8 expression in human lung epithelial cells through a STOML2-dependent JNK/RSK1 pathway

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The World Health Organization estimates approximately that 300 million persons worldwide suffered from asthma, and calculates 250,000 people die each year. The usual characteristics of asthma are airway remodeling and chronic airway inflammation. In addition, thrombin and IL-8/CXCL8 play critical roles in airway inflammation. Recently, stomatin-like protein 2 (STOML2), a mitochondrial protein, has been a putative novel asthma risk gene. In this study, we investigate whether STOML2 is involved in thrombin-induced IL-8/CXCL8 expression and release in human lung epithelial cells. Thrombin-stimulated IL-8/CXCL8 release was abolished by small interfering RNA of STOML2 (STOML2 siRNA) in human lung epithelial cells (A549 and BEAS-2B). Treatment of cells with STOML2 siRNA markedly reduced STOML2 protein expression in A549 and BEAS-2B cells. Moreover, thrombin-induced IL-8/CXCL8-luciferase activity was also inhibited by STOML2 siRNA in A549 cells. Thrombin-induced JNK phosphorylation, but not ERK phosphorylation, was reduced by STOML2 siRNA. Treatment of cells with STOML2 siRNA reduced thrombin-produced C/EBPβ Ser105 phosphorylation and C/EBPβ-luciferase activity. Stimulation of cells with thrombin caused RSK1 Ser381 phosphorylation, which was reduced by SP600125 (a JNK inhibitor) and STOML2 siRNA. Thrombin caused C/EBPβ translocation into nucleus, which was inhibited by STOML2 siRNA. Taken together, these results suggest that the STOML2-dependent JNK and RSK1 signaling pathway play an important role in thrombin-induced C/EBPβ activation and IL-8/CXCL8 expression and release in human lung epithelial cells.

## P-01-049

## Involvement of doublecortin and CaM kinase-like 1 protein in TGF-β-induced connective tissue growth factor and extracellular matrix protein expression in normal human lung fibroblasts

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Airway fibrosis is a prominent feature in patients with chronic obstructive asthma (COA). Mounting evidence showed that transforming growth factor β (TGF-β) played a vital role in severe asthma and correlated with the degree of subepithelial fibrosis, a result of excessive deposition of extracellular matrix (ECM), in airway. Previous study demonstrated that ADAM metallopeptidase domain 17 (ADAM17) was involved in many physiological events including fibrosis. Previously, our study showed that ADAM17/EGFR-dependent ERK activation participates in thrombin-induced connective tissue growth factor (CTGF) expression in human lung fibroblasts. Since previous study reported that doublecortin and CaM kinase-like 1 protein (DCAMKL1) was involved in tissue fibrosis, the aim of this study was to investigate the involvement of DCAMKL1 in TGF-β-induced CTGF and ECM protein expression, including α-smooth muscle actin (α-SMA), collagen I, and fibronectin in normal human lung fibroblasts (NHLFs). Our data showed that pretreatment with a DCAMKL1 inhibitor, LRRK2-IN-1, or knockdown of DCAMKL1 with the siRNA technique both abolished the TGF-β-induced CTGF expression in NHLFs. Moreover, we found that TGF-β-induced α-SMA, collagen I, and fibronectin expression were also abolished by transfection of NHLFs with DCAMKL1 siRNA. In addition, we discovered that treatment with TGF-β induced DCAMKL1 expression in time-dependent manner in NHLFs. However, DCAMKL1 siRNA did not affect TGF-β-induced ERK and c-Jun phosphorylation, suggesting that ERK and c-Jun are not the downstream molecule of the DCAMKL1 in TGF-β stimulation. Taken together, these data suggest that DCAMKL1 may participate in regulating TGF-β-induced CTGF, α-SMA, collagen I, and fibronectin expression in NHLFs.


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## P-01-050
## ADAM17/preadipocyte factor-1 participates in hypoxia-induced CTGF expression and airway fibrosis

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Airway fibrosis and hypoxia occur in patients with chronic obstructive asthma (COA). The overproduction of connective tissue growth factor (CTGF) and -smooth muscle actin (-SMA) contribute to airway fibrosis. Numerous studies demonstrated that hypoxia and A disintegrin and metalloproteinase 17 (ADAM17) play critical roles in tissue fibrosis. ADAM17 cleaves ectodomain of preadipocyte factor 1 (Pref-1) to 50 kDa soluble Pref-1. Soluble Pref-1 reacts with fibronectin via integrin α5β1 receptor to activate the MEK/ERK pathway that regulates adipocyte differentiation. Moreover, several reports revealed that overexpression of Pref-1 participated in lung cancer cell invasion, collagen expression, and liver fibrosis. However, whether Pref-1 is involved in hypoxia-induced CTGF expression and airway fibrosis remains unknown. In this study, we investigated the role of ADAM17/Pref-1 in hypoxia-induced CTGF expression in human lung fibroblasts and airway fibrosis. We found that hypoxia-induced CTGF expression was inhibited by small interfering RNA of Pref-1 (Pref-1 siRNA) in human lung fibroblast (WI-38). Hypoxia-induced Pref-1 protein expression was suppressed by curcumin (an AP-1 inhibitor). In an addition, Pref-1 stimulated CTGF expression in time-dependent manner. We also found that ATN-161, integrin inhibitor, abolish Pref-1-induced CTGF expression. Furthermore, hypoxia and Pref-1-induced α-SMA was inhibited by Pref-1 siRNA and ATN-161. Moreover, in animal model, OVA-induced prominent Pref-1 and airway fibrosis these effects were suppressed in OVA-induced ADAM17 conditional knockout mice. In conclusion, our results suggest that ADAM17/Pref-1 mediates hypoxia-induced CTGF expression and airway fibrosis.

## P-01-051
## Effect of LSD1 inhibition on DSS-induced colitis

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Inflammatory Bowel Disease (IBD) is caused by an acute or chronic dysfunction of the mucosal inflammatory system in the intestinal tract. In our previous study, we elucidated that PKCα-LSD1-NF-κB signaling is involved in the prolonged activation of inflammatory response to LPS. In line with this study, we aimed to investigate the effect of the signaling on colitis. To examine the role of LSD1 in the colonic inflammation, we established a dextran sulfate sodium (DSS)-induced colitis mouse model and compared *Lsd1*$^{+/+}$ and *Lsd1* S112A knock-in (*Lsd1*$^{SA/SA}$) mice harboring deficiency in phosphorylation by PKCα. *Lsd1*$^{SA/SA}$ mice showed less severe colitis symptoms (in terms of the loss of body weight and shortening of colon length) compared with *Lsd1*$^{+/+}$ mice. Histological analysis of colon sections exhibited relatively intact, at least less damaged, colon epithelial lining in *Lsd1*$^{SA/SA}$ mice whereas significant epithelium disruption in *Lsd1*$^{+/+}$ mice. The reduced expression of several pro-inflammatory cytokines and iNOS in *Lsd1*$^{SA/SA}$ mice compared with *Lsd1*$^{+/+}$ mice supported that the inhibition of PKCα-LSD1-NF-κB signaling can protect against colitis by reducing the expression of pro-inflammatory genes. Furthermore, the administration of LSD1 inhibitor alleviated colitis symptoms and reduced the expression of several pro-inflammatory cytokines. Together, the inhibition of PKCα-mediated phosphorylation of LSD1 or the inhibition of LSD1 activity might be a way to protect against the colonic inflammatory response. Thus, LSD1 inhibitor could be beneficial to the treatment of chronic inflammatory diseases including inflammatory bowel disease (IBD).

## P-01-052
## Monoacetyl diacylglycerol has a modulation effect on the monosodium urate-induced chemokine and HMGB1 release

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Monosodium urate (MSU) is an endogenous substance that causes gout, one of the arthritic diseases. MSU-treated cell secrets C-X-C Motif Chemokine Ligand 8 (CXCL8) and recruits neutrophil. In addition, MSU also induces necroptosis signaling and secretes damage-associated molecular patterns (DAMPs) such as high mobility group box 1 protein (HMGB1) in the cells, which leads to an additional inflammatory response. Reducing CXCL8 and DAMP expression will help alleviating gout syndrome via modulation of neutrophil recruitment. We examined the role of monoacetyl diacylglycerol (MADG) in MSU-induced CXCL8 and DAMP secretion. The level of expressed CXCL8 by MSU treatment was measured in THP-1 cells by enzyme-linked immunosorbent assay (ELISA) and reverse transcription–polymerase chain reaction (RT-PCR). In vitro migration assay was used to determine whether chemokines induced by MADG treatment has effects on the activity of neutrophil migration. Necroptosis signaling and DAMP release by MSU were examined by western blot analysis. The enhanced cell permeability was also confirmed by flow cytometry and lactate dehydrogenase (LDH) release assay. MADG had an effect on modulation of CXCL8 expression in the MSU treated THP-1 cells and alleviated neutrophil recruitment. In addition, MADG had activity on attenuation of the induced membrane permeability and DAMP release in the MSU treated cells through modulation of receptor-interacting serine/threonine-protein kinase 1/3 (RIPK 1/3) activity. Our data demonstrate that MADG modulate necroptosis signaling and CXCL8 expression in the MSU treated cells which enable to prevent excessive and successive neutrophil recruitment. This result suggests that MADG has a potential to be a novel therapeutic agent for the acute gouty inflammation.


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## P-01-053
## Mechanisms of ocular surface inflammation associated with iatrogenic damage to the cornea

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Iatrogenic corneal diseases can develop as a complication of ocular surgeries or interventions unrelated to visual system. Ultraviolet-induced corneal damage (UVCD) is a common consequence of photorefractive keratectomy, whereas perioperative dry eye syndrome (PDES) may be caused by prolonged general anesthesia. Importantly, these complications manifest as local inflammation, which being aggravated by infection or autoimmune/systemic factors, can give rise to keratitis or keratouveitis. The standard anti-inflammatory treatment of the cornea involves corticosteroids possessing a number of side effects. Thus, there is a high demand for effective non-hormonal therapy specifically targeting inflammatory mechanisms in each corneal disease. Here, using rabbit models of UVCD and PDES, we demonstrate that the scenarios of ocular inflammation indeed significantly differ in these diseases. UVCD is characterized by a rapid inflammatory response, as loss of corneal epithelium and apoptosis of keratocytes are accompanied by stromal edema, neutrophil infiltration, and exudation of the anterior chamber of the eye. In PDES, inflammation has a slower and less pronounced course: a loss of epithelium is supplemented by inflammatory signs only on the late stages of the disease. Moreover, the differences between inflammatory mechanisms can be seen from distinct patterns of eicosanoids in the tear fluid. In UVCD, there is a pronounced increase in 12-HETE and decrease in 5-HETE indicating the activation of 12-lipoxygenase and 5-lipoxygenase pathways. In PDES, there are less prominent alterations in 12-HETE and 5-HETE, but an increase in 13-HOTrE pointing on involvement of 15-lipoxygenase. Our findings suggest that specific targeting the revealed inflammatory enzymes might be regarded as a prospective powerful therapy of the iatrogenic damage to the cornea induced by general anesthesia and UV-radiation. This study is supported by the Russian Science Foundation (Project no. 16-15-00255).

## P-01-054
## Changes in lymphocytes redox balance and lipid metabolism in development of psoriasis

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Lymphocytes are the most important cells involved in the pathophysiology of psoriasis; therefore, the aim of this study was to assess the lymphocytes redox status and its relation to the lipid metabolism in the lymphocytes of patients with psoriasis vulgaris. The obtained results show in lymphocytes from patients with psoriasis an increased activity of pro-oxidative enzymes and an increased production of ROS above healthy control. These alterations were accompanied by a decreased efficiency of the phospholipid protection by antioxidants (decrease in CAT activity and lower levels of GSH, vitamin E and Nrf2 activation) observed in both groups of psoriatic patients. While the p21 and Nrf2 inhibitor-Keap1 were increased in both groups of psoriatic patients, Bach1 and Nrf2 activators KAP1 and p62 were overexpressed only in patients with psoriasis vulgaris. Lipidomic analysis indicated that prooxidative conditions accompanied to psoriasis led to lipid profile alteration namely decrease in PUFAs associated with an increase of isoprostanes and 4-HNE as were also increased protein modifications caused by lipid peroxidation products. The enzyme-dependent lipid metabolism resulted in elevation of endocannabinoids, while the expression of cannabinoid receptors was enhanced in psoriasis vulgaris and reduced in psoriatic arthritis. Consequently, the decay of lymphocytes of psoriatic patients was manifested by enhanced expression of caspases 8/9/3 and elevated p53 level, while Bcl2 level was decreased in both groups of patients. These findings suggest that oxidative stress and metabolic changes of phospholipids may be considered as important processes involved in pathophysiology of psoriasis. However, a differential response to lipid peroxidation of endocannabinoid system and lymphocytic transcription factors may be at least partially responsible for proteins differential expression in the lymphocytes of patients with psoriasis vulgaris and the onset of psoriatic arthritis.

## P-01-055
## Novel synthetic derivatives of oleanolic acid conjugated with diclofenac modulate Nrf2-ARE signaling pathway in pancreatic cancer cells

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Chronic inflammation is a key factor in the etiology of neoplastic diseases, including pancreatic cancer. Nonsteroidal anti-inflammatory drugs (NSAIDs) were approved for the chemoprevention of colon tumors and suggested for liver and pancreas cancers prophylaxis. Anti-inflammatory and anti-tumorigenic activities also exert naturally occurring and synthetic triterpenoids. Coupling of triterpenoid analogues with NSAIDs may enhance this effect and prevent the unfavorable side effects related to NSAIDs long term use. In this preliminary study the effect of new oleanolic acid (OA) oximes derivatives conjugated with diclofenac on the activation of Nrf2-ARE pathway was evaluated in pancreatic cancer cells. PSN-1 cells were incubated for 24 h with the OA, its derivatives or their conjugates. Cell viability was assessed by MTT test. Muse Cell Analyzer was used to evaluate cells proliferation. The strongest cytotoxic and anti- proliferative effect was observed as result of treatment with 3-hydroxyiminoolean-12-en-28-oic acid morpholide and 3-diclofenacoxyiminoolean-12-en-28-oic acid morpholide. Gene expression was evaluated by RT-PCR and Western blot. The measure of Nrf2 activation was its translocation into nucleus and binding to ARE sequence assessed by ELISA assay. All OA derivatives and their conjugates increased the expression and activity of Nrf2. The most pronounced effect was exerted by 3-hydroxyiminoolean-12-en-28-oic acid morpholide and its conjugate with diclofenac. This effect was confirmed by induction of superoxide dismutase. These results indicate that the conjugation of diclofenac with new OA derivatives may enhance its anti-inflammatory activity through activation of Nrf2-ARE pathway. Acknowledgements: This work was supported by grant no 2016/21/B/NZ7/01758 from the National Science Centre, Poland.


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## P-01-056
## Atorvastatin and Pravastatin affect mitochondrial metabolism and reactive oxygen species generation in endothelial cells

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Vascular injury is the principal complication in patients suffering from metabolic syndrome. Hyperglycaemia, fatty acids, inflammation, and insulin resistance accompanied by hyperinsulinemia, are major inducers of endothelial dysfunction. Statins competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase are highly effective drugs, which are widely used to reduce endogenous cholesterol biosynthesis and restore proper serum lipid profile. However, statins exert various off-target effects in cardiovascular system: They may improve endothelial cell function but on the other hand increase the risk of hyperglycaemia due to impaired glucose uptake from the blood. Since uncontrolled elevation of serum glucose concentration is a cause of severe endothelial complications the question arises how selected statins may influence endothelial cell metabolism. Naturally occurring pravastatin and synthetic atorvastatin are commonly used to reduce serum cholesterol level and a risk of cardiovascular episodes. The aim of our study was to analyse effects of these statins on endothelial cells metabolism, reactive oxygen species generation and nitric oxide synthesis in EA.hy926 cells. We also assume that statins may influence respiratory activity of endothelial cell mitochondria, affect mitochondrial biogenesis and network organization. Except an increase of the level of mitochondrial proteins (Mn-SOD, selected subunits of oxidative phosphorylation complexes) we also found an elevation of nicotinamide N-methyltransferase (NNMT) protein content. This enzyme catalyses nicotinamide (NA) methylation thus one could hypothesize that it reduces availability of substrate for NAD+ synthesis and reduce S-adenosyl methionine content. Results of experiments on cells with siRNA silenced NNMT encoding gene allow assuming that NNMT is an important player in a regulation of endothelial cell function. This work was supported by the National Science Centre, Poland. Grant no. 2015/19/B/NZ3/02302

## P-01-057
## WWOX suppressor gene regulate glucose metabolism of human normal fibroblasts cells under hypoxia and hyperglycemia conditions

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*WWOX* gene is a well known tumor suppressor gene with evidence showing association of carcinogenesis with its decreased expression and genetic alteration. Recent results showed that WWOX protein is directly involved in glucose metabolism regulation via HIF1α interaction. In cancer cell lines, *WWOX* underexpression has been demonstrated to regulate the metabolic switch from oxidative phosphorylation to aerobic glycolysis. The Warburg effect enhances glycolysis rather than oxidative phosphorylation to generate energy. In our ongoing research in the pathogenesis of gestational diabetes on the role of *WWOX* gene; we created an in vitro model to study its contributing role to cellular metabolism in hyperglycemia and hypoxia. We used CRISPR/Cas9 lentiviral system to knockout WWOX protein expression in human skin fibroblasts cell line 1BR3N. The obtained variants of the cell line were examined for 48 h under 4 cultural conditions with 5.5 & 25 mM glucose concentration and 1% & 21% O2 concentration. Subsequently, we analyzed expression levels of *WWOX*, *HIF1A*, glucose transporters *SLC2A1*, *SLC2A4* and glycolytic genes *HK2*, *PFK1*, *PKM2*, *LDHA* using RT-qPCR. Inactivation of *WWOX* in human fibroblast resulted in increased *HIF1A* expression and thereafter, the overexpression of examined genes in all tested conditions. In summary, the results suggest that reduced *WWOX* expression causes upregulation of HIF1α target genes with enhanced glycolysis. These results could possibly suggest that WWOX protein is essential to maintaining homeostasis of glucose metabolism. The hypoxic and hyperglycemic conditions commonly associated with diabetes do not change this relation. The results demonstrate that *WWOX* downregulation critically contributes to enhanced glycolysis and thus *WWOX* inactivation potentially participates in pathogenesis of diabetic complications. Acknowledgements: This study was funded by the National Science Centre, Poland nr 2015/17/N/NZ4/02805.

## P-01-058
## IQGAP1 and IQGAP3 are involved in the development of psoriasis inflammation

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IQGAP family of scaffold proteins consists of three members IQGAP1, IQGAP2 and IQGAP3. Studies have linked the activity of IQGAPs with the vast majority of signaling cascades important for the inflammatory response: NFkB signaling, IFN signaling, growth factor signaling, cell adhesion, cell migration, cell proliferation, nuclear function, Wnt pathway potentiation, epithelial-mesenchymal transition and other. All of the mentioned pathways play roles in the pathogenesis of psoriasis, so we suggested the IQGAP family to contribute to the development of this disease. qPCR analysis of the *IQGAP1, IQGAP2* and *IQGAP3* expression in skin of psoriasis patients showed the genes to be significantly differentially expressed, with *IQGAP1* being downregulated and *IQGAP3* upregulated. Next, in order to identify the protein partners of IQGAPs involved in the development of psoriatic inflammation we have conducted a bioinformatic analysis based on the RNAseq data and the protein-protein interaction database STRING. The research allowed us to develop the IQGAP PPI graphs explaining the differences between lesional and non-lesional psoriatic skin, and to identify the IQGAP protein partners involved in psoriasis. Therefore our studies confirmed the hypothesis on the role of the IQGAP family in the pathogenesis of psoriasis. The research was supported by RSF (project No. 18-75-00126)

## P-01-060
## Putative role of nicotinamide N-methyltransferase in LPS-caused mitochondrial dysfunction

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This work was supported by the National Science Centre, Poland; Grant number: 2015/19/B/NZ3/02302 Vascular endothelium is a highly dynamic and active organ notably exposed to bacterial toxins and its key role such as vasomotor tone control or inflammatory response regulation may be impaired under septic conditions. Nicotinamide N-methyltransferase (NNMT) is an enzyme responsible for methylation of nicotinamide, forming the


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N1-methylnicotinamide (MNA). It has already been shown that NNMT may affect different metabolic pathways through depleting of methyl donors and producing active metabolites in several organs such as liver or cancer cells but its role in the endothelium still remains unknown. Our study aims to shed some light on biochemical mechanisms linking the NNMT activity and mitochondrial energy metabolism in LPS-treated Human Aortic Endothelial Cells (HAECs). Our experimental data clearly show that an incubation of HAECs with LPS results in an elevation of NNMT protein content. Silencing of the NNMT encoding gene with siRNA causes a significant decrease in mitochondrial reactive oxygen species production and an increase in mitochondrial mass. Moreover, we have found that LPS treatment triggers dynamic mitochondrial network fragmentation and its perinuclear localization, while NNMT gene silencing protects mitochondrial from its fragmentation under the same conditions. These affects are in line with time-dependent changes in autophagy-related and mitochondrial dynamics related proteins which we have also observed. This finding may indicate that NNMT is a potential important target involved in endothelial defense response to inflammatory stimuli but the precise role of this enzyme under such challenging conditions needs to be elucidated.

## P-01-061

## Dedifferentiated primary human macrophages can represent a niche for long-term intracellular survival of *Bordetella pertussis*

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The Gram-negative aerobic coccobacillus *Bordetella pertussis*, the agent of whooping cough (also called pertussis), represents a serious health threat especially for infants and pre-school children. For decades the basic research of pertussis pathology was neglected and many fundamental questions regarding biology of *Bordetella pertussis* infections remain unanswered. Among them, any plausible explanation of the very prolonged paroxysmal coughing, typical for whooping cough disease, is missing. In an attempt to identify a potential answer to this question, we revisited the yet controversial issue of long-term intracellular survival of *Bordetella pertussis* inside leukocytes. We infected primary human monocyte-derived macrophages with fluorescent protein-expressing *Bordetella pertussis in vitro* and analyzed the phenotype of the infected cells over time by microscopy and flow cytometry. We report that *Bordetella pertussis* can successfully survive within primary human macrophages for at least two weeks *in vitro* and it can form microcolonies within such cells, in agreement with earlier observations. In order to do this, the bacteria seem to modulate the phenotype of macrophages and appear to survive only in a fraction of specifically shaped infected monocyte-like macrophages. This raises the possibility that infected lung phagocytes might serve as reservoirs for prolonged survival and continuous release of bacteria, or of their virulence factors, such as protein toxins, over prolonged periods after infection. This might offer an explanation of the extremely long whooping cough periods that are the most common manifestation of preceding *B. pertussis* infection.

## P-01-062

## The beneficial effect of regular exercise on obesity and metabolic syndrome: A transgenic mouse study

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It is well-known that inappropriate nutrition and sedentary lifestyle can lead to obesity and proneness to atherosclerosis and metabolic syndrome. However, besides the wide range of medicines, one of the simplest ways to lower the risk of the diseases is regular exercise training. Therefore, we investigated the beneficial effects of regular exercise training on the body by monitoring functional, morphological and gene expressional changes in different tissues of high-fat diet (HFD) fed ApoB100 transgenic mice, a mouse model of atherosclerosis and dyslipidemia. Wild-type and HFD fed ApoB100 transgenic animals were trained by treadmill running five times a week for 7 months. To study the changes in thermogenesis and inflammation we monitored gene expression changes in brown and white adipose tissues (BAT and WAT) as well. We found that the adipose tissue of sedentary male ApoB100 animals showed a high increase in the expression of pro-inflammatory cytokines, stress marker heath shock proteins and leptin, suggesting a disturbed lipid metabolism. However, most of these parameters were reduced by regular exercise. In addition, the level of thermogenesis marker uncoupling protein-1 (UCP-1) was decreased in BAT in contrast to the WAT in which the level of UCP-1 was increased. We also found that triglycerides and fatty acids accumulated in the liver of sedentary ApoB100 transgenic mice and the expression of pro-inflammatory cytokines was significantly elevated. However, this liver damage was prevented by exercise training. Our results showed that moderate intensity exercise training could significantly reduce the bodyweight and the serum triglyceride level of ApoB100 transgenic mice. Based on these data we can conclude that regular physical activity ameliorates certain symptoms of metabolic syndrome and systemic inflammation induced by HFD and dyslipidemia. This work was funded by National Research, Development and Innovation Office, Hungary (GINOP 2.3.2.-15.2016-00040).

## P-01-063

## The MHCII allele HLA-DRB1*01:01, known as a protective for multiple sclerosis, presents peptide fragments of myelin and exogenous antigenic peptides with different velocity and efficiency

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Several alleles of class II human leukocyte antigens (HLA) are described as associated with higher risk of the development of multiple sclerosis (MS), e.g., HLA-*DRB1**15, while other HLA variants, e.g., HLA-*DRB1**01, are associated with protective effect. Here we studied distribution of HLA-*DRB1* locus genes in more than one thousand Russian MS patients and healthy individuals. Analysis of genotypes revealed the compensatory effect


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of risk and resistance alleles *in trans*. We have identified previously unknown $MBP_{153–161}$ peptide located at the C-terminus of MBP protein and $MBP_{90–98}$ peptide that bound to recombinant HLA-DRB1*01:01 protein with affinity comparable to that of classical antigenic peptide 306–318 from the hemagglutinin (HA) of the influenza virus demonstrating the ability of HLA-DRB1*01:01 to present newly identified autoantigen peptides. Measurements of kinetic parameters of MBP and HA peptides binding to HLA-DRB1*01:01, catalyzed by HLA-DM, revealed a significantly lower rate of CLIP exchange for $MBP_{153–161}$ and $MBP_{90–98}$ peptides as opposed to HA peptide. Analysis of the binding of chimeric MBP-HA peptides demonstrated that the observed difference between $MBP_{153–161}$, $MBP_{90–98}$ and HA peptide epitopes is caused by the lack of anchor residues in the C-terminal part of the MBP peptides resulting in a moderate occupation of P6/7 and P9 pockets of HLA-DRB1*01:01 by $MBP_{153–161}$ and $MBP_{90–98}$ peptides in contrast to $HA_{308–316}$ peptide. This leads to the P1 and P4 docking failure and rapid peptide dissociation and release of empty HLA-DM–HLA-DR complex. We would like to propose that protective properties of the HLA-*DRB1**01 allele is directly linked to the ability of HLA-DRB1*01:01 to kinetically discriminate between antigenic exogenous peptides and endogenous MBP derived peptides. The project is supported by Russian Scientific Foundation 17-74-30019

## P-01-064
## MCPIP1 regulates insulin biosynthesis and glucose-stimulated insulin secretion

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Background Pancreatic beta-cells are the only source of insulin in human body. During diabetes development insulin biosynthesis and glucose-stimulated insulin secretion (GSIS) are defective due to inflammation-related and yet unclear mechanisms. The MCPIP1 protein (Monocyte Chemotactic Protein-Induced Protein-1) is a multifunctional protein that regulates the inflammatory response and acts on the transcriptional, post-transcriptional and post-translational levels. The aim of this study was to investigate the role of MCPIP1 in insulin biosynthesis and GSIS. Experimental details The MCPIP1 expression was undetectable in untreated insulin-secreting INS1E cells and increasing glucose concentrations in culture medium failed to stimulate it (qRT-PCR). Cells were transfected with either a wild type $MCPIP1_{WT}$ or a mutated $MCPIP1_{D141N}$ (mutation in the RNase catalytic site of the PIN domain) and then incubated with glucose (3, 10, 30 mM) for 2, 24, or 72 h. A prolonged incubation with 30 mM Glc hampered the responsiveness of cells to acute glucose stimulus. Interestingly, INS1E-$MCPIP1_{WT}$ cells were characterized by a significantly lower insulin gene expression than control cells. *In silico* analysis of the insulin mRNA revealed a presence of a conservative stem-loop structure that can be recognized and degraded by the PIN-domain of MCPIP1. Overexpression of the mutated $MCPIP1_{D141N}$ failed to reduce insulin expression. Additionally, we observed changes in GSIS, ATP content, glucokinase activity and glucose uptake in INS1E-$MCPIP1_{WT}$ cells in comparison to control cells. Conclusions Our results show that MCPIP1 can regulate beta-cell function by affecting insulin biosynthesis. Moreover, MCPIP1-dependent changes in the regulatory mechanism of GSIS suggest an important role of MCPIP1 in the regulation of beta-cell glucose responsiveness. Thus, our findings may open new therapeutic perspectives for diabetes.

## P-01-065
## Blockade of adenosine A2B receptor attenuates diabetic glomerulosclerosis by inhibiting macrophage–myofibroblast transition

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Macrophages (MØ) have been identified as a major source of Myofibroblasts during glomerulosclerosis in diabetic nephropathy (DN) by a process known as Macrophages-Myofibroblast Transition (MMT). This process is dependent on TGFβ1 axis activation. Extracellular adenosine levels increase chronically during DN and the administration of MRS1754, a selective antagonist of the adenosine $A_{2B}$ receptor ($A_{2B}AR$), attenuated glomerulosclerosis in diabetic rats. We aim to investigate the association between $A_{2B}AR$ and MMT during DN. Kidneys and glomeruli of non-diabetic, diabetic (Db), and MRS1754-treated diabetic (Db+MRS1754) rats were processed for transcriptomic, histopathologic, and cellular *in vitro* analyses. Immunofluorescence was performed to detect MØ (CD68 + ), Myofibroblast (αSMA+), and MMT (CD68 + /α-SMA+) in the glomeruli. Conditioned medium from rat glomeruli or human immortalized podocytes (HIP) were used to evaluate MMT and MØ cell migration assays *in vitro*. *In vivo* MRS1754 treatment attenuated clinical and histopathological signs of glomerulosclerosis in DN rats. Transcriptomic analysis demonstrates a decrease in MØ chemokine-chemoattractant in the glomeruli of Db+MRS1754 rats. The number of infiltrated MØ and MMT cells into the glomeruli increased in Db rats, which was reverted by MRS1754 treatment. *In vitro* MMT and cell migration were reduced in MØ stimulated with the supernatant from glomeruli of Db+MRS1754 rats. Similarly, the conditioned medium of HIP previously cultured under TGFβ1 and high glucose conditions induce MMT in human MØ, a process which was attenuated in presence of MRS1754. Pharmacologic blockade of $A_{2B}AR$ in Glomeruli/Podocytes decreases MØ infiltration, MMT, and attenuates the clinic and histopathological signs of glomerulosclerosis in DN rats. Acknowledgements. Financed by grants FONDECYT-Chile 3180749 (A.T.) and FONDECYT-Chile 1171340 (R.S.M.).

## P-01-066
## Effects of simvastatin and simvastatin hydroxy acid on macrophage in vitro behavior

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Statins are widely used antihypercholesterolemic drugs but, at the same time, they display other pleiotropic effects such as the stimulation of the endothelial function, the attenuation of oxidative stress and inflammation, and inhibition of the bone resorption process, as well. In this study, the effects of simvastatin (SV), a commercial drug from statin class, and its metabolically active form – simvastatin hydroxy acid (SV-OH), on the LPS-induced pro-inflammatory macrophage RAW 264.7 cells were investigated. Activation of SV to its hydroxy acid form by NaOH thermal treatment was confirmed by HPLC-MS. Subsequently, the


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effects displayed by SV and SV-OH on RAW 264.7 cells in the concentration range of $10^{-4}$M–$10^{-9}$M were analyzed both in standard and pro-inflammatory (stimulation with 100 ng/ml LPS) culture conditions. For that, RAW 264.7 macrophages were seeded at a cell density of $1x10^4$ cells/$cm^2$, and maintained in culture for 1 day and 3 days. For comparative purposes, the non-treated macrophages have been considered as the control sample. The possible cytotoxic effects were investigated in terms of cell viability (MTT and LIVE/DEAD assays) and cell morphological features (phase contrast microscopy). The non-cytotoxic drug concentrations were further tested for their ability to influence the protein levels of cytokines (TNF-α, IL-1β and IL-6) and NO released in culture media at 24 h and 48 h post-treatment. The results showed that both analyzed compounds induced cytotoxic effects at concentrations higher than $10^{-7}$M. Unexpectedly, they elicited almost similar *in vitro* effects in terms of pro-inflammatory activation of RAW 264.7 cells, suggesting a possible activation of SV at cellular level and targeting a common signaling pathway. Acknowledgements: We gratefully acknowledge CNCS-UEFISCDI for financial support through PCE-55-2017 project.

## P-01-067
## Steady-state systemic aberrant inflammation in paediatric patients with autism spectrum disorders

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Elevated pro-inflammatory cytokines in patients with autism spectrum disorders (ASD) are linked to the behavioral stereotypies and disease regression. Pro-inflammatory mediators and immunoactive complexes can reach higher brain centers from the blood stream and provoke neuroinflammatory processes. This study's aim was to compare plasma cytokine concentrations as well as the transcript levels for NF-kB/pro-inflammatory cytokines in peripheral blood lymphocytes (PBL) of ASD patients and unaffected controls. The study included 64 ASD patients (4–12 years old; 50 boys) and 27 healthy controls. Plasma cytokine concentrations and the transcript levels for NF-kB and pro-inflammatory cytokines in PBL of ASD patients and healthy children have been investigated. Compared to healthy controls, patients with ASD demonstrated increased concentrations of pro-inflammatory cytokines IL-1β, IL-8, and IL-17A in their plasma samples (all $P < 0.01$). It should be noted that in all unaffected controls plasma TNFα contents were below the method detection limit, whereas in most ASD patients it was possible to detect low or moderate concentrations of that cytokine. Accordingly, the transcript levels for NF-kB, IL-1β, IL-8 and TNFα in PBL of ASD patients were also significantly elevated. Thus, the expression levels of IL-1b and IL-8 were by a factor of 1.7 to 2.4 (both $P < 0.01$) higher, whilst the levels of TNFa and NF-kB were 2–4 fold higher (both $P < 0.01$) compared to the control group. In conclusion, elevated plasma cytokine concentrations as well as increased transcript levels for NF-kB/ IL-1β, IL-8, TNFα in PBL suggest the presence of steady-state systemic aberrant inflammation in ASD patients. The work was supported by RFBR grant No. 16-04-00576_A, and as a state task of the Ministry of education and science of Russia. *The authors marked with an asterisk equally contributed to the work.

## P-01-068
## Systemic inflammatory response and changes of the cell-free DNA characteristics during schizophrenia exacerbation

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Immune dysregulation in schizophrenia has been found in numerous studies comparing patients to healthy controls. Previously we have shown that chronic inflammatory disorders are associated with changes in the composition of blood plasma cell-free DNA (cfDNA), in particular with accumulation of GC-rich sequences (mainly ribosomal DNA repeats), which can activate TLR9 and potentiate a proinflammatory response in human cells. Thus, the aim of the study was to compare the characteristics of cfDNA in blood plasma and *TLR9* expression in lymphocytes of patients with schizophrenia during exacerbation compared to healthy controls. The study involved 112 patients with paranoid schizophrenia and 83 healthy volunteers. CfDNA samples were obtained from blood plasma using phenol extraction method. The number of GC-rich ribosomal repeats in cfDNA was determined by non-radioactive quantitative dot hybridization. TLR9 expression was analyzed by flow cytometry. The presence of orosomucoid was determined by ESI-MS/MS. In patients with schizophrenia the signs of systemic inflammatory reaction were revealed. Thus, abnormally elevated levels of orosomucoid (the acute phase proteins) were found in 87% of plasma samples obtained from the patients. In addition, peripheral blood lymphocytes of patients with schizophrenia were characterized by an increased level of TLR 9 expression compared to the cells of the control group ($P < 0.01$). During exacerbation period cfDNA concentrations in plasma samples of the patients were 2-2,5 fold higher than those in healthy controls ($P < 0.01$). CfDNA samples of the patients contained significantly more ribosomal DNA repeats than that of healthy people ($P < 10^{-7}$). In conclusion, schizophrenia exacerbation is accompanied by the development of a systemic inflammatory reaction characterized by the activation of *TLR9* and changes in the cfDNA characteristics. The work was supported by RFBR grant No. 17-29-06017ofi_m. *The authors marked with an asterisk equally contributed to the work.

## P-01-069
## Targeting key signaling factors as a way to control microglial activation and induction of neuroinflammation

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Neuroinflammation is co-occurring phenomenon during pathological processes in the nervous system. Key player in this process is microglia. As moderate activation of microglia is beneficial, excessive one however, leads to more severe degeneration of tissue and inhibition of its endogenous regeneration. One


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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way to prevent this situation is to modulate or inhibit microglia activation. Aim of this study was to use gene silencing technique to influence microglial activation. By targeting key proteins – NF-κB, MyD-88 and TRIF, we intended to decrease inflammatory signaling network. Gene silencing was optimized on stable murine microglia BV-2 cell line. Before stimulation with lipopolysaccharide (LPS), cells were transfected with designed siRNA sequences. Efficacy of transfection was assessed by evaluating expression of NF-κB, MyD-88, TRIF as well as IL-1β, IL-6, TNF-α, TREM1, TREM2 at mRNA and protein level. Optimized sequences of siRNA were then used on primary microglia. Our results showed that siRNA can successfully inhibit activation of microglia *in vitro* after stimulation with LPS. Significant decrease was observed in expression of signaling proteins. However, depending on targeted factor, different decrease patterns were observed for IL-1β, IL-6 and TNF-α. Thus, mixture of siRNA was combined to achieve most successful effect. Our results provide a new method to successfully limit microglia activation with siRNA technique. This approach will be further used in *vivo,* in our models of Parkinson's disease and hypoxia-ischemia encephalopathy, in which severe inflammation is observed. Acknowledgements: The project was supported by the research grant from the Jagiellonian University Medical College: 2015/17/B/NZ5/00294, K/DSC/003575.

## P-01-070
## Some indicators of chronic peroxidal stress in stomach cancer patients

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Stomach cancer is accompanied by histotoxic hypoxia and disorder of tissue respiration. Recent experimental and clinical researches have revealed that several diseases of gastrointestinal tract including malignant tumors are accompanied by membrane-destructive process, resulting in oxidative failures of tissues due to formation of reactive oxygen species (ROS) and stimulation of lipid peroxide oxidation (LPO). In the current study 31 gastric cancer patients after radical surgery were examined. The degree of LPO activity in blood plasma and tissues of gastric cancer patients was estimated by malonic dialdehyde (MDA) level, and MDA induced by iron (Fe-MDA) reflecting antioxidant capacity. The end products of glycolysis lactic acid (LA) and pyruvic acid (PVA) were also detected. Samples of tumoral and normal stomach tissue were taken on the day of surgery. Surgical treatment of these patients resulted in subsequent activation of LPO. MDA exceeded preoperative level by 13.6% and the norm by 118.0%. Fe-MDA did not differ significantly from preoperative level and was 16.6% lower than the norm. This indicator evidences persisting low antioxidant potential. At the same time certain decrease in hypoxia severity indicated in 23.7% lower LA level in comparison with preoperative level was revealed. PVA remained stable compare with preoperative level but exceeded the norm by 65.1%. One of the pathological processes arising as result of stomach cancer regardless of the disease stage is development of histotoxic hypoxia and disturbance of cell respiration what is expressed in alteration of relationship between the end products of glycolysis and free radical reactions in patients' blood plasma and tissues. The surgical intervention is highly aggressive for the entire body since an operative trauma is a powerful factor of activation of various cellular processes and biochemical reactions including oxidative ones. The study was supported by the "RUDN University Program 5-100".

## P-01-071
## *Campylobacter jejuni* Dsb (disulfide bond) network functioning is influenced by thiol oxidoreductase C8J1298

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Introduction of disulfide bonds is an important step in protein folding process. Bacterial Dsb enzymes catalyze disulfide bond generation and act in two pathways: oxidation and reduction/isomerisation. There are many virulence factors amongst Dsb protein targets and as a consequence Dsb proteins impact pathogenicity of bacteria. Human pathogen *C. jejuni* 81116 oxidative pathway consists of two periplasmic thiol oxidoreductases CjDsbA1 and CjDsbA2 (responsible for disulfide bonds formation) and two inner membrane proteins CjDsbB and CjDsbI playing a role in an electron transfer. *In silico* analysis identified two additional potential components of reduction/isomerisation pathway responsible for the rearranging of nonnative disulfide bonds. The periplasmic dimeric thiol oxidoreductase C8J1298 (putative isomerase) was the object of presented research. Our research indicates that *c8j1298*, which expression is controlled by stress envelope CprRS system, plays a key role in the regulation of the *C. jejuni* network. The C8J1298 overproduction results in the lack of CjDsbA1 and CjDsbA2 (probably major oxidoreductases) in the cell proteome. To evaluate hypothesis that C8J1298 compensate for the lack of CjDsbA1 and CjDsbA2, the amount of C8J1298 protein was analyzed in various *C. jejuni* strains (wt, *dsbA1-, dsbA2-, dsbA1-/dsbA2-, dsbA1-/dsbA2-/dsbA1* + , *dsbA1-/dsbA2-/dsbA2* + ) by Western-Blot analysis. Real-time PCR experiments should indicate the level of *c8j1298* transcription in strains lacking *cjdsbA1, cjdsbA2* or both genes as well as in appropriate complements in comparison to wt strain. Because C8J1298 is present in wt cells in both, reduced and oxidized forms, the obtained results imply that under certain conditions it may undertake the function of CjDsbA1 and CjDsbA2. The work was supported by the National Science Centre (grant no. 2015/17/B/NZ1/00230). *The authors marked with an asterisk equally contributed to the work.

## P-01-072
## Initiation and promotion of DMBA/TPA induced papillomatosis in mice lacking epidermal MCPIP1

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The fundamental role of Monocyte Chemoattractant Protein-Induced Protein 1 (MCPIP1) is the control of physiological and pathological processes of inflammation through the degradation of pro-inflammatory cytokine transcripts. MCPIP1 RNase also acts as a modulator of processes related to tumorigenesis and cancer progression in many cell types, particularly by regulating the rate of metabolism and angiogenesis. Furthermore, there is a growing list of evidences that MCPIP1 may play a significant role in the initiation and development of some skin disorders,


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such as psoriasis. To determine the role of MCPIP1 in skin tumor development we generated conditional knockout mice lacking gene encoding MCPIP1 (*Zc3h12a*) in the epidermal basal keratinocytes (Mcpip1$^{EKO}$). Subsequently, we utilized a well characterized DMBA/TPA approach to induce skin carcinogenesis in mice. Our results demonstrated that control and knockout mice treated only with TPA develop epidermal hyperplasia, which is more prominent in the Mcpip1$^{EKO}$ mice. Next, our preliminary studies show that Mcpip1$^{EKO}$ mice developed a large number of papillomas as early as 7 weeks following DMBA treatment, while the control mice developed only one or two lesions after 9 weeks of DMBA application. Moreover, we observed that the growth of the Mcpip1$^{EKO}$ papillomas was suppressed two weeks after initial onset, while in the control mice the tumor growth was continued. At the histological level, skin lesions of both control and Mcpip1$^{EKO}$ mice were characterized by strong epidermal hyperplasia. In the control mice we observed well-differentiated papillomas indicating their exophytic growth and the presence of keratin pearls. In contrast, the Mcpip1$^{EKO}$ mice showed a reduced exophytic growth of papillomas and appearance of clusters of pigment-filled melanocytes. In conclusion, our studies indicate that MCPIP1 play a significant role in the kinetics of both, the initiation and progression of chemically induced skin tumors.

## P-01-073
## Marine 5-thiohistidines as protective molecules from skin damage

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Introduction Marine environment is a great source of bioactive molecules, whose biological properties and applications are often used especially to prevent skin diseases and aging caused by UVA-exposure. Ovothiols are methyl-5-thiohistidines from marine invertebrates, bacteria, and microalgae, which protect cells from environmental stressors. Recently, we have shown that ovothiol, isolated from sea urchin eggs, exerts anti-inflammatory and antioxidant activities on human endothelial cells, and exhibits antifibrotic effect in an *in vivo* model of liver fibrosis. Thanks to their chemical properties, ovothiols represent promising bioactive compounds to use as anti-inflammatory compounds in human diseases. Material and Methods Human keratinocytes (HaCat) and human embryonic kidney 293 cells (HEK293) were used to test the cytotoxicity of these molecules by resazurin-based assay. An *ex vivo* human skin model was used to investigate the anti-inflammatory and dermato-protective properties of these molecules. Results and Discussion In this preliminary study we showed that two types of 5-thiohistidines are not cytotoxic in epithelial cells, indeed the vitality is even greater than in untreated cells. In addition, using specific ELISA assays, we observed that the pre-treatment of *ex vivo* human skin tissue with ovothiol A and the desmethylated form 5-thiohistidine at the concentration of 5 μM, led to a significant decrease in IL-6 and IL-8 production, indicating an anti-inflammatory effect. Finally, pre-treatment at low concentrations with these compounds, was very efficient compared to the pre-treatment with dexamethasone, a type of corticosteroid medication, used as anti-inflammatory in the treatment of many skin diseases. Conclusion These findings indicate that marine 5-thiohistidines have significant anti-inflammatory and dermato-protective properties and can be considered as new marine drugs or dietary supplements for the treatment of skin damage.

## P-01-074
## Searching for calpains beyond the cells; using optimized method for comparing calpain activities in human blood plasma of healthy and arthritic individuals

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Calpains, the ubiquitous, calcium–dependent *cytosolic* cysteine proteases, are found *inter alia* in human peripheral blood immune cells. By limited, non-degrading proteolysis, calpains modulate properties of multiple intracellular substrates, affecting key cell functions like proliferation, apoptosis, signal transduction etc. The only known endogenous calpain inhibitor is calpastatin. Anti-calpastatin autoantibodies are long known to accumulate in blood of some patients with autoimmune diseases, e.g. rheumatoid arthritis. The mechanisms leading to accumulation of these autoantibodies are unknown, but one of the possibilities is that calpains (possibly released or actively secreted) may act on extracellular substrates, including calpastatin. The aims of this study were to find and optimize a method proving that calpains can be active outside the cells (in blood plasma), and to compare the calpain activities between plasmas of healthy controls and arthritis patients using this method as a tool. Cell- and platelet-free plasma (verified by flow cytometry) was assessed for calpain activity using the luminescence-based Calpain-Glo™ Protease Assay, not reported before to be suitable for measuring calpain activities in the extracellular fluids. Human erythrocyte calpain-1 and Calpain Inhibitor I, as well as varying concentrations of $Ca^{2+}$ were used to confirm specificity of the test for human plasma calpain. Optimized test was then applied to the plasma samples from 40 arthritis patients and 20 healthy matched controls. Relative activities (AU) of calpain in healthy controls compared to patients differed significantly ($354 \pm 35.31$ and $536 \pm 53.99$ AU respectively, $P = 0.006$). Concluding, we have managed to optimize the commercial test of calpain activity for use with human plasma and demonstrated this activity to be significantly different depending on health status. Thus, our observations may be useful to develop a diagnostic test, but further experiments are required.

## P-01-075
## Characterisation of protein profile alterations of T lymphocyte subpopulations in depressive patients using mass-spectrometry based proteomic analysis

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There is a huge body of evidence for peripheral inflammation in major depression, i.e. i) increased concentration of inflammatory


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cytokines in the blood and CSF of patients, ii) greater likelihood of treatment resistance occurrence in patients with increased inflammation. There is possibility that immune alterations in major depression primarily derive from immune imbalance in the periphery as it is associated i.a. with impaired function of regulatory T (Treg) cells. However, hitherto documented disturbances of T cells concern merely their production of the anti-inflammatory cytokines. We performed comprehensive mass spectrometry based, label free proteomic characterisation of three subtypes of T cells: CD4 + CD294 + , CD4 + CD294-CD25- and CD4 + CD294-CD25 + Foxp3 + in healthy subjects and in first episode depressive patients as well as the same patients after 2 and 6 weeks of pharmacotherapy. Quantitative statistical analysis of about 5000 proteins performed in MSstats, Bioconductor package and with TPA approach coupled with advanced parametric analysis revealed several hundreds of differential proteins. Gene ontology analysis conducted for differential proteins in IPA software showed statistically significant regulated biochemical pathways. In comparison to CD4 + CD294-CD25- cells the CD4 + CD294 + and CD4 + CD294-CD25 + Foxp3 + cells are much less metabolically active and exhibit inhibition of many cellular functions. Differences between CD4 + CD294 + and CD4 + CD294-CD25 + Foxp3 + cells concern Cdc42 signalling. Depression strongly affects Treg cells metabolism and up-regulates mTOR signaling and seems to transform these cells into functionally impaired helper Th-GM phenotype. Two weeks lasting pharmacotherapy does not reverse the depression effect on protein level in case of Treg, but strongly influences CD4 + CD294 + cells. The alteration in biochemical processes observed after 6 weeks of treatment are significant but they rather do not indicate beneficial changes in T cells functioning.

## P-01-076

## Histone deacetylase 3 is a key epigenetic regulator of gingival fibroblast inflammatory activation in periodontal disease

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Histone deacetylases (HDACs) are important epigenetic regulators of gene expression that are pathologically regulated in several inflammatory and infectious diseases. Periodontal disease (PD) is a chronic inflammatory disease of the periodontium that leads to bone resorption and tooth loss. Activation of resident gingival cells, such as gingival fibroblasts (GFs), significantly contributes to PD pathogenesis and the anaerobic bacterium Porphyromonas gingivalis plays a key role in driving the chronic inflammation. Here, we examined the role of HDACs in inflammatory and antimicrobial responses of primary human GFs. Pan-HDACi significantly reduced TNF- and P. gingivalis-inducible expression and/or production of a cluster of inflammatory mediators in healthy donor GFs (IL1B, CCL2, CCL5, CXCL10, COX2 and MMP3) without affecting cell viability. Selective inhibition of HDAC3/6, but not specific HDAC6i, HDAC8i or HDAC1/2i, reproduced the suppressive effects of pan-HDACi on gene expression induced by TNF and P. gingivalis, suggesting a critical role for HDAC3 in GF activation. Indeed, HDAC3 gene silencing largely recapitulated the effects of HDAC3/6i on inflammatory mediator expression in P. gingivalis-infected GFs. In contrast, P. gingivalis internalization and intracellular survival in GFs remained unaffected by HDACi. Activation of mitogen-activated protein kinases and NFκB signaling was unaffected by global or HDAC3/6-selective HDAC inhibition, and new protein synthesis was not required for gene suppression by HDACi, indicating that disruption of HDAC activity at gene promoters is responsible for the observed effects. Finally, pan-HDACi and HDAC3/6i suppressed P. gingivalis-induced expression of IL1B, CCL2, CCL5, CXCL10, MMP1 and MMP3 in GFs isolated from PD patients. Collectively, these results identify HDAC3 as an important epigenetic regulator of GF inflammatory activation and suggest that therapies targeting HDAC activity may be therapeutically beneficial in PD. *The authors marked with an asterisk equally contributed to the work.

## P-01-077

## Characteristics of the phagocytic activity of macrophages of bone marrow and fetal origin

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Macrophages are key cells that regulate morphogenetic processes in health and disease. Macrophages in mammals originate from different sources of origin, which largely defines their properties and influences the course of inflammation and repair in different organs. A key competence of macrophages is phagocytosis. The purpose of the study is to compare macrophages of monocyte origin to Kupffer cells by phagocytic ability in the non-activated state or under the influence of M1 and M2 inducers. Upon transfer to cell culture, macrophages of monocyte origin and Kupffer cells expressed CD68 at high levels. The CD68 expression did not change under the influence of M1 and M2 inducers. At the same time, the cultures of macrophages of monocyte origin and Kupffer cells showed different dynamics of phagocytic activity. According to the published studies, Kupffer cells have pronounced phagocytic activity. At the same time, several reports claim that phagocytic activity of bone marrow-derived macrophages is more pronounced. This statement was confirmed by us in this study, as the initially non-activated monocytic macrophages showed higher phagocytic capacity than non-activated Kupffer cells. However, at 5 h after the addition of latex particles to the medium, the intensity of phagocytosis for Kupffer cells exceeded by far the capacity of monocyte macrophages; by 24 h, the difference ceased. Despite these differences, monocytic macrophages and Kupffer cells showed similar degrees of maturity of the forming endosomes. This research was supported by Russian Science Foundation, Project no. 17-15-01419.


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## P-01-078
## Anti- inflammatory effects of vitamin K in murine macrophages

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Vitamin K is a name given to a group of fat-soluble vitamins including: phylloquinone (vitamin $K_1$), menaquinones (vitamin $K_2$) and menadione (vitamin $K_3$). The 2-methyl-1,4-naphthoquinone ring is common to all forms of vitamin K but individual members differ in level of the saturation and the length of isoprenoid side chain. Vitamin K plays a key role in blood coagulation and regulation of calcium metabolism. Both of these processes use a reduced form of vitamin K (vitamin K hydroquinone – $KH_2$) as a γ-glutamylcarboxylase (GGCX) cofactor during vitamin K-dependent carboxylation. Recent studies demonstrate that vitamin K functions go beyond coagulation and calcification including regulation of inflammatory response via inhibiting cytokines production. The aim of the current studies was to evaluate wide scope of anti- inflammatory effect of different form of vitamin K in murine macrophages RAW 264.7. The inflammation in murine macrophages RAW 264.7 was induced using LPS. The concentration of nitrite was measured by ENO- 20, NOx Analyzer (Eicom). Quantitative analysis of selected eicosanoids (PGE2, PGD2, PGF2α, 8-iso-PGF2α) was performed using high performance liquid chromatography in combination with mass spectrometry (LC-MS / MS). The concentration of TNFα in cell culture supernatant was determined by the sandwich ELISA method with a commercially available kit from R&D system. Our results suggest that all studied forms of vitamin K inhibit inflammatory response as evidenced by concentration-dependent decrease in $NO_2^-$, $TNF_a$ and pro-inflammatory eicosanoids levels. Our results indicate that vitamin K has a broad spectrum of anti-inflammatory effects in macrophages including the regulation of COX-2 and iNOS activity. Anna Kierońska acknowledges the fellowship with the project no. POWR.03.02.00-00-I013/16.

# DNA variation

## P-02-001
## Analyses of *Streptococcus agalactiae* prophages

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*Streptococcus agalactiae* (GBS, Group B Streptococcus) is an opportunistic pathogen frequently carried in the human gastrointestinal and urogenital tract. This bacterium is a leading cause of sepsis and meningitis in neonates and also causes invasive diseases in adults. Several virulence factors are critical to disease development, and many of them are encoded on mobile genetic elements. Among others, integrated prophages could be beneficial for bacterial survival during colonization and infection. In this work we applied in silico methods for prophage identification from genome sequences of eight S. agalactiae clinical strains isolated from infected patients. These strains belonged to serotypes Ia, Ib, II, III, V and VII and were assigned into seven different MLST sequence types (ST-1, ST-6, ST-12, ST-17, ST-19, ST-23 and ST-130). Our analyses showed that each strain contained one to three prophages with the size range 17–45 kbp. Comparative analysis on nucleotide level distributed all 16 prophages into six groups (A-F). All prophage groups showed significant homology with known phages with the exception of group C. We observed similarity between (i) group A and S. suis phage phi-SsUD.1, (ii) group B and S. pyogenes strain T25-3 phage-like chromosomal island, (iii) group D and bovine S. agalactiae phage JX01, (iv) group E and S. pyogenes phage Str03 and (V) group F and phage P9 derived from a horse-associated S. equi. Group C prophage showed 80% similarity with S. suis temperate bacteriophage phiNJ2 but the prophage covers only 10% of phiNJ2 genome. We designed primers specific to each prophage group and determined prophage content of 75 GBS isolates. PCR based prophages identification revealed that 75% of all strains contained at least one prophage. The most prevalent prophage group was A present in 84% of prophage containing strains. The results show high prophage prevalence in clinical GBS isolates and thus confirmed prophage contribution to the virulence of bacteria.

## P-02-002
## Effects of two kits for amplification of EGFR single nucleotide polymorphisms: -191 C/A (rs712830) and 181946 G/A (rs2293347) which contained high GC rich region

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The investigations of EGFR and EGFR polymorphisms (SNPs) is very important for understanding the carcinogenesis process, regulation of cell proliferation and differentiation and for study of clinical response to TKI inhibitors in different tumors including lung cancer. The PCR technique is used to identify EGFR mutation but it requires setup of specific conditions. We have shown previously that laborious PCR optimization strategy was necessary for amplification high GC rich promotor region of *EGFR* SNPs in lung cancer. In this study we have tested for first time two available kits Biomaster LR HS PCR 2x (BiolabMix) and HS Taq-PCR Color 2x (BiolabMix) for amplification of two *EGFR* polymorphisms: -191 C/A (rs712830) and 181946 G/A (rs2293347). Biomaster HS Taq-PCR Color 2x and Biomaster LR HS PCR 2x, were tested according to recommendations of manufacturer and adjusted with the same PCR-RFLP protocols for both SNPs. Biomaster HS Taq-PCR Color 2x reaction mixture contains all of the components needed for PCR, including highly processive recombinant HS-*Taq* DNA polymerase, deoxynucleoside triphosphate mixture, 2 × PCR buffer, Mg2 + and betaine as additive in previously tested concentration. Restriction enzymes BseRI (New England) and Cfr42I (Fermentas/Thermo Fisher Scientific) were used in this protocols. PCRs were performed in total volume of 25 μl, with 0.4 or 0.5 μl genomic DNA and 0.4 μM of each primer. PCR products for 191C>A polymorphism were detected by gel electrophoresis on 3% agarose gel, and PCR products for -216G>T were detected on 8% polyacrylamide gel electrophoresis. Results indicated that these two products with optimal concentration of additive were effective in amplification of investigated EGFR SNPs including also extremely guanine-cytosine (GC) rich region and that can reduce preparation time as well as decrease contamination risk during the preparation of PCR reaction solution compared to manually prepared reagents.


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## P-02-003
## Variability analysis in the whole-genome sequences of *Staphylococcus aureus* isolates associated with cow's mastitis in Russia

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*Staphylococcus aureus* is a component of normal human and animal microflora. It is often associated with a problem of cow's mastitis, a common infectious disease of dairy cattle. Different factors can trigger switching of the bacterium into a pathogenic form. This work is devoted to the analysis of genome variation *S. aureus* associated with cow's mastitis. Here, we report the genome sequence analysis of *S. aureus* isolated from the milk samples of Holstein cows with subclinical mastitis. Milk samples were collected from farms of different regions of the Russian Federation. Twenty isolates of *S. aureus* were selected for whole-genome sequencing with Illumina MiSeq (Laboratory "Genomed", Moscow, Russia). We used the multilocus sequence type analysis (PubMLST) for species identification and genotyping of *S. aureus* isolates. The analysis of virulence factors was performed with the virulence factor database (VFDB). Genes of enterotoxin family and the genes of cytotoxins (leukocidins and hemolysins) and exfoliative toxin A were detected. Genetic variations have been studied, concentrating at variability in exotoxins sequences in *S. aureus* isolates. The range of SNPs and indels were detected in analysed genome areas. This work was carried out within the framework of grant 15-16-00020 under an agreement with the Russian Science Foundation. *The authors marked with an asterisk equally contributed to the work.

## P-02-004
## The number of cluster DNA damage in A549 cells after short-term UV irradiation

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An increase in the incidence of skin cancer is observed worldwide – according to WHO, about 2–3 million cases are recorded annually. The reason for this is a violation of the hygiene of sunbathing, increasing popularity of tanning beds and other. The aim of this study – determine the possibility of detecting DNA damage during short-term exposure of UV radiation. For the experiment, cell culture of human lung adenocarcinoma A549 was used. Irradiation was carried out for 5 min in the Bio-Rad ChemiDoc XRS system (UV-B, λmax = 302 nm), the control samples were protected from UV by foil.DNA isolation was performed using High Pure PCR Template Purification Kit (Roche). Determination of cluster DNA damage was performed with OCDL method according to Gollapalle, E. (2007), using the APE1 endonuclease. DNA electrophoretic separation was performed under the following conditions: 3 V/cm, 0.5x TBE (pH = 7.6), 240 min. Image analysis was performed according to Sutherland, B.M. (2003). The specific lesions that formed in DNA after UV-B irradiation are cyclobutane-pyrimidine dimers and 6-4 photoproducts. The APE1 allows recognition this lesions and abasic sites, including oxidized and modified. In intact culture, the number of APE1 clusters is 2.46 per 1 million base pairs (Mbp). For comparison, the level of EndoIII clusters is slightly higher and amounts to 3.77/Mbp. The 5-minute exposure to ultraviolet leads to an increase in the number of APE1 clusters by 63% to 4.01 clusters/Mbp. The OCDL method is more sensitive to the detection of DNA damage than the classical methods for determining the genotoxic effect of factors of different nature, including UV. In particular, the micronucleus test reveals differences in the number of cells with micronucleus only with a 30-minute single exposure to ultraviolet radiation. Thus, the results obtained indicate that a 5-minute exposure to UV leads to the formation of DNA damage and the OCDL method is able to detect even such minor changes.

## P-02-005
## Using micronucleus test to assess the genotoxic effects of UV radiation

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According to the WHO, between 2 and 3 million cases of skin cancer are recorded each year worldwide. 9/10 cases occur in people with fair skin. These facts show how relevant is the development of sunscreens with photoabsorbent, anti-inflammatory and antimicrobial properties. The aim of this study – assess the possibility of using the micronucleus test to assess the genotoxic effect of UV radiation. The experiment was performed on the Af line laboratory mice. The animals were irradiated with UV-B for 30 minutes (λmax=313 nm, distance-15 cm, integral power (15 cm) = 1253.1 $\mu W/cm^2$). The irradiated area of the skin was previously depilated. Dimexidum was applied 30 minutes before the UV treatment. The number of skin keratinocytes with micronucleus (MN) was counted 3–4 days after UV-irradiation – at least 1000 cells from each animal in each experimental group. Skin treatment with Dimexidum did not affect the proportion of cells with MN – 0.08 ± 0.04%. At the same time, a 30-minute exposure to the UV-B led to an increase the studying parameter by 3.57 times compared with the Control to 0.5 ± 0.12%. A similar effect in irradiated cells is explained by the free radicals formation, which react with DNA to form various substances, including 6-4 photoproducts and cyclobutane pyrimidine dimers. Also, skin cells death after UV irradiation leads to start of inflammation during which new cells are damaged. Preliminary (30 min before UV irradiation) treatment of the experimental animals skin with Dimexidum allowed reducing the MN level to 0.55 ± 0.05%, which is 2.08 times less than that of irradiated animals. The observed effect is associated with the anti-inflammatory effect of the drug «Dimexidum», which also inactivate hydroxyl radicals, optimize metabolic processes in the treated cells and has an analgesic effect. Thus, the presented data testify to the possibility of using the micronucleus test to assess the genotoxic effect of UV radiation.

## P-02-006
## Accumulation of potential G-quadruplex forming motifs in *Mycoplasma genitalium* genome is closely related to the occurrence of genome fragment copies

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Non-canonical DNA structures are widespread in various organisms including bacteria. One of the most well-known distinctive types of DNA secondary structures is G-quadruplex (GQ).


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Potential quadruplex sequences (PQSs) comply with the classical GQ motif formula Gn-Nm -Gn-Gm-Gn-Nm-Gn, where Gn is a guanine tract ($n \geq 3$) and Nm is an m-nucleotide loop of any sequence ($1 \geq m \geq 7$). However, PQS analogs with single mismatches in G-tracts (imperfect PQSs or imPQSs) are also capable of forming GQs. The mechanisms for the emergence and accumulation of these structures are not clear, so in our work we attempted to find common patterns of PQSs. Since imPQSs are more common in bacterial genomes than PQSs, we studied the imperfect sequences in *Mycoplasma* genus. For the PQS search we used ImGQfinder and for highly similar repeated subsequences we used RepSeek. Sequence comparison and analysis was performed using R. We found that the genomes of *M. mycoides* and *M. capricolum* did not contain any imPQS structures, while genomes of *M. gallisepticum, M. hyopneumoniae, M. canis,* and *M. fermentans* had 4 to 9 imPQSs. We did not find any PQSs that were common for different bacteria. Moreover, in each *Mycoplasma* species most PQSs were unique (we assumed that one sequence was a copy of another if the Levenshtein distance between these sequences was less than 2). Then we analyzed *M. genitalium* because it had the smallest genome, but contained 28 imPQSs, only 9 of which were unique. The other 19 imPQSs were in fact copies of 4 different sequences. All these sequences belonged to repeated genome fragments with average lengths of approximately 700 nucleotides. Moreover, among 132 repeated sequences 52 contained one of 4 repeated PQSs (such redundancy can be explained by the fact that if one sequence has N copies, we have N*(N-1)/2 mentions about it). Our findings suggest possible association between the number of PQSs and copying of genome fragments. This work was supported by RFBR [19-015-00024].

## P-02-007
## Clonal lineages of *Chlamydia trachomatis* strains in Saratov Region (Russia) determined by ompA polymorphism and MLST typing

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Single nucleotide polymorphism (SNP) analysis and genomic multi-locus sequence typing (MLST) are widely used to evaluate and compare genetic variations in bacteria. The aim of this study was to investigate the polymorphism of the *ompA* gene and seven housekeeping genes (*gatA, oppA, hfiX, gitA, enoA, hemN and fumC*) in *Chlamydia,* one of the major bacterial pathogens capable of infecting both humans and a broad range of animals. The *ompA*-based determination of genovars revealed a significant variability among *Chlamydia trachomatis* (CT) strains existing in Saratov city. Overall, there were seven variants of the genovars, such as D (6%), E (50%), F (10%), G (16%), H (6%), J (6%), and K (6%).Following MLST analysis confirmed a marked polymorphism in the housekeeping genes and identified nine sequence types (STs), namely ST4 (51%), ST6 (6%), ST9 (13%), ST10 (6%), ST12 (6%), ST38 (6%), ST118 (6%), and ST13 (6%). The EBURST comparative analysis of our strains with those obtained from PubMLST/Chlamydiales database grouped the Saratov variants into two different clonal complexes. The first complex consisted of ST4, ST12, ST38, and ST118 (ST4 as the founder), and the second one included ST6, ST9, ST10, and ST13 (ST13 as the founder). The most polymorphic were four alleles (*oppA, hplX, gidA and enoA*), which demonstrated a marked difference among the strains studied. In summary, the majority of the Saratov CT strains were assigned to the two different international clonal lineages, ST4 and ST9, respectively of genovars E, G, and F. Grant RSF-No.-17-16-01099

## P-02-008
## Potential role of UCP1 gene variants in cardiometabolic disease development

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Uncoupling protein 1 (UCP1) is an anion-carrier protein, located in the mitochondrial inner membrane, mainly expressed in brown adipose tissue. UCP1 holds an important role in energy metabolism and is considered to be involved in the pathogenesis of cardiometabolic diseases (CMDs). It is proposed that variants of *UCP1* gene might be associated with CMD risk. Existing data though, indicate increased diversity in the prevalence of *UCP1* variants among different populations. Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) of *UCP1* may contribute to the ethnic-specific predisposition to CMD. Ala64Thr, -112AC, -1766AG, -3826AG SNPs in *UCP1* gene were analyzed in Russian, Polish and Greek populations [817 CMD patients (pts.) and 801 controls]. Genotypic results were acquired through PCR-RFLP and/or TaqMan probe assays. Genotype frequencies, for all investigated SNPs, were in Hardy-Weinberg equilibrium. Significant differences in distribution of alleles and genotypes between CMD pts. and controls were found for -3826AG SNP only in Polish population: (G: 0.24 vs. 0.30, $P = 0.04$; AA: 0.57 vs 0.49, AG: 0.38 vs 0.43, GG: 0.052 vs 0.075, $P = 0.046$). In Greek population higher prevalence of the mutant alleles for Ala64Thr and -112AC was observed in controls, while the distribution of alleles and genotypes for those SNPs were similar in CMD pts. and controls in Russian and Polish populations. Of note, these polymorphisms were in strong linkage disequilibrium in all studied populations. Prevalence of -1766AG SNP was extremely low in all ethnic groups. The distribution of alleles and genotypes for studied SNPs differs between populations and further analysis needs to be conducted to evaluate the role of *UCP1* polymorphisms in CMD development in Eastern Europe. This study was supported by: 1) the Russian Foundation for Basic Research (18-34-20065); 2) European Union (U-GENE FP7-PEOPLE-2012 IRSES-319010); 3) Polish Ministry of Science and Higher Education (W15/7.PR/2016). *The authors marked with an asterisk equally contributed to the work.


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## P-02-009
## Investigation of the functional role of UCP1 A-112C (rs10011540) promoter polymorphism – a preliminary study

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Uncoupling protein 1 (UCP1) is a key modulator of thermogenesis and energy expenditure. Recent findings have focused on the involvement of *UCP1* single nucleotide polymorphisms (SNPs) in the risk of cardio-metabolic diseases (CMDs). A-112C SNP located in promoter region of *UCP1* gene is considered to be one of candidate polymorphisms. The aim of this study was to determine, whether A-112C variation influences the promoter activity of the *UCP1* gene. The functional role of A-112C SNP was investigated using of Dual-Glo Luciferase Assay System. Two variants (wild type A and mutant C) of a 1077-base pair proximal promotor were cloned into pGL3-Basic vector upstream of the firefly luciferase gene. HepG2 cells were transiently co-transfected with the respective vectors and pGL4.74 vector expressing Renilla luciferase. Luciferase activity was measured 48 hours after transfection and expressed in relation to Renilla activity and was normalized to the pGL3-Basic. Tests were repeated three times in quadruplicate and statistical analysis was performed using U-Mann Whitney Test. Values are expressed as the means ±SD. We have found no significant difference in promoter activity for wild type and mutant variants. The normalized RLU in HepG2 cells transfected with a –112A construct did not differ significantly compared to the –112C ($17.25 \pm 0.76$ and $17.00 \pm 3.17$, respectively). Moreover, transfected cells were tested in stimulating conditions with cis-retinoic acid and norepinephrine. We have found no effect of both stimulators on promoter activity of investigated variants. On the basis of our results we conclude, that A-112C SNP is not involved in the transcription regulation of *UCP1*, but further experiments in another research model need to be conducted. This work was supported by: (1) European Union (U-GENE FP7-PEOPLE-2012 IRSES-319010); (2) Polish Ministry of Science and Higher Education (W15/7.PR/2016); (3) the Russian Foundation for Basic Research (18-34-20065). *The authors marked with an asterisk equally contributed to the work.

## P-02-010
## Distribution and composition of genic short tandem repeats in the bank vole (*Myodes glareolus*) genome and their role in adaptive evolution

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Short tandem repeats (STRs) are repeated DNA sequences comprising short motifs of nucleotides (generally 1–6 bp) and are highly dynamic regions in the genome and mutations of these loci are a significant source of genetic variation, which can facilitate rapid adaptation. In this study, we analyzed the distribution, composition and frequency of STRs in the genes, both in coding (CDS) and untranslated regions (UTR), at the level of whole genome of the bank vole (*Myodes glareolus*) (genome sequence: Kotlik et al. unpublished). The analysis resulted in a total of 1793 STR loci in 1580 CDS (1.30% of total) and 577 loci in 527 UTR (2.76% of total, 176 in 5′ UTR and 401 in 3′ UTR). In CDS, mono-, di- and tri- nucleotides repeats are most abundant, while in UTR mono- and di- nucleotides are more prevalent. The length of STR was significantly affected by motif size. The STRs were classified as perfect and compound based on the number of motifs present in one locus, which showed higher number of perfect STRs (94.97%) in UTR, in comparison to CDS (89.96%). Mean length of STR loci was 22 nucleotides in CDS and 17 nucleotides in UTR. The functional annotation of STR containing CDS revealed that 414 sequences have high homology with known proteins, of which 234 have gene ontology (GO) terms, which can be categorized into 24 groups. The enrichment of biochemical pathways showed that STRs were overrepresented in the genes involved in specific pathways, such as cell cycle, spliceosome and cancer. The initial bioinformatics results showed the landscape of STRs in the bank vole genome, and provided a basis for further research, in which targeted high-throughput sequencing of these loci will be used to identify genes associated with evolution of high aerobic exercise performance and predatory behavior in artificially selected lines of bank voles (funding: Polish National Science Centre grant no. 2016/23/B/NZ8/00888).

## P-02-011
## Assessment of microsatellite DNA polymorphism impact on the toxic metabolism in the horse body

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The aim of this research is to study the microsatellite DNA polymorphism impact on the toxic metabolism in the horse body. The identification of microsatellite DNA polymorphism in the loci VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1, CA425 (recommended by ISAG) was carried out in the hair bulbs. The metabolic pool of toxic elements was assessed by the number of moles of toxic elements (Sr, Al, Cd, Pb, Sn, Hg) in the mane hair. The study was conducted in mares (n = 214), which were grown in 13 regions of Russia. There were 11 breeds in total. DNA amplification was carried out by PCR reactions using a 17-plex primer set for genotyping horses "StockMarksR". Hair chemical composition was measured by ICP-AES and ICP-MS. Analysis of alleles of DNA microsatellites showed a clear relationship between polymorphisms of alleles and the total number of toxic elements in the mane hair (by the locus HMS7): OQ (n = 4) – $0.96 \pm 0.33$ μmol/g; LO (n = 20)–$1.84 \pm 0.31$; JM (n = 11)–$2.01 \pm 0.38$; NO (n = 10)–$2.49 \pm 0.77$; NQ (n = 3)–$2.76 \pm 1.12$; KO (n = 6)–$3.17 \pm 1.01$; KL (n = 5)–$3.26 \pm 0.77$; LL (n = 34)–$3.32 \pm 0.45$; KM (n = 2) – $3.80 \pm 1.21$; JO (n = 6)–$3.83 \pm 0.85$; JJ (n = 7)–$4.21 \pm 0.74$; JK (n = 4)–$4.55 \pm 0.44$; LN (n = 29) – $4.84 \pm 0.80$; LM (n = 16)–$4.86 \pm 1.33$; MN (n = 8)–$5.16 \pm 1.93$; OO (n = 11)–$5.22 \pm 1.43$; JL (n = 12)–$5.83 \pm 1.35$; KK (n = 3)–$6.43 \pm 2.10$; KN (n = 5)–$6.67 \pm 2.78$; MM (n = 2)–$6.82 \pm 0.46$; JN (n = 8)–$7.34 \pm 1.24$; LQ (n = 3)–$7.83 \pm 1.52$; NN (n = 4)–$8.02 \pm 1.19$; MO (n = 1)–8.34 μmol/g. All the studying breeds had desired polymorphism


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of alleles: OQ, LO, JM, NO, NQ which was associated with a low number of toxic elements in the mane hair. This research was performed with support of the Russian Science Foundation (Project No. 17-16-01109).

## P-02-012

## Analysis of *Babesia canis canis* cytochrome c oxidase subunit 3 coding gene as a potential target of a new molecular diagnostic method of canine babesiosis in Latvia

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Canine babesiosis is a hemoprotozoal life-threatening tick-borne disease caused by several *Babesia* species. In Latvia, the most frequently detected causing parasite is *Babesia canis canis*. Lack of a rapid, specific and sensitive molecular diagnostic method that identifies this pathogen is an important issue in Latvian veterinarian practice. Mitochondrial DNA (mtDNA) of *Babesia* presents in a higher amount than nuclear DNA, and it contains evolutionarily conserved genes. Recent molecular assays targeting mtDNA genes have proved to be highly sensitive in detecting some parasitic protozoa, including *Babesia*. The aim of this study was to explore mtDNA cytochrome c oxidase subunit 3 (COX3) gene of *Babesia canis canis* present in Latvia, to assess its genetic diversity and possible development of a rapid molecular detection method targeting this gene. In total, 36 *Babesia canis canis* positive DNA samples isolated from infected dog's blood during 2016 – 2018 in Latvia were analysed in this study. Presence of this pathogen has been previously confirmed by *Babesia* 18S rRNA gene fragment amplification and sequencing. Primers targeting the full-length Babesia COX3 gene were designed using Primer-BLAST and optimal annealing temperature was determined by gradient PCR. Target gene was amplified by two-step PCR and positive amplicons were sequenced by Sanger method. Sequences were revised and aligned to the reference sequence obtained from the GenBank by using MEGA7 software. Sequence alignment revealed 5 SNP positions: 85C/T, 204C/T, 337C/T, 501C/T and 627C/T. In one sample the combination of first and second mutation was detected, other SNPs were observed as a single mutation in 6 samples. To conclude, this study shows a low mutation level of *Babesia canis canis* COX3 gene which makes it a potential target for such a rapid molecular assay as real-time PCR. This work was supported by the ERDF grant Nr.1.1.1.1/16/A/044.

## P-02-013

## Comparison of sex determination methods for archaeological samples

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Identification of the sex of human ancient remains is not always possible based on morphology, e.g. in cases where the skull or pelvis is missing or damaged, or the person was too young at time of death. Before the advent of modern molecular methods, in such cases sex was determined based on the context of the burial-grave goods, position of the grave etc. However, this can introduce a bias based on the prevailing theory about gender roles in the society, especially for preliterate societies. Molecular methods of sex determination allow the removal of this bias. In this study, we compared three previously described methods in human archaeological samples dated 15th–17th century AD. The classical method uses two separate PCR reactions, one for the AMELY/AMELX genes and one for the Y chromosome SRY gene. An updated method designed specifically for degraded DNA uses separate sets of PCR primers for the sense and antisense strands of AMELY/AMELX/SRY, while the Ry method compares the ratio of reads aligning to the X and Y chromosomes using DNA shotgun sequencing. The updated AMELY/AMELX/SRY PCR method was found to provide better sex determination than the classical one. The effectiveness of the Ry method was strongly impacted by the proportion of endogenous DNA to environmental contaminate (i.e., bacterial) DNA, with low ratios leading to fewer reads and unreliable results. This can be improved by using DNA enrichment methods to increase the ratio of endogenous reads; however, it increases the costs of analysis and does not guarantee a sufficient number of reads for sex determination. In conclusion, next generation sequencing is generally the preferred approach for archaeological samples, which will likely produce enough reads for the Ry method; however, the updated AMELY/AMELX/SRY method can provide extra validation or can be used on its own if sexing is the main question. Acknowledgements. This work was supported by the ERDF grant Nr.1.1.1.1/16/A/101. *The authors marked with an asterisk equally contributed to the work.

## P-02-014

## Polymorphism analysis of *Babesia canis canis* merozoite surface protein coding genes Bc28.1. and Bc28.2. isolated from Latvian domestic dog

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Canine babesiosis is a tick-born disease caused by various species of Babesia, an intraerythrocytic protozoan. Both genes, Bc28.1. and Bc28.2, are members of species-specific Bc28 gene family, coding erythrocyte binding proteins on the pathogen's merozoite surface necessary for invasion and interaction process with host immune system. The aim of this study was to perform DNA polymorphism analysis of Bc28.1. and Bc28.2. genes for B. canis canis samples of dogs in Latvia, to analyse the results in the context with 18S rRNA genotypes and compare to the genotypes reported in Europe. Overall, 36 of 238 clinical samples received from veterinarians in years 2016-2018 were B. canis canis-positive, and the full-length Bc28.1, Bc28.2. and 18S rRNA genes were sequenced. 18S rRNA 610/611GA genotype was detected in 16.7%, and the mixed GA/AG genotype – in 83.3% cases. Bc28.1. genotypes were distinguished on the basis on nucleotide substitutions in 20 positions; the variant IB was detected in 77.4%, genotype IA – in 16.1%, and one sample was heterozygous. Similarly, two main Bc28.2. genotypes were detected based on the basis on nucleotide substitutions in 36 positions: IIA and IIB. A high coincidence between genotypes was observed, as IIA and IIB matched Bc28.1. genotypes IA and IB in all samples but one. Only one sample had IA/IIB genotype, one sample was heterozygous for both Bc28.1 and Bc28.2. genes, and several samples had additional nucleotide substitutions. Only IB genotype matched a sequence available on the GenBank. Overall, the Bc28 genotyping results did not associate with 18S rRNA genotyping results, and high sequence heterogeneity of B. canis canis Bc28.1. and Bc28.2. genes was observed. As these parasite-specific proteins play a significant role in host-parasite interactions, further studies are required to evaluate the possible impact of this variation on the pathogenesis of babesiosis. Acknowledgements. This work was supported by the ERDF grant Nr.1.1.1.1/16/A/044.


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## P-02-016
## The molecular characteristics of *Chlamydia psittaci* strain from cattle isolated in the Southeastern European Region of Russia (Volga Region)

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Zoonotic Chlamydia comprises a group of obligate intracellular bacterial pathogens responsible for a variety of diseases in humans and animals. Here, the *Chlamydia psittaci* strain AMK isolated from cattle with Chlamydial genital infection was investigated by a genome-wide analysis. Chlamydia was grown in infected chicken embryo followed by DNA extraction with the Qiagen DNeasy Blood & Tissue Kit, and sequenced on the Illumina HiSeq 2500 platform. Overall, there were 2,376,938 pair-end reads (Sequence length 230, %CG 42) and 6,884,924 single-end reads (Sequence length 250, %CG 42). The filtration reads with low quality and adapter cutting was carried out with AfterQC software in default settings. For the genome assembly, reads were mapped to the 20 reference genomes showing 99% identity in BLAST analysis with our reads by using Bowtie2. The default Bowtie2 parameters were used to run the mapping, and then the sequences were aligned in Bowtie2 index and assembled using Spades v. 3.13.0. The subsequent BLAST analysis revealed that the chromosome of the AMK strain shared 99.98% nucleotide identity with the plasmid free *C. psittaci* GR9 (GenBank # CP003791.1). In contrast to GR9, the cryptic plasmid was detected in the AMK strain. After the genome assembly, 12 contigs with coverage of 160 were aligned and analyzed in Mauve software. The AMK demonstrated a marked polymorphism with 1,924 chromosomal SNPs versus the reference genome GR9, and 2 gaps in the plasmid in comparison with the reference WS RTE30 strain. The Multi-Locus Sequence Typing (MLST) based on the concatenated sequences of seven housekeeping genes with the use of a database hosted at http://pubmlst.org/chlamydiales/ as signed the AMK to ST28. The MLST analyses differentiated the AMK from GR9 within the same ST28 as both strains belonged to two different clades. In conclusion, this is the first report on detection of C. *psittaci* polymorphism characteristic to the strains in Russia. Grant RSF-No.-17-16-01099.

## P-02-017
## Association of DNMT1 rs8101626 polymorphism with the early miscarriage in Russian women

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Aberrant DNA methylation is an epigenetic change and human early miscarriage is one of its complications. DNA methylation is a process of the addition of methyl groups to cytosines of CpG dinucleotides, catalyzed by DNA methyltransferase (DNMTs). DNMT3a and DNMT3b are responsible for the establishment of de novo DNA methylation patterns whereas DNMT1 is required for the maintenance of DNA methylation patterns during cell divisions after embryo implantation and is considered to be essential for human embryonic stem cell viability. To the best of our knowledge, DNMT1 A>G rs8101626 polymorphism has never been investigated in women with early miscarriage, thus this study aimed to investigate the association of this polymorphism with idiopathic miscarriage in Russian women. 160 peripheral blood samples were collected from 100 women with early miscarriage and 60 healthy women living in Central Russia. The extracted DNA was genotyped using restriction fragment length polymorphism-PCR followed by agarose gel electrophoresis. Our study findings have shown that the minor genotype GG frequency in the patients with early miscarriage was significantly higher when compared with the healthy women (24.0%, 10.9% respectively; $P = 0.037$), but haven't shown significant differences between the other genotype and allele frequencies of patients with miscarriage (AA 28.0%, AG 48.0%; A 52.0%, G 48.0%) and healthy women (AA 36.4%, AG 52.7%; A 62.75%, G 37.25%). Women carrying the genotype GG are at higher risk of early miscarriage by 1.9 fold than non-carriers (OR: 2.64; 95% CI: 1.2–5.76). Consequently, we suggest that the DNMT1 rs8101626 polymorphism might be associated with the early miscarriage and further studies with a big sample size and other populations are required for confirmation. *The authors marked with an asterisk equally contributed to the work.

## P-02-018
## The association of polymorphism of bovine growth differentiation factor-5 gene with development of body measurements

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Variability in the DNA sequence causes intraspecific heterogeneity of traits, which is realized against the background of environmental factors. The search for functional DNA variants contributing to changes in the metabolic and phenotypic characteristics of the organism will provide higher accuracy in selection of farm animals. Thus, different allelic variants in growth differentiation factor-5 (GDF5) gene determine the morphogenesis of bones, ligaments and tendons in humans and animals. Taking into account the role of the GDF5 gene in growth and development of skeletal elements, the study of prevailing alleles and genotypes in cattle is of interest. The aim of the research work was to study the polymorphism of GDF5 gene-marker and identify its association with the variability of body measurements in Bos taurus. We identified single-nucleotide polymorphism (SNP) in the position T586C of exon 1 in GDF5 gene in the study based on the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) with genotyping 149 heads of Hereford bulls. The frequency of TT genotype was 7.38% and the proportion of heterozygous individuals was 12.08% in the studied animal population. At the same time, the following allelic distribution was noted in the GDF5 locus: C – 0.866 and T – 0.134. Genetic variability in the GDF5 gene had a significant effect ($P < 0.001$) on the development of hip height, chest girth, body length, withers height, chest width and hip width in animals at the age of 15 months. The $GDF5^{TT}$ variant is most preferable for breeding purposes. Homozygous genotype CC was accompanied by minimal linear growth of individuals. Thus, the results of the studies allow the use of single nucleotide polymorphism in the GDF5 gene in marker-assisted selection (MAS) in improving the exterior characteristics of beef cattle. This work was


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supported by the State Task No. 0761-2019-0012. *The authors marked with an asterisk equally contributed to the work.

## P-02-019
## Goths and genetics

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For years, the issues related to the origin of the Goths and their early migrations in the Iron Age have been a matter of hot debate among archaeologists. Unfortunately, the lack of new independent data has precluded the evaluation of the existing hypothesis. This report is a continuation of our previous works and concentrates on the human population that lived between the Vistula and Bug Rivers (contemporary eastern Poland) in the Iron Age. The genetic makeup of individuals living at that time in this region has not yet been determined. The biological material was collected from one of the richest Iron Age cemeteries located in east Poland near Maslomecz village. The cemetery is attributed to the Wielbark culture. Our analysis involved 27 individuals. We assigned mitochondrial DNA (mtDNA) haplogroups and determined complete mtDNA genome sequences for all of them. The studied group showed close matrilineal relationship with two other Iron Age populations living, respectively, at the Jutland peninsula and between the Oder and Vistula Rivers. Interestingly our analyses disclosed also the genetic connection between the Maslomecz population and ancient Pontic-Caspian steppe groups. In general, the collected results seem to be consistent with the historical narrative that assumed that the Goths originated in southern Scandinavia; then, at least part of the Goth population moved south through the territory of contemporary Poland towards the Black Sea region, where they mixed with local populations and formed the Chernyakhov culture. Finally, a fraction of the Chernyakhov population returned to the southeast region of present-day Poland and established the archaeological formation called the "Maslomecz group".

## P-02-020
## Selective sweeps patterns in *Neisseria gonorrhoeae* and *Neisseria meningitidis* genomes

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*N. gonorrhoeae* and *N. meningitidis* are phylogenetically closely related species, obligate human pathogens causing diseases in millions of people. The goal of the work was to analyze the genetic diversity of these pathogens and the role of selective pressure in the formation of linkage disequilibrium patterns. The selective sweep characteristics (w values) were calculated for 1302 N. gonorrhoeae and 489 N. meningitidis genomes (taken from https://pathogen.watch) using Omega plus software. The selective sweep revealed changes in SNP frequencies upon DNA recombination and allowed us to identify DNA regions with strong positive selection. Genes bearing sites with w value > 10 were detected including 19 genes for N. gonorrhoeae and more than 50 genes for N. meningitidis. For *N. gonorrhoeae*, there was a group of genes encoding iron-sulfur cluster protein, bacterioferritin and transferrin-binding protein participating in the modulation of cellular iron metabolism, one of the key pathways responsible for gonococcus intracellular survival in macrophages. Other groups included genes encoding proteins participating in the formation and modification of type IV-pili, key gonococcal adhesin: pilin-like protein (fimA gene), pilin glycosylation protein (capD), proteins PilV and PilX; group for efflux pump genes (mexA). Within the promoter region of mtr gene, a highest w was observed in position -10 where the insertion of T and TT causes the increase in the expression of MtrCDE efflux pump associated with the multidrug resistance. Thus, the results indicated that genome variations promoting immune evasion and antimicrobial resistance in *N. gonorrhoeae* are most important for natural selection. For *N. meningitidis*, groups with maximum w included proteins participating in amino acid biosynthesis, proteins belonging to transporters, and proteins for DNA repair and processing. The work was supported by the Russian Science Foundation (Grant No. 17-75-20039).

## P-02-021
## Association of the rs1107946 polymorphism of the COL1A1_1 gene with liver fibrosis

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The causes of liver fibrosis are hepatitis B and C, and alcohol abuse. Increased liver density is detected not only in liver diseases, but also in patients with chronic heart failure (CHF). During fibrosis formation the amount of extracellular matrix, which includes several types of collagen molecules, significantly increases in the liver. The aim of this study was to investigate the association between the COL1A1_1 C/A polymorphism (rs1107946) and liver fibrosis (F0–F4) in patients abusing alcohol and patients with chronic heart failure (F2–F4). The polymorphism of the COL1A1_1 gene was determined using the Real-Time PCR analysis. The stage of liver fibrosis was identified with the elastography (FibroScan, Echosens, France). There were examined 47 patients abusing alcohol and 42 patients with heart failure. Patients with hereditary liver diseases or infected by hepatitis B and C viruses were excluded from the study. The control group was comprised of 40 healthy men and women. It was found that the frequency of allele A among patients with liver fibrosis abusing alcohol is significantly higher than in the control group (38.3% vs. 12.5%; $\chi 2 = 7.6212$, $P = 0.005768$). The frequency of allele A among patients with heart failure is 9.5% that does not differ from the frequency of this allele in the control group ($\chi 2 = 0.817$, $P = 0.367$). Thus, our findings suggest that the presence of the minor allele A of the COL1A1_1 gene (rs1107946) predisposes to the liver fibrosis development in patients abusing alcohol, but not in patients with chronic heart failure. In the group of patients with chronic heart failure the density of the liver significantly decreased during therapy. Increased liver density in CHF is not completely associated with true fibrosis, but is largely due to fluid retention in the liver. The publication was prepared with the support of the "RUDN University Program 5-100".


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## P-02-022
## Distribution of EDN1 Lys198Asn polymorphic marker of cardiovascular diseases among population of South Africa

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Endothelin 1 is a potent vasoconstrictor peptide that acts through binding with ET-A and ET-B receptors of smooth muscle cells. Numerous studies have shown that polymorphism of the gene encoding endothelin 1 (*EDN1*) plays an important role in the pathogenesis of many cardiovascular diseases (CVD); however in other studies, such associations have not been identified, probably due to the ethnic characteristics of the studied populations. Therefore, we investigated the distribution of the *EDN1* Lys198Asn polymorphism among 34 healthy ethnic South Africans with the mean age of 22.52 ± 1.89 years. The genotypes were determined with the use of the real-time PCR. The Lys198Lys, Lys198Asn, and Asn198Asn genotype frequencies were: 70.59%, 29.41%, and 0% respectively, with the allelic frequencies of Lys198 and Asn198: 85% and 15%. The distribution shows a significant difference with the previously obtained results among Russians from Central Russia in genotype frequencies (Lys 198 Lys- 42.37%, Lys198Asn – 54.24% and Asn198Asn- 3.39%; $P = 0.00014$), as well as in allelic distribution (Lys198 – 70% and Asn198 – 30%; $P = 0.01776$). The obtained results demonstrate significant ethnic specificity in frequencies of alleles and genotypes for *EDN1* Lys198Asn gene polymorphism, which should be investigated in larger population samples and considered in further studies associated with cardiovascular diseases.

## P-02-023
## Activation of antioxidant cellular mechanisms and changes in the levels of oxidative cell-free DNA modifications in blood plasma of Wistar rats exposed to stress

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Acute and chronic stresses initiate production of endogenous triggers activating protective antioxidant mechanisms. One of these triggering "alarmins" is seemed to be oxidized cell-free (cf)DNA. The aim of the study was to investigate the changes in the levels of oxidative cfDNA modifications in blood plasma and *NRF2* (antioxidant factor) expression in peripheral blood lymphocytes (PBL) of Wistar rats exposed to stress. Different groups of rats were subjected to acute (2 h), subchronic (2 days) and chronic (11 days) stresses. CfDNA oxidative modifications were assessed by the content of 8-hydroxy-2′-deoxyguanosine (8-oxodG). *NRF2* expression was studied by flow cytometry. In all groups of animals exposed to stress a significant (2.5-8 times) increase in 8-oxodG concentrations in plasma samples has been observed. These findings suggest systemic oxidative stress development. In the same time, a significant increase of *NRF2* expression was found only in PBL of animals exposed to 11-day stress. Rats of this group were characterized with the maximal content of 8-oxodG in their plasma samples: 526.0 ± 215.4 relative units *vs* 64.7 ± 15.0 (control), 201.6 ± 60.2 (animals stressed for 2 hours) and 159.9 ± 90.2 (rats stressed for 2 days)/ In conclusion, our data indicate that both acute and chronic stresses resulted in increase in levels of oxidative cfDNA modifications in blood plasma of antioxidant cellular mechanisms (marked elevation of *NRF2* expression) can be found after chronic stress exposure. The work was supported by RFBR grant No. 17-29-06017 ofi_m, and as a state task of the Ministry of education and science of Russia.

## P-02-024
## Change of the satellite 3 (1q12) content in the genomes of cultivated human skin fibroblasts (HSF) under the genotoxic action of Cr(VI)

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Introduction. 1q12 region belongs to most unstable regions of the human genome. During replicative senescence of HSFs an increase in the content of satellite 3 (SatIII) was observed. One may assume that a genotoxic stress also alters the content of SatIII. Methods. We studied five HSF lines at fifth passage (p) with a known proliferative capacity (the Hayflick limits were 66, 61, 57, 49, 41 p) and a known abundance of SatIII in the genome (15, 14, 23, 25, 26 pg/ng DNA). HSFs were exposed to 4 μM or 6 μM K2CrO4 for 24 h. Further, the medium was replaced with a fresh one, and the cells were incubated for more 72 h. The genotoxic stress intensity was estimated by determining the amount of DNA on the carrier and in the medium and caspase 9 strength. SatIII content was measured in DNA using nonradioactive quantitative hybridization (NQH) of the DNA with a biotinylated probe for SatIII (1q12) sub-fraction. Results. K2CrO4 at 4 μM induced cell damage, which resulted in an arrest of HSF proliferation, but was not followed by an increase in apoptosis (moderate stress). At 6 μM, K2CrO4 stimulated apoptosis (severe stress). HSF-66 and HSF-61 with maximum Hayflick limit were characterized by the lowest Sat III content in the genome. In response to moderate stress, these cells increased the SatIII content by a factor of 1.5 to 2. In severe stress, the effect reduced: the SatIII content elevated by a factor of 1.3 to 1.4. HSF-57, HSF-49 and HSF-41 were characterized by a high content of SatIII in the genome (23–26 pg/ng DNA), and in response to moderate and severe stress they demonstrated a decrease in SatIII content by a factor of 1.2 to 1.5. Conclusions. There can be two scenarios of cell's stress response to a genotoxic impact: (1) cells with a high proliferative capacity increase the content of SatIII (1q12) in the genomes; (2) cells with a low proliferative capacity decrease the content of SatIII. The work is supported by RSF grant #18-15-00437.


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## P-02-026
## The association of bovine growth hormone polymorphism with morphological composition of carcass in cattle

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Regulation of tissues' growth and differentiation in the body takes place with the participation of growth hormone. The bovine DNA fragment encoding the growth hormone is localized on the 19th chromosome and consists of 5 exons and 4 introns (BTA 19, NCBI Reference Sequence AC_000176.1). There are several polymorphisms in somatotropin gene. The *Alu*I polymorphism is associated with the replacement of bases C-G in the 5th exon of the bGH gene, as a result the amino acid *Leu* is replaced with the amino acid *Val* (L127V, rs4192384). There is an association of various polymorphic variants of the bGH gene with different productive traits in cattle. The aim of our work was to study the association of *Alu*I polymorphism of bovine growth hormone gene with morphological composition of carcass and the tissues development in cattle. Hereford bulls were genotyped using technology of polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). Animals were slaughtered in 21 months old. The carriers of $GH^{VV}$ genotype (n = 5) were distinguished by a high content of muscle tissue by 8.10–15.78 kg ($P > 0.05$, $P < 0.05$), adipose tissue – 1.78–2.38 kg ($P > 0.05$), bones – 0.60–2.56 kg ($P > 0.05$, $P < 0.05$) in the half-carcass compared with individuals of other variants of GH gene. Bull-calves (n = 5) homozygous in allele LL were characterized by the minimal development of tissues in the body. There were no significant differences between carriers of different variants in GH gene locus according to the relative yield of studied tissues. Thus, a positive association of $GH^{VV}$ genotype with the growth of muscle, adipose and bone tissues in cattle was found. The obtained data can be further used for MAS-selection in beef cattle of Hereford breed. This work was supported by the task No. 0761-2019-0012. *The authors marked with an asterisk equally contributed to the work.

## P-02-027
## Effects of genetic variations in the downstream regulatory region of human TLR4 gene on transcription in monocytes and B cells

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Data obtained in population studies suggest a number of genomic loci that demonstrate robust associations to susceptibility to various disorders with defects in innate immunity. However, only experimental tests can uncover pathology-associated alleles that directly affect gene functions and cause individual variations in immune responses. As most disease-associated SNPs are located in non-coding regions, their effects are most likely mediated through differential binding of particular transcription factors (TF) to disease-linked allelic variants. Using *in silico* analysis of genetic and epigenetic data, these variations in the TF binding can be predicted with reasonable accuracy. To experimentally verify such predictions, we used immunoprecipitation of DNA-protein complexes from nuclear extracts followed by real-time PCR quantification of the bound DNA. We used such approach to annotate disease-associated SNPs in the 3′-untranslated region (3′-UTR) of human *TLR4* gene that we have previously demonstrated to strongly enhance the activity of *TLR4* promoter in human monocytes. Based on available pathology association data, TF-binding site prediction and epigenetic data, we focused on single nucleotide polymorphisms (SNP) rs41426344, rs77358523 and rs7873784. According to our preliminary data, the minor allele of the latter SNP binds PU.1 and enhances TLR4 transcription. The rs41426344 has been associated with the development of rheumatoid arthritis and juvenile idiopathic arthritis, while the area overlapping rs77358523 harbors strong epigenetic marks of a regulatory region. Effects of these genetic alterations on TLR4-driven luciferase reporter in human B cells have been studied in comparison to monocytes. This project is supported by grant 19-14-00341 from Russian Science Foundation.

## P-02-028
## Absolute quantification of cell free, circulating DNA from plasma, urine and saliva

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The circulating Cell Free DNA (cfDNA) is defined as the cell-independent, naked, double-stranded DNA detected in the blood. It can be obtained from plasma as well as serum, which can also be detected in different body fluids such as urine, synovial fluid, pancreatic canal secretions and saliva. cfDNA, isolated from peripheral blood, is a common procedure in diagnosis of patients with cancer as a tumor biomarker. cfDNA is commonly isolated from plasma; besides, the isolation of the quantity of cfDNA from saliva and urine is discussed in literature. In this study blood, saliva and urine samples were collected from both healthy donors and patients with different pathologies such as osteoporosis, rheumatologic disease and cancer. Healthy donors and patients age ranges were about 18–24, 25–40, 40–60, 60–80 years old. DNA was isolated from all samples using the EURx Bio-Trace DNA purification kit, the Macherey Nagel NucleoSpin tissue kit, and the Qiagen Blood and Tissue kit then measured by real time quantitative PCR (qPCR) and digital droplet PCR (ddPCR). Performed measurements were compared on qPCR and ddPCR, and comparison of cfDNA concentrations between patients' and healthy donors' samples was performed in plasma, urine and saliva samples separately. As a result of the analysis and statistical methods, it was determined that the amount of cfDNA from different body fluids of patients and controls was altered at different percentages. Using the analysis of cfDNA, reliable results were obtained in the diagnosis and treatment of cancer patients in literature. In our study, we can suggest to use the cfDNA analysis and that methodology can be considered to provide an alternative approach for the diagnosis and followup of various diseases instead of routinely used invasive methods.

## P-02-029
## Exome sequencing of acute myeloid leukemia – looking for a needle in a haystack

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Acute myeloid leukemia (AML), one of the most aggressive human cancers, is characterized by the disrupted differentiation and uncontrolled proliferation of hematopoietic progenitors in


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the bone marrow, followed by the infiltration of immature myeloblasts into the peripheral blood. Clinically and molecularly heterogeneous, AML remains one of the most widely studied malignancies. A spectrum of somatic mutations has been identified in genes relevant for AML pathogenesis, e.g. encoding multifunctional protein NPM1, transcription factors (CEBPA, RUNX1, WT1), signaling proteins (KIT, FLT3, JAK2, NRAS, KRAS, PTPN11) epigenetic regulators (DNMT3A, TET2, IDH1, IDH2) or spliceosome components (U2AF1, ZRSR2). The aim of the study was to identify mutations in a group of Polish patients with AML M1 and M2 FAB types, whose proteomes and transcriptomes were previously investigated. DNA was isolated from frozen blood or bone marrow cells collected from 37 patients and subjected to exome-enrichment procedure followed by high-throughput sequencing with Illumina platform. The data were mapped to the human genome GRCh38, genotyped with GATK (Genome Analysis Toolkit, Broad Institute) and filtrated with VQSR (Variant Quality Score Recalibration) method. Results were analyzed with VEP (Variant Effect Predictor, Ensemble). In total, over 800 thousand mutations (SNPs and small indels) were identified, including 66% existing and 34% novel variants. More than 187 thousand mutations were detected within transcripts, and close to 70 thousands within regulatory regions. About 40% variants were localized within introns whereas 2% were missense mutations. Potentially pathogenic mutations were detected in 37 out of 40 genes recurrently mutated in AML. On average, 4 genes were mutated per patient and the most frequently mutated genes were *KIT*, *NPM1*, *DNMT3A*, *NRAS* and *WT1*. Although the number is low, it corresponds with the literature data reporting AML has fewer mutations than other adult cancers.

## P-02-030
## Long-read sequencing of *Bacillus thuringiensis* strains reveals genome rearrangements affecting their virulence effectiveness

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*Bacillus thuringiensis* (*Bt*) is a spore-forming soil bacterium producing highly specific insecticidal toxins widely used as biopesticides. The unique feature of this bacterium is the ability to form so-called parasporal bodies or crystals during sporulation. These bodies contain proteinaceous toxins active against different organisms within Arthropoda, Nematoda and Mollusca phyla. Though *Bt* and their toxins are studied for more than 50 years, still very little is known about the molecular mechanisms controlling the parasporal bodies assembly. In this work, we perform comparative genomic analysis of 17 *Bt* strains obtained from the ARRIAM collection. These strains exhibit specific action against insects belonging to the Lepidoptera, Coleoptera and Diptera orders and are different in their ability to form crystals. Genome sequencing for the strains was performed via both Illumina and Oxford Nanopore platforms. The assemblies of their genomes reached the complete replicon level for most strains. We classified the strains with FastANI v1.1 as belonging to the *thuringiensis, darmstadiensis* and *israelensis* serovars. Further assembly comparison via Mummer v4.0.0 revealed several genome rearrangements mostly related to plasmid loss events to distinguish close strains. These plasmid vanishing acts appeared to be closely connected with virulence loss. However, genes forfeit this way encode not only canonical toxins, but also proteins responsible for cell wall biogenesis, stress response and sporulation as well several transcriptional regulators and RNA polymerase components. These genome rearrangements data suggest expansion of the repertoire of proteins governing virulence and parasporal bodies assembly in *Bt*. This work is supported by the Russian Science Foundation (Grant No 18-76-00028).

## P-02-031
## Genetic control of lipid biosynthesis in a collection of flax (*Linum usitatissimum* L.) cultivars and lines

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Fatty acid composition influences the quality of seed lipids, which are the major source of vegetable oil. The proportion of fatty acids determines the end use of oil, with the ratio of the unsaturated fatty acids, like oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), playing a key role. For human nutrition, flaxseed (*Linum usitatissimum* L.) plays an important role as a major source of essential ω-3 fatty acids. Despite the nutritional importance of fatty acid composition in seed storage lipids, the molecular mechanisms underlying the proportion of fatty acids in seed oil are still not fully established. Fatty acid biosynthesis involves several gene families, with some of them having already been identified, like *FAD* (fatty acid desaturase) and *SAD* (stearoyl-ACP desaturase) gene families. The purpose of our study was to analyze the role of these genes in the level of different fatty acids in seed oil. *SAD* and *FAD* genes were sequenced in 288 flax genotypes with different proportion of fatty acids, obtained from the Institute for Flax (Torzhok, Russia). DNA sequences with an average coverage of 100x for an individual sample were obtained on the Illumina platform and used to identify gene polymorphism among the genotypes. The correlation analysis between identified polymorphism of *SAD* and *FAD* genes and fatty acid composition in flax allows the determination of genetic variation leading to different proportion of fatty acids. These newly identified polymorphisms of flax genes, controlling the levels of fatty acids, will be a useful resource for marker development and marker-assisted selection of flaxseed cultivars with targeted fatty acid composition. This work was performed within the framework of the Program of fundamental research for state academies for 2013–2020 years (No. 01201363824) and was funded by RFBR according to the research project 17-29-08036.

## P-02-032
## Comparative chromosomal microarray analysis of doxorubicin-resistant MCF7 cells

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Drug resistance is the resistance of tumor cells to various functionally unrelated chemotherapeutics and is the major reason of unsuccessful chemotherapy. Since drug resistance drastically


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alters several cellular signaling pathways regulating survival, cell death and migration, it is vital to understand different mechanisms that lead to the drug-resistant phenotype. This study aimed to investigate genome-wide chromosomal copy number changes in doxorubicin-resistant MCF7 cells in order to reveal underlying mechanisms in drug resistance. Doxorubicin-resistant MCF7 cells were developed from drug-sensitive parents by stepwise selection of cells at increasing drug concentrations. Genomic DNA was isolated and chromosomal copy number changes were examined by Agilent Human Genome G3 SurePrint 8x60K ISCA oligonucleotide microarray. Data were analyzed using Agilent CytoGenomics software. Results showed that significant genome-wide changes affecting several cellular events contributed to overall drug-resistant phenotype. For instance, it was shown that there was a duplication in *ABCB1* gene encoding P-gp transporter whose upregulation was one of the predominant resistance mechanisms to doxorubicin in MCF7 cells. Moreover, a homozygous deletion was detected in apoptosis regulator *PDCD10* gene whose downregulation was previously reported as an important contributor of doxorubicin resistance in MCF7 cells by our group. Besides, the restoration of loss of *IL8* and that of heterozygous deletion in *ADAM9* metalloprotease may enhance proliferative and migratory activities of doxorubicin-resistant MCF7 cells. Investigation of genome-wide alterations in drug-resistant cells may not only provide a detailed understanding in drug resistance mechanisms but also propose new targets for chemosensitization studies. *The authors marked with an asterisk equally contributed to the work.

## P-02-033
## Sex-specific genomic locus in *Populus* x *sibirica*

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The genus *Populus* includes dioecious species, whose differences between male and female plants are actively studied at many levels. Recent works on the genetics of poplar sex allowed identification of sex-associated polymorphisms and genome regions of their localization. However, the key genes responsible for sex determination in poplar are still unknown, and the current version of the assembly of reference *Populus trichocarpa* genome does not allow accurate mapping of the sex locus. The present work was focused on the identification of sex-specific DNA polymorphism and high-quality genome assembly of *Populus* x *sibirica*, which is the most common poplar in Moscow. We collected forty samples of *P.* x *sibirica* plants (20 male and 20 female ones) in different regions of Moscow. For these samples, deep sequencing of genes encoding MET1 and homolog of ARR17, which are localized in sex-linked region of poplar and contain a number of sex-associated SNPs, was performed. We identified 17 (11 in MET1 and 6 in ARR17) sex-specific SNPs that were presented in all males but absented in all females. For identified sex-specific SNP sites, females were homozygous, while males – heterozygous. Moreover, colocation of sex-specific SNPs was revealed: in one allelic variant, males had the same nucleotides as females, while in the other one – sex-specific SNPs. Four out of 40 poplars representing typical male and female plants were subjected to the whole genome sequencing. The sequencing was carried out using the Nanopore platform for long reads and the Illumina one for high-precision short reads. Combination of these two platforms enables high-quality genome assembly and accurate mapping of the sex-specific genomic locus in *P.* x *sibirica*. The analysis of the obtained data brings us closer to the understanding of the mechanisms of sex determination in poplar. This work was funded by the RFBR, grant 18-34-20113 mol_a_ved.

## P-02-034
## Variants in TP53 gene associated with carotid paragangliomas

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Carotid paragangliomas (CPGLs) are rare tumors located near the bifurcation of carotid artery. Several genes are known to associate with the development of CPGLs; in particularly, somatic and germline variants were found in *SDHx, RET, VHL, IDH1, NF1, MEN1*, and *KIF1*. Nevertheless, molecular genetic mechanisms behind the tumor initiation and progression remain unclear. In this study, we performed exome sequencing of six tumor and normal tissues (lymph node and blood) from the same patients with CPGLs. Exome library was prepared using TruSeq Exome Library Prep Kit (Illumina). Sequencing was carried out on a NextSeq 500 System (Illumina) under paired-end model with read lengths of 76 bp (for tumor and lymph node tissues (FFPE)) and 151 bp (for blood). Minimum coverage was 300 × . Sequencing data were analyzed with the developed algorithms taking into account type of the samples (FFPE) and genetic feature of the tumor (low-mutated tumor). Germline and somatic variant calling was performed with VarScan and Mutect2. In two patients studied, we found known somatic variant M_000546.5: c.842A>T, p.Asp281Val (chr17: 7,577,096, rs587781525) and novel one NM_000546.5: c.A170A>G, p. Asp57Gly (chr17: 7,579,517) in *TP53* gene. The first variant was previously described in ClinVar with likely pathogenic/pathogenic clinical significance. These variants were co-occurred in the patients. They were also characterized by high allele frequency and were predicted as likely pathogenic variants in silico tools. Moreover, we observed likely pathogenic germline variant in proto-oncogene *RET* and pathogenic germline variant in *SDHD* gene in these patients (separately). Thus, based on the obtained results we assume two significant events that could be responsible for CPGL development – biallelic inactivation of *TP53* gene with genetic alterations of *RET* or that with disruptions in *SDHD* function. This work was funded by the Russian Science Foundation, grant 17–75-20105.

## P-02-035
## The role of *E. coli* AlkB and its human homolog – ALKBH3 dioxygenases and *E. coli* glycosylase AlkA in repair of acrolein adducts to adenine and cytosine

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1,$N^6$-α-hydroxypropanoadenine (HPA) and 3,$N^4$-α-hydroxypropanocytosine (HPC) are six-membered DNA adducts formed in reaction of adenine or cytosine with acrolein (ACR). ACR is a mutagenic agent originated from different sources including cigarette smoke and environmental pollutant. It is also generated endogenously during oxidative stress as a byproduct of lipid peroxidation. *E. coli* AlkB (EcAlkB) and ALKBH3 dioxygenases are DNA repair enzymes that remove alkyl lesions from bases via an


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oxidative mechanism restoring native DNA structure. They belong to the superfamily of 2-oxoglutarate (αKG) and Fe(II) dependent dioxygenases. *E.* coli 3-methyladenine DNA glycosylase II (AlkA) is a DNA-repair enzyme that removes lesions in DNA via the base-excision repair (BER) pathway. AlkA and AlkB are induced within *E. coli* system of adaptive response to alkylating agents (Ada response). Our *in vivo* data show that HPA and HPC have mutagenic properties. We have shown that both lesions are efficiently repaired by EcAlkA, EcAlkB and ALKBH3 enzymes. We have established optimal condition for repair reactions. Our data proved that the protonated form of HPA is preferentially repaired by EcAlkB and ALKBH3 *in vitro*. Moreover, EcAlkB has limited lifetime and reached 38 ± 4 enzymatic cycles before its total inactivation. Molecular modeling of the AlkA/HPA, AlkB/HPA and ALKBH3/HPA complexes demonstrated that substrate fits into the catalytic site of enzymes and the R stereoisomer in the equatorial conformation of the HPA hydroxyl group is strongly preferred. ALKBH3 is overexpressed in different type of cancer. The development of ALKBH3 inhibitors seems to be the appropriate way to support standard anticancer therapy based on alkylating agents. We synthetized several anthraquinone derivatives, potential ALKBH3 inhibitors, and indicated anticancer activity of some of them. Acknowledgements: This study was supported by NCN Poland, grant no UMO-2018/28/T/NZ1/00456

## Cardiovascular diseases

### P-03-001
### Improving patient stratification in systemic lupus erythematosus using endothelial microvesicles as novel biomarkers of cardiovascular risk

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease that confers increased risk of cardiovascular events; up to 50-fold in female patients < 45 years. Traditionally, SLE has not been included in risk prediction algorithms nor is there a standard biomarker for patient stratification. Endothelial microvesicles (EMVs) affect, and provide a snapshot of, vascular health through delivery of miRNA. This project aims to evaluate the use of EMVs as novel biomarkers for cardiovascular risk in SLE. Methods: Risk scores were calculated for 109 patients and 29 controls using QRISK3, and clinical markers of inflammation and vascular health recorded. Plasma EMVs were enumerated using flow cytometry and their miRNA content analysed by quantitative PCR. Gene ontology techniques identified predicted miRNA targets before miRNA overexpression and sponge lentiviral constructs were generated using Gateway cloning technology. Effects of miRNA expression on vascular calcification were elucidated using alizarin red staining and qPCR. Results: EMV numbers were elevated in SLE ($P = 0.001$), and correlated with risk score ($P = 0.001$) as well as vascular and inflammatory markers. MiR-3148 and miR-126-3p were found to be elevated in patient vesicles ($P = 0.009$ and $P = 0.048$ respectively). MiR-3148 overexpression in vascular smooth muscle cells resulted in reduced calcium deposition ($P = 0.002$), which was associated with decreased RUNX2 expression; effects of miRNAs on endothelial cells is ongoing. Gene ontology identified VE-PTP (PTPRB) as a shared target of miR-3148 and miR-126-3p, and RUNX2 as a target of miR-3148; confirmation of binding is underway. Conclusions: Understanding the interactions of vesicular miRNA at the vascular interface in SLE is vital for identifying novel therapeutic targets and supporting biomarker development. The use of EMVs as potential biomarkers shows great promise for personalised medicine, improving patient stratification and preventing premature mortality.

### P-03-002
### Total methylated arginine load as a risk factor in patients with masked hypertension

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Objective: Asymmetric dimethylarginine, symmetric dimethylarginine and L-monomethylarginine are derived from the subsequent proteolysis of methylated arginine residues on different proteins and inhibit the endogenous nitric oxide production. The alterations in total methylarginine load may contribute to hypertension. The aim of this study was to determine serum methylarginine levels in patients with masked hypertension. Materials and Methods: Control group, masked hypertension and hypertension groups consisted of 40 subjects (11 males, 28 females, mean age 48.6 ± 13.1), 28 subjects (14 males, 14 females, mean age 50.9 ± 11.0) and 36 subjects (15 males, 21 females, mean age 54.4 ± 12.3 years), respectively ($P = 0.149$). Serum methylarginine levels were analyzed by liquid chromatography-tandem mass spectrometry. Statistical analysis was performed with IBM SPSS v21 program. Results: While there was no statistically significant difference between control [0.147 (0.03–0.29)] and masked hypertension group [0.144 (0.05–0.42)] for serum symmetric dimethylarginine levels ($P = 0.970$), it was markedly elevated in hypertension group [0.25 (0.07–0.54)] compared to masked hypertension group [0.14 (0.05–0.42)] ($P = 0.001$). There was a significantly increasing trend for "total methylarginine load" for control, masked hypertension and hypertension groups (0.38 ± 0.13, 0.49 ± 0.16, 0.63 ± 0.23, respectively) ($P < 0.05$ for all). Conclusions: Serum symmetric dimethylarginine levels might be useful marker for determining the transition from masked to clinical hypertension. This is the first study evaluating the other methylarginine levels in addition to asymmetric dimethylarginine in hypertension groups. However, further studies with larger clinical groups are necessary to identify the possible relation between total methylarginine load and pathogenesis of masked hypertension. Key Words: Symmetric dimethylarginine, hypertension, total methylarginine

### P-03-003
### Evaluation of 25 hydroxy D3 vitamin levels in obese patients through laboratory data

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Vitamin D is a hormone that is studied on for long time, apart from the effect on calcium, phosphate metabolism, and it acts


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multimeric functions. In this study, it is aimed to investigate the relationship between vitamin D levels and obesity, which is shown in the scientific literature recently. Obesity is a serious syndrome identified for global pandemic. 25 (OH) D3 is a form of vitamin D that is often used to measure body D vitamin state. Data is obtained retrospectively from January 2010 to May 2017 from Kocaeli University Medical Faculty Education and Research Hospital Central Laboratory information system. Statistical evaluation is performed with IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ is considered as sufficient for statistical significance. As a result of statistical analysis, 25 OH D3 levels are found significantly lower in the adult obese group than in the adult patient without chronic disease group ($P < 0.001$). Significantly higher levels of hormones are detected in males compared to females in the analysis ($P < 0.001$). There is a strong positive correlation and significant difference between age groups and D vitamin levels ($r = 0.63$, $P < 0.001$). Season makes significant difference in vitamin D levels ($P < 0.001$). The highest hormone levels are found in autumn and the lowest hormone levels are found in winter. In this research, we find lower D vitamin levels in obese group than non-obese. Besides, further researches are needed to understand the relationship between D vitamin and obesity.

## P-03-004
## Water deprivation shortens primary cilia length in the kidney tubular cells

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The primary cilium is a structure which protrudes from the cell surface and its core is made up of microtubule which consists of a-tubulins. The primary cilium plays an important role for maintenance of cell homeostasis by sensing and transducing extracellular signals. In the kidney, the length of the primary cilium is related to the development of a number of human diseases. Here, we investigated whether water deprivation which is highly associated with renal function affects the primary cilium length and its underlying mechanisms in the kidney tubule cells. Mice were intraperitoneally injected daily with either Tubastatin A (Tub A), a histone deacetylase 6 (HDAC6) inhibitor, or a vehicle from 2 days before water deprivation until sacrifice. In this study, water deprivation increased urine osmolarity and shortened primary cilia length in mouse kidney tubular cells. Water deprivation decreased ac-α-tubulin, sec10, and α-tubulin transferase expression. In Madin-Darby canine kidney (MDCK) cells, treatment of NaCl or mannitol into the culture medium shortened primary cilia length and decreased in ac-α-tubulin, sec10, and α-tubulin transferase expression. Tub A treatment prevented the water deprivation-induced increase of urine osmolarity, shortening of primary cilia, and decreases in ac-α-tubulin, sec10, and α-tubulin transferase expression. These findings demonstrate that primary cilium length is affected by water deprivation and osmolarity changes, suggesting that primary cilium plays a critical role in osmotic response and regulation of water homeostasis.

## P-03-005
## Quercetin attenuates hyperosmotic cytotoxicity

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Polyphenols represent a vast group of natural substances designated as secondary metabolites of plants. They are generally characterized as antioxidants. Nowadays, the group contains more than 6,000 compounds. These substances offer many benefits for human health such as radical scavenging and anticancer or anti-inflammatory properties. The literature also discusses their impact in signalling pathways. One of the most famous representatives is quercetin which is commonly found in fruits, vegetables, beverages and spices. 2,3-Dehydrosilybin is another interesting member of polyphenol group. It is a minor constituent of silymarin, a standardized extract from *Silybum marianum*. Both compounds are frequently tested for their cardioprotective potential. For example, several studies were conducted to investigate pre-conditioning and post-conditioning in ischemia/reperfusion injury experiments. In this study, attenuation of cytotoxicity caused by hypertonic stress, which occurs, amongst other cases, during ischemic disease, was investigated to reveal the effect of these polyphenols and their mode of action. The H9c2 cell line was used as cell model for all experiments. Neutral red assay showed that quercetin has stronger protective effects against hyperosmotic stress than 2,3-dehydrosilybin. On the other hand, antioxidant potential of both compounds is not associated with observed protection because dihydrofluorescein diacetate ROS assay did not reveal any differences between polyphenol-treated samples and non-treated controls. Western blot analysis of expression of PKC-ε, p38 and other proteins revealed divergent effects of quercetin and 2,3-dehydrosilybin. While 2,3-dehydrosilybin retains its effect on PKC-ε phosphorylation, quercetin appears to modulate p-38 in favour of cytoprotective activity. Acknowledgements: This study was supported by grants LO1304 and IGA_LF_2019_015.

## P-03-006
## Plasma platelet polyphosphate level is lowest in thrombocytosis and is not affected by antiplatelet drugs

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Background: Polyphosphates (polyPs), anionic linear polymers of orthophosphate, are present in the dense granules of human platelets and are released following activation. They are supposed to induce thrombosis and infection, linked with the activation of coagulation factor XII; however, their precise biological effects have not been fully investigated so far. The aim of the present study was to measure the platelet polyPs in patients with proper and abnormal platelet count. Patients and methods: 38 patients with hematological disorders and 22 healthy subjects were included in the study. Patients were divided into 3 subgroups according to their platelet count: thrombocytopenia (< 140 G/L),


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thrombocythemia (>440 G/L) and normal platelet count. All of them were on 75 mg of aspirin mainly due to stable coronary artery disease. PolyPs were isolated from lysed platelets, obtained from citrated platelet-rich plasma. The procedure included inactivation of endogenous phosphatases, removal of phosphate units derived from DNA and proteins, and finally hydrolysis into monophosphate units. Quantification was determined using the colorimetric assay utilizes malachite green and ammonium molybdate. Results: The level of polyPs was twice lower in control subjects in comparison to patients (215 vs. 427 μM, $P < 0.001$). Among patients, the lowest level of polyPs was observed in thrombocythemia (273 μM), and the highest in thrombocytopenia (638 μM). Moreover, the polyPs level in thrombocytopenic patients was statistically different from patients with normal and elevated platelet count ($P < 0.05$). Any potential influence of antiplatelet drugs (acetylsalicylic acid or clopidogrel) on the polyPs level was not observed. Conclusions: Level of polyPs differs among patients with hematological disorders and is associated with platelet count. A possible compensation mechanism may exist to prevent bleeding in thrombocytopenia.

## P-03-007
## Conditions for isolation and culture of cells obtained from abdominal aortic aneurysm (AAA) from patients treated surgically

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Abdominal aortic aneurysm (AAA) is a multifactorial clinically heterogenic disease, which leads to blood vessel rupture and patient's death in great measure. Therefore, finding a specific prognostic marker, which is associated with the presence and progression of AAA is very crucial. The aim of the study was to standardize the methods for isolation and culture of cells from AAA specimens and determination of their phenotypes. The material for the research was specimens of aortic walls obtained from patients with AAA subjected to surgical treatment. The AAAs were divided longitudinally into 3 fragments, upper central and lower. Each segment was subjected to separation of the wall layers into inner, medium and external. Isolated cells were cultured in an appropriate standard culture media were analyzed by flow cytometry, immunochemistry and macroscopic methods. The cells from specific specimen were compared to control commercial cell types from healthy human abdominal aortas wall layers. Cells from specific layers of aorta of all patients presented different time to reach primary confluence and different proliferation potential. In all the layers only small fractions of cells presented layer specific cell type markers. Majority of the cells from every layer were the cells positive for CD90 considered as fibroblast marker. The cells from the layers did not show the expected morphology, such as for endothelium in the inner layer, smooth muscle in the middle and fibroblasts in the external layers. The studies here have confirmed that in the wall of the AAA there is a lack of specific cell type composition in comparison to the normal abdominal aorta wall. Further studies are planned on the cells molecular pathobiology in AAA, as well as the cross-talk between the ECM and the cells in AAA. *The authors marked with an asterisk equally contributed to the work.

## P-03-008
## ITGB3 gene PLA polymorphism affects the tolerance to the antihypertensive therapy in patients with dyslipidemia

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Abnormalities in platelet integrin receptors are often associated with cardiovascular diseases. The PLA polymorphism of the beta3 subunit modulates the function of alpha (IIb) beta3a platelet integrin receptor GPIIb/IIIa and attributes to polymorphism 1565 T>C (rs5918) in ITGB3 gene. We examined whether this polymorphism influences cardiovascular complications and drug therapy tolerance to evaluate the doses and duration of antihypertensive and cardiotropic therapies in patients with dyslipidemia. A study group of 92 patients with dyslipidemia aged 40 to 71 years were measured for genotype using genomic DNA extracted from peripheral blood cells. Polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) assay was used to detect PLA polymorphism in ITGB3 gene. The genotypes PLA1/PLA1 and PLA1/PLA2 had ratio of 63% (n = 58) and 37% (n = 34), respectively. In subgroup with PLA1/PLA1 genotype coronary heart disease (CHD) was observed in 28 patients (48.2%), including one complicated by myocardial infarction and/or by rhythm disturbance as in atrial fibrillation – in 10 patients (37%). In the subgroup with genotype PLA1/PLA2 the CHD was observed in 20 patients (58.8%), including one complicated by myocardial infarction and/or by rhythm disturbance as in atrial fibrillation – in 14 patients (70%). Hypertensive heart disease was observed in 48 patients (83%) with PLA1/A1, and in 34 persons (100%) in the group of carries PLA1/A2. Monotherapy or combined therapy with antihypertensive drugs of the main groups was applied for the target blood pressure 140/90 mm Hg. Volume of basic antihypertensive therapy was used in groups of patients with dyslipidemia regarding different genotype status. The results proved evidence that PLA2 allele correlates with cardiovascular complications in patients with dyslipidemia and more complex and durable the antihypertensive therapy. The publication was prepared with the support of the "RUDN University Program 5-100".

## P-03-009
## Variants of FTO and GCKR genes associated with type 2 diabetes-related traits in population from Bosnia and Herzegovina

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BACKGROUND-AIM The aim of this study was to investigate the association of two single nucleotide polymorphisms (SNPs) – *rs8050136* in *FTO* gene and *rs1260326* in *GCKR* gene with parameters of type 2 diabetes (T2D) and diabetic dyslipidemia in the population of Bosnia and Herzegovina (BH). METHODS 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 genes *FTO (rs80501356)* and *GCKR (rs1260326)* were analyzed by using Real Time PCR. RESULTS In group of diabetic patients, significant associations were reported for *rs8050136* in *FTO* gene with very low-density lipoproteins – VLDL ($P = 0.045$) and alkaline phosphatase – ALP ($P = 0.020$). In the control group significant


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associations were observed for polymorphism *rs1260326* in *GCKR* gene with waist circumference ($P = 0.006$; $P = 0.003$) and fasting glucose levels ($P = 0.05$). In the group of diabetic patients, results showed a significant association of *rs1260326* in *GCKR* gene with levels of bilirubin ($P = 0.004$). CONCLUSION This is the first study that examined the impact of variations of candidate genes on a wide range of metabolic parameters in BH population. Our results suggest an association of variations of *FTO* and *GCKR* 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-03-010
## Cardiovascular disease classification using Convolution Neural Network based on deep learning

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Cardiovascular diseases are the major causes of death all over the world, and in case of Korean they are the second causes of death behind cancer. So it is important to manage and treat them. Recently, data related to cardiovascular diseases are collected, and it is possible to combine a variety of data and predict through big data analysis and deep learning algorithm. Also, research for better prediction model is performed. In this paper, using KAMIR data we searched the factors which influence to cardiovascular diseases, and based on them, we developed classification model using CNN (Convolution Neural Network) algorithm of deep learning. Using this model, we classified the patients of STEMI and NSTEMI which are classification standard about cardiovascular disease patients and predicted the accuracy. First, we selected 45 attributes as feature values for input variables among the whole attributes of KAMIR data which is database related to cardiovascular diseases and used definitive diagnosis (STEMI, NSTEMI, UAP, Other) variables with four categories as output variables. The total accuracy is comparatively high as 93.6% after applying Heatmap and CNN algorithm. We proved that the accuracy of STEMI and NSTEMI with a lot of learning data is very high and UAP and others with relatively small learning data has very low accuracy. Based on this research, it is possible to solve the problems that we usually miss important feature selection in case we use general statistics based analysis about a large volume of data and develop the classification model using machine learning algorithm. CNN is deep learning algorithm which has best performance for image classification of clinical and biodata and it is possible to use for early diagnosis and prognosis prediction of a variety of diseases.

## P-03-011
## Intrinsic cardiomyocyte apoptosis activation in acute myocardial ischemia

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Coronary heart disease is the leading cause of death in medium- and high-income countries. Dysfunction of the left ventricle (LV) in acute focal myocardial ischemia is predominantly linked with cardiac remodeling and following development of heart failure. In spite of the availability of considerable amount of data concerning adaptive responses to ischemic injury of the myocardium there is still no definitive concept describing mechanisms of myocardial remodeling. Experiments were carried out on 16 male Chinchilla rabbits with body mass 3…3.5 kg. The animals were divided into 4 groups (4 animals in each): 1 control group (intact animals) and 3 experimental groups (rabbits with 1, 3 and 5 day acute focal ischemia of the LV myocardium). Focal ischemia was modeled by ligation of the descending branch of the left coronary artery between its middle and lower thirds. Intrinsic cardiomyocyte apoptosis activation was examined by estimating the content of anti-apoptotic BCL-2 and pro-apoptotic BAX proteins in cardiomyocytes of the peri-infarct zone using immunohistochemical assay. It was found that the content of these proteins was significantly increased compared with the controls at all the experimental terms. The content of BCL-2 increases on days 1, 3 and slightly decreases on day 5 (26.68, 42.59 and 23.22 vol.% correspondently vs 0.04 vol.% in the controls). The content of BAX increases on day 1 and decreases on days 3 and 5 (42.39, 20.29 and 4.11 vol.% correspondently vs 0.55 vol.% in the controls). BCL-2/BAX ratio showed an increasing grow at all the terms of the experiment (0.63, 2.10 and 5.65 on days 1, 3 and 5 correspondently). This indicates to inhibition of apoptosis at the later stages of the experiment against the background of primary induction of mitochondrial pathway of apoptosis activation. It might be concluded that predominance of BCL-2 over BAX synthesis provides cardiomyocyte survival at the expense of the internal cellular reserves.

## P-03-012
## Correlation of Beclin 1 dependent cardiomyocyte autophagy with morphological changes of the myocardium under acute ischemic injury

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Autophagy occurring under different pathological conditions is considered to be the mechanism either involved in regulated cell death or underlying cell survival due to mobilization of internal cellular resources. In case of myocardial ischemia positive regulation of cardiomyocyte autophagy is characteristic of cardioprotective adaptation. At the same time excessive activity of autophagy may lead to cell death. Experiments were carried out on 16 male Chinchilla rabbits with body mass 3…3.5 kg. The animals were divided into 4 groups (4 animals in each): 1 control group (intact animals) and 3 experimental groups (rabbits with 1, 3 and 5 day acute focal ischemia of the LV). Focal ischemia was modeled by ligation of the descending branch of the left coronary artery between its middle and lower thirds. Cardiomyocyte autophagy was studied using immunohistochemical detection of Beclin 1 protein (BECN1). Morphometric examination of the peri-infarct myocardial area was also carried out. Immunohistochemical study revealed a significant increase in the content of BECN1 on days 1, 3 and 5 with a descending trend ($18.42 \pm 0.03$, $13.98 \pm 0.03$ и $7.87 \pm 0.02$ vol.% correspondently) in comparison with the control group ($2.77 \pm 0.004$ vol.%). The descending tendency had a strong negative correlation with the increasing destruction changes (-0.96) in the myocardium and also a strong positive correlation with a decreasing quantity of morphologically safe myofibrils of the peri-infarct


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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area (+0.97) and with nuclear-cytoplasmic ratio (+0.99) at all the experimental terms. These findings may be explained by a primary reparative effect of autophagy which is reduced under increasing destructive events in the myocardium of the peri-infarct zone.

## P-03-013
## Short-term e-cigarette vapor exposure causes vascular oxidative stress and dysfunction – evidence for a close connection to brain damage and a key role of the phagocytic NADPH oxidase (NOX-2)

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Tobacco smoking is among the leading risk factors for global deaths and life years spent with severe illness/disability. In contrast, the clinical impact of E-cigarette smoking is not characterized in detail but this life style drug also causes vascular damage via oxidative stress and inflammation. The underlying mechanisms, especially, the enzymatic sources of oxidative stress are not well characterized. Here, we investigated effects of phagocyte-type NADPH oxidase (Nox2) knockout on vascular and cerebral oxidative stress and inflammation in response to short-term e-cigarette vapor exposure in mice. C57BL/6j and Nox2-/- (gp91phox-/-) mice were exposed to e-cigarette vapor from liquids without flavor and plus/minus nicotine for 1, 3 and 5d. Adverse effects of e-cigarette vapor on the vasculature and brain were mostly prevented by Nox2 deficiency. The exposure to e-cigarette vapor without nicotine for 3d had the most detrimental effects characterized by endothelial dysfunction, increased markers of vascular/systemic oxidative stress and inflammation. The toxic lipid peroxidation product 4-hydroxynonenal was increased in aorta, heart and lung of exposed wild type but not Nox2-/- mice. E-cigarette vapor also caused increased blood pressure as well as cerebral oxidative stress and inflammation, nNOS mRNA and protein downregulation. Treatment of cultured human endothelial cells with e-cigarette vapor condensate and e-cigarette liquid revealed a more toxic effect of the condensate on cell viability and phenotype (IL-6, NOX-2), which is potentially related to the toxic aldehyde load of the condensate (HPLC and LC/MS analysis). E-cigarette vapor exposure increases vascular and cerebral oxidative stress via NOX-2 leading to vascular inflammation and endothelial dysfunction in mice (and men). Our data point to similar pathophysiological pathways for e-cigarette smoking as reported for tobacco smoking and other metabolic or environmental health risk factors. *The authors marked with an asterisk equally contributed to the work.

## P-03-014
## Role of the IL-10 C819T gene polymorphism in development of coronary artery restenosis

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One of the important limitations after endovascular treatment of coronary artery disease (CAD) is coronary in-stent restenosis (ISR). Recent studies demonstrate the pivotal role of inflammatory responsiveness in the development of restenosis after percutaneous coronary intervention (PCI). The key mediator in the inflammatory process is interleukin (IL)-10, which association with CAD has already been revealed. Our aim was to study the polymorphism C819T in IL-10 gene as predisposing risk factor in the development of ISR in patients with stable coronary artery disease after balloon angioplasty and drug-eluting stent (DES) implantation. 112 patients and 62 healthy individuals participated in the study. All of them were Russians. The patients were divided into two groups – those with significant ISR (n = 52) and those without ISR (n = 60). All patients underwent coronary revascularization with DES and had subsequent coronary angiography performed in a year after primary PCI. Total DNA was extracted from the blood and genotyped using the Real-time PCR. The C819T polymorphism in IL-10 gene was analyzed. The Chi-square test and Fisher's exact test were used to estimate differences between groups. No significant differences between patients with ISR and without it were found. After stratification according to age, we revealed that the TT homozygotes are more frequent among patients > 60 years old with ISR comparing with patients < 60 years old (11% vs 4%, respectively; $P < 0.05$). We suggest that the inflammatory factors involved in the development of restenosis differ in different age groups of patients but to certify this idea it is necessary to continue research in bigger samples.

## P-03-015
## Novel role of HDAC7/MLCP-mediated signaling in vascular injury models

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Acute lung injury (ALI) is an acute systemic inflammatory process arises from a wide range of lung injuries such as toxins or inflammatory mediators. A major cause of ALI is dysfunction of the pulmonary vascular endothelial barrier. Epigenetic mechanisms have emerged as a major therapeutic potential of inhibiting histone deacetylases (HDACs) for the treatment of cardiovascular and inflammatory diseases. Our data indicate that the HDAC7 is involved in the regulation of barrier function in human lung microvascular endothelial cells (HLMVEC) and is regulated by phosphorylation. We showed that lipopolysaccharide (LPS), increases the level of phosphorylation of HDAC7 while barrier protective agents like adenosine, decrease its phosphorylation. We showed that HDAC7 is a substrate for MLCP in HLMVEC. Conversely, HDAC7 directly or indirectly affect MLCP activity. However, the physiological role of HDAC7/MLCP crosstalk in EC barrier regulation is currently unexplored. Our newly published data suggest that MLCP activation is involved in EC barrier preservation via the dephosphorylation of unconventional MLCP cytoskeletal targets such as moesin. Our results represented a direct association between HDAC7 and


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moesin, thus suggesting a novel role of an HDAC7/MLCP/moesin signaling complex in the cytosol. Further, we showed the direct association of MLCP with talin, a cytoskeletal protein that connects actin filaments with integrins. Talin interacts with moesin and we now show that talin regulates stress fiber formation and EC permeability. The role of the HDAC7/MLCP interaction in the ability of MLCP to dephosphorylate cytoskeletal regulatory targets and thus strengthen the EC barrier remains ill-defined and will be characterized in our future experiments. Collectively, these studies will define the role of a novel HDAC7/MLCP-mediated signaling mechanism that regulates the EC barrier and define, for the first time, its importance in vascular injury models *in vitro* and *in vivo*.

## P-03-016
## New recombinant anticoagulants from the salivary cell secretion of medicinal leech

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Thrombosis is the most common pathology underlying many vascular disorders with the high risk of death and, therefore, represents a valuable problem for public health and national economy. Hirudin, one of the most known anticoagulants, is a peptide originated from the salivary cell secretion of medicinal leech. It specifically suppresses thrombin, a key enzyme of hemostasis. Besides hirudin, the salivary cell secretion of medicinal leech contains peptide inhibitors of serine proteases. Many of these inhibitors are known to interact with enzymes of the coagulation cascade. Recently, we have reported transcriptional profiling of the leech salivary cells and proteomic study of the salivary cell secretion. Analysis of the transcriptomic and proteomic data revealed unknown peptides putatively acting as inhibitors of proteases. Among these peptides, we could identify two unknown isoforms of hirudin and several forms of putative antistasins and bdellins. To reveal their effects on hemostasis, the peptides of interest (POIs) were synthetized as recombinant proteins in E. coli. Peptide synthesis was not suitable to produce these POIs because of their high molecular weight (7–10 kDa). When POIs were produced as individual peptides, there was no accumulation of POIs in the bacterial cells. Therefore, to produce three isoforms of hirudin and one isoform of bdellin we fused these POIs with SlyD chaperon. After extraction and purification, the fused POIs did not exhibit suppression of thrombin. Therefore, the fused POIs were cleaved by TEV protease. Separation of individual POIs was performed by ion-exchange chromatography using DEAE-sepharose. Purified individual hirudins exhibited inhibition of thrombin in a test with fibrin gel formation. Similarly, we produce new recombinant putative anticoagulants identified in the salivary cell secretion of medicinal leech to test their effects on hemostasis. This work was supported by the Russian Science Foundation (No. 17-75-20099).

## P-03-017
## Effects of curcumin on the expression of PPARα, MCAD, ACAA2, VLCAD and CPT1 in heart of mice fed with high fructose diet

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Cardiovascular diseases are a global public health problem. The high-fructose diet intake is a risk factor for the development of the metabolic syndrome and cardiovascular diseases by inducing oxidative stress and dyslipidemia. It was suggested that curcumin reduce the oxidative stress and improve the lipid metabolism, exerting a protective effect on cardiovascular system. This study analyzed the effects of curcumin on the expression of proteins involved in lipids metabolism (PPARα, MCAD, VLCAD, ACAA2 and CPT1) in the heart of mice fed with high fructose diet. Six-week-old male C57BL/6 mice were divided in four groups (n = 6) and were treated for 15 weeks as follow: 1) standard diet (C), 2) standard diet + 0.75% (w/w) of curcumin (C+Cur), 3) standard diet + 30% (w/v) of fructose (F) and 4) standard diet + 0.75% (w/w) curcumin + 30% (w/v) fructose (F+Cur). At the end of the treatment, the body weight gain and serum concentration of total cholesterol were determined. PPARα, MCAD, VLCAD, ACAA2 and CPT1 expression in the heart was determined by Western blot. Mice of the group F gained more weight than the group C (p

## P-03-018
## MMP activity and the content of OPG, RANKL and collagen in vascular calcification

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The presented work was devoted to determination of activity levels of MMPs involved in vascular calcification, and to study correlation between MMP activity and OPG, RANKL and type I collagen content. Interaction of OPG with the RANKL/RANK system is important for activation of MMPs. OPG is able to increase the local tissue activity of MMP-2, -3 and -9, and through the activation of MMP, the OPG can affect the extracellular matrix state. Therefore, we tried to find the relationship between the activities of some MMPs and such components as OPG, RANKL and type I collagen. The samples of aortic valve leaflet and part of the aneurysmatically expanded ascending aorta were taken intraoperatively. The main group consisted of patients with aortic stenosis (AS), comparison group – patients with aortic aneurysm (AA). The activity of MMP was determined by zymography method and OPG, RANKL and type I collagen content in tissue was determined by immunoblotting. For patients with AS the level of RANKL was higher in the valve than in the tissue. The data of MMP activity showed that in control the MMP-1 activity is practically zero and maximum value was at AS. The MMP-2 activity is the same for AS and control, and statistically greater at AA. The MMP-9 activity was maximal at AA. At study of correlation between the MMPs activity and


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the content of proteins the strong positive relationship was found for MMP-9 and OPG. The strong positive relationship was found for MMP-2 and RANKL, as well as for MMP-9 and collagen. The strong negative relationship was revealed for MMP-2 and OPG, as well as for MMP-2 and collagen. At AS group, all relations between MMPs and OPG, RANKL and collagen were weaker than in control. At AA the relationships for MMP-1 and RANKL, MMP-1 and RANKL were slightly positive. Given study has two significant limitations: a small number of patients and the lack of aortic valves without calcification for comparison. This work was supported by the RFBR grant 18-015-00016. *The authors marked with an asterisk equally contributed to the work.

## P-03-019
## The changes in the actin-myosin interaction in left and right ventricular myocardium in experimental diabetic cardiomyopathy

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Diabetes mellitus (DM) type 1 leads to diabetic cardiomyopathy. The left (LV) and right (RV) ventricles differ structurally and functionally in norm and pathology. We studied the changes of myocardial structure and mechanical function of single cardiomyocyte and contractile proteins from the LV and RV in diabetic cardiomyopathy induced by DM type 1. The study followed the Directive 2010/63/EU. DM was induced in male Wistar rats and confirmed by increasing the glucose concentration and glycosylated hemoglobin level. The calcium regulation of the myosin interaction with native thin filaments (NTF) was studied in an *in vitro* motility assay. NTF containing actin, tropomyosin, and troponin were extracted from the LV and RV of healthy rats. The calcium dependence of the sliding velocity of NTF (*p*Ca-velocity relationship) was analyzed by Hill equation. We found that DM affects the cardiomyocyte diameter in the LV, but not in the RV. Experiments on single isolated cardiomyocyte showed that DM prolonged contraction and relaxation of cells. Significant differences in the contraction characteristics of cells were found between the LV and RV only in rats with DM. No differences in the sliding velocity of NTF at saturating calcium concentration were found between the ventricles in both control and diabetic groups. The maximal sliding velocity of NTF over myosins of DM rats decreased by 30% compared with the control group. Calcium sensitivity of the *p*Ca-velocity relationship of myosin of DM rats was lower by 0.2 *p*Ca. Thus, DM affects significantly the calcium regulation of the actin-myosin interaction in the LV and RV. The results may be explained by a shift of the myosin heavy chain isoforms and oxidative damage of cardiac myosin. This multi-scale study showed that myocardial structure and function of the LV and RV change differently in diabetic cardiomyopathy induced by DM type 1. Work was supported by the RSF No. 18-74-10059 and performed using the equipment of SRC IIP UB RAS.

## P-03-020
## Recombinant destabilase may cleave isopeptide bonds in stabilized fibrin in aged blood clots in vitro

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A significant disadvantage of existing thrombolytics is the fact that the effectiveness of drugs is determined by the "age" of the thrombus: the older the clot, the more difficult it is to lysis by proteases. Fibrin, which is the basis of the clot, undergoes stabilization due to the formation of strong isopeptide bonds that prevent the penetration of proteases inside the thrombus. Formation of the isopeptide bonds is mediated by factor XIIIa. Destabilase is an enzyme secreted by the medicinal leech salivary cells possessing endo-ε-(γ-Glu)–Lys isopeptidase activity that is responsible for isopeptide bond cleavage. In this work, we describe the mechanism of thrombolysis caused by destabilase action and called "isopeptidolysis" *in vitro*. We used blood clots extracted from patients during surgery. The clots were dried, weighed and treated with a recombinant destabilase within 24 hours. Then they were dried and weighed again. We found that destabilase causes a 27.7 ± 1.8% decrease of weight in comparison to control (9.1 ± 0.6) with significance level $P = 0.0134$. Next, we incubated blood clots in 2% acetic acid and determined the degree of destabilization of blood clots by assessing the amount of protein-peptide material by measuring absorption at 280 nm. We found that the untreated clots barely dissolved in 2% acetic acid, while the clots treated with destabilase dissolved completely. Comparison of the optical density of 2% acetic acid solutions containing destabilized fibrin of residual blood clots showed a significant difference from the control sample ($P = 0.0059$). This approach allows us to assess the direct impact of thrombolytic agent (destabilase-isopeptidase) not only on the weight of the thrombus but also on the level of its stabilization. We believe that treatment with destabilase will increase the overall effectiveness of conventional thrombolytic drugs. This work was supported by the Russian Science Foundation (project No. 17-75-20099).

## P-03-021
## CTRP1 keeps blood pressure through vasoconstriction under dehydration conditions

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Circulating C1q/TNF-α-related protein 1 (CTRP1) levels are increased in hypertensive patients compared to in healthy subjects. Nonetheless, little is known about the molecular and physiological function of CTRP1 in blood pressure (BP) regulation. CTRP1 production was increased to maintain normotension under dehydration conditions, and this function was impaired in inducible CTRP1 knockout mice (CTRP1$^{\Delta CAG}$). The increase in CTRP1 under dehydration conditions was mediated by glucocorticoids, and the antagonist mifepristone prevented the increase in CTRP1 and attenuated BP recovery. Treatment with a synthetic glucocorticoid increased the transcription, translation, and secretion of CTRP1 from skeletal muscle cells. Functionally, CTRP1 increases BP through the stimulation of the angiotensin II receptor 1 (AT1R)-Ras homolog gene family (Rho)/Rho


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kinase (ROCK) signaling pathway to induce vasoconstriction. CTRP1 promoted AT1R plasma membrane trafficking through phosphorylation of AKT and AKT substrate of 160 kDa (AS160). In addition, the administration of an AT1R blocker, losartan, recovered the hypertensive phenotype of CTRP1 transgenic (TG) mice. For the first time, we provide evidence that CTRP1 contributes to the regulation of BP homeostasis by preventing dehydration-induced hypotension.

## P-03-022
## Prilocaine induced epileptiform activity and cardiac toxicity is alleviated by thymoquinone treatment

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Objectives: We aimed to investigate whether thymoquinone (TQ) could alleviate prilocaine-induced central nervous system (CNS) and cardiovascular toxicity in rats. Methods: Rats were randomized to one of the following groups: control, prilocaine (PRL) treated, TQ treated and PRL + TQ treated. Fronto-occipital EEG electrodes and ECG electrodes were placed and the trachea was intubated. Mechanical ventilation was initiated, right femoral artery was cannulated for continuous blood pressure measurements and blood-gas sampling while the left femoral artery was cannulated for PRL infusion. Markers of myocardial injury, oxygen/nitrogen species (ROS/RNS) generation and total antioxidant capacity (TAC) were assayed by standard kits. Aquaporin-4 (AQP4), cleaved caspase-3, nuclear factor κB p65 and p50 subunit in brain tissue were evaluated by microscopy and histopathological scoring. Results: Blood pH and partial oxygen pressure ($pO_2$), was significantly decreased after PRL infusion. The decrease in blood pH was alleviated in the PRL + TQ treated group. PRL produced seizure activity, cardiac arrhythmia and asystole at significantly lower doses compared to PRL + TQ treated rats. PRL treatment caused a significant increase in serum myoglobin and creatine kinase-MB (CK-MB) levels. TQ administration, along with PRL treatment, attenuated levels of myocardial injury. PRL treatment caused increased ROS/RNS formation and decreased TAC in the heart and brain tissue. TQ increased heart and brain TAC and decreased ROS/RNS formation in PRL treated rats. AQP4, p50 and p65 expressions were increased in cerebellar, cerebral cortex, choroid plexus and thalamic nucleus in PRL treated rats. TQ, along with PRL treatment, decreased the expression of AQP4, p50 and p65 in brain tissue. Conclusions: TQ is a protective agent against prilocaine-induced CNS and cardiovascular toxicity. These results indicate that TQ could ameliorate CNS and cardiac toxicity induced by high dose PRL treatment.

## P-03-023
## Determination of hydrolysis of extracellular ATP by vascular smooth muscle cells during differentiation under osteogenic conditions

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Vascular calcification has emerged as a process that profoundly impacts arterial biology and conditions the mortality risk. Calcifying vascular cells usually have high tissue non-specific alkaline phosphatase (TNAP) activity to hydrolyze extracellular pyrophosphate. Extracellular pyrophosphate is an inhibitor of apatite formation formed from extracellular adenosine triphosphate (ATP) or secreted by cells. In the present work we analyzed the ability of TNAP to hydrolyze extracellular ATP. Murine immortal cell line MOVAS, served as model of vascular smooth cells (VSMC). MOVAS were transdifferentiated into osteo-chondrocyte-like cells in the presence of osteogenic medium to mimics the conversion of VSMC into mineralizing cells. In this work, we determined kinetic parameters of ATP hydrolysis by analyzing extracellular medium extracted from MOVAS during their transdifferentiation, using 31P NMR (monitoring nucleotides, pyrophosphate and phosphate) and IR spectroscopy (monitoring phosphate production). We confirmed that specific TNAP activity increased during the transdifferentiation of MOVAS into osteo-chondrocyte like cells. We observed that ATP hydrolysis by MOVAS cells was sequential yielding ADP, AMP and adenosine, without detectable pyrophosphate. Addition of 2–5 mM of levamisole in MOVAS inhibited partially the ATP hydrolysis, indicating that not only TNAP but other phosphatases contributed to the ATP hydrolysis. The number of ATP molecules hydrolysed per cell and per min increased from 138–292 millions at day 0, 276–452 millions at day 7, 477–558 millions at day 14 to 625–1018 millions at day 21 corresponding approximately to a three to seven fold increase of ATP hydrolysis during the transdifferentiation of MOVAS toward calcifying cells, consistent with the specific increase of TNAP activity.

## P-03-024
## Quantitative proteomic assessment of macrophage activation – the influence of dipeptidyl peptidase 8 and 9

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Atherosclerosis is an inflammatory disease, initiated by subendothelial retention of proinflammatory damage-associated molecular patterns (DAMPs). DAMPs-triggered low-grade inflammation involves three main cell types that participate in the development of atherosclerotic lesions: endothelial cells, smooth muscle cells and monocytes. Monocyte-derived macrophages undergo maladaptive cellular death programs and by secretion of proinflammatory mediators are considered to be a key driver of atherogenesis and vulnerable plaque formation. Dipeptidyl peptidases (DPPs) are a family of enzymes with emerging potential as regulators of macrophages activity. In this context, DPP 8 and 9 isoforms are of particular interest since their abundance increases during monocyte to macrophage differentiation. More, DPP8/9 inhibition reduces their M1-like activation efficiency and enhances macrophage apoptosis; however, the precise mechanisms of DPP8/9 actions remain poorly understood. The aim of the study was to comprehensively analyze the role of


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DPP8/9 in the regulation of macrophage activation by means of quantitative proteomic approach. iTRAQ-2DLC-MS/MS method was used to identify differentially regulated proteins upon DPP8/9 inhibition (1G244) in the proteome and secretome of LPS-activated macrophages. Proteomic measurements allowed for quantification of 1727 cellular and 856 secreted proteins, among which 68 and 18 were differentially regulated upon DPP8/9 inhibition in proteome and secretome, respectively. In terms of their molecular function they can be divided into: enzymes involved in the regulation of cellular structure and metabolism (including mitochondria organization), proteins associated with inflammation and oxidative stress, as well as those engaged with protein synthesis and turnover. Collectively, inhibition of DPP8/9 peptidases elicited profound changes in the intra- and extracellular domains of activated macrophages aimed to reduce their pro-inflammatory activation.

## P-03-025
## The effects of cardiomyopathy-associated mutations in the head-to-tail overlap junction of α-tropomyosin on the calcium regulation of the actin-myosin interaction

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Tropomyosin (Tpm) plays a crucial role in the calcium regulation of heart contraction. Tpm molecules bind each other via overlap junction of their N- and C-termini and form a strand that binds the surface of F-actin. The M8R and K15N mutations in the N-terminal part of the junction and the A277V one in the C-terminal part are associated with dilated cardiomyopathy, while the M281T and I284V mutations are related to hypertrophic cardiomyopathy. κ-Tpm (TPM1kappa) is exclusively expressed in the human heart. Its expression increases in chronic dilated cardiomyopathy. We studied the structural and functional properties of α-Tpm with these mutations, and κ-Tpm. We analysed the $Ca^{2+}$ dependence of the sliding velocity of regulated thin filaments reconstructed from human α-Tpm, cardiac troponin, and skeletal F-actin over pig ventricular myosin in an in vitro motility assay. Mutations in the overlap junction had no significant effect on Tpm structure, except the A277V mutation, which increased the thermal stability of the Tpm C-termini. All studied mutations reduced the viscosity of Tpm solution. The K15N and M281T mutations destabilized the Tpm-actin complexes, whereas the A277V mutation stabilised it. The I284V and M281T mutations increased the maximal sliding velocity of thin filaments and the $Ca^{2+}$-sensitivity of the velocity and significantly decreased the cooperativity of pCa-velocity relationship. Mutations M8R and K15N decreased and increased the maximal filament sliding velocity, respectively. All mutations except A277V increased the sliding velocity of thin filaments at low $Ca^{2+}$ concentrations. The results show that mutations in the overlap junction Tpm molecule studied here affect the $Ca^{2+}$ regulation of the actin-myosin interaction and take part in genesis of heart diseases and reveal the effect of κ-Tpm on actin-myosin interaction in ventricles and atria. This work was supported by RFBR (grants 18-34-20085, 17-00-00070, 17-00-00066, and 18-015-00252).

## P-03-026
## Regulation of vascular smooth muscle cell metabolism and vascular tissue remodeling – the role of stearoyl-CoA desaturase

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Increased lipid accumulation in vascular smooth muscles cells (VSMC) is caused by augmented uptake of fatty acids from plasma and by elevated expression of lipogenic genes such as sterol regulatory element-binding protein-1c and fatty acid synthase. Moreover, the increased expression of these genes was found in human atherosclerotic lesion, suggesting that increased lipogenesis and lipid accumulation in VSMC could participate in atherosclerosis development. Stearoyl-CoA desaturase 1 (SCD1) is one of the key regulators of intracellular lipid signaling pathways controlling energy balance. Ablation of SCD1 has a beneficial effect on the metabolic syndrome symptoms course. Therefore, the aim of the present study was to establish the implication of SCD1 in development of VSMC dysfunction in the vascular wall during arterial remodelling following an inflammatory stimuli, and to investigate the potential role of SCD1 in VSMC de-differentiation caused by inflammation-related disease. Our studies have shown that the expression of sm-α-actin increases in aortas isolated from $SCD1^{-/-}$ mice compared to wild type mice. Furthermore, SCD1 deficient mice present the metabolic changes in VSCM of thoracic aorta. Loss of SCD increases protein level of SIRT1 and increases phosphorylation level of AMPK in aorta in physiological condition as well as in mice with high fat diet induced vascular dysfunction. AMPK-mediated inhibition of mTOR was proposed to have a beneficial effect on VSMC. We observed a decrease in phosphorylation of mTOR in aorta of $SCD1^{-/-}$ mice fed high fat diet compared to wild type controls. Taken together presented data suggest that signaling pathways controlled by SCD1 are likely to represent important step in regulation of metabolism of VSMC thus vascular wall morphology and function. This work has been supported by the funding from National Science Centre, Poland grants no UMO-2017/26/D/NZ4/00696 and UMO-2016/22/E/NZ4/00650.

## P-03-027
## Cardiac repair after myocardial infarction – role of macrophages and heme oxygenase-1

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Left ventricular free wall rupture (LVFWR) is a rare, however associated with extremely high mortality, complication of acute myocardial infarction (MI). Still, the impact of genetic and molecular factors on this outcome is not completely understood. Recently, we have demonstrated significantly lower occurrence of LVFWR between days 3–5 post-MI in heme oxygenase-1 (HO-1) knockout (KO) than in wild type (WT) mice, as a result of higher collagen deposition in the infarcted area. HO-1, a stress-inducible protein, was shown to promote polarization of macrophages towards M2-like phenotype. Such macrophages govern the activity of fibroblasts and myofibroblasts and thus, collagen production. The aim of this study was to evaluate the role of HO-1 in macrophage-mediated post-MI cardiac repair. Already in the hearts of HO-1 KO neonates we observed accelerated monocyte


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infiltration and replenishment of embryonically-established macrophages with monocyte-derived macrophages (MDMs). Next, we subjected adult HO-1 KO and WT mice to MI, and performed miRNA profiling of cardiac MDMs ($CD45^+CD11b^+CD64^+$) sorted 4 days after the procedure. Our analysis revealed upregulation of several miRNAs in HO-1 KO cells. Some of them, e.g. miR-146a and miR-21, were shown to promote M2 polarization, what was surprising considering the absence of HO-1. Interestingly, when MI in WT mice was superimposed on clodronate-mediated macrophage depletion, the mortality due to LVFWR was reduced. Additionally, transplantation of bone marrow cells from both HO-$1^{+/+}$/$GFP^{+/+}$ and HO-$1^{-/-}$/$GFP^{+/+}$ donors to lethally gamma irradiated HO-$1^{+/+}$/$GFP^{-/-}$ recipients led to exchange of cardiac macrophage population and entirely protected the WT mice from LVFWR. In conclusion, accelerated turnover of cardiac macrophages naturally occurring in HO-1 deficiency and imposed in WT mice influences the occurrence of LVFWR. Supported by grant 2014/14/E/NZ1/00139 and 2015/17/N/NZ1/00041 (NCN).

## P-03-028
## The effect of palmitate on HUVEC cells as an in vitro model of hyperlipidemia

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Elevated level of lipids and lipoproteins in the blood, which is often associated with sedentary lifestyle and obesity, may lead to type 2 diabetes, and is a risk factor for cardiovascular diseases particularly due to endothelial dysfunction. Palmitic acid is the most abundant circulating fatty acid in the blood and its abnormally increased level is a one of factors responsible for insulin resistance of cells. In this study we have used human umbilical vein endothelial cells (HUVEC) treated with palmitate as an *in vitro* model of hyperlipidemia. We have found that an incubation of HUVECs with palmitate results in an elevated nitric oxide generation and stimulated secretion of the von Willebrand factor (vWF) (ELISA test). It indicates increased exocytosis of the Weibel-Palade bodies (WPB) that contain VWF and P-selectin. Interestingly, stimulation of vWF secretion by palmitate was additive (or even synergistic) to the stimulatory effects of histamine and forskolin on this process. It may suggest different mechanisms of their action. This work was supported by a grant number 2016/23/B/NZ3/03116 from the National Science Center, Poland, and by statutory funds from the Nencki Institute of Experimental Biology.

## P-03-029
## The role of annexin A6 during tissue nonspecific alkaline phosphatase-induced calcification of human coronary artery smooth muscle cells

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Vascular calcification (VC) is a hallmark of atherosclerotic plaques accompanied by inflammation. Expression of tissue nonspecific alkaline phosphatase (TNAP), an enzyme crucial for calcification, in vascular smooth muscle cells (VSMCs) is induced by inflammation, but its activation and interaction with extracellular vesicles (EVs) released by VSMCs, is not fully understood. Recently, it is been shown that annexin A6 (AnxA6), a calcium and membrane-binding protein is abundant in sites of calcification and a depletion of AnxA6 reduces VSMC mineralization. On the basis of above observations, our aim was to determine the molecular mechanisms involved in TNAP-induced calcification of VSMCs and also the proteins involved in TNAP activation and sorting into EVs. We used human coronary artery smooth muscle cells (HCASMCs). These cells were cultured in an osteogenic medium for up to 21 days, promoting their transition to osteochondrocyte-like cells. The role of AnxA6 was analyzed after AnxA6 overexpression by monitoring of HCASMCs protein markers. We found that TNAP activity of cells stimulated for 7 days was 5 times higher than resting cells. Both cell types, after 14 days, produced 2 times more calcium deposits similar to hydroxyapatite, as revealed by AR-S staining, CPC extraction and IR analysis. The interaction of AnxA6 with EVs was $Ca^{2+}$-dependent. A significant amount of AnxA6 was resistant to EGTA treatment and freeze/thaw cycles, suggesting that a certain amount of AnxA6 remained in the EVs bilayer. After 21 days overexpressed AnxA6 accumulated in cytoplasm whereas in stimulated cells was present at the membrane of EVs. In conclusion, a better understanding of mechanisms of the biogenesis of calcifying EVs will allow gaining novel insights into the pathogenesis of VC. The identification of proteins involved in the regulation of TNAP activity is crucial in order to generate novel therapeutic strategies to prevent formation of atherosclerotic plaques.

## P-03-030
## VEGF isoforms in ocular disease

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The vascular endothelial growth factor (VEGF) is a secreted glycoprotein, best known for its ability to induce angiogenesis. VEGF expression is increased in several ocular diseases, where it promotes neovascularisation and drives disease progression e.g. AMD and ischemic retinopathies. Anti-VEGF therapies can be used to limit pathological angiogenesis in the eye, but there is evidence that this may detrimentally inhibit VEGF's non-vascular activities. Specifically, VEGF also acts as a chemoattractant and survival factor for neurons. In fact, VEGF-stimulated neuronal migration can enhance recovery in some ocular disorders, for instance, after corneal injury. It may be possible to selective block pathological angiogenesis in the eye, or promote tissue re-innervation, if it was known which of the three main VEGF isoforms are upregulated in ocular disease. Accordingly, we have determined the expression pattern of VEGF isoforms in the unchallenged mouse eye and in several murine models of ocular disease. Moreover, using mouse strains that alternatively express only one of the main VEGF isoforms, we have demonstrated that loss of the pathologically upregulated VEGF isoform impedes tissue re-innervation in a model of corneal injury. *The authors marked with an asterisk equally contributed to the work.

## P-03-031
## Ethnogenetic differences in susceptibility to essential hypertension in Russians and Buryats

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Essential hypertension (EH) provokes accelerated vascular aging, being at the same time the most common age-related disease. Genetic factors associated with hypertension, as a rule, are


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involved in the general biological processes of regulation of vascular tone and endothelial function. We studied association of *EDN1* rs5370, *NOS3* rs891512, *ADRB3* rs4994, *GNB3* rs5443, and *STK39* rs3754777 gene polymorphisms with EH in Russians and Buryats. Buryats are an Asian ethnic group in the multinational Russia. Materials and methods: The case-control study involved genotyping of the 5 polymorphisms in 172 essential hypertensive patients, 136 Caucasians, ethnic group – Russians, 36 Asians, ethnic group – Buryats, and 143 controls (112 Russians, 31 Buryats). The study was approved by the local ethics committee. Genomic DNA was extracted from the blood samples and polymorphism analysis was performed using real-time PCR. Results: Significant association *EDN1* rs5370 polymorphism and EH was observed at the level of both GG genotype ($\chi2 = 9.99$, $P < 0.05$) and G alleles ($\chi2 = 11.34$, $P < 0.05$) in Russians and G alleles ($\chi2 = 4.44$, $P < 0.05$) in Buryats. T allele *GNB3* rs5443 polymorphism was significantly associated with EH in Russians ($\chi2 = 4.06$, $P < 0.05$). T allele of *STK39* rs3754777 and GG genotype of *ADRB3* rs4994 polymorphisms were significantly associated with EH in Buryats ($\chi2 = 7.27$, $P < 0.05$). Differences in allele frequencies between Russians with EH and Buryats with EH were statistically significant ($\chi2 = 5.19$ $P < 0.05$) only for the *ADRB3* rs4994 polymorphism. Distribution of allele frequencies in controls corresponded to the Hardy-Weinberg equilibrium. Conclusions: Ethnogenetic differences in susceptibility to EH in patients of Russian and Buryat ethnic groups were revealed. The reported study was funded by RFBR according to the research project No. 19-315-80032.

## P-03-032
## Generation and phenotyping of a novel knock-in mouse model of Arrhythmogenic Cardiomyopathy

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Rationale. Arrhythmogenic Cardiomyopathy (AC) is a cardiac disease, mainly caused by mutations in genes encoding desmosomal proteins, accounting for most cases of sudden cardiac death in the young and athletes. AC hearts display cardiomyocyte (CM) death and fibrofatty tissue replacement, which lead to contractile dysfunction and stress-triggered arrhythmias. The AC pathogenesis is unknown and no mechanism driven therapies are available. Most AC models express disease mutations selectively in CM, and fail to entirely reproduce the disease phenotype. Aim. Here, we aim to generate knock-in (KI) mouse, which would faithfully replicate the human disease genetics. Methods. CRISPR-Cas9 technology was used to edit the desmoplakin (DSP) gene and generate two KI strains, harbouring respectively i) the point mutation S311R, which is the murine analogue of the mutation identified in a large cohort of italian AC patients and ii) the point mutation S311A to ablate the phosphorylation site on DSP and determine its functional role. Results. We successfully obtained $DSP^{S311R/WT}$ and $DSP^{S311A/WT}$ KI founders, and expand the AC colonies. While $DSP^{S311R/S311R}$ mutation was embryonic lethal, $DSP^{S311A/S311A}$ weaned normally and were fertile, and were thus phenotyped functionally and morphologically. Echocardiography revealed systolic dysfunction, which progressively worsened between 3 and 6 mo. Consistent with the myocardial histopathologic findings of human AC, $DSP^{S311A/S311A}$ hearts displayed CM death and fibrotic remodeling in both the right and left ventricles. To better define cardiac morphology, IF, EM, molecular and biochemical analyses are in progress. Complete cardiac phenotyping is ongoing in heterozygous $DSP^{S311R/WT}$. Conclusions. We generated two novel DSP mutant KI mouse strains, which may represent preclinical models for a comprehensive study of AC pathogenesis. In addition, our results suggest that ablation of the S311 phosphorylation site may have a role in DSP-linked AC.

## P-03-033
## The influence of diminazene on atherosclerosis and hepatic steatosis in apolipoprotein E knockout mice

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Atherosclerosis and nonalcoholic fatty liver disease (NAFLD) are frequent causes of death in the Western countries. The angiotensin (Ang) converting enzyme 2 (ACE2)/Ang-(1-7)/Mas axis plays an important role in the pathogenesis of both atherosclerosis and NAFLD. ACE2 is a key enzyme responsible for the regulation of Ang II and Ang-(1-7) levels, thus the activators of ACE2 might be useful for novel therapeutic interventions. The aim of our study was to comprehensively describe the influence of prolonged treatment with orally administered diminazene on the development of atherosclerotic lesions and hepatic steatosis in apolipoprotein E knockout ($apoE^{-/-}$) mice fed a high fat diet. We have found that diminazene did not reduce atherosclerotic lesions in $apoE^{-/-}$ mice, but enhanced the stability of plaques by decreasing the content of macrophages and increasing the content of smooth muscle cells in atherosclerotic plaques. Moreover, treatment with diminazene caused the attenuation of hepatic steatosis in $apoE^{-/-}$ mice accompanied by the reduction of triglycerides level in the liver and alanine transaminase (ALT) level in the plasma. Proteomic analysis revealed enhanced expression of cysteine sulfinic acid decarboxylase (CSAD) – an enzyme responsible for the synthesis of taurine – in the liver of diminazene-treated $apoE^{-/-}$ mice. However, the exact molecular mechanisms of diminazene action in atherosclerosis and NAFLD require further investigations.

## P-03-034
## Dichlorvos induces cardiotoxicity in Balb/c mice following subchronic exposure

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Dichlorvos (2,2-dichlorovinyl dimethyl phosphate; DDVP) is an organophosphate pesticide and is widely used for the control of household pests, public health pests and stored product insect Infestations. Meantime, the available information on the cardiotoxic effects of DDVP is very much limited. In the present study, we investigated the toxic effects of DDVP in heart tissues of Balb/c mice after 30 days of exposure. We demonstrated that DDVP increased creatine phosphokinase, lactate dehydrogenase,


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aspartate transaminase, alanine transaminase, total cholesterol and triglyceride levels and induced oxidative stress as monitored by measuring the malondialdehyde level, the generation of protein carbonyls, the catalase and superoxide dismutase activity and the expression of the heat shock proteins (Hsp 70) in a dose dependent manner.

## P-03-035
## Glutamine-dependent metabolic pathways in the regulation of the release of eicosanoids by vascular endothelial cells

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The aim of the study was to investigate the role of glutamine-derived metabolic pathways in the regulation of endothelial cell (EC) functional phenotype, with focus on the production of nitric oxide (NO) and prostacyclin (PGI2) and other endothelial eicosanoids. Human aortic endothelial cells (HAEC) were incubated in DMEM medium with various glutamine concentrations and analyzed based on biochemical profiling (metabolic and functional status of EC). Determination of the intracellular metabolites concentrations was carried out using LC/MS/MS targeted metabolomics. EC functional phenotype was characterized by measuring the release of endothelium-derived mediators. The concentrations of nitrite and nitrate (NO metabolites) were analyzed by HPLC and 6-keto-PGF1α and other eicosanoids by LC/MS/MS methodology. The role of individual glutamine pathways was studied after the addition of specific inhibitors (e.g. azaserine, EGCG, BPTES and others). We report that increasing concentration of glutamine was related with decreasing production of PGI2 (as evidenced by 6-keto-PGF1αa), as well as prostaglandins PGD2, PGE2 and hydroxyeicosatetraenoic acids (12-HETE, 15-HETE). In turn, glutamine deficiency caused a marked increase in the production of these eicosanoids. Metabolomic studies revealed that ECs deprived of glutamine have lower intracellular concentration of polyamines, glutathione, glucosamine-6-phosphate and UDP-GluNAc, what suggests decreased activity of hexosamine biosynthesis pathway, polyamine synthesis and reduced antioxidant defense in glutamine-deficient endothelium. Which of the above mechanism is responsible for increased eicosanoids production is currently investigated. In summary, our results suggest that endothelial cell synthesis and secretion of eicosanoids seems to be negatively regulated by glutamine availability. Acknowledgments: Project financed by the project METENDOPHA (STRATEGMED1/233226/11/NCBR/2015) and Poland Ministry of Science and Higher Education

# Intracellular ion channels and transporters

## P-04-001
## Direct localization of the BKCa channel in the mitochondria of human bronchial epithelial cell line

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Mitochondria play an important role in the energy metabolism within the cell. These organelles are also involved in the regulation of cell death and survival. In mitochondria, potassium transport via channels seems to be a crucial mechanism to control the membrane potential and matrix volume or respiration. It was shown that activation e.g. mitochondrial large-conductance Ca2 + -regulated potassium (mitoBKCa) channel protects against cell death during myocardial infarction or cerebral hypoxia. Possibly, activation of mitoBKCa channel can provide beneficial effects on the epithelia exposed to harmful factors like air pollution. The aim of studies is the biophysical and pharmacological characterization of potassium channels in the inner mitochondrial membrane of bronchial epithelial. In the current study, using the patch-clamp technique we prove the presence of the BKCa channel in the inner mitochondrial membrane of human bronchial epithelial cells (16HBE14o-). The mitoBKCa channel was activated by Ca2 + and 3 μM NS11021 known as a specific activator of the BKCa-type channels. The channel activity was inhibited by 1 μM paxilline, potassium channel blocker. Additionally, the presence in the inner mitochondrial membrane of pore-forming α subunits and auxiliary β subunits of the BKCa channel was confirmed using the Western Blot analysis. Also, gene expression of the BKCa channel subunits was studied with the use reverse transcriptase-PCR analysis. We believe that our findings of the large conducted Ca2 + -regulated potassium channels in mitochondria of human epithelial will help us better understand of their role in global protective mechanisms. This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland (to PB), partially by Nencki Institute. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund; Project number POWER.03.02.00-00-I007/16-00 (to A. Sęk)

## P-04-002
## SLC6A14 – an amino acid transporter $B^{0,+}$ exclusively interacts with SEC24C as a cargo recognizing COPII element

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SLC6A14, a plasma membrane amino acid transporter $B^{0,+}$ ($ATB^{0,+}$), is a Na/Cl dependent transporter for neutral and basic amino acids. It is up-regulated in several types of cancers. Trafficking starts to the plasma membrane with leaving the endoplasmic reticulum (ER) and interaction with a cargo recognizing protein SEC24 within Coatomer II (COPII) complex. We studied


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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trafficking of rat SLC6A14 in a heterologous expression system in HEK293 cells. Western blot, deglycosylation and immunofluorescence demonstrated that the substantial amount of fully glycosylated SLC6A14 appears at the cell surface after 48 h. Only one of four SEC24 paralogues SLC6A14 co-precipitated with SEC24C isoform and has an interaction with SEC24C was confirmed by proximity ligation assay. Co-localization of endogenous SLC6A14 with SEC24C was observed in MCF-7 breast cancer cells. Part of the overexpressed $ATB^{0,+}$ is directed to proteolysis, a process significantly reversed by a proteasome inhibitor bortezomib. Analysis of SLC6A14 amino acid sequence and detected specificity for SEC24C confirms a hypothesis proposed for a neurotransmitter transporters branch of SLC6 family that a hydrophilic residue at + 2 position downstream of the ER export "RI" motif determines interaction with C isoform of SEC24 proteins and promotes further trafficking to Golgi and plasma membrane. There is an equilibrium between export from ER and degradation mechanisms in case of overexpressed transporter. This work was financed with Polish National Science Centre Grant No. 2012/B/NZ3/000225, with the EU Horizon 2020 Research and Innovation Programme under Marie Sklodowska-Curie Grant Agreement No. 665735 (Bio4Med) and with the funds from the Polish Ministry of Science and Higher Education as part of the 2016–2020 funds for the implementation of international projects (Agreement No. 3548/H2020/COFUND2016/2) and grants of the Austrian Science Fund FWF (P31255 and SFB35-10 to Sonja Sucic and Michael Freissmuth, respectively).

## P-04-003

## A role of concentrative nucleoside transporter CNT3, a representative of Solute Carrier Family 28, in drug uptake and efflux

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Solute carrier transporters (SLCs) form a large family of cell and endoplasmic reticulum membrane proteins of diverse structures and mechanisms of action. During SLC-mediated transport a significant reorganization of transmembrane helices from an open to close conformation is observed. An inverted topology of open and close conformations of SLC transporters represents a serious obstacle for common homology modeling methods. What is more, being membrane proteins often composed in large homo or heteromeric systems, SLCs represent also a challenge for modern crystallography. Despite inadequate structural characterization of this distinct protein family SLCs are important targets for pharmacokinetics research due to their role in drug absorption, excretion and cytotoxicity. A sodium-dependent concentrative nucleoside transporter 3 (CNT3) plays a crucial role in a flux of natural nucleosides or their pharmacologically active derivatives. The distribution pattern of the CNT3 transporter transcript (mainly: pancreas, trachea, mammary gland, bone marrow, intestine) is surprisingly widespread comparing pyrimidine-specific CNT1 and purine-specific CNT2 and has been shown to correlate with clinical responsiveness to gemcitabine. CNT3, of the highest concentrative capacity, and other transporters belonging to the SLC28 family are under strong negative selection leading to preserving their amino-acids sequence. Few single nucleotide polymorphisms (SNPs) have been proved to modulate patients' response to treatment, e.g., by preventing hemolytic anemia in ribavirin pharmacotherapy of hepatitis C due to the reduced drug uptake into red blood cells. The current study is focused on computational modeling of the CNT3 homotrimer structure, its mechanism of action and molecular basis for observed SNPs (Latek D., 2017, doi: https://doi.org/10.1186/s12900-017-0078-8).

## P-04-004

## Altered expression of SR-BI/CLA1, SR-BII and LDLr lipid receptors, ABCA1 transporter and HSL in human sperm impact obese infertility

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Dyslipidemia in obese patients is associated with male secondary hypogonadism and infertility. High cholesterol levels are negatively correlated with sperm quality. Objective: To study the cellular location and expression of receptors (SR-BI/CLA-1, SR-BII and LDLr) and lipid transporters (ABCA1) involved in uptake and efflux of cholesterol in human spermatozoa and whether obese lipid alterations are related to infertility. Methods: We used controls and obese patient's semen samples to determine concentration, motility and morphology of spermatozoa; protein expression by western blot and localization by immunohistochemistry. Results: Concentration and spermatozoa's motility decreased in obese patients. 65% obese patient's spermatozoa presented altered morphology. SR-BI/CLA-1, SR-BII, LDLr and ABCA1 are located in head, neck and tail spermatozoa's plasma membrane; and localization do not change between groups. Control spermatozoa showed intense positive staining and expression for SR-BI, and tenuous for the others receptors analyzed. Obese patient's spermatozoa showed less SR-BI/CLA-1 expression than the controls, and more intense staining for SR-BII, LDLr and ABCA1. Human sperm expresses the 130 and 82 kDa HSL isoforms. The 130 kDa HSL isoform is not expressed in obese patients' sperm. Conclusions: Obese lipid homeostasis alterations of receptors/transporters and proteins involved in cholesterol uptake, efflux and mobilization from stored intracellular pools, significantly alter normal spermatozoa function and the process of maturation/capacitation of human spermatozoa. The lack of the 130 kDa HSL isoform in obese patients' sperm prevents the hydrolysis of cholesterol esters internalized by these receptors and favours accumulation of cholesterol esters in cytoplasm contributing to steatosis and infertility in obese patients. Acknowledgement: CIBEROBN. Financed by PI16/00154. Instituto de Salud Carlos III. Ministerio de Economía y Competitividad, Spain.

## P-04-005

## The effect of dopamine on the Cl-ATPase

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The anion activated ATPases represent the enzyme systems which are activated by anions. Cl-anion dependent Mg-stimulated ATP hydrolysis is reported in the literature. It plays a principal role in the regulation of nerve excitability and determination of rest potential. Cl-anion activated $Mg^{2+}$-dependent ATPase satisfies the necessary kinetic conditions of transport ATPases and it belongs to the P-type ATPases group. It has been assumed that Cl-ATPase, like Na,K-ATPase, takes part in the regulation of


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synaptic transmission mechanisms. The neurotransmitters 5-hydroxytryptamine (5-HT), dopamine (DA) and synaptosomal factor (SF) represent modifiers of Na,K-ATPase system. In the cytosol of nerve ending endogenous factor Synaptosomal Factor (SF) with MW 60kD was found, which regulates the synaptic Na,K-ATPase system. It is remarkable that this factor along with neurotransmitters activates the synaptic Na,K-ATPase. The effects of dopamine (DA) and endogenous synaptosomal factors on anion ATPase (Cl-activated ATPase E.C.3.6.3.11) were studied in the rat brain fraction, greatly enriched in synaptic junctional complexes (SJC). DA and SF separately inhibit Cl-ATPase, whereas their combined action (SF/DA) results in the withdrawal of the inhibitory effect and passes into activation. Dopamine does not alter stoichiometry of Cl- transport. The number of the Cl- sites intended for the essential activator and full inhibitors remains 1. The neurotransmitter's regulation by synaptic factor is universal for those transport enzyme systems, which are involved in synaptic transmission and does not extend to other membrane enzymes.

## P-04-006
## $Ni^{2+}$-dependent Mg-ATPase activity

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Bivalent cations, and among them nickel, play a significant role for the functioning of living organisms. Extracellular and intercellular concentrations of $Ni^{2+}$ differ from each other. It is natural, that there exist some specific mechanisms by which the execution of $Ni^{2+}$ transport through the membrane is possible and it manages to keep its unequal distribution. It is known, that in the microorganisms the transport of $Ni^{2+}$ is executed on the one hand by the fast and the non-specific carriers and on the other hand by P-type ATPase. $Ni^{2+}$ stimulated change of Mg-ATPase activity has been found in the synaptic fraction of rat brain and was named Ni-ATPase. Investigation has shown that Ni-ATPase is a multi-sited enzyme system; its activity is described by kinetic curves of complicated geometric shape that makes analysis hard enough. It does not obey the classical Michaelis-Menten kinetic analysis and the formula of transformation utilized for the single-sited enzyme systems does not allow linearization of the function. For deciphering molecular mechanism of Ni-ATPase we have applied the method of kinetic analysis of multi-sited enzymes systems, that is a single method used for kinetic investigation of multi-sited enzyme systems. The kinetic study of the Ni-ATPase has shown that Its substrate is the MgATP complex; $Mg^{2+}$ and ATP represent modifiers for the given enzyme system with a constant affinity for the substrate and $Mg^{2+}$ bound forms of the enzyme have no catalytic activity. The number of the $Ni^{2+}$ sites intended for the essential activator and the full-effect inhibitor are equal. By using the kinetic method of complex geometric shape curves, theoretical analyze of kinetic curves and its compare to experiment, the "minimal model" for Ni-ATPase has been created.

## P-04-007
## IP3 and the anti-apoptotic Bcl-2 compete for the ligand-binding domain of IP3Rs modulating $Ca^{2+}$ signaling

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Bcl-2 protein has emerged as a critical regulator of intracellular Ca2 + dynamics by directly targeting and inhibiting the IP3 receptor (IP3R), a major intracellular Ca2 + -release channel. Here, we demonstrate that such inhibition occurs under conditions of basal, but not high IP3R activity, since overexpressed and purified Bcl-2 (or its BH4 domain) can inhibit IP3R function provoked by low concentration of agonist or IP3, while fails to attenuate against high concentration of agonist or IP3. Surprisingly, Bcl-2 remained capable of inhibiting IP3R1 channels lacking the residues encompassing the previously identified Bcl-2-binding site (a.a. 1380-1408) located in the Dom 3, part of the modulatory region. Using a plethora of computational, biochemical and biophysical methods, we demonstrate that Bcl-2 and more particularly its BH4 domain bind to the ligand-binding domain (LBD) of IP3R1. In line with this finding, the interaction between the LBD and Bcl-2 (or its BH4 domain) was sensitive to IP3 and adenophostin A, ligands of the IP3R. Consistent with this, the BH4 domain of Bcl-2 counteracted the binding of IP3 to the LBD. Collectively, our work reveals a novel mechanism by which Bcl-2 influences IP3R activity at the level of the LBD. This allows for exquisite modulation of Bcl-2's inhibitory properties on IP3Rs that is tunable to the level of IP3 signaling in cells.

## P-04-008
## Br-Cl flav – a novel synthetic flavonoid with potent antibacterial properties

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The emergence of pathogenic multidrug-resistant bacteria demands new approaches in finding effective antibacterial agents. Synthetic flavonoids could be a reliable solution due to their important antimicrobial activity. We report here the potent *in vitro* antibacterial activity of Br-Cl flav – a novel synthetic tricyclic flavonoid with bromide as halogen substituent at the benzopyran core. The antimicrobial effects were evaluated using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time kill assays. DNA fragmentation assay, gel-based nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), scanning electronic microscopy were employed to study the mechanism of action. The tested flavonoid displayed a strong antimicrobial activity with MIC values as low as 0.24 μg $ml^{-1}$ against *Staphylococcus aureus* ATCC 25923 and 3.90 μg $ml^{-1}$ against *Escherichia coli* ATCC 25922. Flavonoid Br-Cl showed antibacterial properties, causing not only the inhibition of bacterial


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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growth up to 12 hours, but also killing bacterial cells within 2 hours. The mechanism of action is related to the impairment of membrane integrity, cell agglutination as well as the reduction of protein abundance in the cytoplasm as shown by SDS-PAGE and nanoLC-MS/MS experiments. Br-Cl flav was found to have a stronger antibacterial effect at lower concentrations than those described in the earlier reports. Based on these findings, Br-Cl flav has a good potential for the design of new antibacterial agents.

## P-04-009
## Redox regulation of the growth, hydrogenase activity and bioenergetic properties of *Ralstonia eutropha* upon different carbon source utilization

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*Ralstonia eutropha* is a facultative chemolithoautotrophic bacterium able to grow on organic substrates or $H_2$ and $CO_2$ under aerobic conditions. *R. eutropha* H16 possesses 4 different $O_2$-tolerant [NiFe] hydrogenases (Hyds) involved in $H_2$ turnover. Hyds of *R. eutropha* are attractive targets for potential application in biochemical hydrogen fuel cells. Redox regulation of growth of *R. eutropha* and oxidation-reduction potential (ORP) kinetics, bioenergetics properties (intracellular pH ($[pH]_{in}$), ATPase activity) and activities of membrane-bound Hyd (MBH) and cytoplasmic soluble Hyd (SH) upon different carbon source (fructose and glycerol) utilization were investigated. Bacteria were grown on 0.4% fructose (FN) and 0.2% fructose and glycerol (FGN) with the yield of 2.3 ± 0.02 and 3 ± 0.05 g cell dry weight/L, respectively. The growth rate was stimulated ~1.2 fold by 1 mM $K_3[Fe(CN)_6]$, whereas prolonged, reduced growth was observed upon addition of 3 mM DTT, which then recovered after 48 h. The ATPase activity upon utilization of only fructose (FN) was 92 ± 5 nMol Pi (min μg protein)$^{-1}$ and 0.2 mM DCCD inhibited ATPase activity ~2.3 fold. Compared to growth in FN, membrane vesicles demonstrated ~4.2 and ~2.5 fold lower $F_OF_1$ -ATPase activity, respectively, upon fructose and glycerol co-utilization (FGN). Compared to FN, ORP, measured by Pt and Ti-Si electrodes were lower upon bacterial growth on FGN and 0.4% glycerol and 0.05% fructose (GFN). The $[pH]_{in}$ measured by the 9-AA quenching was 8.00 ± 0.05. Highest activities for MBH (6.00 ± 0.03 U·mg$^{-1}$) and SH (2.30 ± 0.05 U·mg$^{-1}$) were determined upon bacterial growth on GFN. Our results suggest that reductive conditions and low ATPase activity might be signals for energy depletion, which, in turn, leads to increased hydrogenase biosynthesis. *The authors marked with an asterisk equally contributed to the work.

## P-04-010
## Regulation of the mitoBKCa channel by cardioprotective flavonoids

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Flavonoids belong to a large group of polyphenolic compounds that are widely present in plants. Certain flavonoids, including luteolin, naringenin or cyanidin, have been shown to be cardioprotective, although the antioxidant effect of flavonoids has long been thought to be a crucial factor accounting for cellular cardioprotection. Also, mitochondrial pathways (including mitochondrial large-conductance Ca2 + -regulated (mitoBKCa) channel) are presently emerging potential targets for a specific pharmacological action of flavonoids in anti-ischemic strategies. The aim of these studies is the characterization of interactions between cardioprotective flavonoids and the mitoBKCa channel present in the inner mitochondrial membrane of the endothelial cells. Single channel activity of the mitoBKCa was measured with patch-clamp of the mitoplasts isolated from endothelial cells (EA.hy 926). Application of micromolar concentration of naringenin leads to an increase in mitochondrial BK channel activity. Open probability increase from 0.02 to 0.29 at + 40 mV in low calcium condition. In contrast, 3 uM cyanidin has an inhibitory effects. In the presence of luteolin, changes of open probability of the mitoBKCa channel were not observed. Furthermore, regulation of the mitoBKCa channel by flavonoids was studied in the presence of 0.5 mM Dithiothreitol. Changes in the redox state causes that luteolin and cyanidin have activatory properties. Open probability of the mitoBKCa increase from 0.02 to 0.36 at -40 mV in the presence 10 uM Cyanidin. We expect that our studies describing the regulation of mitochondrial potassium channels by the natural substances will bring us closer to a better understanding of flavonoid-induced cytoprotective mechanisms. This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund (to Aleksandra Sęk).

## P-04-011
## ABCA1-mediated mechanism of resistance to amphotericin B toxicity in mammalian cells

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Amphotericin B (AmB) belongs to a group of polyene antibiotics commonly used in the treatment of the systemic mycotic infections. A widely accepted mechanism of action of AmB is based on the formation of an oligomeric pore structure within the plasma membrane (PM) by interaction with membrane sterols. Despite that AmB binds preferentially to ergosterol, it can also bind to cholesterol in the mammalian PM and cause severe cellular toxicity. The lipid content and lateral organization of the PM appear to be significant in this process. ATP binding cassette (ABC) transporters, including ABCA1, play a crucial role in lipid translocation, cholesterol redistribution and efflux at the cell PM. Here we demonstrate that cells expressing an active ABCA1 transporter are more resistant to AmB treatment, while cells lacking ABCA1 expression or expressing non-active ABCA1MM mutant display increased sensitivity. Moreover, a decrease of cellular cholesterol content abolishes resistance of ABCA1-expressing cells to AmB. Furthermore, FLIM analysis reveals the formation of bulk cholesterol-AmB structures at the cell surface of ABCA1-expressing cells but not on the ABCA1MM mutant cells. Therefore, we propose that ABCA1-mediated cholesterol efflux from cells immobilizes AmB out of the cell preventing its cytotoxicity. This work is financially supported by a research grant No. 2016/21/B/NZ3/00343 from Polish National Science Center (NCN).


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## P-04-012
## MmpS5/MmpL5 efflux pump provides imidazo [1,2-b][1,2,4,5]tetrazine resistance to *Mycobacterium smegmatis*

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The emergence and spread of drug-resistant *Mycobacterium tuberculosis* strains is the main threat to global tuberculosis (TB) control, urging the need to develop anti-TB drugs with novel mechanisms of action, thus active against drug resistant strains. We have previously described a number of substituted imidazo [1,2-b][1,2,4,5]tetrazines, that showed promising minimal inhibitory concentrations (MICs) on both drug-susceptible and drug-resistant *M. tuberculosis* strains, and were able to obtain *M. smegmatis mc2 155* spontaneous mutants resistant to 4 different imidazo[1,2-b][1,2,4,5]tetrazines on 3.5-4x MIC. Whole-genomic sequencing revealed 7 different mutations in 11 strains in the *MSMEG_1380* gene, encoding a TetR-family transcriptional regulator. Overexpression of the wild-type *MSMEG_1380* gene on the pMIND plasmid increased *M. smegmatis* susceptibility to imidazo[1,2-b][1,2,4,5]tetrazines, while overexpression of mutant variants didn't affect the phenotype. *MSMEG_1380* is adjacent to *mmpS5/mmpL5* operon, encoding a trans-membrane transporter. The structure of this operon is conserved in mycobacterial species, and it has been previously reported that overexpression of the *mmpS5/mmpL5* operon, caused by mutations in TetR repressor gene, modulates resistance to the derivatives of thiacetazone in *M. abscessus*, and cross-resistance to bedaquiline and clofazimine in *M. tuberculosis*. We used real-time PCR to confirm the role of *MSMEG_1380* gene in the regulation of *mmpS5/mmpL5* operon in *M. smegmatis*: we observed an 85- to 540-fold increase in expression of *mmpS5* in mutant strains, while it was repressed by overexpression of *MSMEG_1380*. Thus we show that the primary mechanism of drug resistance to imidazo[1,2-b][1,2,4,5]tetrazines in *M. smegmatis* is mediated by efflux, and confirm that MmpS5/MmpL5 efflux system is an uprising concern in mycobacterial drug resistance. This work is supported by the Russian Science Foundation grant 17-75-20060.

## P-04-013
## The influence of selected chalcones on ABCB1 transport activity in human colon cancer cells

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Colorectal cancer is third most commonly diagnosed malignancy and the fourth leading cause of cancer death in the world (Arnold et al. Gut 2017. 66, 683–691). Moreover, nearly all patients with colon cancer suffer from drug resistance that leads to decrease in the therapeutic efficacy of drugs (Hu et al. World J Gastroenterol. 2016, 22, 6876–6889). Therefore, studies on the activity of compounds which are able to kill cancer cells, especially cells resistant to drugs, or to improve treatment of patients with colon cancer, are desirable. One of the mechanisms of drug resistance overexpression of ATP-binding cassette (ABC) proteins known as transporters. ABCB1 (P-glycoprotein) is the most extensively studied transporter widely distributed in the intestinal epithelium. The high expression of this protein is related to the active efflux of drugs (e.g. doxorubicin) following by the reduction of drug concentration within the cell. In our studies we focused on the potential ability of selected chalcones to inhibit the activity of ABCB1 in human colon cancer cells: LoVo and HT29. Chalcones are a group of plant-derived polyphenolic compounds extensively studied for their numerous pharmacological properties. It is postulated that methoxy substitutions of the chalcones depending upon their positions in the aryl rings (A or/and B) appear to influence anti-cancer activities. In our work the concentration-depended cytotoxic effects of some methoxylated chalcones were introduced. An ability of the compounds to increase the cytotoxicity of drug - doxorubicin in studied cells was investigated. Also, the changes in the accumulation of doxorubicin and the level of gene coding ABCB1 (*Mdr1*) in the presence of the chalcones were checked. We found that the number and position of methoxyl groups may influence on the efficiency of chalcone used as ABCB1 inhibitor. *The authors marked with an asterisk equally contributed to the work.

## P-04-014
## Cytotoxic and multidrug resistance reversal activity of oxicam derivative is strongly enhanced by theobromine, a phytochemical from cocoa

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The MDR phenotype (multidrug resistance) cells often manifest altered properties such as genome instability or loss of the cell cycle control points, which also hinders effective chemotherapy. Because of the frequent occurrence of the resistance caused by the activity of the MDR transporters, it is essential to find the effective and – at the same time cell-non-toxic – inhibitors of those proteins. Their use would allow making cancer cells sensitive to the activity of the drugs administered in chemotherapy. The approaches relying on the use of a single chemosensitizer have never resulted in a clinical success. Therefore, the application of drug combinations of two or more compounds with different mechanisms of action might be an alternative approach to increase the success rate. Oxicams belong to a diverse group of non-steroidal anti-inflammatory drugs (NSAIDs). Apart from the inhibition of cyclooxygenase 2 (COX-2) and the reduction of the pro-inflammatory concentration of PGE2 (prostaglandin E2), they affect various molecular targets. It results in both the anti-inflammatory and chemo-preventive effects because, it has already been proven, there is a strong correlation between COX-2 expression and the ABCB1 protein responsible for the MDR phenotype. In the present study the cytotoxic potential of the oxicam derivative, PR29, was evaluated. PR29 was demonstrated to be an effective doxorubicin-resistance modulator in human adenocarcinoma cell line LoVo/Dx. In the presence of PR29 cytotoxicity of doxorubicin was elevated, and its intracellular accumulation increased. Strong synergism occurred between PR29 and Dox. PR29 diminished also the expression of ABCB1 transporter (P-glycoprotein) by affecting NF-KB pathway. Theobromine, a phytochemical from cocoa, which was barely active itself, strongly augmented MDR reversal potency of PR29. Co-administration of these modulators allowed for the reduction of MAE-TPR concentration needed to obtain anti-MDR effect.


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## P-04-015
## New regulatory mechanism of mitochondrial large-conductance calcium-activated potassium channel

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Mitochondrial large-conductance calcium-activated potassium channel (mito$BK_{Ca}$) is formed by DEC splice variant of KCNMA1 gene, which encodes also plasmalemmal $BK_{Ca}$. The activity of mito$BK_{Ca}$ is regulated by many modulators, including activators such as $Ca^{2+}$, NS1619, and inhibitors such as paxilline, heme and its oxidized form hemin (Fe(III)-protoporphyrin IX). It is known that heme/hemin is a receptor for gasotransmitters like carbon monoxide (CO) and hydrogen sulfide ($H_2S$). $H_2S$ mechanism of action is complex and includes persulfide formation with -SH groups of cysteines, sulfide-metal interactions in heme proteins and redox reactions. It was shown that $H_2S$ regulates the activity of $BK_{Ca}$ from the plasma membrane, but literature data are equivocal. However, nothing is known about its effects on the mito$BK_{Ca}$ activity. In this study, we performed patch-clamp experiments on mitoplasts what allows recording of the activity of single mito$BK_{Ca}$. To check the effects of $H_2S$ on the activity of mito$BK_{Ca}$ we applied sodium hydrosulfide (NaHS) as a $H_2S$ donor. We found that $H_2S$, when applied alone, does not change the activity of mito$BK_{Ca}$. Next, we applied hemin and other metal ion-substituted protoporphyrins IX (PP IX) followed by NaHS application. We discovered that NaHS activates mito$BK_{Ca}$ inhibited for example by hemin and Sn(IV) PP IX. In addition, we also found that for example, Mg(II) PP IX and PP IX without metal do not inhibit the activity of mito$BK_{Ca}$. In addition to patch-clamp technique, we also investigated by absorption spectroscopy the impact of NaHS on the formation of the complex between metal-ion substituted protoporphyrins IX and a model peptide containing heme binding motif (–CXXCH–). In general, we found that $H_2S$ is an important molecule for regulation of the mito$BK_{Ca}$ activity and it seemed to depend on the metal center ion of PP IX. This work was supported by Polish National Science Center, grant no. 2015/17/B/NZ1/02496.

## P-04-016
## Optimization of growth conditions and substrates used as a tool altering the mode of metabolism of *Rhodobacter sphaeroides*: the role of membrane-bound systems in the mechanisms of regulation

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Application of phototrophic microorganisms to obtain molecular hydrogen ($H_2$) has been considered as one of the promising ways to generate this clean and highly efficient fuel. *Rhodobacter sphaeroides* isolated from Armenian mineral springs is a purple non sulfur bacterium capable of producing $H_2$ with high rate from variety of organic substrates. The growth conditions and substrates used have been shown to impact the mode of metabolism and consequently the biohydrogen production by these bacteria. Current work is an attempt to reveal the input of $F_0F_1$-ATPase and membrane conductivity in regulation of $H_2$ production of *R. sphaeroides* through variations of growth conditions and substrates used. Factors, such as initial pH, temperature and light intensity were optimized for maximal $H_2$ production based on our previous research. Proton conductance of bacterial membrane was measured using acid-pulse technique. Based on an estimated cellular buffering capacity the proton conductance of *R. sphaeroides* cells grown using succinate as a substrate was 15 nmol of $H^+$ /s/pH unit/mg protein. Addition of $F_0F_1$-ATPase inhibitor DCCD lowered this value, suggesting the involvement of $F_0F_1$-ATPase. However, the decrease of proton conductance in the presence of DCCD was less than 10% showing the involvement of other proton leakage pathways. Membrane conductance has considerably decreased during the bacterial growth in various light-dark alterations, suggesting its role in bacterial metabolism change. The research on the $F_OF_1$-ATPase activity of *R. sphaeroides* membrane vesicles indicated its important role in the increase of $H_2$ production efficiency during mixed carbon source photofermentation. Further experiments will be performed to clarify the specific mechanisms responsible for enhanced $H_2$ production from mixed carbon sources and to exploit the potential of the process for industrial waste utilization.

## P-04-017
## Characteristics of the BK-DEC channel formed in mitochondria

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Ischemia of the brain or heart tissue is the one of the most common causes of death in most Western countries. It is known that a group of several potassium channels in the inner mitochondrial membrane have been identified to lead to cytoprotection during ischemic event. According to previous studies, activation of mitochondrial large conductance calcium activated potassium channel (mito$BK_{Ca}$) preserves brain and heart muscle cells. Mito$BK_{Ca}$ channel's molecular identity was described in recent past. Against that background the $BK_{Ca}$-type channels α subunit's splice variant – BK-DEC – has been demonstrated to localize in mitochondria. However it is not known whether this isoform is able to form a functional channel in mitochondria. In our study we used transiently transfected HEK293T cells with plasmid encoding human BK-DEC splice variant. We isolated mitochondria and run electrophysiological recordings on swelled inner mitochondrial membrane (mitoplast). Our experiments revealed presence of the large conductance and voltage dependent ion channel. What is important, this type of activity was not present in mitoplasts isolated from untransfected cells. Recorded channel showed all basic pharmacological properties typical for the mito$BK_{Ca}$ channels: $Ca^{2+}$ sensitivity, regulation by potassium channel openers like NS1619 and inhibition by paxilline. Moreover, the channel activity was inhibited by hemin. Conductance of observed channel was around 290pS and corresponds to the properties of the mito$BK_{Ca}$ channels described previously in various tissues. In summary, using HEK293T cells as a model cell line we show directly for the first time that the BK-DEC splice variant forms a fully functional channel in the inner mitochondrial membrane. This work was supported by the Polish National Science Centre grant No. 2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.


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## P-04-018
## Reconstitution of ROMK1/2 proteins into native nanodiscs

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ROMK2 (Kir1.1b) is a mitochondrial isoform of plasmalemmal ROMK1 channel (Kir1.1a), which is missing the first 19 amino acids. It is believed that ROMK2 is a part of the mito$K_{ATP}$ channel. However, the data directly linking the protein to this channel activity is limited. The small amount of Kir1.1 protein in mitochondria of H9c2 cells prompted us to express the Kir1.1 protein in E. coli and purify Kir1.1 channels for further studies. To obtain purified ROMK1/2 channels for further studies, we expressed the ROMK1/2 protein in *Escherichia coli*. The sequence of several expression tags was fused to codon-optimized ORFs or chimera between cytoplasmic N- and C-termini of ROMK1/2 and transmembrane part of bacterial potassium channel KirBac1.3. These tags (MISTIC, SUMO and OMPF) could assist membrane insertion and folding of proteins expressed in bacteria. The fusion proteins contained also affinity tags for purification (6xHis and STREP-Tag). The ROMK1/2 with C-terminal 6xHis construct was chosen for further studies, because it exhibited the highest membrane expression level and low degradation. Large amount of *E. coli* expressed ROMK1 and ROMK2 proteins gave us opportunity to reconstitute these channels in membranes and also in nanodiscs. We decide to solubilized membranes containing ROMK-His with different detergents, such as DDM, OG, digitonin and other. For solubilization we also used three copolymers of styrene and maleic acid (SMA) of different stoichiometry of 3:1, 2:1, and 1:1, as well as with copolymer of diisobutylene and maleic acid (DIBMA). These polymers produce membrane protein-containing particles (nanodiscs, SMALPs – SMA lipid particles and DIBMALPs – DIBMA lipid particles) without the need for the use of classical detergent. After initial experiments we decided to use nanodiscs of SMA 2:1 and DIBMA for further studies. Here, we evaluate the applicability of these polymers for solubilization, purification and recording of channel activity of ROMK1/2 protein.

## P-04-019
## Are BK(Ca) channels located in mitochondria of mouse embryonic fibroblast (MEF) cells?

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$BK_{Ca}$ channels are involved in many cellular processes. Being in the plasma membrane $BK_{Ca}$ channels regulate for instance the resting membrane potential, smooth muscle contraction, and neuronal firing. The activity of $BK_{Ca}$ channels was found both in mitochondria designated as mito$BK_{Ca}$ but also in lysosomes, where it is called Lyso$K_{vCa}$. In mitochondria, activation of mito$BK_{Ca}$ seems to be an important factor for recovery of cardiac function after ischemia/reperfusion injury. It was found that gain-of-function mutation that results in activation of $BK_{Ca}$ reduces ROS produced by complex I and complex II/III in transgenic mice after ischemia/reperfusion. In lysosomes, Lyso$K_{vCa}$ forms a complex with TRPML1 and provides positive feedback for $Ca^{2+}$ release by TRPML1 allowing the influx of $K^+$ into the lysosomes and leading to hyperpolarization. Lysosomal localization and Lyso$K_{vCa}$ activity was described in mouse embryonic fibroblast cells (MEF). MEF cells are used in multiple studies concerning mitochondria, i.e. studies on fusion and fission. In this work we attempt to answer the question whether $BK_{Ca}$ channels are localized also to mitochondria of MEF cells. To this end we use cell fractionation, immunocytochemistry and patch-clamping of mitoplasts.

## P-04-020
## Naringenin as an opener of mitochondrial potassium channels

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Certain flavonoids, including naringenin, have cytoprotective properties. Although the antioxidant effect has long been thought to be a crucial factor accounting for the cellular effects of flavonoids, mitochondrial channels have emerged recently as targets for cytoprotective strategies. In the present study, we characterized interactions between naringenin and the mitochondrial $BK_{Ca}$ channels recently described in dermal fibroblasts and endothelial cells. Our path-clamp study shows that naringenin in micromolar concentrations leads to an increase in mito$BK_{Ca}$ channel activity. The opening probability of the channel decreased from 0.97 in the control conditions (200 mM $Ca^{2+}$) to 0.06 at a low $Ca^{2+}$ level (1 mM) and increased to 0.85 after the application of 10 mM naringenin. Additionally, the activity of the mito$K_{ATP}$ channel increased following the application of 10 mM naringenin. To investigate the effects of naringenin on mitochondrial function, the oxygen consumption of dermal fibroblast cells was measured in potassium-containing media. The addition of naringenin significantly and dose-dependently increased the respiratory rate from $5.8 \pm 0.2$ to $14.0 \pm 0.6$ nmol $O_2$ x $min^{-1}$ x mg $protein^{-1}$. In this study, we demonstrated that a flavonoid, naringenin, can activate $BK_{Ca}$-type channels present in the inner mitochondrial membrane of dermal fibroblasts and endothelial cells. This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland. Work implemented as a part of Operational Project Knowledge Education Development 2014–2020 cofinanced by European Social Fund (to Aleksandra Sek).

## P-04-021
## Changes in large conductance potassium channel and its auxiliary subunits expression upon stress of endoplasmic reticulum

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Endothelium is composed of single layer of cell line interior surface of blood and lymphatic vessels covered with glycocalyx from lumen side. Glycocalyx covered with plethora of enzymes responsible for interaction of blood constituencies (e.g. leucocyte and thrombocyte). Endothelial cells per se are responsible for blood pressure regulation, clotting (thrombosis and fibrinolysis), and inflammation. Endothelial dysfunction leads to development of atherosclerosis, coronary artery disease, hypertension etc. One of the reasons which can lead to endothelial cells dysfunction is mitochondrial and endoplasmic reticulum stress. It is well known that mitochondrial potassium channels – especially mito$BK_{Ca}$ – are protective element in ischemia-reperfusion processes. Our task in these studies was to establish how endoplasmic reticulum (ER) stress can influence the expression of $BK_{Ca}$ channels (especially mitochondrial form) and its auxiliary subunits. To establish the role of ER stress in endothelial $BK_{Ca}$ expression and its four (ß


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1-4) auxiliary subunits we have used stable line of EA.hy 926 cells, which were treated with compounds casing ER stress that influence the glycosylation (Tunicamycin), calcium homeostasis (Thapsigargin), and oxidative stress. The levels of protein expression and mRNA for $BK_{Ca}$ were measured. Also changes in morphology of mitochondria and its activity were established with confocal microscopy and oxygen consumption measurements, respectively. The results have shown that upon ER stress level of $BK_{Ca}$ channels pore forming subunits is unchanged however the level of its auxiliary subunits is unevenly affected. The expression of ß1 subunit was about four times increase but the expression of ß4 subunit was decreased by four fold. Furthermore, the ER stressor causes changes in morphology of mitochondria on the level of its fragmentation and elongation. This project was supported by Polish National Science Center grant No. 2015/18/E/NZ1/00737

## RNA processing

### P-05-001
### The role of Pbp1, the yeast ortholog of human Ataxin-2, in the cell growth on non-fermentable carbon source media

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The fate of a newly transcribed mRNA is highly dependent on post-transcriptional control in which 5' cap and 3′ poly (A) tail of mRNA are critical for mRNA stability and translation. Pbp1, the yeast ortholog of human Ataxin-2, is a protein that binds to poly(A)-binding protein (Pab1) and regulates the Pan2-Pan3 deadenylase complex, thereby modulating the mRNA stability and translation efficiency. However, since PBP1 deletion yeast strain (pbp1Δ) grows similarly to wild-type (WT) strain, the physiological function of Pbp1 remains unclear. Recently, our laboratory found that the pbp1Δ mutant grows similarly to WT cells on normal YPD medium containing glucose as a carbon source but shows slower growth on YPGL media containing non-fermentable carbon sources, glycerol and lactate. This suggests that Pbp1 has a role in the growth on non-fermentable carbon sources. In this study, we aim at elucidating the regulatory role of Pbp1 in non-fermentable carbon source media. Microarray data, verified by quantitative PCR, is employed to compare gene expression profiles between WT and pbp1Δ in YPD and 4 hours after being transferred into YPGL. Deletion of PBP results in more than two-fold decrease in mRNA level of several genes involved in gluconeogenesis pathway and in mitochondria function in YPGL but not in YPD. These results indicate that Pbp1 regulates the expression of those genes involved in gluconeogenesis and mitochondria function in YPGL. To identify regulatory stages at which Pbp1 controls the level of each mRNA, the reporter plasmids harboring GFP gene driven by the promoter of each gene are constructed, transformed into both WT and pbp1Δ. The level of GFP driven by PCK1p shows similar decreased expression pattern with endogenous PCK1, suggesting that Pbp1 regulates PCK1 expression through PCK1 promoter. Meanwhile, the levels of GFP driven by COX10p are similar between WT and pbp1Δ in YPGL, suggesting that Pbp1 regulates COX10 expression not through COX10 promoter.

### P-05-002
### Functional analysis of the ribosomal protein eS12 in *Saccharomyces cerevisiae*

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Ribosome synthesis involves the concomitance of pre-rRNA processing and ribosomal protein assembly. In eukaryotes, this is a multistep process that takes place successively in the nucleolus, the nucleoplasm and the cytoplasm. About 300 trans-acting factors and the 80 ribosomal proteins participate in this complex process. The role of most ribosomal proteins in the biogenesis of each ribosomal subunit has been addressed. However, there are still some ribosomal proteins that require characterization. Herein, we have analyzed the contribution of ribosomal protein eS12 in ribosome biogenesis. eS12, together with 18S rRNA helix H33 and ribosomal proteins uS10 and eS31, forms a structural landmark of the 40S ribosomal subunit named the "beak". eS12 is a *quasi*-essential ribosomal protein encodes by the *RPS12* gene. Deletion of this gene results in a strong deficit in 40S ribosomal subunit. Pulse-chase and northern hybridization show that 20S pre-rRNA accumulates in the absence of eS12. FISH and fluorescent microscopy indicate that cytoplasmic processing of 20S pre-rRNA is indeed impaired. Interestingly, the deletion of the *UBI3* gene, which encodes eS31, leads to similar phenotypes. We will present data on the genetic analysis with an *rps31* null allele and loss-of-function mutants of *trans*-acting factors involved in cytoplasmic maturation of pre-40S ribosomal particles. No synergy is observed when we simultaneously delete *RPS12* and the *UBI3* gene. A very slight translational defect could be found for the rps12Δ mutant. We conclude that formation of the beak is a prerequisite for the efficient maturation of 40S r-subunits and its proper function. *The authors marked with an asterisk equally contributed to the work.

### P-05-003
### Human RNA cap1 methyltransferase CMTr1 cooperates with RNA helicase DHX15 to modify RNAs with highly structured 5′ termini

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The 5′-cap structure, characteristic for RNA polymerase II-transcribed RNAs, plays important roles in RNA metabolism. In humans, RNA cap formation includes post-transcriptional modification of the first transcribed nucleotide by RNA cap1 methyltransferase (CMTr1). We found that CMTr1 activity is hindered towards RNA substrates with highly structured 5′ termini. CMTr1 binds ATP-dependent RNA DHX15 helicase and that this interaction, mediated by the G-patch domain of CMTr1, has an advantageous effect on CMTr1 activity towards highly structured RNA substrates. The effect of DHX15 helicase activity is consistent with the strength of the secondary structure that has to be removed for CMTr1 to access the 5′-terminal residues in a single-stranded conformation. This is, to our knowledge, the first demonstration of the involvement of DHX15 in post-transcriptional RNA modification, and the first example of a molecular process in which DHX15 directly affects the activity of another enzyme. Our findings suggest a new mechanism underlying the regulatory role of DHX15 in the RNA capping process. RNAs with highly structured 5′ termini constitute a significant fraction


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of the human transcriptome. Hence, CMTr1–DHX15 cooperation is likely to be important for the metabolism of RNA polymerase II-transcribed RNAs. *The authors marked with an asterisk equally contributed to the work.

## P-05-004
## The role of hypoxia in alternative splicing of neurodegenerative disease-related genes

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The most wide-spread neurological disorders such as Alzheimer's and Parkinson's diseases are characterized by progressive loss of brain cognitive function. There are several neuropathological hallmarks common to these diseases: i) accumulation of extracellular beta amyloid (Aβ) peptides in the brain cells, which are produced of amyloid precursor protein (APP); ii) accumulation of hyper-phosphorylated microtubule-associated protein Tau that forms neurofibrillary tangles. Recently it has been shown that the reduction of oxygen tension (hypoxia) in the brain cells promotes development of neurodegenerative diseases. Reduced oxygen levels in cellular microenvironment induce the stabilization of hypoxia-inducible transcription factors (HIFs) which activate the transcription of genes involved in cells' ability to adapt to the altered conditions. Changes in alternative pre-mRNA splicing also occur under hypoxic conditions, producing different mRNA isoforms that are translated to proteins promoting cell survival under unfavorable conditions. This study is focused on the influence of hypoxia on alternative splicing of pre-mRNAs that are associated with neurodegenerative diseases (APP and Tau) and initial analysis of factors involved in hypoxia dependent alternative pre-mRNA splicing regulation of these pre-mRNAs. Our results revealed that hypoxia influences Tau and APP mRNAs isoform formation in hypoxic cells. We are also currently elucidating the role of individual SR proteins on hypoxia dependent alternative splicing regulation of these genes. This research is funded by the European Social Fund under the No 09.3.3-LMT-K-712 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities".

## P-05-005
## Biochemical and structural bioinformatics studies of fungal CutA nucleotidyltransferases explain their unusual specificity towards CTP

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Eukaryotic RNA molecules are modified at ends, which has profound effects on their fate. The most widespread 3′-end modification is an addition of non-templated nucleotides by RNA nucleotidyltransferases (NTases), which display variable substrate specificity. mRNAs are polyadenylated by canonical poly(A) polymerases, which is essential for their stability and efficient translation. In turn, non-canonical Trf4-like poly(A) polymerases synthesize oligo(A) tails at the 3′-end of different RNAs, which mark they for rapid degradation by the exosome. Some other Trf4 family members, including Cid1 from S. pombe add 3′-terminal oligouridine tails, which triggers mRNA degradation in either deadenylation-independent decapping-mediated pathway or stimulates 3′-5′ exoribonucleolytic decay by DIS3L2. A related enzyme adding C/U-rich extensions promoting mRNA decapping and degradation, named CutA, was identified in Aspergillus nidulans. All non-canonical NTases contain PAP/OAS1 substrate-binding domain (SBD) with inserted nucleotidyltransferase (NTase) domain, encompassing active site. Here, using enzymatic assays and high-throughput sequencing of the 3′-RACE products for tails generated by CutA proteins, we show that they indeed contain an unprecedented number of cytidines interrupted by uridines, and that majority end with two cytidines. The molecular basis of such NTP specificity has not been defined. Comparison of our CutA 3D model to selected non-canonical NTases of known structures revealed substantial differences in the nucleotide recognition motif (NRM) within PAP/OAS1 SBD. We demonstrate that CutA specificity towards CTP can be partially changed to PAP or PUP by mutagenesis within NRM and, analogously, Cid1 PUP can be converted into C/U-adding enzyme. Collectively, our data indicate that a short cluster of amino acids within NRM is a major determinant of the non-canonical NTases substrate preference, which may allow to predict their specificity

## P-05-006
## Structural and biophysical studies of the plant m6A methyltransferase complex

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Recently, widespread N6-methyladenosine (m6A) modification of messenger RNA (mRNA) and non-coding RNA was discovered in yeast, flies, mammals and plants. It was shown that this modification could affect mRNA fate within the cell: changing its splicing pattern, promoting transport to the cytoplasm, increasing degradation rate and enhancing translation. Moreover, m6A modification of primary miRNA transcripts promotes miRNA biogenesis in human cell lines. At the functional level m6A plays an important role in many biological processes, including: stem cell differentiation, circadian clock in mammals, functioning of the nervous system and development in plants. In mammals, two methyltransferases: METTL3 and METTL14, together with the adaptor protein WTAP are required for m6A methylation. In plants MTA and FIP37, identified as homologs of METTL3 and WTAP, respectively. Here we present the results of SAXS studies of WTAP and FIP37 proteins in solution. Our data showed that WTAP and FIP37 proteins form in solution high-order flexible oligomers. Using yeast two-hybrid system we showed that C-terminal part of FIP37 and N-terminal part of MTA are responsible for the interaction. Moreover we discover that N-terminal part of MTA encodes plant specific domain with no sequence homology to other protein domains. Structural and biophysical characterisation of this domain will be presented. This work was supported by the international grant POLTUR2/3/2017 from National Center of Research and Development and research grant UMO-2017/27/B/ST4/00485 from the National Science Centre.


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## Signal transduction

### P-06-001
### Class I phosphoinositide 3-kinase is responsible for neutrophil extracellular traps formation upon stimulation with reactive nitrogen species

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During previous decade, the release of neutrophil extracellular traps (NETs), the newly-described antimicrobial strategy employed by neutrophils, has been placed in the spotlight. Although mechanisms of NETs formation are extensively studied, some aspects of this process, e.g. the role of reactive nitrogen species (RNS), remain poorly understood. The aim of this study was to analyze the role of autophagy (a natural, self-degradative process) in RNS-induced NETs formation. Human blood neutrophils were stimulated with nitric oxide (NO) donor – SNAP (S-Nitroso-N-acetylpenicillamine) or peroxynitrite. NETs release and the synthesis of reactive oxygen species (ROS) were assessed by fluorometry and fluorescent or light microscopy. Autophagy was analyzed by western blotting as accumulation of LC3-II protein and with electron microscopy. We found that RNS stimulate NETs release without accumulation of LC3-II. Inhibitors of PI3 kinases (wortmannin and 3-methyladenine, used as inhibitors of autophagy), but not inhibitors of the autolysosome formation, drastically reduced NETs formation upon RNS treatment. Specific inhibitors of class I PI3K effectively diminished NETs release. RNS only slightly increased ROS production by neutrophils, but 3-MA and wortmannin significantly decreased ROS production by RNS-stimulated neutrophils. Finally, using chemical inhibitors and neutrophils isolated from patients suffering from chronic granulomatous disease, unable to produce ROS, we found that activity of NADPH oxidase was necessary or contributed to RNS-induced NETs formation. We conclude that RNS efficiently stimulate NETs formation and class I PI3K activity, but not autophagic flux, is necessary for this process. PI3K influences NETs formation via regulation of NADPH oxidase activity. Acknowledgments: This work was supported by the National Science Centre, Poland (AMH, Preludium 2015/19/N/NZ6/01317) and the Foundation for Polish Science (MDW, AMH, POWROTY/2016-2/7).

### P-06-002
### Involvement of aromatase and estrogen receptors in the effects of Cannabis tetrahydrocannabinol: Implications for steroidogenesis dysregulation in placenta

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Cannabis has become a hot topic due to its growing legalization for medical purposes. Due to its popularity, the number of pregnant consumers is also increasing, despite the adverse pregnancy outcomes such as prematurity, low birth weight and intrauterine growth restriction. Placental steroid synthesis is fundamental for gestation, with estradiol (E2) being involved in embryo implantation, angiogenesis and myometrial contractions. This molecule is produced through the conversion of testosterone by aromatase. Due to the concerning increase in pregnant consumers and the lack of clarification on the estrogenic effects of THC, we studied its influence on estrogen signaling. Term placenta explants and the trophoblastic cell line BeWo, were treated with THC (10 to 40 μM) and the expression of aromatase and estrogen receptor α (ERα) was analysed by immunohistochemistry, Western blot and qRT-PCR. E2 levels were assessed by ELFA. Results show that both aromatase and ERα are expressed in placental trophoblast cells, although no significant differences were found with THC treatment in the pattern of expression. Furthermore, we demonstrate that 40 μM of THC increased both aromatase transcription and protein levels at 24 h and 72 h, with increased E2 secretion in the latter time span. Likewise, in BeWo cells, aromatase expression was also increased at 24 h. Cannabinoid treatment (20 μM) induced ERα expression in both placenta and BeWo cells at 24 h. These findings reveal that THC has a disruptive behaviour on placental estrogen signaling, which may be one of the molecular mechanisms contributing to the negative effects observed in pregnant cannabis users. This work is financed by the FEDER Funds through the Operational Competitiveness Factors Program-COMPETE and by National Funds through FCT within the scope of the project PTDC/DTP-FTO/5651/2014-POCI-01-0145-FEDER-016562. João Maia thanks FCT for the PhD grant BD/136105/2018.

### P-06-003
### Osteoblast and osteoclast communication in the response to a mesoporous bioactive glass designed for bone repair

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In the field of bone tissue engineering, more and more attention is being attracted to the mesoporous bioactive glasses (MBGs), whose design with a highly ordered mesoporous structure confers high surface area and porosity that results in a high ionic exchange rate of mainly silicon and calcium soluble species with the surrounding fluids. Its composition also stimulates the expression of several genes of osteoblastic cells and induces angiogenesis both in vitro and in vivo. To evaluate in vitro the effects of these materials on bone remodeling and repair, it is necessary to design optimal culture models that mimic the physiological situation of the bone tissue in which they will be implanted. In the present study, a coculture model has been developed with osteoblasts and osteoclasts, the two cell types involved in bone remodeling, whose imbalance produces osteoporosis, one of the most prevalent diseases in today's society. Thus, osteoblasts (main cells responsible of bone regeneration) and osteoclasts (involved in bone degradation) have been cocultured allowing their communication with each other in the presence of powdered MBG-75S. Different cells parameters have been evaluated: cell proliferation, viability, apoptosis, cell cycle, osteoclast differentiation and osteoclast resorptive activity. The effects of MBG-75S on macrophage polarization towards M1 and M2 phenotypes have also been studied. The results show that MBG-75S allows the


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differentiation of osteoclast but decreases their resorptive activity and this effect is increased when osteoclasts are cocultured with osteoblasts. This MBG does not induce on macrophages their polarization towards the M1 proinflammatory phenotype, increasing the M2 repair phenotype expression. This behaviour indicates that MBG-75S could be used for treatment of osteoporotic bone due to the decrease of osteoclast resorptive activity without inducing further inflammatory complications.

## P-06-004
## AKT kinase phosphorylates PHF10, a subunit of PBAF chromatin remodeling complex

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Remodeling complexes play an important role in the regulation of gene expression during development and in the adult organism. The PBAF remodeling complex is the subfamily of SWI/SNF complexes in mammals that change the structure of chromatin, providing access for transcription factors to regulatory sequences of genes. Regulation of the work of the complexes is largely due to changes in the subunit composition and to post-translational modifications that affect the functions of the entire complex. One of the important subunits of the PBAF complex is the PHF10 protein, which determines the interaction of the PBAF complex with chromatin. In mammalian cells, PHF10 is represented by four isoforms that are alternatively incorporated in the PBAF complex and have different effects on the genes remodeled by the complex. We have shown that all isoforms of PHF10 are significantly phosphorylated. Each isoform has the unique phosphorylation pattern that is dependent on the domain structure. In this work, we studied the phosphorylation of the N-terminal domain of the long PHF10 isoforms, which we designated as Y-cluster phosphorylation. We have shown that phosphorylation of the Y-cluster is triggered by two key serines. Mutation of these serines leads to the complete elimination of phosphorylation of the Y-cluster. These serines are the part of the Akt and kinases motif of the FGF / EGF signaling cascade. We have demonstrated the interaction between active form of AKT kinase and PHF10 in HEK293. Also, we have identified a minimal PHF10 sequence that undergoes phosphorylation, and it has been shown that the most phosphorylated isoforms of PHF10 are included in the PBAF complex. Thus, it can be assumed that phosphorylation of the Y-cluster of the long isoforms PHF10 by Akt kinase affects the functioning of the PBAF complex and regulates its participation in gene activation. This study was supported by the Russian Foundation for Basic Research [grant number 18-04-00885\18].

## P-06-005
## Mitochondrial STAT3 regulates cell proliferation by promoting mitochondrial gene expression

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Stat3 is a transcription factor that, acting both in the nucleus and mitochondria, is needed to maintain embryonic stem (ES) cells pluripotency and promote proliferation. In this work, using zebrafish Stat3 signaling pathway reporter, mutant lines and by manipulating the Stat3 coding sequence, we could show *in vivo* that Stat3 activity correlates with proliferation of embryonic, adult and cancer tissues. In particular, Stat3 is needed to maintain intestinal tissue homeostasis, by resulting highly active in few proliferating cells, named Fold Base Columnar (FBC) cells, located at the base of intestinal folds. Notably, we observed that the highly proliferating Stat3-positive FBC cells are considerably expanded in intestinal adenomas of *apc* mutants. Next, we enquired *in vivo* the molecular needs for Stat3 to promote proliferation. We propose that Stat3 mitochondrial translocation and transcriptional activity, mediated by Tyrosine-705 and Serine-727 phosphorylation respectively, are a prerequisite for Stat3 to activate cellular proliferation through mitochondrial transcription.

## P-06-006
## C2 domain of Copine1 plays a pivotal role as protein interaction region for its function

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Copine1 (CPNE1), known as calcium-dependent membrane-binding protein, has tandem C2 domains and a von Willebrand factor A domain (A domain). It was known that C2 domains are related with calcium-dependent phospholipid binding activities and A domain is related with protein binding region. We have previously demonstrated that CPNE1 directly induces neuronal differentiation via Protein kinase B (AKT) phosphorylation in the hippocampal progenitor cell. To better understand its cellular function, yeast two-hybrid screening was performed to find CPNE1 binding partners. Some binding candidates were identified as 14-3-3γ and Jab1 proteins. Between CPNE1 and 14-3-3γ/JAB1, the physical interaction as well as the specific binding regions of CPNE1 was confirmed *in vitro* and *in vivo*. These interaction increase AKT phosphorylation, neurite outgrowth and expression of the neuronal marker protein. In addition, only C2A domain of CPNE1 is the binding site of these proteins for their function related neuronal differentiation. Collectively, 14-3-3γ and JAB1 activates the neuronal differentiation ability of CPNE1 through the binding of C2A domain in CPNE1. Our findings suggest that C2 domain of CPNE1 also plays important role as a protein-protein interaction domain.

## P-06-007
## The functional analysis of long noncoding RNAs affected by oncogenic Ras signaling

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Long noncoding RNAs (lncRNAs) range in size from 200 nucleotides to over 10 kb and are spliced and polyadenylated post-transcriptionally. Several lncRNAs are involved in biological processes, including differentiation, proliferation, senescence, apoptosis, and cancer development. Recently, we reported that expression of multiple lncRNAs fluctuated by forced expression of activating H-Ras mutant including G12V amino acid substitution. Activating Ras signaling (called oncogenic Ras signaling) leads to oncogenic transformation. Among the lncRNAs regulated by oncogenic Ras signaling, functional lncRNAs associated with malignant transformation may be present. *ANRIL* is one of lncRNAs downregulated by oncogenic Ras signaling. *ANRIL* is located in the *INK4* locus, which encodes three tumour


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suppressor proteins: the cyclin-dependent kinase inhibitors p15 and p16, and alternative reading frame(ARF). We showed that silencing *ANRIL* increased *p15* and *p16* expression, leading to cell growth arrest of human normal fibroblasts. RNA immunoprecipitation assay demonstrated that *ANRIL* associates with SUZ12, a component of polycomb repression complex 2 (PRC2) which is involved in gene silencing. Chromatin immunoprecipitation assay demonstrated that silencing *ANRIL* disrupts the binding of PRC2 on the *INK4* locus. Collectively, these results suggest that *ANRIL* represses the transcription of *INK4* locus via associating with PRC2, leading to the promotion of cell proliferation. We also showed that *OIP5-AS1* is another one of lncRNAs downregulated by oncogenic Ras signaling. Silencing *OIP5-AS1* caused G2/M phase cell cycle arrest, leading to the repression of HeLa cell proliferation. These results suggest that *OIP5-AS1* positively regulates cell proliferation, via promoting G2/M phase progression. I will present on the functions of *ANRIL* and *OIP5-AS1* in the regulation of cell proliferation.

## P-06-008
## Lysosomal cathepsin C as crucial player for the release of cathepsins from lysosomes in LLOMe-triggered apoptosis

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Lysosomal membrane permeabilization (LMP) was believed to have great therapeutic potential to induce cell death in cancer cells. However, the exact mechanism connected to LPM-dependent cell death still remains not completely clear. In our study the usage of lysosomotropic detergent L-leucyl-L-leucine methyl ester (LLOMe) as a model led to a very early increase in lysosomal pH in immune and non-immune cells, followed by lysosomal leakage and the release of cathepsins into the cytosol. Both the increase in lysosomal pH and cell death were almost completely prevented by the cathepsin C-selective inhibitor. Further one, consistent with this idea, the ablation of the major cytosolic inhibitor of cysteine cathepsins, stefin B, resulted in sensitizing primary PyMT cancer cells to cell death without affecting the release of cathepsins. Even more, simultaneous ablation of two major lysosomal cysteine cathepsins, cathepsins B and L, largely protected MEFs against cell death, confirming our idea, that released cathepsins trigger the cell death cascade in caspase-dependent manner. Within this study, we showed as first that the different sensitivity of cells to LLOMe is conditioned by the expression levels of lysosomal cathepsins, their endogenous inhibitors, pro/anti-apoptotic proteins, and, possibly, lysophagy. Even more, due to the different response of immune and non-immune cells to LLOMe triggering, we confirmed our belief that usage of LLOMe for treatment is inappropriate.

## P-06-009
## A novel regulatory mechanism of p38 MAPK by protein arginine methylation impacts erythroid differentiation

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p38 MAPK plays critical roles in various cellular processes under normal and pathological conditions. The activation of p38 MAPK requires dual phosphorylation on Thr180 and Tyr182 residues via a phosphorylation cascade involving MAPKKs (MKK3, 6) and MAPKKKs. Our study has shown p38α activation is required for EPO- and AraC-induced erythroid differentiation. Furthermore, protein arginine methyltransferase 1 (PRMT1) is essential and responsible for the induced p38α activation and erythroid differentiation, suggesting a novel PRMT1/p38α axis in regulating erythroid differentiation. PRMT1 methylates many intracellular proteins by modifying arginine residues and is involved in modulating a number of cellular processes, such as gene expression, DNA repair, RNA metabolism, and signal transduction. Our results have prompted us to investigate the potential arginine methylation of p38α and its functional role in erythroid differentiation. PRMT1 can methylate p38α *in vitro* as shown by methylation assay. Further, PRMT1 is associated with p38α in K562 cells. We have identified several methylated arginine by mass spectrometric analysis. The p38a mutant cannot be methylated by PRMT1 and is unable to stimulate erythroid differentiation. PRMT1 can no longer promote p38α-mediated stimulation of erythroid differentiation when these arginine residues are mutated to lysine residues. These results demonstrate that there is a functional role of p38α arginine methylation. This study unveils a novel regulatory mechanism of p38α by protein arginine methylation which impacts erythroid differentiation.

## P-06-010
## Antioxidant and whitening effects by sulforaphane mixed-composition

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Sulforaphane is an organic isothiocyanate (ITC) found in vegetables such as broccoli, kale, cauliflower and cabbage, and has potent antioxidant effects to suppress the NOS induction. PF-3758309 is an inhibitor for p21-activated kinases (PAK), and has reported that regulates melanogenesis through inhibition of PAK4 in skin keratinocytes. In this study, we analyzed two components, sulforaphane and PF-3758309 to prove that the mixed composition of them has more effective antioxidant and whitening functions compared to each component. To analyze the possibility using this composition in skin, we assayed the cellular toxicity of each component and also the mixed composition to find the safe concentration level for cell viability. Combination treatment of 10 μM sulforaphane and 1 nM PF-3758309 did not affect the cell viability. In this condition, the LPS-stimulated NO generation was significantly reduced by the mixed composition of sulforaphane and PF-3758309. Melanogenesis by α-melanocyte stimulating hormone (α-MSH) was also inhibited by the mixed composition. In order to confirm the possibility as the cosmetic material, we carried out clinical studies for the mixed composition samples. Skin safety evaluation using patch test was judged to be unstimulated, skin whitening effect was increased, and melanin deposition was suppressed by treatment of mixed composition samples. These results provide us with the opportunity for applying it into the development of new functional cosmetics.

## P-06-011
## The evaluation of (anti)androgenic effects of mycotoxin citrinin with the use of recombinant yeast-based in vitro bioassay

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Dietary exposures to food pollutants such as mycotoxins have assumed greater significance due to their adverse effects on


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production and reproduction of animals and humans. Citrinin is a secondary metabolite produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. This mycotoxin contaminates long-stored food of plant origin and may cause adverse health problems in humans and animals *by* targeting *different* organs and systems. The aim of the study was to assess the androgenic and antiandrogenic effects of citrinin with the use of *in vitro* yeast-based reporter bioassay that has been developed to study the ability of compounds to induce human androgen receptor (hAR)-mediated transactivation. Citrinin was studied at 9 concentrations ranging from 0.1 nM to 200 μM alone or in binary mixtures with 100 nM and 5 μM 17β-testosterone (17βT). Each bioassay was performed two times, for each concentration 6 replicates were applied. The cytotoxicity, $EC_{50}$, relative androgenic potency (RAP) and antiandrogenic activity of the compounds were assessed. Results showed that citrinin was not cytotoxic in the selected concentration range. The compound showed partial androgenic activity in the yeast androgen bioassay ($EC_{50}$=36.62 mM, RAP = 0.229%). Moreover, citrinin showed dose-dependent antiandrogenic response in the presence of 50 and 100 nM of 17βT with the strongest inhibitory effect at the concentration of 50 mM. Our study reveals that citrinin is an endocrine active compound that may disturb androgen receptor-mediated signaling. Further research with the use of *in vivo* models is warranted to fill current gaps in knowledge regarding hormonal activity of citrinin and the related health effects in humans and animals. The study was supported by the Leading National Research Centre (KNOW), Scientific Consortium "Healthy Animal- Safe Food", Decision of Ministry of Science and Higher Education No 05-1/KNOW/2015.

## P-06-012
## Mechanism of signal transduction in a two-component system formed by a globin-coupled histidine kinase, AfGcHK (a heme-based oxygen sensor)

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The heme-based oxygen sensor, a globin-coupled histidine kinase, *Af*GcHK is an element of a two-component signal transduction system in bacteria. The $O_2$ binding to the Fe(II) heme complex of its N-terminal globin domain significantly stimulates autophosphorylation at His183 in its C-terminal kinase domain. The phosphate group is subsequently transferred from His183 of the *Af*GcHK to Asp52 and Asp169 residues located on its response regulator partner protein. As it was shown earlier, the oligomerization state of *Af*GcHK is very important for its phosphorylation activity. *Af*GcHK kinase function can be observed only in the case that the protein forms a dimer. It is important to state that the overall dimer structure of the full-length protein is probably maintained by dimer formation within its two domains. Namely, there are two separate dimerization interfaces of the kinase and globin domains. Our results suggest that Tyr15 residue plays a key role in the globin domains dimerization and subsequently, protein autophosphorylation activity. This effect was confirmed by examination of properties of four *Af*GcHK full-length mutants: Y15F, Y15W, Y15G and Y15A. Two of these mutants (Y15F, Y15W) show slightly lower activity than wild type protein. Other two mutants (Y15G, Y15A) are basically without enzymatic activity. These results show that Tyr15 is without any doubt important for proper *Af*GcHK function/kinase activity and probably plays a crucial role in protein dimerization. Recently, the kinetics results of the full-length protein were supported by investigation of isolated globin domain as well as isolated kinase domain of *Af*GcHK. All these data together suggest a pivotal steps in signal transduction system with Tyr15 playing an important part. *Supported by Charles University (GAUK 704217).*

## P-06-013
## Signal transduction via adenosine receptors in human primary cells – detection of the second messenger

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Adenosine is an important metabolite present in the intracellular as well as extracellular compartment. It triggers cellular response through plasma membrane purinergic receptors. Activation of these GPCRs' (G protein-coupled receptors) is transduced by cAMP pathway but the effect depends on the type of α subunit in particular receptor type. Four adenosine receptors can be distinguished: A1 and A3 (Gαi), A2A and A2B (Gαs) which differ with affinity towards adenosine – A1, A2A, A3 can be activated within physiological adenosine concentrations (≤1 μM) whereas low affinity receptor – A2B – only if ligand concentration exceeds 24 μM. These data were predominantly obtained with use of cell lines overexpressing adenosine receptors. Most of the tests were conducted on HEK (Human Embryonic Kidney) or CHO (Chinese Hamster Ovary) cells. Our research is focused on adenosine as an immunosuppressive agent having direct impact on immune cells via ADORAs (adenosine receptors). According to our knowledge until now there were no data presenting adenosine potency with use of human primary immune cells. We were able to measure the level of cAMP – second messenger in GPCR pathway – in human lymphocytes both CD4 + and CD8 + . The final measurement of cAMP concentration with TR-FRET method was preceded with detailed optimization process which included not only reaction factors but also physiological state of the cells. We found out that adenosine receptors are significantly more active in activated lymphocytes than in those in resting phase. The assay was used to determine potency of ADORAs antagonists which was also lymphocyte type-dependent. Data were supported with the downstream effects measurements such as CREB (cAMP response element-binding protein) phosphorylation and cytokine production. For the first time adenosine EC50 values calculated from proper cell type can be transferred and related to other studies in immunological field. *The authors marked with an asterisk equally contributed to the work.

## P-06-014
## FGF1 protects cells against p53-dependent apoptosis independently of FGFR activation

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Fibroblast growth factor 1 acts through activation of specific cell-surface tyrosine kinase receptors (FGFRs). Besides this classical mode of action, under stress conditions FGF1 is translocated into the cytosol and nucleus of the cell. Recently, we found that exogenous FGF1 (as well as FGF2) demonstrates an anti-apoptotic activity, independent of receptor activation and


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downstream signaling. Here, we show that both transient and stable FGF1 overexpression increases survival of U2OS cells treated with different apoptosis inducers. These results are in clear contrast to FGF1 effect in G292 cells, a p53-null osteosarcoma cell line. In the presence of FGFR inhibitor anti-apoptotic activity of FGF1 was not observed in p53-negative cells, either treated with externally added growth factor or transfected with FGF1. Therefore, we postulate that intracellular FGF1 protects cells against p53-dependent death. Acknowledgments The work was supported by the National Science Centre, Poland (Sonata Bis 2015/18/E/NZ3/00501). *The authors marked with an asterisk equally contributed to the work.

## P-06-015
## MPK38 promotes p27-mediated cell cycle arrest, apoptosis, and inhibition of adipocyte differentiation by Ser83 phosphorylation

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Murine protein serine-threonine kinase 38 (MPK38)/MELK has been shown to interact with p53 and to stimulate p53 signaling. Here, we identify cyclin-dependent kinase (Cdk) inhibitors $p27^{KIP1}$, a downstream target of p53, as another interacting partner of MPK38. The association between MPK38 and $p27^{KIP1}$ is mediated through the C-terminal domain of MPK38 and the cyclin-dependent kinase (CDK) binding region of $p27^{KIP1}$. This interaction is increased by 5-fluorouracil or doxorubicin treatment. MPK38 phosphorylates $p27^{KIP1}$ at $Ser^{83}$, implying a potential role for $p27^{KIP1}$ phosphorylation in $p27^{KIP1}$-mediated activity. Indeed, MPK38 potentiates $p27^{KIP1}$-mediated apoptosis, cell cycle arrest, and inhibition of adipocyte differentiation in a kinase-dependent manner by increasing stability of $p27^{KIP1}$. These results suggest that MPK38 acts as a positive regulator of $p27^{KIP1}$ activity.

## P-06-016
## Actin cytoskeleton disruption leads to enhanced activity of TRPC6 channels in podocytes

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Currently, more than 347 million people worldwide suffer from diabetes mellitus (DM), and by 2030 the number of people suffering from diabetes and its complications will exceed 450 million. Diabetic nephropathy (DN) is one of the most common complications of insulin – dependent and insulin-independent forms of diabetes, which often leads to the development of chronic renal failure. DN is characterized by impaired renal function and manifested with proteinuria, glomerulosclerosis, etc. Previously, it was shown that the abnormal activity of TRPC calcium-permeable channels in renal glomerular podocytes plays a critical role in the development of diabetic nephropathy. The calcium entry into podocytes via TRPC may lead to rearrangements of the cytoskeleton. It can be assumed that there is feedback and the cytoskeleton in turn modulates the activity of TRPC6 channels. To examine the relationship between the cytoskeleton and calcium entry via TRPC6 channels the experiments with cytochalasin D that destructing the actin cytoskeleton was made. Electrophysiological analysis of the functions of native channels in podocytes of decapsulated glomeruli from Dahl salt-sensitive rats demonstrated enhanced TRPC6 activity under cytochalasin D action. Similar results were obtained in CHO cells with exogenous TRPC6 expression. We propose the existence of a relationship between the dynamic rearrangements of the cytoskeleton, which determine the filtering properties of podocytes and calcium entry through TRPC6 channels. This work was supported by the RSF No. 19-14-00114

## P-06-017
## Regulation of vitamin D receptor expression by FGFR receptor signalling pathway

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Vitamin D receptor (VDR) is present and active in many blood cells, and the correct level of vitamin D is necessary for proper function of the immune system. Active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 (1,25D) supports differentiation of monocytes and innate immune functions. 1,25D is able to induce differentiation and to suppress proliferation in these acute myeloid leukemia (AML) cells which express VDR and have sufficient level of VDR protein. Our recent studies revealed that aberrant signal transduction from constitutively active fusion protein FOP2-FGFR1 is responsible for downregulation of expression of VDR gene in AML cells, which results in resistance to 1,25D-induced differentiation. This fusion protein consists of the FGFR1 oncogene partner 2 (FOP2) and of the portion of fibroblast growth factor receptor 1 (FGFR1). Disruption of the fusion FOP2-FGFR1 gene restored the expression of VDR and sensitivity to 1,25D in AML cells. The FGFR1 gene belongs to the subfamily of receptor tyrosine kinases and comprises of four family members. The FGFR1 was also found fused to numerous other genes because of chromosomal translocations. The common feature of these translocations is disruption of the FGFR1 gene resulting in a fusion gene and fusion protein with constitutive activation of the FGFR1 tyrosine signaling cascades. In addition, constitutively active FGFRs cause downstream activation of signal transducer and activator of transcription (STAT) pathways. To investigate whether or not the crosstalk between FGFR signaling and VDR expression is observed only in AML cell lines the experiments we used human osteosarcoma cell line U2OS which shows high VDR expression level and is 1,25D-sensitive. The U2OS cell line was transfected with expression plasmids encoding the FGFR1–FGFR4 genes. Data showed that overexpression of FGFR1 even in the absence of FGFR ligand is responsible for downregulation of expression of VDR gene. OPUS 2016/23/B/NZ5/00065 NSC Poland. *The authors marked with an asterisk equally contributed to the work.

## P-06-018
## Neurotransmitter vesicles redistribution during the activation of the neuron acetylcholine receptor

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Somatic exocytosis in central neurons of vertebrates and invertebrates is triggered by transmembrane depolarization through the mobilization of internal pools of vesicles towards the plasma membrane, with which vesicles continue to fuse even for several minutes after the end of the depolarization. We studied these


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finely-regulated processes to understand a general aspect of exocytosis and the redistribution of vesicles in the neurons cytoplasm during the activation of acetylcholine receptor (AChR). Studies were performed on isolated Retzius neurons, which synthesize and secrete serotonin. We used the method of laser interference microscopy, which allows conducting real-time measurements on living object without additional effect on it, such as inserting dyes or probes. We measured the optical heterogeneity of the neuron cytoplasm. The neuron optical phase profile (OPP) is determined by the refractive index of the components of the cytoplasm, their size and, their quantity and, most importantly, depends on the redistribution of vesicles and the state of subcellular structures (mitochondria, reticulum, nucleus, etc.). After AChR activation maximal OPP changes were observed in near-membrane region of the neuron including the membrane and near-membrane layers of the cytoplasm, and also in perinuclear space. This results correlates with location of two pools of serotonin vesicles: a perinuclear pool near Golgi apparatuses, from which vesicles apparently form, and a peripheral pool with vesicle clusters at a distance from the plasma membrane. It is likely that during the ACh action, the release of the vesicles with serotonin from the neuron does not occur evenly throughout the neuron membrane, and there are areas for the formation of clusters of vesicles with serotonin and these areas correspond to the areas with the significant change in membrane potential. The research was carried out at the expense of the Russian Science Foundation grant (project No. 19-79- 30062)

## P-06-019
## LIM kinases, new therapeutic targets to treat cancers, neurological disorders and Neurofibromatosis: development of small molecule inhibitors

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LIMK1 and LIMK2 (LIM kinases or LIMKs) are kinases playing a crucial role in cytoskeleton dynamics by regulating both actin filament and microtubule remodelling. They have been shown to be involved in cancer development and metastasis, resistance of cancer cells to microtubule targeted treatments, neurological diseases, viral infection, and Neurofibromatosis type I and type II. LIMKs have thus recently emerged as new therapeutic targets. Based on molecular modelling and docking experiments combined with LIMK inhibitor chemical structures described in the literature, we have synthesized a library of 130 small molecule inhibitors of LIM kinases. We have tested their activity and selectivity *in vitro*. Over fifty of our molecules are very active *in vitro* on purified LIMKs, with Ki under 50 nM. Seven of them are highly specific showing low inhibitory activity on a panel of 100 kinases. We have also tested the *in cellulo* activity of our compounds on actin cytoskeleton dynamics, by measuring the level of phosphorylated cofilin. 25 of our compounds show better activity than the reference inhibitor LX7101, inhibiting cofilin phosphorylation at more than 85%. We finally tested the cytotoxicity of our compounds on different cell lines. They show a broad range of cytotoxicity (from 5 mM to no toxicity), most of them exhibiting low cytotoxicity. We want to further characterize the biological activity of our compounds with different *in cellulo* tests: microtubule remodelling, cell cycle progression, cellular migration, and to determine their pharmacokinetic properties. We also want to characterize LIMK level and activation in different cancer cell lines. Our ultimate goal is to perform pre-clinical assays on the three more promising compounds on the most appropriate model.

## P-06-020
## Neuregulin 2 leads to the occurrence of a phosphorylated, truncated form of EGFR in a human breast cancer cell line

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Neuregulins (NRGs) belong to the EGF family of growth factors. NRG1 and NRG2 have a single EGF-like domain, which is responsible for binding to their cognate receptors of ErbB family: c-erbB3 and c-erbB4. c-erbB3 may signal only as heterodimers with other family members, c-erbB4 signals as homo- or heterodimers. We have studied the effects of NRG1 and NRG2 EGF-like domains on ErbB family receptors activation in various mouse and human cell lines. EGF-like domains of NRGs are considered sufficient to activate ErbB receptors dimerization and phosphorylation of specific tyrosine residues within the receptor cytoplasmic tail. Indeed, recombinant EGF-like domain of NRG1 readily increased tyrosine phosphorylation of all ErbB receptors in mouse and human cell lines expressing c-erbB3 and/or c-erbB4. In contrast, we found little or no ~180 kDa ErbB phosphorylation in a number of c-erbB3 and/or c-erbB4-expressing cell lines stimulated with EGF-like domain of NRG2. The same negligible effect was obtained when the cells were stimulated with NRG2 extracellular domain expressed in mammalian cells. However, in the human breast cancer cell line, MDA-MB-468, the EGF-like domain of NRG2 led to extensive phosphorylation of EGFR-truncated fragments. We found that these fragments comprised products of proteolytic cleavage rather than alternatively spliced EGFR variants. EGFR proteolysis was effectively blocked by the serine protease inhibitor AEBSF. However, lack of inhibition of EGFR proteolysis by aprotinin, another serine protease inhibitor, ruled out the involvement of aprotinin-sensitive matriptase-prostasin cascade or hepsin – enzymes that had been shown to cleave EGFR within its extracellular domain. Our results indicate that NRG2 may induce non-canonical activation of EGFR receptor; further experiments are required to elucidate whether phosphorylated, truncated EGFR fragments are signaling-competent or provide yet another pathway of ligand-induced degradation of EGFR.

## P-06-021
## Endoplasmic reticulum (ER) chaperone GRP78 is required for epidermal self-renewal and differentiation during skin morphogenesis

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Glucose regulated protein 78 kDa (GRP78), is a major endoplasmic reticulum (ER) chaperone and a master regulator of ER stress signaling. As a key effector of the protective arm of the unfolded protein response (UPR), GRP78 facilitates protein folding, protecting cells from unfolded and misfolded proteins in ER and exhibits potent anti-apoptotic properties. GRP78 deletion results in early embryo lethality at the peri-implantation stage at E3.5 from proliferation defects and apoptosis of the inner cell mass (ICM). However, even though GRP78 is essential for initial development of ICM, still little is known about the role of GRP78 in different tissues development and differentiation at


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later stages. In this study, we aim to unveil the role of GRP78 during tissue morphogenesis using the skin as a model system. Here, we report creation of a novel mouse model where GRP78 was specifically ablated in the basal layer of the developing epithelium. GRP78 knockout (KO) mice fail to form the stratified layers of the skin epidermis and in consequence skin barrier function is compromised. We show that GRP78 KO cells lack proper epithelial differentiation as they undergo apoptosis, demonstrating that GRP78 is crucial for proper epithelial differentiation and skin development. Additionally, we reveal that GRP78 regulates epidermal self-renewal via p63 pathway. Together, these studies add new insights into the role of GRP78 later in tissue morphogenesis. *The authors marked with an asterisk equally contributed to the work.

## P-06-022
## Comparison of chemiluminescence immunoassay and biosensor methods for measurement of saliva glucose levels in adults

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Background: Glucose is a very important carbohydrate involved in energy metabolism, representing the stress response of body. Determination of blood glucose levels and HbA1c levels are the most important diagnostic criteria for diabetes. The need for daily measurement of glucose levels in many diabetic patients has made glucose the most analysed biomarker. The aim of this study was to compare the biosensor and autoanalyzer compliance for saliva glucose measurement and to identify its potential for use. Materials and Method: Salivary samples of volunteers were collected by SARSTEDT brand saliva collection tubes by providing oral hygiene, and after 1 minute oral of the cartridge, 30 minutes waiting and 15 minutes, 3200 g centrifugation. Glucose levels of all samples were determined by an autoanalyzer (UniCel DxI 800 ACCESS Immunoassay System) working with chemiluminescence principle and biosensor methods. The advantages and disadvantages of those methods were discussed. Results: The glucose results of the twenty-six study samples, which were obtained by using two different methods were correlated using algorithm described by Passing and Bablok, regression equations, confidence intervals (CIs) of the slope and the intercept, as well as the *P*-value were calculated. Salivary glucose level was found to be %95 Cl: intercept 26,566; slope 0,578; p:0,046 at the comparison study of biosensor and chemiluminescence immunoassay. Conclusions: The outcomes of methods compared in our study are; salivary glucose measurements were found to be compatible with chemiluminescence immunoassays (autoanalyzer). The biosensor provides low cost, rapid results and specificity according to the frequently used autoanalyzer method. Keywords: Saliva, glucose, biosensor

## P-06-023
## Cellular signaling pathways involved in arginine deprivation response in the human pathogen *Leishmania donovani*

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*Leishmania donovani* is a kinetoplastid protozoan parasite that causes a deadly disease visceral leishmaniasis or kala-azar. During infection, parasites activate macrophage nitric oxide synthase and arginase activity, both of which use arginine as a substrate. These elevated activities depleted macrophage arginine pools, a situation that invading *Leishmania* cannot tolerate as this is an essential amino acid. The *L. donovani* parasite imports exogenous arginine via a mono-specific amino acid transporter (AAP3) and utilizes it primarily to provide precursors for trypanothione biosynthesis. The depletion of arginine from promastigote and amastigote growth media induced a rapid up-regulation in AAP3 expression and activity, as well as a few other genes by activation of a signaling cascade of arginine deprivation response (ADR) pathway. A previous phospho-proteomic analysis revealed that the arginine sensor activates a MPK2-mediated response pathway (ADR) that induces arginine transport up-regulation via an mRNA stability mechanism. Treatment of arginine starved promastigotes with protein kinase A inhibitor (PKI) resulted in downregulation of ADR pathway and AAP3 expression suggesting role of PKA-mediated pathway in ADR. CRISPR-CAS9 mutants of MAPK2 and AGC kinase suggest role of these proteins in ADR pathway. Our data suggests that ADR pathway involves interplay of MAPK2 and PKA signaling cascades.

## P-06-024
## The CRK5 kinase as an essential signaling protein in regulation of senescence and stress responses

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A characteristic feature of plant genomes in unusually large number receptor-like kinases (RLKs), with more than 600 representatives in *Arabidopsis*. These membrane-localized proteins possess both external receptor domain and internal kinase domain, thus they are able to perceive various stimuli and proceed signaling pathways controlling development and stress response. One of the largest subgroups of RLKs is formed by cysteine-rich receptor like kinases (CRKs). Expression profiling has linked the CRKs with reactive oxygen species and ROS signaling, which might result from the redox regulation of their extracellular thiol groups. One member of this family, CRK5, appeared as negative regulator of cell death. Its recessive mutant shows striking premature leaf aging phenotype, which is reverted in complementation lines. The data obtained from next generation sequencing studies in aging plants revealed a significant upregulation of many senescence-related genes in *crk5* mutant compared to wild type. We crossed *crk5* with plants disrupted in hormonal signaling under stress conditions (*sid2*, *abi1*, *abi2*, *ein2*) and affected in regulation of leaf aging (*sag12*). Most of them were able to minimize or recover *crk5* phenotype, suggesting that this receptor kinase might act a molecular hub linking multiple diverse processes related to stress response and plant senescence.


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## P-06-025

## Autologous tumor-derived microvesicles influence gene expression profile and enhance protumorigenic chemotactic potential, signal transduction and cellular respiration in gastric cancer cells

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Background: Tumor-derived microvesicles (TMVs) interact with different cells of the immune system (lymphocytes, monocytes, dendritic cells, etc.) as well as tumor cells that they have originated from. In the present study, we examined the effect of autologous-TMVs (auto-TMVs) on gene expression, chemotaxis, intercellular signaling and cellular metabolism in cancer cells of the gastric cancer cell line 1415 (GC1415). Methods: The effect of auto-TMVs on mRNA gene expression profile in GC1415 cells was assessed using pathway-focused PCR arrays. Chemotaxis assay was performed using the HoloMonitor M4 System. Signaling pathways were evaluated using Western blotting method. Cellular respiration was measured using the Seahorse XF Cell Mito Stress Test. Results: Exposure of GC1415 cells to auto-TMVs led to the overexpression (75) and under-expression (96) of genes engaged in signal transduction, metabolism, chemotaxis, angiogenesis and metastasis. The auto-TMVs were able to induce chemotaxis and activate the PI3K/AKT, but not the MAPK/ERK, signaling pathway in GC1415 cells. Moreover, studies on cellular respiration in GC1415 cells exposed to auto-TMVs revealed a metabolic shift towards glycolysis. Conclusions: The obtained data support the clause that auto-TMVs, besides other types of extracellular vesicles (EVs), may exert a profound impact on tumor cells functions.

## P-06-026

## Anti-apoptotic activity of FGF1 and FGF2 in PC12 cells

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Fibroblast growth factor 1 and 2 (FGF1 and FGF2) act as a survival factors both *in vivo* and *in vitro* in various type of cells. However, no detailed information is currently available about the mechanism of their anti-apoptotic activity in nervous or neural precursor cells. Here, we investigated the effect of FGF1 and FGF2 on apoptotic rat pheochromocytoma PC12 cells. First the expression of FGF receptors (FGFRs) was evaluated. Then, the ability of FGF1 and FGF2 to activate the FGFR as well as signaling pathways was determined in the presence or absence of specific FGFR inhibitor PD173074. Apoptosis of PC12 cells was induced by etoposide or hydrogen peroxide treatment and evaluated using various methods. After different time points cell viability was measured. Additionally, the levels of cleaved PARP as well as p53 and phospho-p53 were determined. Moreover, for selected agents caspase-3/7 activity was measured. We demonstrated that externally added FGF1 and FGF2 protected PC12 cells from p53-dependent apoptosis. FGF1 and FGF2 increased survival of PC12 cells, decreased caspase-3/7 activity, PARP cleavage as well as inhibited p53 phosphorylation and stabilization. Our data indicate that FGF1 and FGF2 protect PC12 cells from apoptosis. Acknowledgments: The work was supported by the National Science Centre, Poland (Sonata Bis 2015/18/E/NZ3/00501).

## P-06-027

## Structural basis of the signal transduction via transmembrane domains of type I receptors in norma and pathology

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The human epidermal growth factor (EGFR/HER) and growth hormone (GHR) receptors serve as excellent models of type I receptors to illustrate how ligand-induced conformational rearrangements and specific dimerization of extracellular domains lead to the allosteric activation of the cytoplasmic domains via single-span transmembrane domain (TMD). We determined the alternative dimeric conformations of the EGFR and GHR TMDs in different membrane-mimicking environments using high-resolution NMR spectroscopy combined with MD-relaxation in explicit lipid bilayer. Based on the location of pathogenic transmembrane mutations, observed conformations correspond to the dormant and active states of both receptors, assuming an impact of intramembrane interactions to the cell signaling dysfunction in human organism. Fine adaptation of intermolecular polar and hydrophobic contacts that we found to accompany the different EGFR TMD dimerizations suggests that certain membrane properties can govern the TMD helix-helix packing and, thus, their alteration can trigger the receptor state. Whereas two distinct dimeric modes of GHR TMD revealed the functional role of juxtamembrane region rearrangements in alternation between protein-protein and protein-lipid interactions that can be initiated by ligand binding. Observed the TMD helix-helix packing diversity appears in favor of the lipid-mediated rotation-coupled activation mechanism, which implies that the sequence of structural rearrangements of EGFR and GHR domains is associated with perturbations of the lipid bilayer in the course of ligand-induced receptor activation, considering the receptor together with its lipid environment as a self-consistent signal transduction system. Bioengineering work, NMR and SAXS studies were supported by the Russian Foundation for Basic Research (projects 17-00-00489 and 18-04-01289). MD simulations and computational data analysis were sponsored by the Russian Science Foundation (project 18-14-00375).

## P-06-028

## Effects of ursodeoxycholic acid on lipopolysaccharide-induced epithelial mesenchymal transition in biliary epithelial cells (BECs)

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Epithelial-mesenchymal transition (EMT) of biliary epithelial cells (BECs) plays an important role in biliary fibrosis. Lipopolysaccharide (LPS) can promote EMT in BECs. Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid extracted from animal bile and has a wide range of biological


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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functions. Currently, UDCA is the only drug approved as primary biliary cirrhosis (PBC) that appears to have beneficial effects on hepatocyte and liver damage and has been proposed as a treatment for cholestatic liver disease. This study investigated the effect of UDCA on EMT induced by LPS (1 μg / mL) in BECs. We analyzed TLR4 and EMT markers after application of UDCA (500 μM) alone and LPS plus UDCA for 1 to 24 hours. At the mRNA level, LPS -induced TLR4, slug, and Zeb1 were reduced by UDCA treatment. However, when UDCA was applied for more than 2 days, apoptosis of BEC cells was observed. LPS treatment in BECs induced phosphorylation of LPS-induced JNK and p38 MAPK and this phosphorylation was further enhanced by UDCA. In cell cycle analysis, UDCA arrested at the G0 / G1 stage in BECs. In the presence of low concentrations of UDCA (200 μM), no apoptosis was observed. The depolarization of mitochondrial membrane potential was inhibited with lower UDCA concentration. When five times subcultures were performed in low UDCA concentrations, E-cadherin mRNA was significantly increased and vimentin was slightly decreased. In summary, at appropriate concentrations and time of UDCA induces cell cycle arrest and apoptosis through the JNK / p38 MAPK pathway. The above results suggest that UDCA is a potential new drug for the prevention of biliary fibrosis in BECs

## P-06-029
## ALOX15 participates in re-sensitization of doxorubicin resistance in MCF7 and HeLa cells by re-organizing cell membrane, inducing apoptosis and regulating intracellular redox states

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Lipoxygenases (LOXs) are the enzymes generally metabolizing polyunsaturated fatty acids. As a member of LOX family, ALOX15 specifically metabolizes arachidonic acid and linoleic acid. Thus, it associates with both physiological and pathophysiological conditions as it regulates protein-lipid interactome, intracellular redox state and production of reactive lipid metabolites. ALOX15 has also been shown to have a role in tumorigenesis, particularly in colon carcinoma. However, there are not any studies combining ALOX15 and cancer drug resistance. In this study, we, for the first time, aimed to show the potential role of ALOX15 in doxorubicin resistance in human breast cancer cell line, MCF7 and cervical cancer cell line, HeLa, which were resistant to doxorubicin in an acquired manner. Our results showed that ALOX15 was transcriptionally down-regulated in doxorubicin-resistant cells compared to their drug-sensitive parental cells. Moreover, restoring ALOX15 levels in the drug-resistant cells resulted in re-sensitization of those cells to doxorubicin in a tumor origin-dependent manner. ALOX15 was proved to induce cell apoptosis by activating PPARG and promoted accumulation of doxorubicin in resistant MCF7 cells by re-organizing cell membrane dynamics; however, it triggered re-sensitization by putatively regulating intracellular redox states in the resistant HeLa cells. These results underlines the role and importance of ALOX15 in cancer drug resistance, and points the mechanisms that ALOX15 is involved as a novel therapeutic approach to overcome cancer drug resistance.

## P-06-030
## FGF12 is able to activate signaling in cells expressing FGF receptors

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FGF12, also known as a fibroblast growth factor homologous factor (FHF1), belongs to FGF11 subfamily (FHFs), which shows sequence and structural similarity with other members of FGFs family but exhibits different biological activities. In contrast to canonical FGFs, FHFs are not secreted, have no identified interaction with FGF receptors (FGFRs) and, due to the lack of mitogenic potential, are believed to be non-signaling proteins. They function as regulators of voltage gated sodium channels. Here, we show that recombinant FGF12 can induce FGFR activation and initiate signaling pathways, including MAPK and Akt, in cell lines expressing FGF receptors, while remaining defective in the induction of cell proliferation. Preincubation of cells with specific FGFR inhibitor (PD173074) abolished FGFR phosphorylation and downstream signaling, strongly indicating that FGF12 is able to interact with FGFR. Furthermore, we found that exogenous FGF12 was able to reduce caspase 3/7 activity in starved cells and cells treated with staurosporine. Our results suggest that FGF12 not only activate FGFR but can also protect the cell against apoptosis. Acknowledgments: The work was supported by the National Science Centre, Poland (Sonata Bis 2015/18/E/NZ3/00501).

## P-06-031
## Binding of pleiotrophin to vascular endothelial growth factor receptor 2 regulates endothelial cell migration

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Pleiotrophin (PTN) is a heparin-binding growth factor that through its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) regulates normal and pathological angiogenesis, in the presence or absence of vascular endothelial growth factor $A_{165}$ (VEGF-$A_{165}$). We have previously shown that VEGF-$A_{165}$ binds to RPTPβ/ζ and the latter is required for VEGF-$A_{165}$-induced endothelial cell functions. PTN competes with VEGF-A for RPTPβ/ζ binding and affects VEGF-$A_{165}$-induced endothelial cell migration. In the present work, by using immunoprecipitation/ Western blot, immunofluorescence, proximity ligation assays, molecular dynamics and surface plasmon resonance assays, we show that PTN binds with strong affinity to VEGF receptor type 2 (VEGFR2) in human endothelial cells and competes with VEGF-A for VEGFR2 binding. Binding of PTN to VEGFR2 affects VEGFR2 Tyr1175 phosphorylation and down-stream signaling and mediates PTN-stimulated endothelial cell migration. Collectively, our data suggest that VEGFR2 is involved in the stimulatory effect of PTN on cell migration and the interplay between VEGFR2 and RPTPβ/ζ will be discussed. Acknowledgements: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening


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Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY), scholarship to PK and EC), and by a Marie Curie Intra European Fellowship within the 7th European Community Framework Programme (ALTangioTARGET, grant agreement No 626057). The authors thank the Advanced Light Microscopy facility of the Medical School, University of Patras for using the Leica SP5 confocal microscope.

## P-06-032
## Formyl peptide receptor 1-mediated transactivation of high affinity nerve growth factor receptor TrkA in SH-SY5Y neuroblastoma cell line

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Formyl peptide Receptor 1 (FPR1) belongs to GPCR family and is coupled to pertussis toxin (PTX)-sensitive Gi proteins. FPR1 shows high affinity for N-fMLP and other formylated peptides and mediates intracellular calcium mobilization, NADPH oxidase-dependent reactive oxygen species (ROS) generation, cellular migration and proliferation. Recent evidence has unveiled high expression of FPR1 in cancers and neurodegenerative processes, among which FPR1 functions seem to be correlated with cellular proliferation and migration. In this study we observed that FPR1 is functionally expressed in SH-SY5Y neuroblastoma cell line and that stimulation of 24-hours serum-starved cells with N-fMLP induces NADPH oxidase activation. Consistently, this event was inhibited by preincubation with PTX or with the FPR1 antagonist Cyclosporin H. Signalling cascades triggered by GPCRs can induce tyrosine kinase receptors (TKRs) transactivation through different molecular mechanisms. In this study we observed that FPR1 stimulation induces TrkA transactivation in SH-SY5Y neuroblastoma cell line. TrkA is the high affinity receptor for Nerve Growth Factor (NGF), a neurotrophin that influences neuronal cell survival, axonal growth and synaptic plasticity. Western blotting experiments showed that N-fMLP treatment induces Erk1/2, Akt and TrkA phosphorylation, in absence of NGF stimulation. Furthermore, these phosphorylations were prevented either by preincubation with PTX, Cyclosporin H, or by TrkA inhibitor GW441756. We also observed that pretreatment with Apocynin, a selective NADPH oxidase inhibitor, prevents Erk1/2, Akt and TrkA phosphorylation, suggesting that NADPH oxidase-dependent ROS generation plays a crucial role in FPR1-mediated TrkA transactivation. Taken together, these results can contribute to identify new potential therapeutic targets to modulate cellular proliferation and migration in cancer and neurodegenerative diseases.

## P-06-033
## Investigating the regulation and function of AMPKalpha1 vs. AMPKalpha2 complexes

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AMP-activated protein kinase (AMPK) is a key regulator of cell energy homeostasis. Although AMPK has been studied extensively, little is understood about the specificity of its upstream kinases calcium calmodulin dependent protein kinase kinase 2 (CAMKK2) and liver kinase B1 (LKB1) or its catalytic subunit alpha1 and alpha2 isoforms, to downstream substrates. To begin exploring these specificities, AMPKalpha1, AMPKalpha2 and AMPKalpha1/alpha2 KO HEK293T cells were generated using CRISPR/Cas9 system. AMPK phosphorylation and activity in response to activation by Ionomycin and 2-Deoxy-D-glucose, which activate AMPK predominantly by CAMKK2 and LKB1 respectively, was compared between cell lines. Quantitative phosphoproteomic analyses were performed under these same conditions and detected phosphosites were subsetted to obtain a high confidence list of potential AMPK substrates. 25% of these were common to both treatments, many of which have already been identified from previous studies. However, potential novel AMPK substrates were identified, specific to activation by either CAMKK2 or LKB1 and potentially executed specifically by AMPKalpha1 or AMPKalpha2 containing complexes. Four of these were further validated and the regulation of their function by AMPK phosphorylation was investigated. Identification of isoform and/or upstream kinase specific substrates of AMPK reveals a functional purpose for the presence of two upstream kinases which phosphorylate AMPK at the same residue and/or for the presence of two catalytic subunits of similar but not identical sequences.

## P-06-034
## Lipid-derived loss of pancreatic β-cell identity – the role of stearoyl Co-A desaturase 1

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Type 2 diabetes (T2D) is one of the major challenges which biomedical research is facing nowadays. The progression of T2D goes along with dysregulation of lipid homeostasis. It has been shown that disruption of stearoyl-CoA desaturase 1 (SCD1) activity results in reduced β-cell insulin secretory capacity and survival, thereby contributing to pancreatic islets failure and development of T2D. Recent studies indicate that not only β-cell death, but also loss of β-cell identity, is a major cause of T2D. In the present study we investigated the role of SCD1 in epigenetic control of pancreatic β-cell identity. Our data showed that pancreatic islets of SCD1 knock-out mice are characterized by different microarchitecture and lower expression of transcription factors (TFs) involved in maintenance β-cell identity (Pdx1, FoxO1, Isl1) in comparison with wild type mice. Furthermore, we noticed that SCD1 inhibition/gene silencing leads to changes in global DNA methylation profile in β-cells. The hypermethylation of promoter regions of TFs responsible for maintenance of β-cell identity such as Pdx1 and MafA were also reported. Moreover, our analyses indicate that treatment of β-cells with anti-diabetic drug metformin can prevent these cells from lipid accumulation by downregulation of lipogenesis. Nevertheless, metformin treatment decreases SCD1 protein level and activity in β-cells, and in consequence does not protect these cells from lipid-derived loss of expression of TFs responsible for maintenance β-cell identity. Obtained results suggest that SCD1 affects expression of identity TFs in pancreatic β-cells via alterations in DNA methylation pattern, therefore indicating that SCD1 may be a novel player in regulation of β-cells functional identity. Research supported by National Science Centre, Poland grants no UMO-2017/27/N/NZ3/01987 (AMD) and UMO-2013/10/E/ENZ/00670 (AD) and by NCBiR grant STRATEGMED no 3/305813/2/NCBR/2017.


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## P-06-035
## Nrf2 signaling pathway in skin keratinocytes treated with cannabidiol

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Skin keratinocytes are characterized by well developed cytoprotective system that includes Nrf2 pathway. However, everyday exposure to harmful environmental factors causes redox imbalance and induces the disorder in skin cells functioning. That leads to constant search for natural compounds with cytoprotective properties. Therefore, the aim of this study was to estimate the effect of plant cannabinoid – cannabidiol [CBD] on UVA/UVB irradiated main epidermal cells – keratinocytes. Obtained results indicate that CBD increases the level of phosphorylated Nrf2 (100%), as well as its main target HO-1 expression in both control (90%) and UV irradiated keratinocytes (20%), compared to non-treated cells. Moreover UVA and UVB radiation enhances the expression of Nrf2 activators – p21 (20%) and p62 (100%), while 2-fold increase in p21 in all CBD treated cells and in p62 in control keratinocytes is observed. Simultaneously, CBD decreases by 20% the expression of Nrf2 main cytosolic inhibitor – Keap1 that level is reduced by UV radiation. As a result of CBD-induced Nrf2 activation the significant decrease in ROS generation and lipid peroxidation product 4-HNE is observed in control and UV irradiated cells. Therefore 4-HNE-protein adducts diminished level is indicated. All these changes suggest that cannabidiol improves the anti-oxidative/anti-inflammatory effects of Nrf2. The protective action of cannabidiol in relation to the redox balance of the treated keratinocytes takes place through enhanced transcription of the antioxidant genes activated by Nrf2. However, this also leads to inhibition the NFκB pathway. In conclusion, it can be suggested that the protective effect of cannabidiol on keratinocytes associated with activation of the Nrf2 pathway may be helpful in describing the mechanism of action of cannabidiol. In addition, cannabidiol may be indicated as a potential compound that protects the skin against pro-oxidant/pro-inflammatory factors.

## P-06-036
## Syndecan-4 plays a role in cytoskeleton organization and nitric oxide production

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Syndecan-4 (Syn4) is a transmembrane heparan sulfate proteoglycan that has been shown to regulate focal adhesions and to play a crucial signaling center for proper communication between intracellular, cell-surface and cell-extracellular matrix. Normal vascular endothelial cells (EC) from rabbit aorta and Syn4 knockdown cells (ShRNA-Syn4-EC) were transfected with vectors encoding beta-actin, a protein-KDEL fusion to retention in endoplasmic reticulum, caveolin1, clathrin light chain and histone H2B, all of them targeted with fluorescent protein. Cells were cultured in glass coverslips (12 mm, 3 days) in F12 medium supplemented with 10% fetal serum bovine, streptomycin and penicillin (both 100 IU/mL) at 37°C in a humidified atmosphere (2.5% of $CO_2$). MitoTracker were used to stain total mitochondria. DAF-FM diacetate were used to fluorescent detected intracellular nitric oxide. DAPI and PicoGreen were used to stain nucleus and wheat germ agglutinin (WGA) were used to stain plasma membrane. Images were captured to *in vivo* cells with a confocal scanning microscope equipped with a Plan-Apochromat x63 objective (numerical aperture 1.4) under oil immersion. Total RNA were extracted from EC and ShRNA-Syn4-EC cells and both semi-quantitative (RT) and quantitative (q; real-time) polymerase chain reaction (PCR) were performed. Total proteins were extracted from EC and ShRNA-Syn4-EC cells and western blot were performed. Actin filaments, endoplasmic reticulum, mitochondria distribution and nitric oxide production are altered in ShRNA-Syn4-EC cells. Syn4 knockdown also induces the expression of the C-MYC transcription factor and inhibit expression of GATA3 transcription factor and CAVEOLIN1 protein. The gathered data shows the pivotal role of Syn4 in cell signal transduction and further pinpoint its importance for cytoskeleton organization.

## P-06-037
## The reversible platelet aggregation is caused by an inhibition of iPLA2-dependent TXA2 synthesis in the presence of extracellular calcium

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Platelets prevent blood loss upon vessel wall disruption. Since 1962 the main technic to investigate platelet functionality is light transmission aggregometry (LTA). LTA is based on platelet suspension optical density measurement. There is an intriguing phenomenon that aggregation is weaker in the presence of physiological concentration of calcium. Its mechanisms are still unclear. Here we aimed understanding of mechanisms of reversible aggregation. Blood of healthy donors was collected into tubes with hirudin, citrate or ACD. Washed platelets were resuspended in buffer with or without calcium. Platelet aggregation was performed with Chronolog turbidimetric aggregometer and flow cytometry (FACSCanto), platelets were activated by ADP, adrenalin or serotonin with or without aspirin. Platelets disaggregate in response to ADP, serotonin combined with adrenalin or low doses of TRAP-6 in suspension with calcium. In the absence of calcium, only aspirin-preincubated platelets disaggregate. So the main cause of the irreversible aggregation is TXA2 synthesis in the absence of calcium. At the same time the irreversible aggregation in response to arachidonic acid is independent of extracellular calcium. IPLA2 is known to be inhibited by calmodulin and to be activated by a depletion of intracellular calcium stores (ICS), but platelets ICS could not be depleted in response to weak agonists. We show that ICS are depleted in weak agonists induced aggregometry. The model was constructed in Python describes platelet activation and TXA2 synthesis when aggregates attain the certain sizes. It confirms experimental data. Therefore, this work shows that extracellular calcium-free medium provides depletion of the intracellular Ca2 + store. It results iPLA2 activation in response to weak agonists. This leads to TXA2 synthesis that causes irreversible aggregation in the absence of calcium. All parts of this study were supported by the Russian Science Foundation grant 17-74-20045

## P-06-038
## A link between a potential transcription factor SaoC and survival of *Staphylococcus aureus* in adverse conditions

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*Staphylococcus aureus* is a clinically relevant opportunistic pathogen responsible for an increasing number of infections worldwide.


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Its remarkable ability to respond to environmental cues appears indispensable to its success as a pathogen. Well tuned alterations in metabolism allow the bacteria to survive in hostile environments. The signalling pathways providing such adaptations are still not fully understood. The aim of this study was to investigate the potential involvement of *saoABC* operon in regulation of gene expression and in adaptive response to environmental cues by *S. aureus*. Knockout strains were examined for changes in their growth dynamics, the number of persister cells formed and the ability to survive upon internalization into human cells. Furthermore, pull-down assays were performed to investigate the binding of proteins to conserved sequences within *saoABC* operon. The *ΔsaoB* mutant exhibited decreased growth rate in a nutrient-limiting medium as well as better survival after exposition to antibiotics. On the other hand, the disruption of *saoC* gene led to decreased survival in human fibroblasts. Moreover, SaoC protein was demonstrated as a factor binding a conserved, putative regulatory DNA sequence. The results suggest the involvement of *saoABC* operon in transduction of environmental signals, which allows the bacteria to adapt to adverse conditions. The project was partially financed by funds granted to MB by the National Science Centre in Poland No. 2016/21/D/NZ1/00273

## P-06-039
## Calculation of lag-time for platelet activation in healthy vessel based on a mathematical model of protein kinase A

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In circulating platelets, protein kinase A (PKA) is persistently active due to prostacyclin-induced stimulation of cAMP levels, keeping multiple key targets phosphorylated to prevent inordinate platelet activation. Upon vessel injury circulating platelets have to decide whether or not to adhere to the lesion site before blood flow drags them away from it. This is a time-sensitive process, taking at most 10 s, and it is unclear whether PKA inactivation is has a significant part in it. Since measuring kinetics of cAMP, PKA activity, or substrate phosphorylation is impossible in this scenario, we studied them in silico, describing the binding of PKA subunits to each other and binding of cAMP, substrates, and products to them, with a system of ordinary differential equations, assuming mass-action rate law. Model parameters were estimated automatically from literature data, and then validated by comparing predictions with an independent dataset. The half-time of PKA inactivation in PKA-cAMP system was 1 s. We complemented this model with equations for phosphatase, adenylate cyclase, and phosphodiesterase in platelets, and fitted it to data of VASP and GSK3 phosphorylation in response to iloprost (Beck et al., 2014). Estimated half-dephosphorylation time for VASP was in 1–5 s range. We also found out that substrates competing with regulatory subunit of PKA and each other produces significant interactions at least in platelets due to high concentrations of VASP, pleckstrin, and filamin-A. Dephosphorylation half-times for all PKA substrates lie in the range of 1–150 s depending on PKA and phosphatase's $K_m$ and $k_{cat}$ towards them and the aforementioned "major" substrates. Altogether we can conclude that a significant dephosphorylation of platelet's PKA substrates could be achieved within ten seconds after a drop in adenylate cyclase activity. The study was supported by Russian Foundation for Basic Research grant 17-00-00138.

## P-06-040
## Activation of the ATM/Akt/CREB/eNOS signaling axis by aphidicolin increases nitric oxide production, attenuating endothelial cell death

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We reported that aphidicolin acutely increases nitric oxide (NO) production in bovine aortic endothelial cells (BAEC) by increasing phosphorylation of endothelial NO synthase at serine 1179 (p-eNOS-Ser1179) and decreasing p-eNOS-Ser116 without altering eNOS protein expression. Unlike the acute effects, prolonged treatment with aphidicolin (over 24 h) increased eNOS protein expression and NO production, which was accompanied by increased eNOS dimer/monomer ratio and BH4 levels. Levels of eNOS mRNA expression were also increased, and a promoter assay using 5′-serially deleted eNOS gene promoters revealed that the Tax-responsive element (TRE) site, a CRE-like site, located at −962 to −873 of the eNOS promoter, was responsible for aphidicolin-stimulated eNOS gene expression. Ectopic expression of a dominant-negative (dn-) inhibitor of CREB, A-CREB, repressed the stimulatory effects of aphidicolin on eNOS gene expression and its promoter activity. We also found that aphidicolin increased CREB activity, as evidenced by an increased level of p-CREB-Ser133. Co-treatment with LY294002, a PI3K inhibitor, decreased the aphidicolin-stimulated increase in p-CREB-Ser133 levels, eNOS expression, and NO production. Furthermore, ectopic expression of a dn-Akt construct attenuated aphidicolin-stimulated NO production. Aphidicolin also increased p-ATM-Ser1981 and the knockdown of ATM using siRNA attenuated all stimulatory effects of aphidicolin on p-Akt-Ser473, p-CREB-Ser133, eNOS expression, and NO production. Lastly, aphidicolin significantly decreased EC viability, which was further decreased by co-treatment with the NO scavenger, PTIO, or L-NAME, or transfection with eNOS siRNA, suggesting a protective role for NO in EC death caused by aphidicolin. In conclusion, our data suggest that aphidicolin increases NO production in BAEC by increasing eNOS expression via an ATM/Akt/CREB signaling cascade, which contributes to the attenuation of aphidicolin-induced EC death.

## P-06-041
## Cinnamaldehyde protects human umbilical vein endothelial cells against hydrogen peroxide-induced oxidative stress and apoptosis through the regulation of the Nrf2/HO-1 signaling pathway

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Cinnamaldehyde (CA) is a natural compound of *Cinnamomum cassia,* which has various functional properties, such as anti-cancer, anti-microbial, anti-inflammatory, and anti-oxidant activities. Here, we investigated the cytoprotective and anti-inflammatory effects of CA on hydrogen peroxide- or TNF-α-treated human umbilical vein endothelial cells (HUVECs). The results demonstrated that CA and its natural derivative 2-methoxycinnamaldehyde (MCA) strongly induced the cellular protein level of heme oxygenase-1 (HO-1). CA-mediated HO-1 induction largely


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inhibited the levels of ROS and protected the HUVECs from hydrogen peroxide-induced apoptotic cell death. Moreover, HO-1 depletion by siRNA abrogated the cytoprotective effect of CA against oxidative stress. Upon CA treatment, Nrf2 rapidly translocalized to the nucleus and induced the HO-1 expression. By showing that the SB202190 inhibited nuclear translocation of Nrf2, we suggested the involvement of p38 signaling pathway in CA-induced Nrf2/HO-1 activation. Interestingly, CA largely inhibited the TNF-α-induced U937 cell adhesion on HUVECs by decreasing the expression level of VCAM-1. Furthermore, we demonstrated LPS-induced inflammatory cell infiltration was markedly attenuated by CA in Sprague-Dawley rats. These observations provide a scientific basis for the cytoprotective and anti-inflammatory effects of CA and suggest CA as a potential anti-atherosclerotic agent.

### P-06-042
### Brain as an endocrine source of circulating norepinephrine in ontogenesis in rats

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Development of individual organs and the whole organism is under the control of morphogenetic factors over a critical period of ontogenesis. The aim of this study was to test our hypothesis that the brain operates as an endocrine organ during the period of morphogenesis, in rats during the perinatal period. Norepinephrine (NE), which is a morphogenetic factor, was used as a marker of endocrine activity of the developing brain, although it is also secreted by peripheral organs. In this study, it was first shown that the concentration of NE in the peripheral blood of perinatal rats is sufficient to ensure the morphogenetic action of NE on the peripheral organs and the brain itself. Using pharmacological suppression of the NE production in the brain, it was shown that NE is delivered from the brain into the general circulation in neonatal rats, i.e. during the critical period of morphogenesis. In fact, even partial suppression of NE production in the brain of 3-day-old rats resulted in a reduction of NE concentration in the plasma more than 30%, suggesting that at this time the brain is the source of circulating NE. Conversely, suppression of NE production in the brain of the prepubertal rats – after cessation of morphogenesis, was not accompanied by a change in the NE concentration in the plasma, apparently because of the closure of the brain blood barrier. Noteworthy is that the NE concentration in the peripheral blood of perinatal rats is sufficient to provide morphogenetic action of NE on target cells. The above data confirm our hypothesis that the developing brain secretes into the bloodstream potential morphogenetic factors, including NE, in the critical period of morphogenesis.

## Mitochondria and signaling

### P-07-001
### Mitochondrial glutaredoxin 2 activation upon oxidative stress in HeLa cells

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Glutaredoxin 2 (Grx2) is a small protein principally located in the mitochondrial matrix that receives reducing equivalents by both glutathione and thioredoxin reductase. Grx2 main role is the post translational modification of proteins through glutathionylation/de-glutathionylation activity. Moreover, Grx2 can coordinate an iron-sulfur cluster (2Fe-2S), forming inactive dimers stabilized by two molecules of glutathione. In the present study, Grx2 monomeric and dimeric state was analyzed in HeLa cells incubated in different redox conditions. Interestingly, after cell treatment with sodium selenite, oxidative stress was found to induce Grx2 activation through the disassembly of the dimer only in mitochondria. Interestingly, Grx2 monomerization was induced by the concomitant impairment of both the thioredoxin and glutathione systems. In fact, together with a decrease of total cellular thiols and glutathione levels, a large decline of thioredoxin reductase activity was observed in the mitochondrial compartment. Grx2 monomerization and activation, by releasing the iron-sulfur cluster, increased also the amount of free iron ions in the mitochondrial matrix. Consequently, induction of lipid peroxidation and decrease of mitochondrial membrane potential were observed in cells upon selenite treatment. Moreover, sodium selenite-triggered lipid peroxidation was hindered by the addition of deferiprone, an iron chelator with mitochondriotropic properties, suggesting a role of the iron and sulfur cluster release in the observed impairment of mitochondrial functioning. Thus, in cells, Grx2 could sense changes in the overall cellular redox condition getting activated after oxidative stimuli in mitochondria and releasing the iron and sulfur cluster following oxidative stress induction.

### P-07-002
### The proteome of *Yarrowia lipolytica* yeast during adaptation to a combined (cross) pH and heat stress

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The phenomenon of cross-adaptation to several unfavorable environmental factors, when the yeast cell has to respond to two simultaneous stress factors, radically differs from its responses to the single stress factor. In the presented study, we assessed some changes in the proteome of *Y. lipolytica* yeast during the adaptation to a combined pH and heat stress. *Y. lipolytica* W29 cells were raised in YNB media up to the stationary growth stage at two pH values (5.5 and 9.0) and two temperatures (28-30°C and 37-39°C). The culture grown under optimal conditions (pH 5.5, 28–30°C) was used as the control. The cell homogenates from the cells grown in various conditions were separated using 2D-electrophoresis and the proteins from the gels were identified by MALDI-TOF method. Under alkaline conditions (pH 9.0, 28–30°C) the expression of the mitochondrial porine VDAC (YALI0F17314p) and Cu-Zn SOD (YALI0E12133p) was significantly increased. Furthermore, the increased expression of 20 kDa (YALI0C03443p) and 70 kDa (YALI0C17347p) heat shock proteins (HSP) was shown. Under the heat stress condition the expression of HSP70 and its chaperones, belonging to the Ssa and Ssc families (hypothetical protein of CPAR2_700380, YALI0D00220p) evidently rose. The cytoskeleton protein expression (YALI0F20856p, YALI0F27049p) and glycolysis enzymes (hypothetical protein CPAR2_401230, hypothetical protein CPAR2_602950) also increased under combined influence. The amount of mitochondrial proteins (XP_003868331.1, DQ447218.1) decreased and VDAC disappeared. The data obtained contradict the assumption of the VDAC involvement into the hypothetical adaptation to variable unfavorable conditions, which was supposed before for the adaptation to pH- and oxidative stresses. The performed study was supported by RFBR "grant mol_ev_a" # 19-34-80012.


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## P-07-003
## Sirtuin 3 as a mediator of mitochondrial function upon estradiol treatment in hormone-dependent breast cancer cells

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Breast cancer is the most frequent cancer among women, and 70% of all breast cancer cases are estrogen receptor (ER) positive. Sirtuin 3 (Sirt3) has a promising role in cancer tumorigenesis and treatment, but there have been controversies about its role as oncogene or tumor suppressor in different types of cancer. We have recently shown that de novo expressed Sirt3 in ER-α positive MCF-7 breast cancer cells causes higher expression of ER-α, but lowers tumorigenic properties of the cells. Therefore, we proposed that Sirt3/ER-α crosstalk may be responsible for the reduction of proliferation and invasiveness of MCF-7 cells. To investigate this crosstalk in estrogen ($E_2$)-responsive MCF-7 cells, we use combination of treatments ($E_2$ and its antagonist, ICI), overexpressed Sirt3 and Sirt3 silencing to alter the expression and activity of different proteins involved in mitochondrial biogenesis, metabolic regulation and cell cycle progression. We hypothesize that this crosstalk between Sirt3 and ERα is achieved via proteins which are being activated in mitochondria and then translocated to nucleus. So far we observed that $E_2$ addition induced metabolic activity and increased gene and protein ER-α level in control MCF-7, but had no effect in Sirt3 clones. Upon silencing Sirt3 protein with siRNA we observed suppressed ER-α protein level in Sirt3 clones, while silencing ER-α had no effect on Sirt3 protein level. We are also investigating the effects of different treatments and Sirt3 on proliferation, cell cycle, ADP/ATP radio, mitochondrial and redox status of the cells. Furthermore, we are testing intracellular distribution of ER-α and potential Sirt3 targets under different treatments by using cellular fractionation and confocal microscopy. Successful completion of these experiments will contribute to mechanistic understanding of the role of Sirt3 in hormone-dependent breast cancer cells, as a potential pharmacological target.

## P-07-004
## Development and characterization of human cell line models of the mitochondrial protein import failure

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Mitochondria are essential eukaryotic organelles with bacterial ancestry involved not only in oxidative energy conversion but also in many other, often essential, cellular functions. The mitochondrial proteome is estimated to consist of 1000–1500 different proteins with only around 1% of these proteins encoded in the mitochondrial genome. Thus, building mitochondria and sustaining their functions requires import of proteins encoded by nuclear DNA and produced in the cytosol as mitochondrial precursor proteins. Precursor proteins are transported into mitochondria utilizing specialized import channels. To fit through major entry gate to mitochondria, the TOM translocase, proteins must be largely unfolded. Import of proteins that fold prematurely in the cytosol is inefficient and may lead to the blockade of the translocase. To investigate mechanisms by which human cells respond to such events we developed cell line based models. We use controlled expression of fusion proteins that combine mitochondrial targeting signal sequences with rapidly folding domains to challenge translocation machinery. We found out that such fusion proteins localize to mitochondria with different efficiencies, depending on the targeting sequence used. The expression of such fusion proteins negatively affects the proliferation of the cells, especially in the growth conditions that are highly dependent on the oxidative phosphorylation. Proliferation decrease was also more pronounced for fusion proteins that contained most effective mitochondrial targeting signals. From that, we conclude that developed fusion proteins directly affect mitochondrial function and can be used to model mitochondrial protein import failure to improve our understanding of quality control mechanisms related to the mitochondrial biogenesis.

## P-07-005
## Effects of the endocrine disrupting chemical, DEHP, on the mitochondrial metabolism of the detoxification organs

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Humans are exposed to the various synthetic chemicals via industrial products including cosmetics, food packaging, toys, medical devices and pharmaceuticals. DEHP is the most commonly used plasticizer which has both endocrine disrupting and peroxisome proliferator effects on the human and wildlife. Also, endocrine disrupting chemicals are known for their contribution to the elevated levels of oxidative stress and the formation of various diseases including cancer, diabetes, and obesity. There is an increasing concern about human exposure to the DEHP because of its adverse health effects. On the other hand, mitochondria are the major source of the oxidative stress and most affected organelle from disturbance in the oxidative stress metabolism. Impact of the DEHP on the reproductive and endocrine system has been widely studied, however there is no data about its effect on the mitochondrial metabolism of the detoxification organs including liver and kidney. Therefore, we aimed to investigate influence of the DEHP on the mitochondria metabolism of liver and kidney of rats. Therefore, 24 prepubertal male Wistar albino rats were randomly divided into four groups based on the DEHP administration as 0, 100, 200 and 400 mg/kg/day dose groups. Mineral and trace element levels, anti-oxidant enzyme activities, and electron transport chain complexes including complex I-V were investigated in the mitochondria of kidney and liver as the detoxification organs. Our data showed that DEHP can induce changes in the anti-oxidant enzyme activities and mitochondrial complexes involving in the mitochondrial oxidative stress metabolism. Moreover, we observed differences in the mineral and trace element levels upon DEHP administration that may damage biochemical reactions in the cell. In conclusion, DEHP can impair mitochondrial metabolism of the detoxification organs.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-07-006
## The role of heat shock protein 72 in regulation of skeletal muscle–adipose tissue crosstalk

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Recent evidence has identified skeletal muscle as a secretory organ and released cytokines have been classified as myokines. As production of many proteins in skeletal muscle is dependent upon contraction, physical activity leads to an altered myokine response. Some studies show that physical training increases expression of brown adipocyte marker uncoupling protein 1 (UCP1) in subcutaneous adipose tissue, what can be mediated by released myokines. Moreover, heat shock protein 72 (HSP72) is elevated during physical training and have been identified as important regulator of muscles metabolism. Therefore, the aim of the study was to investigate the effect of HSP72 on skeletal muscle secretome and browning of white adipose tissue. Herein it was shown that overexpression of HSP72 both *in vitro* in C2C12 myotubes, and *in vivo* in skeletal muscle upregulates 5'AMP-activated protein kinase, energy homeostasis regulator in cell, that is naturally activated by exercises. Muscle cells with overexpression of HSP72 are characterized by increased β-oxidation rate and mitochondrial biogenesis, decreased lipid content, as well as increased activity of enzymes involved in oxidative metabolism. Overexpression of HSP72 in C2C12 myotubes increases gene expression of interleukin 6, which is known myokine that increases content of UCP1 mRNA in white adipose tissue. Furthermore, conditioned media from C2C12 cells overexpressing HSP72 increase browning of white adipocytes. Overall, so far the study showed that HSP72 regulates skeletal muscle metabolism by increasing oxidative capacity of the cells and also suggests that overexpression of HSP72 in skeletal muscle potentially can affect myokine profile and browning of adipose tissue. Acknowledgements: This research was supported by the Foundation for Polish Science, grant TEAM/2010-5/2 and National Science Centre (NCN), grant NCN UMO-2011/03/B/NZ3/00693 and UMO-2017/24/T/NZ4/00275.

## P-07-007
## Transcriptional regulation of plant mitochondrial biogenesis in abiotic stress

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For the proper biogenesis of plant mitochondria, coordinated regulation of mitochondrial and nuclear gene expression is expected. We studied such coordination at the transcriptional level in cauliflower (*Brassica oleracea* var. *botrytis*) mitochondria under acclimation and de-acclimation to cold, heat and drought. For that, whole transcriptome high-throughput sequencing on Illumina NovaSeq 6000 platform was employed. Key transcript alterations were further validated by reverse transcription-quantitative PCR (RT-qPCR). Some mitochondrial mRNAs (ex. *nad9*, *coxII*) were regulated inversely comparing to the protein level, suggesting more efficient use those transcripts for translation in cold acclimation and de-acclimation. Furthermore, we detected the lack of coordination of accumulation of messengers for subunits of the same protein complexes (ex. complex I and ATP synthase). However, changes in abundance of transcripts for various alternative oxidase (*AOX*) isoforms compensated unfavorable proteomic alterations in stress de-acclimation. *AOX* messengers were differentially accumulated depending on stress conditions and the extent of stress pre-acclimation. Variations of *AOX1a* transcripts suggested only partial utilization of mRNA pool in translation due to the altered availability of transcripts for translation, mRNA/ribosome interactions and miRNA action. Stress responding transcripts included inter alia mRNAs for some matrix enzymes and transcription factors (ex. from ETHYLENE RESPONSE FACTOR family). We detected downregulation of transcripts coding for enzymes of proline catabolism in cold and heat de-acclimation. De-acclimation was not always accompanied by return of transcript abundancies to the control level. In conclusion, some alterations suggest serious perturbations in mitochondrial biogenesis lasting after stress de-acclimation. Presented results extend our knowledge on new candidates participating in abiotic stress response of plant mitochondria at RNA level.

## P-07-008
## Venturicidin-type macrolides as FoF1-ATPase inhibitors: structure-activity relationships

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Venturicidins (also known as aabomycins) are quite rare glycosylated 20-membered macrolide antibiotics isolated from *Streptomyces* species in the 1960s. It was described as perspective for agricultural use against plant pathogenic fungi. Antifungal activity of venturicidins is linked with inhibition action on FoF1-ATP synthase localized in mitochondrial membrane. Three compounds of this type, known irumamycin and X-14952B, as well as new isoirumamycin, were isolated from culture *Streptomyces* sp. INA-Ac-5812. Structure elucidation and identification were done by using extensive spectroscopic analysis: 1D and 2D NMR, HRESI-MS, UV and IR. Stereo configuration of irumamycin and X-14952B hemiketal (C3-C7) and tail (C23-C24) fragments was deduced from NMR data (ROESY and HSQMBC). Structure of isoirumamycin differs from known congeners in the size of macrocyclic core (18-membered) according to key HMBC and ROESY correlations. Cytotoxicity of all isolated compounds was in the 5–15 μM range for various tumor cell lines and human postnatal fibroblasts. Despite this, the antifungal activity was strongly dependent on structural variations. Maximum inhibition activity was detected for irumamycin; activity decreased in the following order: irumamycin > X-14952B > isoirumamycin. Obtained data can contribute to the rational design of FoF1-ATPase inhibitors based on macrocyclic compounds.

## P-07-009
## MCT-mediated lactic acid import regulates mitochondrial oxidative phosphorylation via the PDK/PDH axis

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Aerobic glycolysis and mitochondrial dysfunction are key metabolic features of cancer cells, but their interplay during cancer development remains unclear. We previously reported that human hepatoma cells with mitochondrial defects exhibit lactate dehydrogenase subunit B (LDHB) downexpression. Here we


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investigated how LDHB suppression regulated mitochondrial respiratory activity and contributed to liver cancer progression. We found that transcriptional LDHB downexpression was an effective upstream event of suppressed OXPHOS activity. LDHB knockdown increased inhibitory phosphorylation of pyruvate dehydrogenase (PDH) via lactate-mediated PDH kinase (PDK) activation, thereby attenuating OXPHOS activity. Interestingly, monocarboxylate transporter 1 (MCT1) was the major lactate transporter of hepatoma cells and its expression was essential for the PDH phosphorylation through modulating intracellular lactate level. Finally, analysis of the hepatocellular carcinoma cohort from The Cancer Genome Atlas revealed that low LDHB/LDHA ratio was significantly associated with poor prognostic outcomes and high gene set enrichment of glycolysis hallmarks, and negatively correlated with PDK1 and 2, supporting a close link between LDHB suppression and PDK/PDH axis. These results suggest that LDHB suppression is a key mechanism in glycolysis enhancement, and critically involved in the maintenance and propagation of mitochondrial dysfunction via lactate release in liver cancer progression.

## P-07-010
## Mitochondrial alternative cyanide-resistant oxidase and alternative NAD(P)H dehydrogenases are involved in a resistance of spring and winter wheat to temperature stress

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The plant mitochondria contain the alternative enzymes of respiration that do not contribute to the generation of a proton-motive force, but it is assumed that activation of some of them can allow the plant to withstand of the stress influence. The alternative enzymes are located in the inner mitochondrial membrane and presented by the alternative cyanide-resistant oxidase (AOX) and alternative «external» (NDB) and «internal» (NDA) NAD (P)H dehydrogenases. There is little information about the effect of elevated and heat shock temperatures on AOX, NDB and NDA abundance. At low temperature, we see more information about AOX, NDB and NDA, but it often is conflicting data. The functional role of these proteins remains poorly understood. In this work, the comparative analysis of the protein content of AOX, NDB, and NDA in mitochondria of the spring and winter wheat after heat and cold adaptation and subsequent stress was studded. We used the purified mitochondria from the leaves after heat and cold treatments of wheat. All temperature treatments were done in present of the continuous light. It was noted, that the content of AOX protein are different in mitochondria after heat and cold treatments. After heat treatment the content of AOX, as compared with low temperature, was higher and presented by 4 isoforms in mitochondria. The content of NDA and NDB are also increased after heat adaptation and subsequent heat shock. We also detected that the content of AOX and NDB protein increased after influence on adaptive plant the subsequent cold stress. It was concluded that alternative enzymes play an important role in the acclimation of wheat to high and low temperatures. But contribution of the AOX, NDB and NDA is higher under the action of heat treatments. This work was supported by the Russian Science Foundation [No 17 74-10096].

## P-07-011
## Inhibition of oxidative phosphorylation by semisynthetic oligomycins in rat liver mitochondria

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Bioactive compounds targeting the oxidative phosphorylation are of considerable value for an application in biochemistry investigations as well as for the drug discovery. Oligomycin A (1) is widely used in current biochemical researches of mitochondrial autophagy and cell respiration due to its ability to inhibit $F_OF_1$ATP-synthase. However, there is only desultory data about exact mechanism of oligomycin A binding to $F_O$ c-subunit and kinetics of this process in literature. In effort to reveal some patterns we have investigated inhibition effects of oligomycin A and series of novel semi-synthetic oligomycin A derivatives on rat liver mitochondria respiration during ATP synthesis. Correlation of the inhibition potency and biological activity was also considered. In observation lasted for 1 minute, the mode of oligomycin A action was proved to be sharp: the difference in $IC_{50}$ (55 nM) and $IC_{100}$ was approximately 10 nM; concentrations lower than 5 nM not effected on respiration even over a period of several minutes. Semi-synthetic oligomycin A derivatives, modified in the side chain ((33S)-oligomycin A 2) and in the macrolactone core (Diels-Alder adduct of oligomycin A with N-benzylmaleimide 3) were approximately in 10 times less potent ($IC_{50}$ 0.35 and 0.55 μM respectively) than parent antibiotic, as well as their differences in $IC_{50}$ and $IC_{100}$ values were more noticeable (about 15–25 μM). Compounds 2, 3 were still highly active against tumor cell lines, sensitive to oligomycin A, but their toxicity for normal cells were in several times lower in comparison with parent antibiotic 1. Thus, the diminishing activity for normal cells might be the result of decreased ability of these derivatives to inhibit the mitochondrial oxidative phosphorylation. This work was supported in part by Russian Science Foundation grant 15-15-00141 (chemistry and biology) and Russian Foundation for Basic Researches grant 19-04-00835\19 (inhibition of ATP-synthase). *The authors marked with an asterisk equally contributed to the work.

## P-07-012
## Bioenergetics of primary liver sinusoidal endothelial cells (LSECs)

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The aim of the study was to characterize bioenergetics and substrate preferences of primary mouse liver sinusoidal endothelial cells (LSECs), which constitute a highly specialized hepatic non-parenchymal cell sub-population. LSECs display unique morphological and phenotypic features and contribute to the regulation of liver metabolism. However, bioenergetics of LSECs has not been characterized yet. LSECs were isolated from mouse liver and examined using Seahorse XF technique allowing for measurements of oxygen consumption and extracellular acidification rates. We revealed that LSECs rely on both mitochondrial respiration and glycolysis, as inhibitors of these pathways (oligomycin, rotenone and antimycin A, 2-deoxyglucose, iodoacetic acid) limited ATP production. Moreover, we demonstrated a lack of


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effects of several individual inhibitors for specific metabolic pathways on basal mitochondrial respiration. Only an inhibitor of mitochondrial pyruvate carrier (UK-5099) decreased maximal respiration measured after addition of FCCP, what confirmed a significance of glucose as a metabolic substrate in energetically-stressed mitochondria in LSECs. Combination of UK-5099 with inhibitors of glutaminase (BPTES, CB-839, DON, azaserine) demonstrated that glutamine plays a role of anaplerotic substrate. Interestingly, we did not observe inhibition of mitochondrial respiration after etomoxir, an inhibitor of long-chain fatty acids utilization, and only a slight inhibition after ranolazine, which inhibits β-oxidation. In summary, given that endothelial cells are considered to rely mainly on glycolysis, our results demonstrate a remarkable metabolic plasticity of LSECs, featuring their adaptation to rich in metabolic substrates microenvironment of the liver. Acknowledgments: Project financed by Polish National Science Centre (DEC-2015/16/W/NZ4/00070) and Poland Ministry of Science and Higher Education

## P-07-013
## Impact of gene silencing on energetic state and viability of INS cells

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Insulinoma is a high secretion of insulin cells that does not cause backward regulation when reducing the free glucose concentration and leads to hypoglycemia. This is the functional pancreatic neuroendocrine tumor (pNET), which 2/3 of causes are benign and 1/3 are malignant tumors. Changes in the beta-cell physiological state may be due to an imbalance in metabolic and genetic processes (expression of glucose transporter-1 (GLUT-1) correlates with expression of hypoxia-inducible factor-1a (HIF-1a) and malignant potential of pNET). Our goal was to stimulate mitochondrial activity by gene silencing of HIF-1a and miR-210 (induced by pseudohypoxia) and invoke apoptotic processes that eliminated these damaged cells and prevented their "imbalance". Material and methods: We targeted oxidative phosphorylation by genetically suppressing the hypoxic factors HIF-1a and miR-210. We have been referred to in mitochondrial activity (Seahorse XF 96), cytosolic glycolysis (Peredox Assay) and cell viability (Trypan Blue Staining Assay). Results and Conclusions: We have identified the activation of apoptotic signals by increasing of an activity in respiratory complexes (I, III) and ATP production ($P < 0.001$) evoked by reduce of the influence of pseudohypoxic environment, (probably by increasing ROS). We observed a decrease in the ratio of free NADH/NAD+ in the cytosol ($P < 0.001$), increase in basal respiration, proton leakage, ATP-coupled respiration (ATP production) and maximum respiratory capacity ($P < 0.001$). Monitoring the growth of the population of the affected cells has shown a significant reduction in proliferation ($P < 0.001$). Our experimental data show potential course of targeted effecting on respiratory complexes to evoke self-degradation of damaged cells.

## P-07-014
## Understanding role(s) of phosphatidylethanolamine in H+ ATPase activity, temperature and acetic acid tolerance in *Saccharomyces cerevisiae*

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Background: Biological membranes are highly selective barrier for permitting flow of molecules and primary target for various external and internal signals for proper functioning of living systems. Phospholipids are the key components of membranes. Phosphatidylethanolamine $^{PE}$ and phosphatidylcholine $^{PC}$ have been identified as major lipids of eukaryotic cell membrane. The PE has a preference of forming non-bilayer structures and thereby facilitating proteins to adapt to their native conformations and proper functions. It has already been confirmed that the synthesis of PE significantly decreased in *psd1,2* double mutant cells. In the present study we analysed the lipid composition of wild as well as mutant cells *vis-à-vis* activity of plasma membrane ATPase, tolerance to acetic acid $^{200\ mM}$ and higher temperature. Such factors are critical for cellular physiology during bio-ethanol formation when acid hydrolysates from lignocellulosic biomass and apoptosis. Methods: Pma1 activity and $H^+$ efflux was done as reported by Serrano (1980). Lipids were extracted by Bligh and Dyer method (1959).Phospholipids were separated by 2D HPTLC and inorganic phosphorus estimated by Ames procedure (1966). Proteins were estimated by Bradford's method (1976). Results: It was found that mutants have about 30% decreased level of PE which was compensated by increase in phosphatidylserine 20%, phosphatidylglycerol by 10% and PC 15% with respect to wild type cells. There was a slight decrease in plasma membrane ATPase activity. However, the mutant cells showed sensitivity to acetic acid and died within six h. The cells with depleted PE content could not grow at 37°C. These results showed probable increase in apoptosis as a consequence of diminished amount of PE during acetic acid and temperature stress. Conclusion: The findings suggest that PE content in the plasma membranes plays an important role for growth and adaptation to changed environment and physiological condition).

## P-07-015
## Hypoxia increases the rate of renal gluconeogenesis via hypoxia-inducible factor-1 (HIF-1) dependent activation of phosphoenolpyruvate carboxykinase (PEPCK) expression

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Although liver is considered to be the main glucose buffer in a mammal organism, up to 25% of glucose released into circulation in the postabsorptive state comes from renal gluconeogenesis. One of the most important functions of hypoxia-inducible factor-1 (HIF-1) is shifting energy metabolism from oxidative phosphorylation to anaerobic glycolysis. The stimulatory effect of HIF-1 on hepatic gluconeogenesis was also reported, but there are no data on similar HIF-1 action in kidneys. Thus, the aim of the present study was to examine if HIF-1 might be involved in the regulation of renal gluconeogenesis. It was found that in HK-2 cells (immortalized human kidney proximal tubules, capable of


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gluconeogenesis / glycogen synthesis) incubated with glucose precursors: 1) either under hypoxia (1% $O_2$) or in the presence of DMOG (an inhibitor of HIF-1α degradation) glycogen content increases despite lowered glucose intake, compared to cells cultured without gluconeogenic substrates, and these effects are reversed in the presence of echinomycin (an inhibitor of HIF-1 binding to HRE sequence); 2) under conditions of hypoxia / increased HIF-1 level the activity of phosphoenolpyruvate carboxykinase (PEPCK) is elevated, as concluded from the analysis of the intracellular content of gluconeogenic intermediates; 3) both under hypoxia and in the presence of DMOG the expression of cytosolic isoform of PEPCK is increased, while echinomycin prevents it. Moreover, chromatin immunoprecipitation (ChIP) proved HIF-1 ability to bind to the promoter region of PEPCK-C gene. It is concluded that hypoxia / HIF-1 accelerates the rate renal glucogenesis *via* the mechanism engaging activation of PEPCK-C expression. These observations might be useful in terms of *e.g.* diabetes treatment, as under diabetic conditions kidneys and liver seem to be equally important sources of glucose synthesized *de novo*. The study was supported by the grant of the National Science Centre, Poland, 2016/21/B/NZ3/00365.

## P-07-016
## Gαq activation induces mitochondrial arrest by interacting with Alex3, Miro1 and Trak1/2 proteins

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G not only transduce a myriad of signals from receptors at the plasma membrane, but also at the mitochondria and other endomembranes to regulate the physiology of these organelles. In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane, among other functions. A mass-spectrometry analysis based on Gαq immunoprecipitates from cellular endomembranes has provided evidence that G proteins regulate the mitochondrial dynamic process through the interaction with the mitochondrial armadillo domain proteins (Alex3 and 10). Subsequent immunoprecipitation and pull-down studies demonstrated a specific interaction of Gαq with the mitochondrial Rho GTPase 1 (Miro1) and both Milton adaptor proteins TRAK1 and 2, that couple mitochondria to Kinesin and Dynein motor proteins and constitute the main regulators of mitochondrial transport in neurons. To analyze the physiological role of those interactions, we have performed tracking analysis of mitochondria along the axons of hippocampal neurons overexpressing Gαq or its constitutive-active mutant, GαqR183C, as well as activating a Gαq-exclusive DREADD receptor with a specific agonist. The results of these studies reveal a significant arrest of mitochondrial motility upon Gαq activation, which in long term affects neuronal complexity and dendritic branching. In turn, depletion of Gαq using short-hairpin RNAs increases the number of motile mitochondria and their speed. With this data, our group postulates a new non-canonical mitochondrial function of Gαq acting as a molecular switch in neurons that would stop mitochondria with its activation at the point of the synapsis and would allow mitochondrial movement during its inactive state.

## P-07-017
## Defects in cytochrome c oxidase biogenesis negatively affects the activity of complex III respiratory chain

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Mitochondria play an essential role in cellular metabolism, mainly by producing majority of the cellular ATP by means of oxidative phosphorylation (OXPHOS). This process depends on OXPHOS complexes localized in the internal mitochondrial membrane. The biogenesis and function of OXPHOS complexes depends on genetic information encoded by two genomes – mitochondrial and nuclear. Only 13 out of 90 structural subunits of respiratory chain complexes are encoded in the human mitochondrial genome. All other structural subunits as well as biogenesis factors are encoded by nuclear genome, translated by cytosolic ribosomes and then imported into mitochondria. Experiments performed on human cell lines have shown correlation between incorrect formation of complex III or IV, and functional impairment of complex I. Using yeast *Candida albicans* as a model we found that maintenance of the activity of complex III depends on proper biogenesis of complex IV. This link was revealed by targeted deletion of nuclear genes encoding structural components of complex IV as well as inactivation of nuclear-encoded factors which are required for expression of mitochondrial encoded components of this complex. Our data extends view on strong functional/biogenesis interconnection between OXPHOS complexes. Results obtained with the yeast model as well as our attempts to validate these observations in higher Eukaryotes will be presented. *The authors marked with an asterisk equally contributed to the work.

## P-07-018
## ROS production by mitochondrial complex III versus bacterial cytochrome bc1 and its mechanistic implications

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The mitochondrial cytochrome $bc_1$ complex, also known as complex III or ubiquinol cytochrome c oxidoreductase is one of the key components of the electron transport chain in eukaryotic mitochondria and several prokaryotic organisms. It is the only complex releasing superoxide anion into the intermembrane space of mitochondria, with its implicated involvement in apoptosis, aging process and cellular signaling. Superoxide production in cytochrome $bc_1$ is commonly considered a side reaction of unstable semiquinone intermediate of the quinol oxidation site ($Q_o$), with molecular oxygen. This reaction is enhanced under redox conditions slowing the electron flow and is often associated with mitochondrial diseases. Here we reveal that mitochondrial cytochrome $bc_1$ exhibits increased propensity for superoxide generation compared to its bacterial counterpart. This is reflected by higher percentage of ROS measured under a set of defined redox conditions. We also show that mutational lowering of heme $c_1$ redox midpoint potential ($E_m$) in bacterial cytochrome $bc_1$ to mimic $E_m$ of mitochondrial heme $c_1$, increases ROS production to the levels characteristic for native mitochondrial enzyme. This result implicates that the difference in $E_m$ between heme $c_1$ and the Rieske cluster (FeS) and the resulting change in the relative proportion of reduced vs oxidized FeS occupying the $Q_o$ site, can


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affect the level of ROS production. We discuss this observation in the context of molecular mechanism evoking kinetic competition between catalytic and side reactions taking place at the $Q_o$ site.

## P-07-019
## Mitochondrial dynamics in yeast with repressed adenine nucleotide translocator PET9

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Mitochondrial network structure depends on the functional state of mitochondria but the mechanisms of this dependency are still not clear. In particular, dissipation of mitochondrial transmembrane potential by protonophores induces fragmentation of mitochondrial network. This can be a result of a direct regulation of OPA1/Mgm1 processing by the transmembrane potential. At the same time, mitochondrial depolarization can decrease NTP/NDP ratio in the cytoplasm due to the activities of ATP-synthase and adenine nucleotide translocator (ANT). As mitochondrial fusion machinery requires cytosolic GTP, the decrease of NTP/NDP ratio can potentially induce mitochondrial fragmentation. To evaluate the relative contributions of these mechanisms, we studied mitochondrial dynamics in the cells with inhibited ANT using *Saccharomyces cerevisiae* yeast as a model. We generated a strain with repressed major yeast ANT — *PET9*. Mitochondria isolated from this strain showed negligible ANT activity. We found that the protonophores induce fragmentation of mitochondria in the wild type but not in *PET9*-minus strain. Moreover, we found that the inhibitor of ATP-synthase oligomycin A prevented the effect of the protonophores in the wild type cells. However, mitochondrial network in the untreated *PET9*-minus cells was more fragmented compared to the one in the wild type cells. Nonetheless, we found that *PET9* repression did not prevent mitochondrial fusion in yeast zygotes. In particular, selfish mtDNA from *Rho-* yeast strain was able to expand into mitochondrial network of *PET9*-minus strain. Together, our data suggest that the protonophores-induced mitochondrial fragmentation is not a direct consequence of the drop in the transmembrane potential and requires ATP-ase and ANT activities.

## P-07-020
## Role of mitochondria in regulation of intestinal permeability

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Crohn's disease and ulcerous colitis represent the major forms of chronic inflammatory bowel disease (IBD). During IBD various inflammatory mediators disrupt intestinal epithelial junctions leading to increase in epithelial permeability and intestinal barrier dysfunction. Epithelial dysfunction is thought to play a central role in IBD pathogenesis. Understanding of IBD causes and mechanisms of inflammatory reactions in the intestine has both theoretical and practical values. Previously we have shown that mitochondria play a key role in the inflammatory response of vascular endothelium. We have found that mitochondria-targeted uncouplers of respiration and oxidative phosphorylation protect endothelium against deleterious effects of major proinflammatory cytokine TNFα *in vitro* and *in vivo*. The goal of current research was to study the role of mitochondria in regulation of intestinal permeability under damaging conditions *in vitro*. Caco-2 cell line was used as a model of intestinal epithelium. TNFα and lipopolysaccharide (LPS) along with ethanol were used as damaging agents. These compounds induced the degradation of intercellular contacts (tight junctions, mediated by ZO-1, and adhesion junctions, mediated by E-cadherin), increase of trans-cellular permeability and decrease of E-cadherin content. Mitochondrial uncouplers (DNP and C4R1) prevented the degradation of intercellular contacts and the loss of E-cadherin. The increase in trans-cellular permeability also was reduced. The loss of mitochondrial potential was not significant indicating that the effect of uncouplers was most possibly mediated by alteration of cell signaling but not cell metabolism. These data suggest that mitochondria play important role in regulation of condition of epithelium and mitochondria-targeted compounds may be considered as possible therapeutic agents. The project was supported by RFBR grant No. 19-34-70056.

## P-07-021
## A Warburg-like metabolic profile underlies early pulmonary branching morphogenesis

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Lung branching morphogenesis is an intricate process governed by epithelial-mesenchymal interactions and extremely regulated by a complex signaling. To sustain high proliferative rates, free energy and building blocks are necessary. However, the metabolic requirements/changes that occur during early pulmonary branching are still unknown. In this work, we characterized, for the first time, the metabolic profile of early stages of chick lung branching: b1, b2, and b3 (1, 2 or 3 secondary bronchi, respectively). *Ex vivo* lung explant culture was performed, and the medium was collected to analyze the production/consumption of metabolic intermediates associated with glucose catabolism (lactate, acetate, alanine) by $^1$H-NMR spectroscopy. *In situ* hybridization and qPCR were performed to assess the expression patterns/levels of key enzymes and transporters from the correspondent metabolic pathways. Lactate dehydrogenase protein expression levels were evaluated by Western blot. Results revealed an increase in lactate and acetate production as lung development proceeds. Still, glucose consumption is maintained, implying a constant consumption throughout pulmonary branching. Furthermore, glucose transporters (*glut1*, *glut3*, and *glut8*), monocarboxylate transporters (*mct1* and *mct8*), hexokinase (*hk1*), lactate dehydrogenase (*ldha* and *ldhb*) and pyruvate dehydrogenase (*pdha* and *pdhb*) transcripts, are present in the embryonic lung. *ldha* is mainly expressed in the proximal region of the lung whereas *ldhb* is restricted to active branching sites and growing tips. LDH protein levels increased in the three stages studied. This study


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describes the temporal metabolic changes associated with early chick pulmonary branching. Throughout this period, Krebs cycle metabolism appears to be impaired with branching morphogenesis dependent on a glycolytic lactate-based metabolic profile. Additionally, lactate and acetate are potential developmental biomarkers of lung branching.

## P-07-022
## NMN – a bioactive nucleotide with beneficial pharmacological activities in preclinical studies – added to *S. cerevisiae* mitochondria changes adenylate energetic charge, thus modulating cellular survival

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Because $NAD^+$ is biomolecule with multitasking functions, insight into the $NAD^+$ World, can provide clues on cellular energy status and as possible marker for healthy aging. With aging, it was reported a bottleneck in $NAD^+$ content and it was associated to increase NCDs. NMN is naturally found in foods and it might be absorbed/synthesised also from microbiota thus helping to sustain $NAD^+$ biosynthesis via NMN adenylyltransferases 1-3 (i.e. NMN+ATP↔$NAD^+$+PPi). Recently it was identified the transporter for NMN (*Slc12a8*gene). Up to date, NMN uptake, $NAD^+$ biosynthesis and catabolism in yeast mitochondria is far to be clarified. Thus, this study reports pre-clinical and *in vitro* research by means of *S. cerevisiae* with different phenotypic landscape induced by nutritional stress in which $NAD(P)^+$/NAD(P)H pool and adenylate compounds were determined in spectrofluorimeter microplate reader and via HPLC. Moreover, NMN was added to bioenergetically active mitochondria and mitochondrial functions -ICR, $\Delta\psi$ and key physiological parameters (i.e. Adenylate Energy Charge, Adenylate pools, $[PP_i]$)-were measured via polarographic. HPLC and fluorimetric experiments. Data show that *S cerevisiae* changes the intramitochondrial purine nucleotides profile thus that NMN uptake influences Energetic Charge and modulates survival fate

## P-07-023
## Impairment of mitochondrial ATP production down-regulates Wnt signaling

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Wnt signaling affects fundamental development pathways by regulating cell differentiation and proliferation. Aberrant activation of this pathway promotes the development of several cancers. Wnt/β-catenin signaling is known to influence mitochondrial function, but the possibility that the mitochondrial energetic state affects Wnt signaling has not been explored so far. Here we show that sub-lethal concentrations of different pharmacological compounds acting on mitochondrial fitness downregulate Wnt/β-catenin signaling in HEK293 cells and colon cancer lines. Accordingly, impaired respiratory chain complex III function in human fibroblasts from a GRACILE syndrome patient led to reduced Wnt signaling with respect to healthy cells. The above data was further validated *in vivo* in zebrafish reporter lines. Other signaling pathways were not affected, indicating specificity of the mitochondria-Wnt signaling axis. We identified a mechanism whereby a decrease in mitochondrial ATP reduces uptake of calcium to the endoplasmatic reticulum (ER), leading to ER stress and, as a consequence, to inhibited Wnt signaling. In turn, recovery of ATP level restored Wnt activity. These findings reveal an unexpected mechanism related to the control of Wnt pathway activity by mitochondrial ATP and underline the importance of mitochondrial function and energetic state in cellular signaling.

## P-07-024
## Relationship between reactive oxygen species formation, membrane potential and coenzyme Q reduction level in rat heart mitochondria

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Coenzyme Q is an important mobile electron carrier in the mitochondrial electron transport chain that participates in aerobic respiration producing energy in the form of ATP. Main sites of superoxide anion formation of respiratory chain, including complex I and complex III, are associated with coenzyme Q. Therefore, coenzyme Q takes part in mitochondrial reactive oxygen species (ROS) production, contributing to oxidative stress and damaging mitochondria and cells. Recently, we have proposed that the coenzyme Q pool reduction level (endogenous coenzyme Q redox state) could be a useful endogenous reporter that allows indirect assessment of overall. ROS production in mitochondria[1]. The aim of present work was to study the relationship between respiratory rate, membrane potential, ROS formation, and coenzyme Q reduction level in isolated rat heart mitochondria. These mitochondrial parameters were measured under various conditions, i.e., with different respiratory coenzyme Q-reducing substrates, under phosphorylating, uncoupling, and non-phosphorylating conditions. The rates of the coenzyme Q-reducing and coenzyme $QH_2$-oxidizing pathways were titrated by substrate availability and/or respective respiratory chain component inhibition. Our results indicate that relationship between ROS, membrane potential, and coenzyme Q reduction level depends on involved superoxide formation site. The present study is important because disorders related to coenzyme Q and ROS production play an important role in oxidative stress, aging and the development of many pathological diseases including heart diseases. [1]Dominak K, Koziel A, Jarmuszkiewicz W (2018) Redox Biology 18, 256-265. This work was supported by the National Science Centre, Poland (2016/21/B/NZ3/00333)

## P-07-025
## Nucleo-mitochondrial interactions – retrograde regulation in yeast *Candida albicans*

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The mechanisms of nucleo-mitochondrial interactions differ among diverse yeast species, reflecting the co-evolution of the nuclear and mitochondrial genome. We study the regulatory response of the nuclear genome to the dysfunction of mitochondrial oxidative phosphorylation in *C. albicans*, comparing it to


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the well-known *S. cerevisiae* pathway. In silico analysis showed that both the major effector (*CIT2* gene) and regulators (Rtg2p and Mks1p) of this pathway in *S. cerevisiae* are not found in *C. albicans*. Orthologues of the major transcription factors: Rtg1p and Rtg3p are, however, present. Deletion of *CaRTG1* or *CaRTG3* in a strain with functional mitochondria (WT) did not affect its respiratory capacity. On the other hand, when one of these two genes was deleted in a strain with dysfunctional mitochondria - Δ*Caaep3*, deficient in the expression of mitochondrial ATP synthase, the respiratory phenotype was suppressed. In *C. albicans*, one of the alternative respiratory pathways besides the classical respiratory chain is the alternative oxidase – AOX, which is not present in *S. cerevisiae*. AOX is constitutively expressed at a low level in WT, and its main function is reducing ROS stress in mitochondria. Our RNA sequencing results showed that in Δ*Cartg1* and Δ*Cartg3*, *CaAOX2* was not expressed at all. More interestingly, *CaAOX2* expression level was significantly elevated in Δ*Caaep3* to compensate for the respiratory deficiency, while in a double mutant Δ*Caaep3* Δ*Cartg1* it was decreased even below the WT level. These results suggest a functional relationship between the alternative oxidase and the retrograde pathway in *C. albicans*. In *S. cerevisiae*, ScRtg3p and ScRtg1p localize to the nucleus in response to various stresses. In *C. albicans*, these two proteins constitutively localized in the nucleus, both in strains with functional, and dysfunctional mitochondria. Our results also suggested that the nuclear localization of CaRtg1p could depend on CaRtg3p.

## P-07-026
## Mistargeted mitochondrial proteins influence proteasome activity and assembly

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The majority of mitochondrial proteins are encoded by the nuclear genes and synthetized in the cytosol prior to their transport to mitochondria. Accumulation of these proteins in the cytosol, leads to the activation of the proteasome machinery. The 26S proteasome is highly conserved among eukaryotes and it is primarily responsible for selective degradation of proteins. It consist of 20S core particle that has proteolytic activity and the 19S regulatory particle that capes the core on one or both ends of 20S particle. The 19S regulatory particle is further divided to the base and the lid components and it is responsible for substrate recognition and further processing to the 20S core. Taking into consideration that mistargeted mitochondrial proteins stimulate the proteasome, we have postulated that these proteins can also influence the assembly process of proteasome formation in the cytosol. To test this hypothesis we have followed the effect of the overexpression of mitochondrial proteins transported through the mitochondrial intermembrane assembly (MIA) pathway and the mitochondrial proteins targeted to the organelle by the presequence signal. We have observed that the MIA pathway substrates and the mature forms of the mitochondrial proteins lacking the presequence selectively increase proteasome activity. We have discovered that this phenomenon is not caused by the increase of the individual proteasome components levels in the cytosol, but it is rather due to enhancement of 26S proteasome assembly. Using proteasomal chaperones deletion strains in yeast, we have further characterized chaperones involved in the response. In summary, our studies suggest that an increase of proteasome activity required for degradation of mitochondrial proteins is achieved by the stimulated assembly of 20S core and the base of 19S regulatory particle prior to further association of 19S lid.

## P-07-027
## The influence of chromium(III) and molybdenum(III) and their mixture on mitochondrial metabolism

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Chromium and molybdenum play an important role in the cell metabolism. They can affect on various cell organelles, i.e. mitochondria. In the presented study BALB/3T3 and HepG2 cells were incubated with chromium chloride or molybdenum trioxide at concentrations range from 100 to 1,400 μM. The cells were incubated in mixtures of chromium chloride at 200 μM plus molybdenum trioxide at 1,000 μM or chromium chloride at 1,000 μM plus molybdenum trioxide at 200 μM. After 24 hours of incubation MTT reduction and mitochondrial transmembrane potential (MTP) assays were performed. Additionally DCFDA Cellular ROS Detection Assay, TBARS Assay, SOD Assay, Catalase Assay and Glutathione peroxidase Assay were performed. Cytotoxicity assessed by MTT reduction assay was observed in BALB/3T3 and HepG2 cells after incubation with chromium(III). Moreover, molybdenum(III) was non toxic in both cell lines. A concentration dependent statistically significant decrease in the level of MTP was observed in both cell lines after exposure of both microelements. The results obtained from both cell lines show that HepG2 cells are more sensitive when compared to the BALB/3T3 cells. Additionally, chromium(III) and molybdenum(III) induce oxidative stress, by the induction of ROS production. Antioxidant activity of superoxide dismutase and catalase increased in low concentration of chromium(III); however, they decreased in higher concentrations. The same enzymes decreased after molybdenum(III) treatment in dose dependent manner. The activity of glutathione peroxidase decreased in dose dependent manner in both used microelements. The protective effects of Cr(III) in pairs of Cr(III) at 200 μM and Mo(III) at concentration of 1000 μM were observed. Chromium(III) and molybdenum(III) impair mitochondrial function by the induction of oxidative stress and lowering antioxidant activity. Chromium(III) protects from molybdenum (III) toxicity by activation of antioxidant enzymes. *The authors marked with an asterisk equally contributed to the work.

## P-07-028
## Molecular effect of mitochondrial-related mutation G171H in cytochrome b in *Rhodobacter capsulatus* and its influence on ROS production

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Cytochrome $bc_1$ plays an essential role in electron transport chain. It takes part in proton motive force formation. Cytochrome *b* subunit of $bc_1$ complex, being encoded by mitochondrial DNA, is more prone to mutations compared with other subunits encoded by nuclear DNA. Some of these mutations are associated with mitochondrial disorders in humans. In this work we examined the effects of mutation Y155H, which was found in cytochrome *b* in a Prader–Willi Syndrome patient. Using a


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purple bacterial model we introduced an analogous mutation G171H in *Rhodobacter capsulatus*. To assess the functionality of mutated $bc_1$ complex we performed biochemical analysis, electron paramagnetic resonance (EPR) experiments and flash – induced electron transfer measurements. The mutated enzyme shows reduced activity at higher pH values, however the $K_M$, defining the affinity of the enzyme to quinone, is the same for G171H and wild type protein (WT). Interestingly, the kinetics of reactive oxygen species (ROS) formation in G171H mutant significantly differs from WT. For the first few seconds of enzymatic reaction the mutant, unlike WT, displays reduced free radicals production. So far, this phenomenon has not been described in any other mutants of cytochrome $bc_1$. To explain this observation we perform detailed spectroscopic analysis of this mutant. It is expected to provide further insight into mechanism of ROS production.

## P-07-029
## How does the activated Mec1/Tel1/Rad53/Dun1 pathway control the mitochondrial DNA stability?

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We study cells of the budding yeast as a model eukaryotic organism to elucidate the mechanisms by which the activation of nuclear DNA damage response (DDR) influences the stability of mitochondrial DNA (mtDNA). The DDR signalling is initiated by two conserved protein kinases: Mec1/ATR and Tel1/ ATM. Upon their activation, the two kinases phosphorylate several kinase mediators (Rad53/ CHK2, yeast Chk1 and Dun1) which subsequently activate many effector proteins to initiate cell cycle control and DNA repair, but also modify a considerable part of the cellular proteome and reprogram transcription of hundreds of genes. Rad27, a conserved nuclease that plays important roles in the maintenance of nuclear DNA stability, has been shown to reside also in mitochondria. However, our current analysis indicates that the mitochondrial mutator phenotype in *rad27* null cells depends on the DDR-controlled checkpoint kinase Dun1. We propose that Rad27 deficiency causes the mitochondrial mutator phenotype mainly via activation of DDR checkpoint kinase Dun1 and the consequent increase of dNTP pools. Here we show evidence that the influence of DDR pathway activation on mitochondria is much broader than the Dun1-dependent mitochondrial mutator phenotype in *rad27* null cells. In the census of yeast proteins possessing characteristic amino acid motifs that may be phosphorylated by Mec1 and Tel1 kinases, there are mitochondrial proteins or protein regulators that are functionally linked to mitochondria. For those proteins which will prove to be substrates of activated Mec1 or Tel1, our long-term aim is to check the functional significance of those DDR-dependent modifications for the maintenance of mtDNA stability. Our current analysis indicates that levels of selected target proteins and their cellular localizations alter upon DDR activation. These findings suggest that functions of analyzed proteins may be regulated by activated DDR kinases.

## P-07-030
## Insights into dynamics of mitochondrial network in primary fibroblasts derived from patients diagnosed with sporadic form of Alzheimer's disease

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Mitochondria are recognized as highly dynamic and interconnected organelles, which are characterized by repeated cycle of fusion and fission, turnover (biogenesis and mitophagy), as well as movement along the cytoskeleton. Tight control and proper functioning of these interdependent processes are essential for maintenance of vital functions of the cell. Recent studies revealed, that abnormal mitochondrial dynamics may contribute to pathological conditions and play a crucial role in many diseases. Moreover, wide events connected to impaired mitochondrial dynamics are one of the most early and prominent features in various neurodegenerative diseases. Detailed mechanisms of these deteriorations are still lacking and have a far-reaching significance in studies of health and disorders. In our investigation we analyzed three following aspects of mitochondrial physiology: mitochondrial turnover, transport and dynamics of mitochondrial network. Our study was conducted on primary fibroblasts derived from patients diagnosed with sporadic form of Alzheimer's disease (AD). Previous results showed diminished mitochondrial turnover, changes of the level of proteins involved in mitophagy and decreased level of factors engaged in biogenesis in AD cells. Furthermore mitochondria in AD cells were functionally older and created diverse mitochondrial network. Recent data indicate that mitochondrial network in AD is less fragmented, contain longer branch length and has an increased quantity of junctions. Additionally, the analysis of the dynamic of mitochondrial network architecture revealed some alterations in the number of fusion and fission events that occur during mitochondrial trafficking. We observe also changes in the track duration, track displacement, and track straightness. To summarize, our results indicate that mitochondrial dynamics in fibroblasts derived from Alzheimer's disease patients is distinct than in control cells.

## P-07-031
## Sestrins regulate respiration and cell death through interaction with mitochondria

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The members of sestrin protein family, composed of sestrin 1, sestrin 2 and sestrin 3, are critical regulators of metabolism that work through control of mTORC1/2 kinase and reactive oxygen species. Different stress insults activate expression of sestrins through induction of transcription factors p53, FOXO family members, ATF4 and NRF2. However, the mechanisms of regulation of metabolism and cell death by sestrins are not well defined. We have shown that sestrins are involved in the regulation of mitochondrial respiration and cell death in response to glucose starvation. In some cell types, such as immortalised fibroblasts, inactivation of sestrins leads to aggravation of cell death


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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associated with decreased mitochondrial respiration. However, as we found recently, in some lung adenocarcinoma cell lines sestrins can support cell death in response to metabolic stress. Many of these effects can be explained by translocation of sestrins to the mitochondria. Surprisingly, these effects were not dependent on the GATOR2 protein complex, the major known sestrins' interactor in cells. This work was supported by Russian Science Foundation grant 17-14-01420.

## P-07-032
## The effect of (-)-nicotine on basic cellular and mitochondrial functions in SH-SY5Y neuroblastoma cells

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Nicotine is the main addictive constituent of cigarette smoke. It acts as an agonist of nicotinic acetylcholine receptors, which are present mostly (but not exclusively) in the central and peripheral nervous system. Nicotine is detectable in the brain within few seconds following cigarette smoke inhalation. There is a constant interest in nicotine action not only due to its addictive effect, but it is also postulated to mediate neuroprotective effects in some neurodegenerative diseases, such as Parkinson's disease. The aim of this study was to characterize the impact of (-)-nicotine on neuronal cells. We tested the effect of (-)-nicotine (at concentrations ranging from 1 μM to 3 mM) on SH-SY5Y cells morphology and function: cell viability, proliferation, cytosolic calcium, reactive oxygen species (ROS) levels and mitochondrial function. When SH-SY5Y cells were treated with (-)-nicotine for up to three days, we observed toxic effects at the highest, 3 mM concentration. It slowed down cellular proliferation, which was accompanied by an accumulation of cells in the S-phase, an increased cytosolic calcium and a decreased ROS levels. Mitochondrial membrane potential was decreased, while respiration rates were increased both in basal state, and upon mitochondrial depolarization with protonophore CCCP. Examination of cellular and mitochondrial morphology revealed cell shrinkage, cytoskeletal reorganization and collapse of the mitochondrial network. Taken together, our results with SH-SY5Y cells show that (-)-nicotine exerts toxic effects only at relatively high, millimolar concentrations. The research was financed by Philip Morris International.

## P-07-033
## The unusual effect of exogenous citrate on HepG2 cells

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Citrate, the first product of the Krebs cycle, is an essential intermediate located at the crossroads of metabolic pathways and a crucial sensor of ATP level by inhibition of strategic glycolytic enzymes through negative feedback. In mitochondria, citrate may either be oxidized via the Krebs cycle or exported outside of mitochondria. Cytosolic citrate is processed by ATP citrate lyase to produce cytosolic acetyl CoA, which sustains the cell proliferation by lipid synthesis or histone acetylation. The role of citrate and ATP citrate lyase might be particularly important in reprogramming of cancer cell metabolism. Cancer cells exhibit reprogrammed pathways of nutrient acquisition and metabolism in order to support the bioenergetic, biosynthetic, and redox demands. The metabolic phenotype of cancer cells is aerobic glycolysis (or Warburg effect) characterized by increased uptake of glucose and increased rate of glycolysis to lactate in the presence of oxygen. With this aim we have treated HepG2 cells, maintained in medium with high and low concentration of glucose, with different concentration of sodium citrate. The results show the effects of citrate on viability, ROS and lipid levels, and the mitochondrial membrane potential. Furthermore, the results show that the expression of ATP citrate lyase is correlated at the histone acetylation.

## P-07-034
## Changes in the transcriptional activity of genes under the action of radiation on skin fibroblasts culture of patients with Leigh-like syndrome with the m14441T>> C mutation in the ND6 gene of mtDNA

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It was shown that radiation can cause damage not only to the nuclear DNA, but also induce breaks and deletions in the mitochondrial circular DNA. The role of mitochondria in the response to small and medium doses of radiation is major issue. The role of mitochondria in the response to low (0.1–0.5 Gy) and medium (0.5–1 Gy) radiation doses on cell cultures of skin fibroblasts with the described mutation m14441T> C in the ND6 gene of mitochondrial DNA was investigated. Healthy donors' skin fibroblasts and fibroblasts of patients with a Leigh-like syndrome with the m14441T> C mutation in the mtDNA ND6 gene were used. The expression level of the NRF2 and BCL2 genes was determined by real-time PCR. In healthy donors the *NRF2* expression level increases 1.5–2-fold ($P < 0.01$) under the action of low doses of radiation (0.1–0.5 Gy) and 4–4.5-fold ($P < 0.001$) with 1 Gy of radiation. In the cell culture with the mutation m14441T> C in the ND6 mtDNA gene, a significant decrease in the expression level of the NRF2 gene is observed (2-3-fold, $P < 0.001$). In healthy donors the level of the anti-apoptotic BCL2 gene increases 1.5-fold ($P < 0.05$) only under action of 0.1 (24 h), while in cell culture with mutation m14441T> C in the ND6 mtDNA gene, a significant 2.5–3-fold increase in the BCL2 expression level ($P < 0.001$) is observed 24 hours after the exposure to 0.1–0.5 Gy of radiation. Thus, it was shown that exposure to small and medium doses of radiation on cell culture with the m14441T> C mutation in the ND6 gene of mtDNA causes abnormal activation of these cells compared to the control. The antioxidant response is not activated – a significant decrease in the expression level of the NRF2 gene is observed and the anti-apoptotic gene BCL2 is activated. The work was performed as part of the state assignment of the Ministry of Education and Science of the Russian Federation with the support of the Russian Foundation for Basic Research (project No. 18-34-00878).


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## P-07-035
## The influence of mutations located close to quinone reduction Qi site of cytochrome $bc_1$ on $Q_o$ site-mediated ROS production

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Cytochrome $bc_1$ (mitochondrial complex III) one of the key enzymes of the respiratory electron transport chain, catalyses two types of reactions, quinol oxidation at the $Q_o$ site and quinone reduction at the $Q_i$ site. It is also considered (apart from the complex I) to be a potent producer of reactive oxygen species (ROS) in mitochondria, and the only one that releases ROS to intermembrane space. Although molecular mechanism of ROS generation by cytochrome $bc_1$ is not fully understood, it is generally agreed that ROS are formed as a product of the side reactions of the quinol oxidation at the $Q_o$ site. In this work we studied the effects of mutations at positions 48 and 49 of cytochrome *b* subunit of complex $bc_1$ located within the $Q_i$ site in the vicinity of heme $b_H$. Some of these mutations were found to be related to human mitochondrial diseases such as myopathies and exercise intolerance (G48S, I49P). We observed an increased level of ROS in the mutants in the presence of $Q_i$ site specific inhibitor – antimycin A. This result is interesting given that the $Q_i$ site is not considered to be directly involved in the generation of ROS. To explain this observation we performed detailed examination of structural and kinetic properties of these mutants. Spectral analysis suggests that the mutations might increase redox midpoint potential of heme $b_H$. We discuss these observations in the context of possible influence of changes in the rates of hemes b reduction/oxidation on ROS generation in the $Q_o$ site.

## P-07-036
## The effect of anatabine, anabasine and nicotine on mitochondrial bioenergetics and mitochondrial permeability transition pore

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The aim of our study was to evaluate the effects of tobacco alkaloids like nicotine, anatabine and anabasine on the mitochondrial function. Mitochondria occupy a central position in the biology of most eukaryotic cells and are among the first responders to various stress factors that challenge cell and tissue homeostasis. *In vitro* and *in vivo* studies have shown that tobacco smoke can influence the function of the mitochondrial respiratory chain complexes. Interestingly, several studies revealed also an inhibitory effect of nicotine on the OXPHOS machinery. In our studies, we evaluated the effects of nicotine, anatabine and anabasine on the mitochondrial bioenergetic parameters as well as their effects on the opening of the mitochondrial permeability transition pore (mPTP). The studies were performed with the use of isolated mice liver and brain mitochondria. The mitochondrial oxygen consumption rate was measured with the use of Clark electrode. $\Delta\Psi$ was determined by measuring the accumulation in mitochondria of lipophilic (membrane-penetrating) cationic probe safranine O. Accumulation of safranine O inside energized mitochondria is accompanied by fluorescence quenching and hence increase of $\Delta\Psi$ is depicted by a decrease of fluorescence. The opening of the mPTP was determined by measuring changes of mitochondrial suspension optic properties. We found that nicotine, anatabine and anabasine over a wide range of concentrations have no significant effect on the mitochondrial membrane potential as well as on the maximal oxygen consumption rate in both brain and liver mitochondria. The studied compounds showed the inhibitory effect on non-ADP-stimulated respiration rate at concentrations of 1.2 μM and 1 mM. Interestingly, anatabine at 1 mM concentration slow down the swelling (mPTP induction) rate of mice brain mitochondria induced by calcium. This work was funded by Philip Morris Product SA (a member of Philip Morris International group of companies).

## P-07-037
## Mitochondrial physiology and biogenesis in primary fibroblasts derived from patients with sporadic Parkinson's disease

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Parkinson's disease (PD) is the second most common age-related neurodegenerative disease. Mitochondrial dysfunctions have been the focus of the pathogenesis of PD. We characterize mitochondrial physiology in the fibroblasts derived from patients suffering from sporadic Parkinson's disease (sPD) and control fibroblasts derived from healthy, age-matched people. Our research is concentrated on the mitochondrial biogenesis and dynamics of mitochondrial network. We observed differences in the levels of oxidative phosphorylation (OXPHOS) proteins, in particular the level of complex I is lower in patients than in control cells. Mitochondrial membrane potential ($\Delta\psi$) and concentration of ATP are lower in sPD cells than in controls. We show that the level of TFAM (Mitochondrial transcription factor A) protein is significantly reduced in sPD cells. Mitochondrial mass is also lower in sPD cells. The levels of proteins involved in the regulation of mitochondrial network dynamics (Drp1, Fis1, Mfn1, Mfn2, Opa1) are altered between control and patients cells. Confocal microscopy observations showed different morphology of mitochondrial network in sPD patients and control fibroblast.

## P-07-038
## The role of cofilin ubiquitylation in regulation of mitochondrial function in the nerve cells

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Ubiquitylation is the post-translational modification process implicated in a variety of cellular functions. Mitochondria play a central role in ATP production and generation of reactive oxygen species (ROS). Neurons critically depend on mitochondrial function. Cofilin (CFL) is a regulator of actin filament assembly/disassembly. The role of CFL ubiquitylation in the regulation of mitochondrial function in the nerve cell is little studied. The aim of this investigation was to analyze relationship between CFL ubiquitylation and mitochondria in the nerve cells. Hippocampal cells were isolated from freshly dissected embryonic mouse hippocampus. For CFL ubiquitylation study, primary hippocampal culture was treated with the ubiquitin-proteasome inhibitor,


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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MG132. ROS/Superoxide Detection Assay Kit was used to measure intracellular ROS level. The mitochondrial respiration was studied by the method of high-resolution respirometry. Western blotting was applied for the detection of cofilin, ubiquitin (linkage-specific K63), and LC3B. The treatment of the nerve cells with MG132 led to the changes in expression of CFL modified by K63-linked polyubiquitin chains. K63-linked chains were established to facilitate the clearance of ubiquitylated proteins via autophagy. Indeed, the level of both LC3B-I and LC3B-II was elevated under MG132 action that indicates the autophagy activation. Moreover, it was accompanied with increase in the rate of mitochondrial respiration and ROS production. Thus, there was shown a complex relationship between mitochondrial function (ATP synthesis, ROS content, autophagy) and CFL ubiquitylation that represents one of the quality control systems in the nerve cells. Understanding of mechanisms that regulate the crosstalk between mitochondria and CFL ubiquitylation is essential for discovery of therapeutic tools in the treatment of the nerve system disorders. This work was supported by RSF (project # 17-75-10202) and RFBR (project # 18-34-00690). *The authors marked with an asterisk equally contributed to the work.

## P-07-039
## Mechanisms of increased mitochondria-dependent necrosis in Wiskott-Aldrich syndrome platelets

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Wiskott-Aldrich syndrome (WAS) is associated with microthrombocytopenia of unclear origin. We investigated mechanisms of cell death and phosphatidylserine (PS) exposure in platelets of 24 patients with WAS. Real-time cytosolic calcium dynamics, mitochondrial membrane potential and PS exposure were investigated in single fibrinogen-bound platelets using confocal microscopy. WAS platelets spontaneously lost mitochondrial membrane potential followed by PS exposure (21.4% vs 3.6% in healthy donors, $P < 0.001$). This phenomenon was inhibited by mitochondrial permeability transition pore inhibitor, cyclosporin A. The number of mitochondria was decreased in WAS platelets and was a predictive parameter for this mitochondria-mediated necrosis: 44 + -26% of platelets from WAS patients with 4 or less mitochondria exposed PS, while only 14 + -19% did among those who had 5 and more. Healthy donor platelets with fewer mitochondria also more readily became procoagulant upon PAR1/PAR4 stimulation. Collapse of single mitochondria led to greater cytosolic calcium increase in WAS platelets if they had 1–3 mitochondria compared with a higher number. Computer systems biology model of platelet calcium homeostasis revealed that the smaller platelets with fewer mitochondria could have impaired calcium homeostasis due to higher surface-to-volume ratio and greater metabolic load. This cell death mechanism could contribute to the development of thrombocytopenia in WAS: indeed, there was significant correlation ($C = 0.81$, $P < 0.02$) between the mean platelet size and platelet count in the WAS patients. We conclude that WAS platelets readily expose PS via a mitochondria-dependent necrotic mechanism caused by their smaller size (which is the immediate cause of increased surface-to-volume ratio and decreased mitochondria number). This work was supported by grants 17-00-00140, 17-04-01309, 18-34-20026 from the Russian Foundation for Basic Research and by foundation "Doctors, Innovations, Science for Children.

# DNA architecture

## P-08-001
## Opbp is a new architectural protein involved in organization of the housekeeping TRF2 dependent promoters in *Drosophila melanogaster*

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Transcription of protein-coding genes is highly dependent on the RNA polymerase II core promoter. The core promoter includes the transcription start site (TSS) and functional core promoter motifs that facilitate recruitment of the general transcription complex. In Drosophila, TBP homolog named TBP-related factor 2 (TRF2) is responsible for activity of many housekeeping promoters. Transcription factors DREF, Z4 and CP190 are present in the TRF2 complex. Unlike TBP, TRF2 fails to bind DNA suggesting existence of proteins that recruit TRF2 to the promoters. Here, we describe a new architectural protein Opbp as a potential recruiter of the TRF2 complex to the promoters. Opbp binds to a long sequence motif located near TSS of the housekeeping promoters controlled by TRF2. By using the CRISPR/Cas9 approach, the *opbp* gene was substitute by the *att*P site that allows introduction of different mutations into the *opbp* gene. We showed that Opbp is essential for viability and inactivation of Opbp decreases expression of target genes. Opbp directly interacts with the CP190, Z4, DREF and TRF2 proteins. The CP190 and Z4 proteins interact with two domains in N- and C-terminal regions of Opbp. Deletion of either of these domains in Opbp does not severely affect fly viability. Deletion of both regions leads to lethal phenotype suggesting inactivation of the Opbp function. These results suggest a role of Opbp in recruiting the proteins involved in the TRF2 complex to the promoters. Opbp displays all the characteristics of an architectural/insulator protein. The multimerized Opbp binding sites form functional insulator-like elements that support distance interactions and display enhancer blocking activity. These activities might be supported by a dimerization domain mapped near the N-terminus. Deletion of the dimerization domain in Opbp does not affect viability but causes bobbed phenotype that is associated with reduced expression of rRNA genes. Supported by RFBR 17-00-00285.

## P-08-002
## Cooperation between architectural proteins is required for boundary function in Drosophila bithorax complex

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Boundaries in the bithorax complex delimit autonomous regulatory domains that drive parasegment-specific expression of the Hox genes *Ubx*, *abd-A*, and *Abd-B*. The boundaries consist of two types of regulatory elements: insulators and Polycomb Response Elements (PRE). The *Fab-7* boundary is located between *iab-6* and *iab-7* domains and has two key functions: 1) it blocks crosstalk between these domains; 2) it allows *iab-6* to communicate with the *Abd-B* promoter. Previously was shown that multimerized binding sites for Pita, Su(Hw), and CTCF, when inserted instead of *Fab-7* boundary, can function as


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conventional insulators that block communication not only between the *iab-6* and *iab-7* domains, but also between the *iab-6* and *Abd-B* promoter. In these transgenic assays we used four CTCF sites ($C^{x4}$). Interestingly, three CTCF sites ($C^{x3}$) failed to block crosstalk between the *iab-6* and *iab-7*. qChIP showed that the CTCF protein binds to $C^{x4}$ stronger than to the $C^{x3}$ site. A combination of $C^{x3}$ with PRE restored blocking activity. PRE by itself does not possess boundary function suggesting that cooperation between PRE and CTCF forms a functional boundary. The core 340 bp *Mcp* boundary contains sites for Pita and CTCF proteins and functions as a boundary, while the 66 bp subfragment including Pita and CTCF sites is devoid of boundary activity. Adding PRE to 66 bp Pita+CTCF fragment restored blocking activity. qChIP showed that Pita bound to this subfragment in all cases, while CTCF needs Pita and some additional factor for binding to its site. Similarly, when the fully functional 209 bp *Fab-8* fragment containing two CTCF sites was reduced to 106 bp subfragment including these two sites, the boundary function was lost. Combination of the *Fab-8* subfragment with PRE restored boundary function. These results suggest that cooperative binding of several architectural proteins is required for formation of the effective boundary and PRE have new boundary function.

## P-08-003
## Studies of PARP inhibitors in the mononucleosome system

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Currently there are more than 200 completed or running clinical trials for the PARP inhibitors (PARPis). Four of them (olaparib, rucaparib, niraparib and talazoparib) have been approved by FDA for treating ovarian and breast cancers. It is assumed that their anti-cancer effect is associated with inhibition of the PARP catalytic activity. Recent studies have shown that PARPis also exert their cytotoxicity by trapping PARP-DNA complexes. Potency to trap PARP–DNA complexes varies widely among PARPis and often does not correlate with their ability to inhibit PARP catalytic activity. This differential trapping potency can potentially originate from an allosteric change in protein conformation leading to an increase in binding activity. We have studied the mechanism of PARP1 trapping using fluorescently labeled mononucleosomes as "minimal" experimental system that recapitulates interaction of PARP1 with DNA in chromatin. PARP trapping by three PARPis and gel-shift analysis. Our studies revealed that PARP1 forms three types of complexes with the mononucleosome; one of them induces partial nucleosome unfolding that we have described previously. In the presence of NAD+, only the unfolded PARP-nucleosome complex was trapped by PARPis, suggesting that the PARPis-induced PARP trapping strongly depends on the structure of PARP-nucleosome complex. The new proposed approach to identification of the trapped complexes can be useful for the development of efficient PARPis with increased trapping and cytotoxic activity. This study was supported by RFBR grant 17-54-33045

## P-08-004
## G4 DNA structures as possible traps for histone chaperons

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We report on interactions between G-quadruplex (G4) DNA structures and histone chaperons with FACT-like activities. The heterodimeric complex FACT (FAcilitates Chromatin Transcription), which promotes nucleosome disassembly/reassembly and relieves the nucleosomal barrier for Pol II, is reportedly sensitive to non-B DNA conformations. In a recent DNA-protein interaction profiling assay, we identified FACT and BRD3 (the hyperacetylated chromatin-recognizing protein that facilitates transcription in the absence of FACT) among top candidates for efficient binding to several model G4s. We noticed that other functional analogs of FACT – nucleolin (the multifunctional protein that enhances histone transfer and may partially relieve the nucleosomal barrier) and ATRX (the chromatin remodeler that assists H3.3 histone positioning and promotes telomere transcription) also interact with G4 DNA, according to the literature. We concluded that affinity to G4s may be a biologically significant common feature of the FACT-like histone chaperons. We next verified G4-chaperon binding for a set of genomic G4 structures using microscale thermophoresis and other methods. All of the tested chaperons were found to distinguish G4s of different topologies, and for several G4s affinities in the low micromolar to nanomolar range were shown. These findings encourage future investigations of G4 contribution to transcription control and epigenetic alterations (including nucleosome positioning). We hypothesize that persistent G4s in R-loops can recruit and probably trap histone chaperons analogously to the recently reported recruitment of DNMT1. Apart from these fundamental aspects, we analyze implications of the revealed G4-histone chaperon interactions for aptamer design. For instance, the well-known nucleolin binding aptamer AS1411 exhibited cross-reactivity against BRD3. This sheds new light on cancer-selective antiproliferative effects of AS1411. This work was supported by RFBR [19-04-00050 A].

## P-08-005
## 3D genome organization as a potential chemotherapeutic target

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Cancer cells are characterized by multiple epigenetic alterations resulting in changes of gene expression. Oncogenes are activated in cancer by enhancers and super-enhancers, usually distant regulatory genomic elements, which control gene expression via formation of DNA loops bringing them to the close proximity with the target gene promoter. Inhibition of cancer-specific enhancer activity is a novel strategy for cancer treatment. Recently, new class of anti-cancer compounds, curaxins, was discovered. They suppress transcription of oncogenes by affecting the packaging of a chromatin fiber. In this study, we demonstrate that effects curaxins exert on physical properties of DNA can prevent efficient long-distance enhancer-promoter communication *in vitro* and *in vivo*. Hi-C technique identified that curaxins strongly affect spatial organization of the genome. Particularly, curaxins compromise topologically associating domains (TADs) and


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disrupt chromatin loops, thus altering enhancer-promoter communication in living cells. This effect is mediated, at least in part, by curaxins-induced depletion/dissociation of CTCF from its binding sites. This work was supported by the Russian Science Foundation (17-74-20030) and the Russian Foundation for Basic Research (18-29-07001). *The authors marked with an asterisk equally contributed to the work.

## P-08-006
## DNA analyser, a new powerful tool to identify and analyse local DNA structures in genomes

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Local DNA structures play important roles in the regulation of basic biological processes. Whilst computational approaches to identify these structures are available, the number of easily accessible and user-friendly tools is limited, especially for analysis of different types of local structures within long nucleotide sequences. We have developed a web-based server, DNA analyser, which is a user-friendly application for analysing local DNA structures. At first we implemented a tool for analyses of inverted repeats in various nucleic acids sequences including genome sequences and oligonucleotides. It allows users to search and retrieve desired gene/nucleotide sequence entries from the NCBI databases, and provides data on length, sequence, locations and energy required for cruciform formation. We have recently included new tools for analyses of quadruplex-forming sequences – an interactive web-version of the G4Hunter algorithm – as well as a tool to search for p53 target sequences. DNA analyser also features an interactive graphical representation of the distribution of local DNA structures and options for sorting and data export. DNA analyser allows whole genome analyses and it is freely available at http://bioinformatics.ibp.cz. Acknowledgment: This work was supported by The Czech Science Foundation (18-15548S).

## P-08-007
## DNA replication machinery and topoisomerases – good targets for new antibiotics development

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Due to spread of the resistance to classic antibiotics, development of the new antibacterial agents becomes one of the principal goals of the modern biotechnology. Fluoroquinolones (FQ), one of the first discovered inhibitors of the bacterial topoisomerases (Topo IV and gyrase) are effective against wide range of gram-negative and gram-positive bacteria. Meanwhile resistance to these drugs due to mutations in the targets significantly increases during the last years. Recently, we developed high-throughput fluorescent approach for discovering antibiotics, affecting DNA replication machinery (1). Nybomycin, old, but not well-studied antibiotic, was one of the compounds, which induced our reporter in *E. coli* cells. We demonstrated, that Nybomycin inhibits activity of both wild-type and FQ-resistant gyrase (gyrA: Ser83-Leu), but another mutation (gyrA: Tyr87Asp) gives gyrase resistance to FQ and Nybomycin. Molecular docking experiments predicts binding place on gyrase for Nybomycin close to FQ, but position of the drug is different. The mechanism of nybomycin action is also different from FQ, latter inhibit DNA ligation after double break, but Nybomycin prevents double break formation. Like FQ, Nybomycin acts on bacterial DNA topoisomerase IV, but unlike latter it also inhibits eukaryotic topoisomerase 2α. In addition to type II topoisomerases, which cut both strands of the DNA, Nybomycin acts on type I topoisomerases from bacteria and eukaryotes. Our work demonstrates, that natural antibacterial agent Nybomycin inhibits activity of different DNA topoisomerases, so it is good candidate for antibacterial agents design, because simultaneous resistant mutations in different targets are a rare event. Inhibition of eukaryotic topoisomerase could be used for development of the anticancer agent. Work was supported by the Russian Fund for Basic Research (grant 18-34-20055) for I.A.O. 1. Osterman, I.A., *et al.* (2016) Sorting Out Antibiotics' Mechanisms of Action. *AAC*

## P-08-008
## qPCR inhibition in six stacked G-quadruplex forming sequences

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In 2018 we have, for the first time, described sequences capable of formation of six-stacked G quadruplexes. By the means of bioinformatic methods we have found their non-random distribution throughout human genome. They are located in genes or promoter regions of genes responsible, i.e., for cancer and neurodegenerative disorders. By the means of electrophoretic and CD measurements of G6 forming oligonucleotides we have proven their ability to form six stacked structures. In present work we have studied ability of these structures to affect biological processes. We have cloned sequences capable of forming G6 structures into plasmid DNAs and used qPCR to analyse ability of G6 structures to inhibit DNA polymerase. We have also checked the thermostability of G4 and G6 structures by the means of HRM. Our results indicate, that in the conditions of standard qPCR mastermix the qPCR reaction was inhibited by average of 3.5 cycles in wide range of plasmid DNA concentrations both in standard G4 and newly tested G6 structures, showing the formation of G6 structures in plasmid DNAs and ability of these structures to inhibit DNA polymerases. Results were even more significant in elevated KCl concentrations. We conclude, that such inhibition might be the mode of operation of these structures on regulation of gene expression. HRM measurements show differences in melting profiles dependent on KCl concentration in reaction buffer, confirming formation of quadruplex structures that are stabilized by monovalent cations. Stabilisation of G6 structures might be found as potent targeted therapeutic strategy, as in comparison of G4 targets, there are only dozens of sequences capable of forming G6 structures in the human genome. Our next goals are searching for chemicals binding specifically to G6 structures and *in-situ* confirmation of G6 structures role in gene expression. *The authors marked with an asterisk equally contributed to the work.


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## P-08-009
## Python toolkit for DNA geometry analysis and modeling

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The structure of DNA largely determines its function and affects the degree of chromatin compactization. With the development of new experimental techniques, an increasing number of structures of macromolecules containing DNA become available. Modern computational methods allow us to obtain the molecular dynamics trajectories of macromolecules that exceed tens of microseconds. This new data can be analyzed with a number of state of the art software packages for DNA geometry analysis. However, the growth of data volumes requires the adaptation of well-established tools and the development of new tools for data processing. On the other hand, in order to achieve reproducible research, the data processing toolchain must be openly accessible, including the source codes of the libraries and executable files. We developed a set of tools for the rapid and high throughput analysis of DNA structures. This toolkit is written in Python with Numpy stack and MDAnalysis module for molecular structures handling. Thus it is easily extensible and can open source by design. The current version of the software can be used for DNA molecular dynamics simulations analysis in a streamlined workflow. The developed software module also contains visualization tools for internal parameters of DNA geometry (such as reference frames and base-pair step parameters). Besides the analysis, software is able to create molecular models of DNA geometry. It is capable of homology modeling of DNA structure and assessment of DNA bending energy. The latter, coupled with a set of external geometric restraints, can be used to produce molecular models of complex DNA molecules. Overall the developed software simplifies and accelerates the process of DNA geometry analysis. This work was supported by the Russian Science Foundation Grant No. 18-74-10006.

## P-08-010
## Studying the interactions between *Arabidopsis thaliana* Fen1 and PCNA1/2 proteins

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DNA replication is one of the fundamental processes for all living organisms. There are several proteins engaged in DNA replication, including proliferating cell nuclear antigen (PCNA) and Flap endonuclease 1 (Fen1). PCNA acts as a universal platform for many DNA transacting proteins. Fen1 is characterized by 5′-flap endonuclease as well as 5′-3′ exonuclease activities and is involved in OKAZAKI fragment maturation during DNA replication. Moreover, Fen1 plays also a crucial role in responses to abiotic stress as a component of DNA repair systems. Fen1 activity is stimulated by the interaction with PCNA. Despite the fact that the interaction of PCNA and Fen1 has already been described, the knowledge about the Fen1/PCNA interplay in the model organism *Arabidopsis thaliana* is still scarce. It is particularly interesting whether *Arabidopsis* PCNA1 and PCNA2 proteins may differently affect the function of Fen1. Fen1 as well as its mutated variant with a *disrupted* canonical PCNA-interacting protein (PIP)-box motif was overexpressed and purified from *E. coli* BL21 cells. BIACORE analysis, yeast two hybrid and pull-down assays were used to study the interactions between Fen1 and PCNA1 as well as Fen1 and PCNA2 proteins. Moreover, we performed crystallization and preliminary X-ray diffraction studies of crystals of PCNA2 complexed with a peptide derived from the *Arabidopsis* Fen1 containing a PIP-box motif. In this study we analyzed the interaction between *Arabidopsis* Fen1 with both PCNA proteins. Dissociation constants for the studied complexes were determined. Although the amino acid sequence identity between PCNA1 and 2 is 97%, we found that the interaction of Fen1 with PCNA1 was significantly stronger than with PCNA2. The obtained crystals of PCNA2 belonged to the hexagonal space group $P6_3$, while those in a complex with the Fen1 peptide belonged to the rhombohedral space group *H3*. This work was supported by NCN grant Sonata-Bis3/UMO-2013/10/E/NZ1/00749 to WS.

## P-08-011
## Multiple PHD domains of mixed lineage leukaemia protein (MLL1/KMT2A) target active chromatin regions

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SET/MLL proteins Lysine (K) Methyltransferases (KMT2) are the core proteins of the multi-subunit hCOMPASS-like complexes, which catalyze H3K4 methylation and therefore favour an active chromatin state. The best studied human KMT2 protein is KMT2A (known also as MLL1) due to its involvement in the pathogenesis of mixed lineage leukaemia (MLL). KMT2A within hCOMPASS-like complex has the ability to di- and trimethylate H3K4 localized mostly at gene promoters. KMT2A proteins contain several domains, including DNA mark reader – CXXC domain and histone mark reader – PHD domains. Recently we showed that CXXC domain of KMT2A recognized specifically only non-modified CpG. Interestingly, in contrast to majority of PHD domains present as a single domain, KMT2 protein family possesses PHD domains, mostly arranged in triples. So far, the detailed recruitment of MLL-containing complexes to targeted genes as well as characterization of multiple PHD domains is not well known. In order to address the contribution of PHD domains to KMT2A targeting, we performed proximity ligation assay in HeLa cells to investigate the co-localization of stably expressed triple PHD1-3 domains of KMT2A with several chromatin marks. We found a stronger association of the PHD domains with eu- than heterochromatin marks. In addition, histone peptide array experiments with purified GST-tagged triple PHD1-3 domain of KMT2A protein showed affinity to multiply modified histone tails. As for promoter-targeting protein, GST-KMT2A-PHD1-3 was observed to be recruited to histone tails containing modification of H3K4, especially H3K4me3. In summary, KMT2 multiple PHD domains contribute to MLL


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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targeting by recognizing combinations of chromatin marks in contrast to single PHD domain which is known to bind a single (H3K4me3) chromatin mark. Funding: National Science Centre (NCN) – HARMONIA and Polish National Agency for Academic Exchange (NAWA) grants to M.B.

## P-08-012
## The insulator protein Suppressor of Hairy-wing (Su(Hw)) positions replication proteins in 66D and 7F Drosophila amplicons in follicle cells (DAFCs) during oogenesis

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To date insulator proteins are known to take part in wide variety of processes in the cell, from canonical transcription regulation to stress response, replication, DNA reparation, RNA-polymerase pausing, RNA splicing and transport to cytoplasm. Earlier we have shown that Su(Hw) insulator protein recruits to chromatin SAGA and Brahma complexes and constitutes part of Origin Recognition Complex (ORC) binding sites in the Drosophila genome. We have recently found that Su(Hw) binds sites in Drosophila amplicons in follicle cells (DAFCs), known as convenient model for DNA replication studies. DAFCs contain chorion membrane genes and undergo DNA amplification starting from stages 10AB of egg chambers during oogenesis. As knock out of *su(hw)* gene cause female sterility and violations in structure of mature egg chambers, for investigations we selected only ovaries of immature flies, containing egg chambers of 1–7 stages. We have used mutant flies, expressing Su(Hw) unable to bind DNA ($su(hw)^{V/E8}$), and wild type flies as control ($su(hw)^{+/+}$). In ChIP-seq experiments we have investigated Su(Hw) influence on chromatin remodelers and replication proteins binding. We have shown that Su(Hw) knock out disrupts Brahma, ISWI, CHD1 and Mi2 chromatin remodelers binding and ORC2 and CDC45 positioning on 66D and 7F DAFCs. Also in FAIRE experiments we have shown that Su(Hw) depletion leads to compaction of chromatin in 66D and 7F amplicons. These results allow us to conclude that Su(Hw) insulator protein positions replication proteins in DAFCs through the influence on chromatin remodelers binding and, as a consequence, through chromatin state. The work was supported by the Russian Science Foundation grant No 17-74-10211.

## P-08-013
## Inhibition of DNA replication by an anti-PCNA aptamer/PCNA complex

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PCNA is an essential protein involved in DNA metabolism. The results of previous studies revealed its multifunctional role in many cellular processes such as: DNA replication, repair, and cell cycle control. Recently, PCNA was proposed as a non-oncogenic target of anti-cancer therapy. Therefore, the aim of this study was to develop a DNA aptamer that could block DNA replication by inhibiting the interactions of PCNA with its protein partners. Using the SELEX method, we found an anti-PCNA DNA aptamer. The dissociation constant of the anti-PCNA aptamer/PCNA complex was in the range of 2–5 mM. The DNA polymerase assay showed that the activity of human DNA polymerase d or e was strongly inhibited in the presence of PCNA and the anti-PCNA aptamer. Surprisingly, electrophoretic mobility shift assay revealed that the anti-PCNA aptamer did not inhibit the binding between PCNA and DNA polymerase d or e, but rather formed a complex with those proteins. We demonstrated that the observed inhibition resulted from a competition between the anti-PCNA aptamer and primer-template DNA for binding to the PCNA/DNA polymerase d or e complex. Based on the observations, a model of inhibition of DNA replication by the anti-PCNA aptamer /PCNA complex was proposed.

## P-08-014
## *S. medicae* genomes screened for genomic islands

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*Sinorhizobium medicae* is a root nodule bacteria taxonomically closely related to *S. meliloti* species. *S. medicae* form effective symbiosis with annual medics and frequently noneffective with perennial alfalfa species. Genome architecture of both rhizobia species are similar since they contain chromosome with a size at about 3.7 Mbp and two megaplasmids with a sizes varied from 1.2 to 1.7 Mbp. Chromosome genes are mainly responsible for housekeeping processes, while genes located on megaplasmids are involved in symbiotic fitness. Accessory elements of chromosomes of model strains *S. medicae* WSM419 and *S. meliloti* Rm1021 are represented by two (Sme4.42S and Sme4.51K) and three (Sme21T, Sme19T and Sme80S) genomic islands (GIs), correspondently. Each of these islands is site-specifically integrated in particular tRNA gene according to the ISLANDER database. For the first time the 23 sequenced genomes of *S. medicae* (GenBank) were screened for GIs and theirs insertion sites in order to design primers for PCR-GIs detection among 29 isolates native to alfalfa centers of diversity located at Caucasus and at the north part of Kazakhstan. One of two GIs of WSM419 (Sme4.51K) was detected more frequently (0.70) in genomes of native isolates. The second island (Sme4.42S) of WSM419 was revealed twice rare, as well as other two GIs (Sme21T and Sme19T) of *S. meliloti* Rm1021. The third island (Sme80S) of Rm1021 was not detected in the *S. medicae* tested genomes at all. *S. meliloti* and *S. medicae* species significantly differed in the presence and composition of GIs tested ($P = 7.8 \cdot 10^{-3}$). Unique for each species and common for the two species GIs insertion sites (recombination hotspots) were identified. We propose that these particular sites are actively involved in horizontal genes transfer process occured within or between species. This work was supported by the RSF 17-16-01095. *The authors marked with an asterisk equally contributed to the work.


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## P-08-015
## Accessory elements of rhizobia chromosome: site-specific integration, structural and functional diversity

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Chromosomes of rhizobia forming symbiosis with legumes are possessed "core" genes encoding cell vital metabolic functions inherited by vertical evolution pathways. But a number of extend "accessory" elements are occurred in chromosome due to the horizontal gene transfer. Among them are genomic islands (GI) with great gene pool plasticity. The classification of GIs does not reflect the mechanisms of their formation or evolution. Classically GIs are site-specifically integrated phage related sequences known for GC content which is lower than neighboring chromosomal sequences. GIs identified in chromosomes of nitrogen-fixing slow-growing rhizobia of genera Bradyrhizobium and Mesorhizobium are harbored genes determining symbiotic activity («symbiotic islands») and their sequences have more than 500 kbp in length. GIs are also identified in chromosomes of fast-growing rhizobia of *Sinorhizobium meliloti* species. Thus GIs of *S. meliloti* native isolates from centers of alfalfa diversity next to Aral Sea area and at the NE of Caucasus are greatly varied in number per genome and in sizes (from 10 to 80 kbp) as well. GIs of *S. meliloti* harboured various virulence factors, genes of archaea and bacteria of different phyla and blocks of genes determining a whole metabolic pathways related to bacteria fitness. GIs sequences analyzed in silico provide new data support that theirs evolution occurs, apparently, through intragenomic rearrangements, mutations, by lost and acquisition of new functionally significant sequences and mobile elements. GIs of *S. meliloti* could be assigned to some new type in-between of pathogenic and symbiotic islands, as they simultaneously contain elements of different types of islands. GIs are module elements of bacteria evolution and their role in response to contemporary global agro climate changes should be under special focus. This work was supported by the RSF 17-16-01095. *The authors marked with an asterisk equally contributed to the work.

## P-08-016
## New geometrical restraints for nucleic acids

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The reference dictionary of nucleic acid stereochemistry was compiled from data contained in the CSD in 1996, as presented by Parkinson. 23 years later and with 10x expanded CSD, it is time to re-assess the validity of those standards and to see if more sophisticated paradigms (e.g. conformation-dependent functions) might be used. We based our analysis on rigorous statistical treatment of suitable CSD fragments, divided into three main classes of the principal nucleic acids constituents. The covalent geometry of the phosphodiester group is indeed dependent on conformation, albeit in a discrete way, where the O-P-O bond angles and distances are grouped into six conformational categories. The same clustering results were obtained by manual analysis and by automatic machine-learning algorithms, confirming that artificial intelligence can be successfully applied for the discovery of complicated structural patterns. The nucleobase geometry is not conformation-dependent but in this class we tested different hypotheses, e.g. if the CSD data correctly represent WC base pairs, or if advanced quantum mechanical (QM) calculations could provide sufficiently accurate restraints. While the QM models are remarkably good, they are still inferior to high-quality CSD data. For the glycosidic moiety, the situation is complex because some of the geometrical parameters (e.g. the glycosidic bond) depend on conformation, while others do not. Our results confirm that the Parkinson library is still remarkably valid and can be used without compromising the quality of macromolecular models. However, since there are some parameters that require adjustment, and as our statistical and analytical tools are more elaborate, we suggest to use the current library for refinement/modeling of nucleic acids structure. For convenience, a RestraintLib webserver has been created (http://achesym.ibch.poznan.pl/restraintlib/) to allow easy generation of external restraints for refinement.

## P-08-017
## Genome structure of highly effective strain *Sinorhizobium meliloti* AK555

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The genome of *Sinorhizobium meliloti* AK555 widely used for the production of biologicals was completely sequenced by MiSeq (Illumina) and nanopore (Oxford Nanopore) technologies as a part of project RSF 17-16-01095. The strain AK555 was originally recovered from nodule of wild-growing *M. falcata* at the NW Kazakhstan. It was characterized as a salt tolerant strain well growing in liquid TY medium with 750 mM NaCl. The AK555 forms highly effective symbiosis with a number of different varieties of *M. sativa* subsp. *varia* as it was proved by plots and field trials done by Geographical Experiment Network with biologicals in Russia. The average plant dry mass was increased on 215.6% in comparison with control non-inoculated *M. sativa* subsp. *varia* plants. In model plant tests AK555 formed an effective symbiosis with *M. truncatula* and ineffective with *M. polymorpha* plants. The genome of AK555 consists of five replicons: the chromosome (3.68 Mbp), two megaplasmids (SMa and SMb of 1.33 and 1.66 Mbp, respectively), and two cryptic plasmids (SMd of 31.2 kbp and SMe, assembled from 3 contigs: 440.0 kbp, 790 and 787 bp). The AK555 chromosome was longer by 21.2 kbp than the reference strain Rm1021, whereas the megaplasmids SMa and SMb, on the contrary, had smaller sizes (by 25.3 and 24.5 kbp, respectively). The nucleotide sequence which could be predicted as genomic island (41 kbp) was identified in the chromosome of AK555. It is different from genomic islands of the reference strain Rm1021 by length, internal structure and insertion site. Another nucleotide sequence (51.4 kbp in length) is an intact prophage according to PHASTER tool. Further studies of the AK555 genome will be aimed at finding and analysis of determinants of efficiency and stress tolerance. Studies of the symbiotic and cultural characteristics were supported by research grant RFBR 17-04-02011a; genome analysis of the strain was carried out according to the RFBR 18-04-01278a program. *The authors marked with an asterisk equally contributed to the work.


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## P-08-018
## The genome architecture of the strain *Ensifer meliloti* AK89, a highly effective symbiont of *Medicago lupulina*

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*Ensifer meliloti* is a soil saprophytic bacterium able to form nitrogen fixing symbiosis with legumes of *Trifolieae* tribe. The AK89 was originally isolated from nodule of wild-growing *Medicago lupulina* in Kazakhstan. It was shown that seed inoculation with strain AK89 led to a 50% increase of green mass of *Medicago lupulina* var. Mira. Strain contribution into the symbiosis formation was shown to increase by seven times in moderate acid soils, typical for temperate zone of Russia. Using NGS sequencing (Illumina and nanopore) we have shown that AK89 complete genome was 6.79 Mbp and it consists of 4 replicons represented by chromosome (3.58 Mbp, 62.9% GC) and three plasmids (1.22 Mbp, 60.4%; 1.65, 62.5% and 0.35 Mbp, 59.1%). A comparative genomic analysis made for AK89 and Rm1021 showed 87.56% nucleotide homology between these genomes. The "ancient part" of the AK89 genome was 70.73% according to COG analysis of the proteomes data obtained for AK89 and the α-proteobacterial ancestor. No genomic islands similar to those in genome of the reference strain Rm1021 were determined in the chromosome of AK89 according to Islander algorithm. The homology (blastN) between chromosomes of AK89 and Rm1021 was 99.9% with an exception of two unique potential genomic islands. They are 10.1 kbp (60.3%) and 40.1 kbp (59.3%) in length sequences integrated in tRNA-Lys and tRNA-Met, respectively. The second one contains proteins related to *Sulfitobacter* phage pCB2047-A, according to Phaster tool. Intact phages were not detected in the AK89 genome, but incomplete phages sequences of different species were found in all four replicons. Thus, genome architecture of AK89 is probably more mobile than those of Rm1021 as cryptic plasmid, genes related to different phages and genomic islands were found out in it. This work was supported by the RSF 17-16-01095.

## P-08-019
## Pipeline for prediction and classification of retrotransposons as putative markers for selection

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Retrotransposons forms one of the subclasses of transposons, which are the genetic elements that can amplify themselves in a genome and are essential component of nuclear DNA. Also, retrotransposons that have not lost transposition and expression functions can influence the expression of nearby genes as expression enhancers or silences and influence the adaptability of plants to various environmental conditions. We developed a software package that allows identifying and classifying LTR transposons in plant genomes, identifying portions of the genome adjacent to transposons that are potentially susceptible to the expression effect of LTR transposons, and their mutual arrangement. Inside the package, special programs in the R and bash languages and the built-in utilities LTRharvest and BLAST are used, allowing you to work with a large amount of input information and create user-friendly tabulated files with the obtained results. The first step is to identify potentially containing LTR transposons of the sites using the LTRharvest program, then determine the exact presence of transposons and their class using the BLAST program for aligning the sequences to the TREP nucleic and protein bases, then determining the relative location of neighboring genomes. Using this package, we were able to predict and classify lists of transposons in the chickpea genome and annotate LTR transposons with regulatory sequences of genes, which will allow the use of retrotransposons as selection markers. The project was supported by the RFBR grant 18-34-00728 mol_a

# RNA transcription

## P-09-001
## Targeting gene transcription: mechanisms of antitumor potency of Olivomycin A and its preclinical derivative

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Olivomycin A (1) and other aureolic acid derived antibiotics have been known for a high cytotoxicity attributed to the formation of stable complexes with GC-rich regions in the DNA minor groove. We dissected the binding sites for 1 with single nucleotide accuracy and found the number and sequence of C and G optimal for drug-DNA interaction. Divalent cations were dispensable for 1:DNA complex formation. In support of the DNA binder's ability to interfere with template syntheses, 1 down-regulated dozens of genes in human colon carcinoma and leukemia cell lines. The *c-Myc* oncogene was the most sensitive target (~50-fold decrease after 3 h with 50 nM of 1). The *c-Myc* promoter contains the GC-rich site-84 bp to -64 bp that binds the transcription factors Sp1 and NFAT. This site is preferable for 1 since the binding constant was 15-fold bigger than average value for 1:DNA complexes. Nevertheless, 1 inhibited both basal transcription of the endogenous *c-Myc* and activation of this gene in the context of an inducible promoter free of Sp1 and NFAT sites. These data reveal a detailed molecular mechanism of transcriptional inhibition by DNA ligands. Among our series of derivatives the amide of the 'short acid' of 1 (compound 2) demonstrated a retained cytotoxicity for tumor cell lines and the ability to inhibit *c-Myc*. Importantly, the therapeutic effect of 2 in an animal model was achievable at tolerable doses. Such an attenuated efficacy is associated with minor differences in drug-duplex interaction between 1 and 2. Thus, 2 emerged as a drug candidate in which the high antitumor efficacy (due to altered gene transcription) is combined with a controllable tolerance. The study was supported by the Megagrant (Agreement No. 14.W03.31.0020 between the Ministry of Science and Education of the Russian Federation and Institute of Gene Biology, Russian Academy of Sciences).


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## P-09-002
## The effects of genotoxic stress factors on DNA-repair system in Mycobacteria

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Tuberculosis is still among the most challenging global health problems. One of the main reasons for that is the quick and efficient adaptation of the pathogen *Mycobacterium tuberculosis* to the variety of harsh conditions in the host. Mycobacteria uncommonly develop resistance only by chromosomal mutations, in particular, single-nucleotide polymorphisms. Based on the remarkable genetic diversity of mycobacteria in isolates from tuberculosis patients, a great mutation rate could be expected in the bacteria. Although, in vitro studies show a very low basal mutation rate in mycobacteria (~$10^{-10}$/generation/cell). These contradictory results suggest that genomic stress factors like the immune pressure and antituberculotic drugs within the patients may provoke increased mutational rates in the bacteria. Cells provide the genome stability and low mutation rates by presenting countless DNA surveillance and correction processes. Mycobacteria has a unique, yet not quite understood DNA repair system with several redundant enzymes, absent canonical mismatch repair pathway, and proteins with special functionality. Our aim is to study the activation pattern of mycobacterial DNA repair system upon *in vitro* genotoxic stress with the help of qPCR. In our experiments, we use the non-pathogen *Mycobacterium smegmatis*, which shares the same metabolic and repair routes as the medicinally relevant strains. Our results will hopefully contribute to a better understanding of the adaptive events resulting in the resistance of mycobacteria.

## P-09-003
## The role of ATR and ATM in hypoosmotic stress-induced silencing of nucleolar transcription

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The contribution of nucleoli to the cellular stress response has been discussed for over a decade. Stress-induced inhibition of RNA polymerase I-dependent transcription is hypothesized as a possible effector program in such a response. In this study, we report a new mechanism by which ribosomal DNA transcription can be inhibited in response to cellular stress. Specifically, we demonstrate that mild hypoosmotic stress induces stabilization of R loops in ribosomal genes and thus provokes the nucleoli-specific DNA damage response, which is governed by the ATR kinase. Activation of ATR in nucleoli strongly depends on Treacle, which is needed for efficient recruitment/retention of TopBP1 in nucleoli. Subsequent ATR-mediated activation of ATM results in repression of nucleolar transcription. The study shows that in the course of cellular response to hypoosmotic stress DNA damage-dependent pathway of nucleolar transcription silencing can cooperate with the long noncoding RNA-dependent one. This work was supported by the Russian Science Foundation (17-74-20030) and the Russian Foundation for Basic Research (17-00-00098). *The authors marked with an asterisk equally contributed to the work.

## P-09-004
## Identification of human mRNAs affected by expression levels of short isoform of securin (PTTG1) but not by the full length PTTG1

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Securin (*PTTG1*) is a separase inhibitor and transcriptional regulator. PTTG1 expression reaches peak before anaphase, when it is rapidly degraded to free separase and initiate chromosome divergence. We recently reported a short *PTTG1* isoform that lacks APC/C recognition site (D-box) and DNA-binding domain but retains the N-terminal transactivation region. Using overexpression we've shown previously that short isoform is able to influence only a subset of genes regulated by full-length isoform. The aim of this study was to find genes, which are regulated by short isoform but not by the full-length *PTTG1*. To measure the transcriptional influence of securin we overexpressed both isoforms in HEK293T cell line. mRNA levels were measured by RNAseq. Our data revealed that genes regulated by both isoforms are predominantly associated with the extracellular matrix, intercellular signal transduction, development of the pituitary gland and cell migration. Genes, which are controlled solely by short isoform enrich categories associated with excretion, vasoconstriction and neuroactive ligand-receptor interaction. In a knock-down study, several genes that were identified as transcriptional targets in the overexpression experiment, were significantly altered by transfection of an siRNA specific for the short *PTTG1* isoform. Thus, we were able to identify genes that specifically depend on the expression level of the short isoform of *PTTG1*. This work was supported by grant 18-34-00628 from Russian Foundation for Basic Research and by the Program of fundamental research for state academies for 2013–2020, research topic 01201363823.

## P-09-005
## Interplay between BER and NHEJ in BER-deficient cells

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Ionizing radiation induces both DNA single- and double-strand breaks that are repaired by base excision repair (BER) and nonhomologous end joining (NHEJ), correspondingly. Unrepaired strand breaks promote cell-cycle delay, which facilitates DNA repair prior to replication. On the other hand, in response to persistent DNA strand breaks, ATM-dependent degradation of transcription factor Sp1 leads to downregulation of BER genes expression, further accumulation of SSBs and renders cells susceptible to elimination via apoptosis. However, the regulation of other DNA repair pathways in response to BER downregulation is not thoroughly investigated. In this study we found that BER deficiency leads to simultaneous downregulation of NHEJ repair pathway of DNA double strand breaks. We demonstrated that downregulation of BER by knockdown of the *XRCC1* gene


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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expression leads to simultaneous downregulation of Lig4/XRCC4 and Ku70/80 at the transcription and protein levels. We propose that this coordinated response of BER and NHEJ to persistent DNA damage is Sp1-dependent and aimed to facilitate elimination of genetically unstable cells. This study has been supported by RSF according to the research project No 19-74-20069. *The authors marked with an asterisk equally contributed to the work.

## P-09-006
## Analysis of RNA polymerase interactome in the radioresistant bacterium *Deinococcus radiodurans*

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*Deinococcus radiodurans* is a well-known radio-resistant and stress-resistant bacterium. Most previous studies of the nature of radioresistance were focused on the analysis of DNA repair and antioxidative systems in *D. radiodurans*, while highly coordinated transcriptional regulation could also play a major role in the stress resistance. We hypothesize that protein factors associated with RNA polymerase (RNAP) can orchestrate the interactions between the transcription, DNA repair, replication and translation machineries. To analyze the RNAP interactome, we constructed *D. radiodurans* strain with tagged beta'-subunit of RNAP and used it for pull-down of RNAP and associated proteins for further mass spectrometry analysis. We found that under optimal conditions RNAP in *D. radiodurans* cells is bound with a set of partner proteins. In order to find stress-specific interactions between RNAP and transcriptional regulators we are performing pull-down of RNAP from *D. radiodurans* cells exposed to gamma-irradiation, UV and hydrogen peroxide treatment. Identification of specific changes in the RNAP interactome will help to understand how the transcriptional machinery operates in *D. radiodurans* under stress conditions and will shed light on the interplay between transcription and other genetic processes in stress resistant bacteria. This work was supported by the Russian Foundation for Basic Research (grant No. 18-34-00905).

## P-09-007
## Ecdysone-dependent genes use different modes of transcriptional regulation at embryonic and larval stages of Drosophila development

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Transcriptional activation of eukaryotic genes is a multistep process which allows them to regulate gene expression on its different stages. The purpose of our study is to compare mechanism of transcriptional activation for the same genes at various developmental stages using Drosophila ecdysone-dependent genes as a model. We carried out a ChIP-Seq analysis of different forms of RNA polymerase II (total Pol II, Pol II CTD S5P and S2P) using collected nuclear extract material of Drosophila embryos (2–4 h and 6–8 h), L3 larvae, and pupae (0 h and 10 h). We focused on studying the genes which transcription is directly induced with 20E (20-hydroxyecdysone) both at embryonic and metamorphosis stages (e.g. hr3, hr4 and eip75b). We found out that activation of these genes in embryos proceeds through the mechanism of stimulation of Pol II recruitment (we observed a significant increase in the binding level of all Pol II forms with the promoters during the transition from 2–4 h to 6–8 h of the embryogenesis). But the same genes demonstrated a different way to be activated during metamorphosis. During puparium formation, we did not observe any changes in binding of total Pol II and Pol II CTD S5P form with the promoters of ecdysone-dependent genes, but detected a significant increase of Pol II CTD S2P form. So, the transcriptional activation of ecdysone-dependent genes is carried out by the mechanism of stimulation of transcription elongation. Additional ChIP-Seq experiments with Spt5, NELF-E, Brd4 and PAF1 regulators of the RNA polymerase II elongation showed no significant binding of the NELF-E and Brd4 proteins with the regulatory regions of the ecdysone-dependent genes in embryos. But both proteins were present at a great amount at promoters and enhancers of ecdysone-dependent genes during larval stage. We believe that transcriptional activation of the ecdysone-dependent genes during metamorphosis is mediated with the action of NELF and Brd4 regulators. *The authors marked with an asterisk equally contributed to the work.

## P-09-008
## Myb and osteogenesis: focused on mandibular/alveolar bone formation

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c-Myb is a transcription factor well known for its role in haematopoiesis and cancer where high levels of expression correlate with high proliferation and low differentiation status. Recently, several novel functions of Myb have become apparent, including a role in osteogenesis. Our present studies have focused on the early stages of the mandibular/alveolar (m/a) bone formation using temporospatial detection of Myb protein and quantification of myb RNA by qRT-PCR. Additionally, PCR Array/RNASeq analyses of myb gene knock-out embryos are underway. Mice homozygous for the myb gene knock-out die in utero at around the day 14. Unlike long bone germs at this stage, the m/a bone anlage already contains differentiated osteoblasts stemming directly from mesenchymal precursors without a cartilaginous inter-step. Immunohistochemistry was used to localise Myb protein within the cell condensation representing the first morphologically visible stage of the m/a bone formation (prenatal day 13). These cells are precursors of osteoblasts but not yet positive for osteocalcin. During the next two days of development, osteocalcin positive osteoblasts become abundant and the first sclerostin positive osteocytes appear. Myb protein was localized in osteoblasts as well as osteocytes, and the expression was maintained also at later stages of development. Relative myb RNA expression in the early bone at prenatal days 13, 14, and 15 was compared to expression levels of osteocalcin, the marker of osteoblasts, and sclerostin, the marker of osteocytes. At stage 13 myb RNA expression was 6 times higher than osteocalcin and comparable to sclerostin. By stage 15, when osteocalcin and sclerostin dramatically increase, myb RNA expression was at a relatively constant level. Our results demonstrate the presence of Myb at the earliest stages of m/a development. The consequence of Myb deficiency on osteogenic profiles has been investigated. Supported by GACR (19-15272Y) and AZV (NV18-07-00073).


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## P-09-009
## The role of components of the DNA-repair complex DNA-PK in transcription regulation

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The trimeric complex DNA-PK, composed of DNA-binding heterodimer Ku and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), is a participant of DNA double-strand breaks repair pathway through the non-homologous end joining (NHEJ). The Ku heterodimer formed by Ku70 and Ku80 subunits acts as the main sensor of DSBs which triggers a cascade of phosphorylation events required for the subsequent DSB repair. In addition to NHEJ, Ku is involved in various cellular processes such as V(D)J recombination, AP-site repair, telomere maintenance, apoptosis, transcription, and translation. Also, Ku is suggested to participate in human immunodeficiency virus-1 (HIV-1) replication at the stages of integration and transcription although the exact mechanism of Ku-dependent transcriptional regulation is unclear. To clarify the way of Ku-mediated regulation of HIV transcription a set of HEK 293T derived sublines with a stable depletion of either Ku70, Ku80 or DNA-PKcs subunits was established using CRISPR/Cas9 technology. Then using a luciferase reporter system with firefly luciferase under the control of promoters (viral promoters CMV, SV40, TK and HIV promoter LTR and also cellular promoter PGK), we observed a strong reduction in luciferase expression from all tested promoters under depletion of Ku70 and especially Ku80 subunit. Surprisingly, the influence of DNA-PKcs knockout on the transcription efficiency from all promoters and particular HIV promoter was not detected. To elucidate the influence of the DNA-PK subunits on the cellular transcription, we performed a transcriptome analysis of wild type HEK 293T cells and those with depletion of either Ku70, Ku80 or DNA-PKcs. The genes regulated by each subunit were defined, and the genes dependent on the all DNA-PK complex were separated. The work was supported by the RSF grant 17-14-01107.

## P-09-010
## Transcription of damaged DNA by bacterial RNA polymerase

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DNA lesions can significantly affect DNA replication and transcription and must be repaired to avoid mutations. During transcription-coupled repair, RNA polymerase acts as a sensor of lesions in the template DNA strand. According to the prevalent model, mostly based on studies in *Escherichia coli*, the Mfd translocase then dislodges the stalled transcriptional complex and recruits the nucleotide excision repair enzymes to the site of the lesion. However, many molecular details of translesion transcription by bacterial RNA polymerase remain unclear. In this work, we studied RNA synthesis on damaged DNA templates by RNA polymerases from *E. coli* and *Deinococcus radiodurans*. We found that despite the great difference in stress tolerance of these two organisms their RNA polymerases behave similarly on DNA lesions. DNA modifications greatly affecting its structure (thymine dimers, 1,N6-ethenoadenine, AP-sites) significantly inhibit transcription while 8-oxoguanine and O-6-methylguanine decrease the RNA polymerase fidelity. We further showed that the Mfd protein from *D. radiodurans* can dissociate transcription elongation complexes paused at the sites of lesions. Moreover, Gfh transcription factors increase this pause and stimulate transcription complex disassembly by Mfd. Interestingly, the transcription elongation factor GreA, which normally reactivates backtracked transcription complexes, has an inhibitory effect on translesion synthesis by *D. radiodurans* RNA polymerase. This suggests that elongation complexes blocked at the lesions do not undergo backtracking and GreA binding may stabilize an enzyme conformation unfavorable for nucleotide addition. Thus, we conclude that diverse types of lesions in the template DNA strand differently affect transcription and may lead to transcriptional mutagenesis or blockage of RNA synthesis, which can be modulated by regulatory factors. This work was supported by the Russian Science Foundation (grant 17-14-01393).

# DNA editing and modification

## P-10-001
## Transcription factor Ets-2 regulates the expression of key lymphotropic factors

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Ets-2 is a transcription factor (TF) that functions either as an activator or as a repressor of transcription and is involved in diverse biological functions, such as cell mitosis, development, differentiation, apoptosis and the regulation of immunity. Recent work in our laboratory has shown that in naive T helper cells IL-2 expression is blocked by Ets-2. In this work we studied the role of Ets-2 in the regulation of expression of key lymphotropic factors that play a pivotal role in the expression of genes involved in activation and differentiation of T and B cells; in particular, we examined the TFs NFAT2, NF-κB p65, c-Jun and the kinase CDK10. To this end, Jurkat, H938 (T lymphocytic cell lines) and HEK cells (embryonic kidney cell line), were transfected with increasing amounts of an Ets-2 overexpressing vector (pCDNA-ets2), in the presence (P/I) or absence (CM) of mitogens. Assessment of Ets-2 overexpression at the transcriptional level was performed by real time PCR. Ets-2 overexpression and lymphotropic factor expression at protein level were assessed by Western blot. Overexpression of Ets-2 in Jurkat and H938 cells induced the levels of NFAT2, NF-κB p65 and c-Jun under both CM and P/I conditions. In contrast, in stimulated HEK cells, the overexpression of Ets-2 resulted in a reduction in c-Jun and in CDK10 levels, whereas in non-stimulated HEK cells it resulted in an increase in c-Jun and CDK10 levels. Overexpression of Ets-2 in unstimulated H938 cells resulted in reduced CDK10 protein levels, whereas in stimulated cells Ets-2 over-induced CDK10 levels. In conclusion, Ets-2 is involved in the regulation of expression and synthesis of key lymphotropic factors. Our results set the stage for further studies to elucidate the role of Ets-2 in the regulation of signaling pathways involved in the activation and differentiation of T and B lymphocytes.

## P-10-002
## Application of plant ROS1 5-methylcytosine-DNA glycosylase from *Nicotiana tabacum* as tool for human epigenome editing

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DNA methylation is a reversible epigenetic mark for transcriptional gene silencing in diverse organisms including plants and animals. In higher eukaryotes, two general modified DNA bases 5-methylcytosine (mCyt) and its oxidized derivative 5-hydroxymethylcytosine (hmCyt) play epigenetic roles. In mammals, active


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DNA demethylation can occur by oxidation or deamination of mCyt, catalyzed by TET dioxygenases and AID/APOBEC deaminases respectively followed by base excision DNA repair. On the other hand, in plants mCyt can be directly removed by specialized bifunctional DNA glycosylases DEMETER and REPRESSOR OF SILENCING 1 (ROS1). However, the exact functions and substrate specificity of these plant DNA glycosylases remain unknown. Furthermore, ROS1 and DEMETER is a potential instrument for epigenome editing. In this study, we cloned and purified a catalytically active fragment of ROS1 from *Nicotiana tabacum*. ROS1 activity was investigated on substrates with a CpG dinucleotide, in which cytosine was methylated, hemimethylated or hydroxymethylated. Besides, we transfected HEK293 cell line by a plasmid coding for wild-type *ROS1* or D1445N to detect of mCyt level variation. It was shown that ROS1 possessed an enzymatic activity to remove hmCyt and mCyt residues from DNA substrates. Moreover, substrates containing mCyt were digested more efficiently then substrates containing hmCyt in both CpG sites with only one modified strand and with fully modified site. ROS1 was expressed in human cells and caused global DNA demethylation. The results obtained in the investigation suggest that plant ROS1 DNA glycosylase can contribute to change of mCyt level in human DNA and can be used as tool for quick and specific epigenome editing.

## P-10-003
## CMGE complex influences the stability of repetitive DNA sequences in yeast cells

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For years studies of the mechanisms providing high fidelity of DNA replication has been focused mainly on determining the influence of catalytic elements of the replisome. However, recent results show that also noncatalytic proteins could play an important role in maintaining genome stability. One of such elements is the GINS complex -a component of the CMG helicase (Cdc45-Mcm2-7-GINS). GINS is composed of 4 essential subunits: Psf1, Psf2, Psf3, Sld5 and interacts with many replication proteins, including a major DNA replicase- polymerase epsilon. Previously we showed that strains possessing mutated forms of Psf1 subunit exhibit mutator phenotype for base substitution and frameshift mutations. We are interested whether the proper functioning of GINS might be crucial for genome stability, particularly the stability of repetitive DNA sequences. We tested the stability of mono-, di-, tri-nucleotide, and other repetitive sequences in yeast strains carrying the *psf1-1* mutator allele (kindly provided by H. Araki). The Psf1-1 destabilizes interactions within the GINS complex, what may influence the stability of the whole CMGE complex as well as its interactions with DNA polymerase epsilon or DNA. Using 2 different assays, we investigated the mechanism and measured the instability of repetitive tracts in *psf1-1* mutant strains. The obtained data showed that impaired GINS leads to high instability of mono-, di-, tri-nucleotide, and other repetitive tracts. Our current results enhanced our knowledge about the role of the CMGE in the proper functioning of the replication apparatus and its role in ensuring microsatellite stability. These findings are particularly important in the context of a recent report on severe genetic disorders caused by mutations in human homolog of PSF1 gene. This work was supported by the National Science Centre Grant 2015/17/B/NZ1/00850 "Contribution of DNA polymerase delta in the leading strand replication in *Saccharomyces cerevisiae* cells".

## P-10-004
## Mechanistic insights on the DNA methylation by DNMT3a mutants observed in AML patients

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DNA methylation is an epigenetic modification essential for regulation of cell processes such as chromatin condensation, transcription, gene imprinting and cell differentiation. In mammals, DNA methylation at CpG sites is established by cytosine-C5 DNA methyltransferases (MTases) which form specific DNA methylation patterns. During malignant diseases progression, different multiple changes both in DNA methylation patterns and in MTase genes were observed. Human *de novo* MTase DNMT3A is most frequently mutated in acute myeloid leukemia (AML) with striking prevalence of R882H mutation. R882H has been extensively studied and its potential carcinogenic effect has been suggested. Here, we investigate the role of the other missense mutations in DNMT3A catalytic domain found in AML (S714C, R635W, R736H, R771L, P777R, and F752V) using accordingly mutated murine Dnmt3a catalytic domain and short CpG-containing DNA substrates as model system. The 3-5-fold decrease of initial methylation rates was observed for R181L (R771L), S124C (S714C) and P187R (P777R) with conserved ability to bind DNA. In the case of F152V (F752V), R45W (R635W) and R146H (R736H) a complete loss of the methylation activity was observed accompanied with the loss of DNA binding for R45W and R146H. Strikingly, all the mutations except S124C (S714C) are not located in the DNMT3A catalytic loop. The importance of these amino acids for the proper DNMT3A inner contacts formation was suggested. The ability of the DNMT3A partner protein DNMT3L to restore the methylation activities of S124C (S714C) and R181L (R771L) was revealed. The role of aberrant DNMT3a activity in AML was discussed on the basis of our knowledge of how these mutations affect methylation function and via the computer modelling. This work was supported by the RFBR grant 19-04-00533. *The authors marked with an asterisk equally contributed to the work.

## P-10-005
## Effects of single-nucleotide polymorphisms in human NEIL2 gene on functional role in DNA repair

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To prevent the consequences of DNA damages all living organisms have protective mechanisms named DNA repair. Base excision repair (BER) is necessary for removal of damaged nucleobases from the genome DNA and initiated by specific enzymes DNA glycosylases. Altering sequence of the DNA glycosylase genes can lead to changing enzyme function, accumulation of DNA damages and association with risk of developing cancer. In mammalian cells DNA glycosylase NEIL2 has unique ability to repair damages from "bubble" structures formed during transcription and replication. There are data on single nucleotide polymorphisms (SNPs) of the *NEIL2* gene are associated with risk of developing cancer, particular, one of the most dangerous – lung cancer. However, the corresponding functional protein variants of NEIL2 are not characterized. Using bioinformatics


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approaches we found some SNP *hNEIL2* in available SNP database related to switch amino acid class in protein variants. Two of them hNEIL2(R103W) and hNEIL2(P304T) were located in conservative regions responsible for catalysis and DNA binding respectively. The appropriate recombinant proteins were obtained and their substrate specificity was compared to the reference protein. All proteins demonstrate increasing DNA glycosylase activity observed in the following chain: two-stranded DNA, single-stranded DNA, and DNA containing "bubble" structure, and polymorphic variant hNEIL2(P304T) has reduced AP-lyase and DNA glycosylase activities. Understanding the functions and properties of the polymorphic variants of the protein NEIL2 will provide an actual opportunity for predicting increased risk of cancer in carriers of the corresponding alleles. This study has been supported by RFBR according to the research project No. 18-44-540029 and partially under SB RAS Integrated scientific program, No. II.1/16.

## P-10-006
## New oligomycin A biotargets in *Streptomyces fradiae* (*xinghaiensis*) ATCC 19609 – DNA replication and repair proteins

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Oligomycin A is a macrolide antibiotic that has high activity against pathogenic microorganisms, as well as high antitumor activity. Currently, only one oligomycin A biology target is well-known F0F1 ATP synthase, however, there are data on the presence of several biotargets of the action of oligomycin A. To search for other biotargets we used supersensitive to oligomycin A strain of *Streptomyces fradiae* (*xinghaiensis*) ATCC 19609. Attempts were made to obtain mutant strains of *S. fradiae* resistant to oligomycin A. However, even with a mutation rate of $10^{-11}$, this was not possible. This made it possible to suggest the presence of several oligomycin A biotargets in the cells of this strain. We have carried out the synthesis of a number of oligomycin A derivatives and have chosen those with reduced activity relative to oligomycin A. We managed to obtain oligomycin A resistant mutants in two steps. First, was obtained a strain of *S. fradiae* Olg2R, which is resistant to oligomycin A derivative – (33S)-azido-33-deoxyoligomycin A. From it, we obtained a strain that is resistant directly to oligomycin A. To search for SNP, we performed complete genome sequencing of this strain (QFBD00000000). During bioinformatics analysis of the *S. fradiae* OlgR genome, we found seven single nucleotide polymorphisms (SNPs). First, ATP synthase F0 subunit A (previously known oligomycin A biotarget). Four genes whose products can potentially be included in the processes of DNA replication and repair: helicase, histidine kinase, DNA ligase, RecB, and two hypothetical proteins. Four of them were also found in the previous strain by Sanger sequencing (*S. fradiae* OlgR2). Thus, we detected 3 additional mutations that may be involved in the formation of oligomycin A resistance in the mutant strain S. *fradiae* OlgR Mechanism of formation of oligomycin resistance is the subject of our further research. This work was supported by the Russian Science Foundation (grant number 15-15-00141-П).

## P-10-007
## Interaction of plasmid Rep proteins with ssDNA – structural and functional analysis of nucleoprotein complexes

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Formation of nucleoprotein complexes by replication initiator proteins triggers the cascade of events resulting in synthesis of new DNA molecule. Although the prokaryotic replication initiators differ in their structure, they all bind specific nucleoprotein sequences within the origin region of double-stranded DNA (dsDNA). Next, after the dsDNA melting within a DNA Unwinding Element (DUE) region, they bind single-stranded DNA (ssDNA) of DUE. The data concerning DnaA protein, the bacterial chromosome replication initiator, revealed that it binds to the specific sequence of dsDNA (DnaA-boxes) via DBD domain (DNA binding domain) and ssDNA via AAA+ domain (ATPases Associated with diverse Activities). Rep proteins, the iteron plasmids' replication initiators, instead of DBD and AAA+ domains possess Winged Helix domains (WH). WH domains specifically bind to dsDNA, and due to lack of other domains in Reps, they should also bind to ssDNA. However the structure of Rep-ssDNA complex is unknown as well as its function for replication initiation process. Here, based on the obtained crystallographic data as well as bioinformatic analysis, we show the structure of this nucleoprotein complex and propose the amino acid residues of Rep proteins, responsible for the interaction with ssDNA within DUE region. Using different biochemical approaches we also try to define the role of Rep proteins interactions with ssDNA for plasmid DNA replication.

## P-10-008
## Tick-borne encephalitis virus inhibits production of ribosomal RNA in human cells of neuronal origin

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Ribosomal RNA (rRNA) contributes to the structure of ribosomes and thus is essential for the protein synthesis process. Transcription of human rRNA is carried out by RNA polymerases I and III (POLR1, POLR3). In more detail, POLR1 yields a single transcription unit 45S pre-rRNA, which undergoes a complex maturation process resulting in the generation of 5.8S, 18S, and 28S rRNA molecules. POLR3 is responsible for the transcription of 5S rRNA. Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus* and can cause serious infections in humans which may result in encephalitis/meningoencephalitis. Our transcriptomic data from TBEV-infected neural cell line DAOY suggested a possible link between the infection and rRNA production. We therefore analysed the levels of mature 5S, 18S and 28S rRNAs in DAOY cells infected by TBEV. Surprisingly, only levels of POLR1 transcripts, 18S and 28S rRNAs, were significantly reduced upon TBEV infection.


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Following metabolic labelling experiments using Click chemistry revealed that TBEV decreases the production of nascent RNA, particularly the 45S pre-rRNA precursor. In order to determine whether the rRNA decrease is a result of virus-induced translational shut-off, which was described earlier, we treated cells with cycloheximide (CHX), a translation elongation inhibitor. Subsequent analyses of rRNA levels showed that unlike TBEV, CHX decreased the production of both, POLR1 and POLR3 rRNA transcripts. These data therefore suggests that TBEV may specifically target POLR1 transcription process. This is the first report of flavivirus-dependent decrease of host rRNA levels, which may contribute to the viral pathogenesis. Further experiments are in progress in order to describe the exact mechanism of observed rRNA decay. This study was supported the Grant Agency of the Czech Republic (18-27204S). Access to instruments and other facilities was supported by the Czech research infrastructure for systems biology C4SYS (LM2015055).

## P-10-009
## New fluorescent nucleosides from chemo-enzymatic synthesis

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Tricyclic analogs of the natural purine bases (adenine, guanine, xanthine and hypoxanthine) and their nucleosides show frequently intense fluorescence, what enables their application as fluorescent probes in the investigations of structure and function of nucleic acids (DNA, RNA) and related enzymes. We are working on an enzyme purine-nucleoside phosphorylase, responsible for the regulation of the various nucleosides concentrations within the living cells, it is also utilized in chemo-enzymatic synthesis of various nucleoside analogs of pharmaceutical significance. Our research have shown that PNP isolated from bacteria E. coli, which is known to possess broad specificity toward various base and nucleoside analogs [1], is also active towards a tricyclic adenosine derivative, 1,$N^6$-etheno-adenosine. The reaction of sugar removal was almost equally rapid as an analogous reaction with adenosine or guanosine. In the absence of phosphate ions it is possible to observe the reverse reaction, that is, attachment of the sugar moiety to the tricyclic base, where the second substrate is a phosphorylated sugar (ribose-1-phosphate). This reaction runs similarly rapidly as the phosphorolytic reaction. The aim of the present work was to examine if any other similar tricyclic bases also undergo similar reactions and whether it is possible to obtain in this way highly fluorescent compounds, useful for the future research. In total, 8 bases structurally similar to etheno-adenine, including 3 strongly fluorescent, were investigated. Most of these were found to be fairly good substrates for PNP from bacteria (*E. coli*), and some were also ribosylated by the calf thymus PNP, the latter structurally similar for the human enzyme [1]. The ribosylation sites differed for various forms PNP which were used as bio-catalysts [2]. [1] Bzowska A. et al. (2000) *Pharmacol. Therap.* 88, 349-425 [2] Stachelska-Wierzchowska et al., 2018, *Nucleosides, Nucleotides & Nucleic Acids* 37, 89-101.

## P-10-010
## Various modes of nucleic acid processing by mesophilic bacterial Argonaute proteins

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Argonaute (Ago) proteins are key components of RNA interference in eukaryotes, where they function as site-specific RNA nucleases guided by small noncoding RNAs and participate in post-transcriptional regulation. Many bacteria also encode Ago proteins but their functions and the mechanisms of action in bacterial cells remain unclear. We characterize *in vitro* biochemical properties of Ago proteins from several mesophilic bacteria, which can potentially be used as a tool for genome editing. Some of these proteins can bind small guide DNAs and act as DNA-dependent DNA nucleases, preferably acting on single-stranded DNA targets. We show that the preferred length of the guide DNAs bound by the Ago nucleases is 15–20 nucleotides, and that single-nucleotide mismatches between the guide and target DNA strands can significantly affect the slicing activity, depending on the mismatch position. In particular, mismatches in the 3′-supplementary guide region decrease the cleavage efficiency, while mismatches in the seed region have a mild effect on the target cleavage. 5′-Phosphorylation of guides increases the rate and accuracy of target cleavage. The Ago proteins can catalyze DNA cleavage at the physiological range of temperatures (from < 25 $^0$C to 60 $^0$C), depending on the type and concentration of divalent cations in the reaction. Interestingly, some Agos can also utilize small RNA guides and/or cleave RNA targets. Thus, mesophilic Ago nucleases that have slicer activity at physiological temperatures are perspective candidates for development of new tools for manipulation with nucleic acids. This work was supported by the grant 14.W03.31.0007 of the Ministry of Science and Higher Education of the Russian Federation.

## P-10-011
## hADA3 mutations in acquisition of hrHPV infection

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High-Risk Human Papilloma Virus (hrHPV) is involved in cervical cancer development. E6 and E7 viral proteins exhibit transforming properties through complex mechanisms. HPV16 E6 has been shown to induce p53 degradation and immortalization activity. HPV16 E6-mediated hAda3 degradation represents an alternate way of p53 inhibition. hADA3 protein has been shown to be involved in a number of pathways, but one of the most important roles in cancer pathology are related to its ability to co-activate p53, oestrogen receptor, retinoic X receptor and its involvement in stabilization of p27. In this purpose, this study aims to identify the occurrence of mutations in hADA3 gene sequence and hrHPV acquisition and cervical lesion progression. 42 cases of patients (age median = 35, range: 21–56) with precursors lesions (LSIL and HSIL, n = 15), tumour samples (n = 8), NILM hrHPV positive (n = 9), versus negative NILM (n = 10) were included in the study. DNA was isolated from patients' samples. Further on, a region (exon 3) that includes *in silico*


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prediction potential sites for residues susceptible to PTM (SUMOylation, phosphorylation, acetylation and ubiquitination), has been amplified through PCR using custom primers. The amplicons obtained were separated through gel electrophoresis. Sanger sequencing was performed on purified amplicons. The results were analyzed with BioEdit, Blastn and EMBL (NCBI Reference Sequence: NC_000003.12). The results of the study showed that the patients positive for hrHPV 16 and 18 (HSIL and cervical cancer) present 9789777delC and 9789938 delA, respectively. We also observed two mutations characteristic for HPV66 acquisition (9789763delA and delA9789793). 9789763delA was identified as a frameshift mutation rs988079177. These mutations affect the phosphorylation domain of the hADA3 protein, which in turn affect its capacity to activate p53. Patients presenting the aforementioned mutations may have a greater risk to develop cervical cancer.

## P-10-012
## Catalytically active Argonaute nuclease from *Synechococcus elongatus*

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Argonaute proteins, which are found in almost all eukaryotes and in many prokaryotes, use small nucleic acid guides for the recognition and cleavage of complementary nucleic acids. While the role of eukaryotic Argonautes in RNA interference is well understood, the functions of prokaryotic Argonautes remain largely unknown. It was proposed that they may provide defense against invading nucleic acids, preferably acting on DNA targets. In this work, we studied the SynAgo protein from the cyanobacterium *Synechococcus elongatus*. We expressed affinity-tagged SynAgo in *S. elongatus*, purified the protein, and sequenced and mapped associated nucleic acids. We showed that SynAgo is bound with ~18 nt small DNAs coming from all genomic regions with no obvious gene specificity. Mass-spectrometry of co-purified proteins from *S. elongatus* also revealed several possible protein partners of SynAgo. Biochemical analysis demonstrated that SynAgo is an active nuclease that can cleave both target DNA and RNA with varying efficiency, depending on the reaction conditions and the presence of mismatches between the guide and target strands. Finally, we introduced the SynAgo gene in the *E. coli* genome and tested its effects on plasmid maintenance and phage infections. This work was supported by the grant 18-29-07086 of the Russian Foundation for Basic Research.

## P-10-013
## The non-catalytic components of the CMGE complex and their influence on genome stability

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Faithful duplication of the genome, coordinated with the progression of cycle progression is essential for all organisms. Specialized multiprotein complexes assembled before DNA synthesis start are involved in the process of DNA replication. Numerous studies of mechanisms involved in high fidelity of DNA replication were focused mainly on the role of catalytic elements of the replisome. However, recently it has been shown that the non-catalytic proteins are also important. An essential role is played by the helicase complex CMG composed of Cdc45, Mcm2-7 helicase subunits, and GINS composed of four essential subunits: Psf1, Psf2, Psf3, Sld5. GINS interacts with numerous proteins involved in replication, including DNA polymerase epsilon – Polε (composed of Pol2, Dpb2, Dpb3, and Dpb4 subunits), one of the major DNA replicases. Together, Cdc45- Mcm2-7-GINS, and Polε form the CMGE complex which is assembled at the entry of the S phase. In our laboratory, we are analyzing mutants in the Psf1 subunit of GINS and the Dpb2 non-catalytic subunit of Polε. We identified phenotypes associated with impaired functioning of these essential subunits of CMGE, which affect DNA replication process and genomic stability e. g., impaired progression through the cell cycle and the participation of DNA polymerase delta (Polδ) in DNA synthesis on the leading strand. This work was supported by the National Science Centre Grant No. 2015/17/B/NZ1/00850 "Contribution of DNA polymerase delta in the leading strand replication in *Saccharomyces cerevisiae* cells" and the National Science Centre Grant No. 2017/26/M/NZ3/01044 "CMGE helicase-polymerase complex as a factor integrating the regulation of cell cycle progression and DNA replication".

# RNA transport and translation

## P-11-001
## Studies on transformation of 2-thiouridine to 2-selenouridine catalyzed by *Escherichia coli* tRNA 2-selenouridine/geranyl-2-thiouridine synthase and its analogs

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Transfer RNAs (tRNAs) constitute a special subclass of natural RNAs because of a substantial content of modified nucleosides. The 2-thio- and 2-seleno-modified uridines are located in a wobble position (the first position of the anticodon) of several transfer ribonucleic acids ($tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Gln}$) and play a fundamental role in tuning the decoding of genetic information in protein biosynthesis. Bacterial tRNA 2-selenouridine synthase (SelU, recently renamed as tRNA 2-selenouridine/geranyl-2-thiouridine synthase) is responsible for two post-transcriptional modifications in tRNA chains, leading to the formation of recently discovered S-geranyl-2-thiouridine (geS2U) and 2-selenouridine (Se2U). Using simplified anticodon-stem-loop (ASL) tRNA oligonucleotide fragments (17-mer RNAs) containing 2-thiouridine (S2U) or geS2U units, we documented that SelU transforms in vitro S2U-ASL to Se2U-ASL in a two-step process consisting of geranylation of S2U to geS2U, followed by selenation of the resultant geS2U to Se2U. A direct conversion of S2U-RNA to Se2U-RNA, in the absence of any source of the geranyl moiety, was not observed. Our results suggest that the in vivo S2U→geS2U and S2U→Se2U transformations should follow this scheme, although to date these reactions are claimed to occur independently, yet in the same domain of the SelU protein. Furthermore, we show that a single mutation in 67 position of the wtSelU polypeptide chain (Gly replaced by Glu) increases the otherwise low geranylation activity of the wild type SelU with the concomitant switching off the selenation activity of geS2U. Acknowledgements: This research was financially supported by The National Science Centre in Poland, Grant number [UMO-2014/13/B/ST5/03979] for years 2015-2018 and Grant entitled "Why Nature introduced selenium to wobble nucleosides in transfer RNA?" for years 2019–2022.


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## P-11-002
## RNA-binding proteins as tools for molecule selection with potential diagnostic applications

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The activity of many RNA-binding proteins (RBPs) depends on the identity of the 5′ group on the target transcript. Such RBPs could potentially be used for specific selection of molecules from a mixture of RNAs with different 5′ ends. This principle could be applied to detect RNA of bacterial or viral origin in patient samples since their 5′ ends often differ from mammalian cytosolic RNA. In this study we investigated one of such RBPs and its activity towards RNA molecules with different 5′ moieties. First, the 5′ ends preferentially bound by the protein were determined experimentally by RNA pull-down assay using *in vitro*-transcribed RNA. Next, in order to assess whether the protein could be applied for detection of pathogens, our experiments aimed to identify *Listeria monocytogenes* RNA bound by the protein. Total RNA isolated from Listeria monocytogenes (EGDe), cultured in either optimal or stress-inducing conditions stimulating transcription of virulence-related genes, was subjected to pull-down procedure. We demonstrated successful enrichment of several transcripts of interest using RT-qPCR. Identification of molecules effectively bound by the investigated RBP helped to select RNAs specific for virulent Listeria which could be promising targets for detection of listeriosis. The results obtained in the study enabled to create a basic idea of a novel procedure which could be applied in molecular diagnostics of bacterial diseases. *The authors marked with an asterisk equally contributed to the work.

## P-11-003
## Drosophila CPEB protein Orb2 participates in cell polarization

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Cell polarity is a phenomenon important for development and functioning of any multicellular organism. One of the ways to establish cell polarity is asymmetrical mRNA localization. Some localized mRNAs contain motifs at their 3′ UTRs called cytoplasmic polyadenylation elements (CPEs). Cytoplasmic polyadenylation element binding proteins (CPEBs) are translational regulators that can recognize these elements. Orb2 is one of the CPEB proteins in Drosophila. Previous studies have showed its functions in asymmetric cell division, viability, motor function, learning, and memory in Drosophila. Recent experiments indicated that Orb2 regulates its own expression in at least two different contexts, in the nervous system in the processes of learning and memory formation and in the testis in the spermatogenesis. Mature Drosophila sperm is a highly polarized cells and useful model for studying cell polarization. To study Orb2 functions in more details we have generated novel allele $orb2^R$ using CRISPR/Cas9 approach. $orb2^R$ carries 3′-UTR deletion, thus it is lacking CPE elements what disrupts its auto-regulation. $orb2^R$ has little effect on viability; however, $orb2^R$ males are sterile. Confocal studies of testis suggest that malfunction of spermatogenesis is a consequence of sperm differentiation defects. The defects are due to the failure of spermatid nuclei bundling and condensation at the final stages of sperm maturation. Orb2 could regulate expression of genes involved in assembly of the individualization complex. Preliminary experiments indicated that $orb2^R$ flies has CNS malfunction as well. A plausible interpretation of our current results is that Orb2 negatively regulates its own expression in the CNS while it positively regulates its own expression in testes. Using different approaches, we are testing these hypotheses. This study was supported by the Russian Science Foundation 18-74-10051.

## P-11-004
## Messenger RNA delivery to mitochondrial ribosomes: hints from a bacterial toxin

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During post-transcriptional RNA processing in human mitochondria, large primary transcripts are processed giving rise to mature RNA species. It is remarkable that the mechanisms of messenger RNA handling and loading on mitochondrial ribosomes are still obscure. Furthermore, what happens when large and small mitoribosomal subunits are disassembled? With the aim of dissecting this mitoribosomal pathway, we set out to selectively remove the large ribosomal subunit (mt-LSU) in human cells. Structural analyses revealed a strong similarity between the SRL (Sarcin:Ricin Loop) of *E. coli* 23S rRNA and the mitochondrial 16S rRNA. Based on this prediction, we targeted VapC20, a *Mycobacterium* ribotoxin that specifically cleaves the 23S rRNA within the SRL, to the human mitochondrial matrix. Using a combination of molecular biology techniques and mt-RNA FISH, we showed that mtVapC20 specifically localises to the mitochondrial matrix and cleaves the 16S mt-rRNA, which in turn causes a selective loss of the mt-LSU and subsequent degradation of many but not all mt-mRNA species. By contrast, 12S mt-rRNA and mt-tRNA steady-state levels remained unaltered. Intriguingly, we found that LRPPRC/SLIRP complex, which should facilitate the ordered association of mature mRNAs with the mitoribosome, was maintained in mtVapC20-induced cells even in the absence of mt-mRNA. We suggest that nascent mature mt-mRNAs associate with the mt-SSU prior to recruitment of the mt-LSU to form the translationally active monosome. This thesis prompted us to also deplete the small ribosomal subunit (mt-SSU) in mtVapC20 cells. The concomitant loss of both large and small ribosomal subunits partially recovered mt-CO1 mRNA steady-state level, possibly due to their maintained association with LRPPRC/SLIRP in the absence of the mitoribosome. We propose an intricate pathway of mt-mRNA ribosomal loading and degradation, which involves LRPPRC/SLIRP complex protecting the nascent mt-mRNA and presenting it to the mt-SSU.

## P-11-005
## Regulation of ribosomal protein synthesis in mycobacteria

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Autogenous regulation of ribosomal protein (r-protein) synthesis plays a key role in maintaining the stoichiometry of ribosomal


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components in bacteria. Mechanisms underlying autogenous control have been studied mainly for *E. coli* and its close relatives, and to a lesser extent for *Bacillus* species. In fact, at present very few experimental data exist concerning r-protein regulation in other bacterial phyla. Such information is indispensable for our knowledge of the basic principles of regulation of ribosome biogenesis. The main goal of the present work was to develop techniques allowing examination of the protein synthesis control in mycobacteria, gram-positive organisms with a high G+C content. We used *E. coli* as a surrogate organism to study the in vivo regulation of the *rpsO* gene encoding r-protein S15 from *M. smegmatis* (Msm). We found the mycobacterial *rpsO* promoter was inoperative in *E. coli*. For that reason, we constructed the *rpsO-lacZ* fusions driven by the *E. coli rpsO* promoter, while the *rpsO* 5′-UTR and several first codons were from Msm. These constructs were incorporated in the *E. coli* chromosomal *lac* region to drive *lacZ* expression, and their activity was measured in the presence of the plasmid pS15Msm or an empty vector. The plasmid pS15Msm expressed the *M. smegmatis rpsO* gene under the control of both the promoter and 5′-UTR of *E. coli rpsO*, providing sufficient expression level to measure the autogenous control in the β-galactosidase assay. The results revealed significant (ca 9-fold) decrease in the reporter activities in the presence of pS15Msm as compared to the control vector, indicating that mycobacterial *rpsO* expression is feedback regulated at the translation level like it occurs in *E. coli*. Moreover, *E. coli* S15 in trans was also able to repress expression of the Msm *rpsO-lacZ* reporter, suggesting close resemblance of the autocontrol mechanisms. This work was supported by RFBR grant 18-04-00743.

## P-11-006
## Influenza A virus NS1 protein expression level depends on NS gene RNA secondary structure

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The influenza A virus (IAV) non-structural protein 1 (NS1) is a key antagonist of the cell's interferon system. The NS1 protein is translated from the 8th IAV gene segment, and NS1 expression in infected cells varies between IAV strains. Earlier, it has been shown that NS1 mRNA contains specific secondary structures that differ in shape and stability, especially in pandemic IAVs. At least two NS regions have stable (RNA) secondary structures (both located near 3′ and 5′ splice sites). The first region (82–148 nt) forms multi-branch and stem-loop structures. Transfection of plasmid encoding NS1 sequence with a disrupted 82–148 hairpin resulted in decreased NS1 expression [Ilyinskii et al., 2009]. The second region (497–564 nt) has a tendency to fold into pseudoknot and stem-loop structures; in highly virulent avian H5N1, the equilibrium favors stem-loop over pseudoknot [Gultyaev et al., 2007]. The aim of this research was to investigate whether NS1 secondary structures modulate NS1 expression during infection. Using reverse genetics, we obtained 4 viruses (based on A/PR/8/34; H1N1) featuring different NS mRNA secondary structures (82–148, 497–654). They differed by several single mutations which have predictable effects on structural stability. These mutations occur naturally among IAV strains [Vasin et al., 2016]. For each virus, NS1 expression level was observed during the first cycle of infection (MDCK; moi = 10; ELISA using anti-NS1 mouse polyclonal sera). It was found that NS1 level was about 2x higher at 4–6 hpi for viruses featuring stable secondary structures at the 82–148 positions. Mutations in the second region had no obvious effects; however, the 497–564 hairpin may be involved in other processes apart from NS1 protein regulation. Based on the data obtained, we assume that NS1 expression level depends on mRNA hairpin (82–148) stability. This work was supported by Russian Science Foundation grant 18-74-00130.

## P-11-007
## Undercover translation: hidden secrets of the ribosomes

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Ribosomes are a centre of every living cell. For decades, they were perceived as homogeneous macromolecules carrying a constant set of ribosomal RNAs and proteins. Consequently, they were not considered to actively participate in the regulatory role of gene expression. The hypothesis of specialized ribosomes assumes the existence of a subpopulation of ribosomes carrying unique structural properties allowing fast and precise response to environmental stimuli throughout selectivity for distinct mRNAs. We use sporulation process in Bacillus subtilis bacteria as a model to study regulation of gene expression on translational level. Using combination of ribosome profiling, genetics, biochemistry and microscopy, we aim to identify factors modulating translation and accounting for ribosomal selectivity towards mRNAs. Initial data shows massive global rearrangements in proteins synthesis profile and unveils interesting events, like expression of previously unannotated genes, occurrence of paralogues of ribosomal proteins or rearrangements in the ribosomal structure – implying a presence of distinct sub-sets of ribosomes. This work will shed more light on how translation contributes to the gene expression regulation during sporulation. Finding specialised ribosomes will add a new level of regulation of gene expression with a ribosome as an active element.

## P-11-008
## A challenge of Next Generation Sequencing data analyses

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The Next Generation Sequencing (NGS) technology has revolutionized the biological sciences. With its ultra-high throughput, scalability, and speed, NGS enables researchers to perform a wide variety of applications and study biological systems at a level never before possible. Due to the increasing popularity and lower price we get more and more data to analyze. The most important analysis we are interested in is identification of differentially expressed genes (DEGs) between specific conditions. High-throughput transcriptome sequencing (RNA-Seq) has become the main technique for these studies. Thus, the number of methods and software for differential expression analysis from RNA-Seq data also increased rapidly. There are several different approaches to analyzing such data, each consisting of several steps. Each step of the analysis can be carried out by many different tools. Most of them give slightly different results for the same input. The question is which approach and tools we should use, since there is no consensus about the most appropriate pipeline or protocol. In our research we use data from bacteria and yeasts to find the optimal pipeline for this type of analysis, by comparing the results obtained from different tools.


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## P-11-009
## Quick ribosome profiling in *Bacillus subtilis*

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The ribosome profiling (RIBO-seq) is a powerful method allowing for direct monitoring of the exact position of the ribosome on transcripts – the so-called translatome. Unlike RNA-seq (total mRNA sequencing), RIBO-seq not only provides information about the mRNA composition in the cell at a given time, but also tells us about the rate of translation of each mRNA, number of copies of a synthesized protein, unveils stalling events (regulatory mechanisms), evaluates the character of small-RNAs (coding versus non-coding) or reveals cryptic open reading frames which may lead to discovery of novel proteins and pathways. By employing this method we aim to identify specialized ribosomes in *Bacillus subtilis* – a subpopulation of ribosomes carrying unique structural properties allowing fast and precise response to environmental stimuli. During translation, approximately 28–30 nucleotides of the mRNA are buried within the ribosomal small subunit. Upon nuclease treatment, these nucleotides are protected from degradation. Such nuclease-protected mRNA fragments can be converted into a DNA library and characterized by deep sequencing. For this project, the original protocol published by the Weissman lab has been restructured to render a simple, robust, reliable and reproducible protocol which has been successfully applied in the group to study translation in various bacteria, including *Escherichia coli*, *Bacillus subtilis* and *Streptomyces* spp.

## P-11-010
## The human ribosome as a possible mRNA quality controller that recognizes the abasic site by protein uS3

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The quality of mRNAs in eukaryotes is controlled in several pathways, one of which, no-go decay, is intended for disassembling the stalled ribosomal complexes with subsequent degradation of mRNAs, whose translation prematurely stopped. One of the reasons for ribosome stalling is the presence of damaged nucleotides in mRNAs, in particular, abasic (apurine/apyrimidine, AP) sites. To obtain data on the molecular basis of ribosome stalling on AP site-containing mRNAs, we investigated interactions of human ribosomes with synthetic mRNA analogues bearing the AP site protected by a photocleavable group at the 3′-terminus. It was found that these mRNA analogues can form specific complexes with 80S ribosomes and 40S subunits, where undamaged parts of oligomer sequences are placed at the mRNA binding channel by interaction with the Peptidyl (P) site $tRNA^{Phe}$ cognate to the UUC triplet at their 5′-ends. For all that, the ribosome-unbound downstream parts of mRNA analogues were cross-linked through AP sites to an exposed fragment 55–64 of the uS3 protein of the 40S subunit. These findings could reflect the features of the mechanism ensuring the participation of 40S subunits in the mRNA quality control during translation, thereby highlighting the possible functional assignment of the ability of the above uS3 peptide to react with AP sites in unstructured RNAs. This work was supported by the Russian Foundation for Basic Research (grants 16-04-00241 and 19-04-00098 to D.G.) and partially by the State funded budget project 57.1.2 (0309-2014-0002),and the Russian Ministry of Science and Higher Education within Grant 14.W03.31.0034 (megagrant).

## P-11-011
## Viral tRNA-like structures and pre-miRNA can mediate RNA phloem transport

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The long-distance RNA transport in plants is shown to be essential for plant development and responses to different stresses; however molecular mechanisms of this process are largely unknown. Recently, analyses of *Cucurbita maxima* phloem exudate revealed the presence of various RNA including mRNA, siRNA, miRNA, tRNA and rRNA. In addition, the functional significance of phloem transport for some RNAs such as miR399, miR395, miR827 and miR2111 was experimentally demonstrated. While, in general, signals in mobile RNAs enabling their phloem transport remain unknown, there are experimental data demonstrating significance of tRNA sequences embedded into the 3′- terminal regions of some mRNAs. It is important to note that presence of tRNA sequences in many phloem-mobile mRNA correlates with the presence of tRNAs in the phloem sap. At the same time, tRNA-like structures (TLSs) are present at the 3′-ends of genomic RNAs of certain plant viruses with positive-stranded RNA genomes and potentially may enable their phloem transport. Here, we developed an experimental PVX-based system for visualization of RNA phloem transport based on a modified potato virus X RNA capable of replication, but not encapsidation and movement in plants, and used this system for analysis of the ability of different RNAs to direct RNA phloem transport. We demonstrated that the Brome mosaic virus TLS, as well as the TLSs of Tobacco mosaic virus and Turnip yellow mosaic virus, contain signals of RNA systemic transport. Additionally, we revealed that unprocessed pre-miRNA can direct the phloem transport of heterologous RNA. This work was supported by the Russian Science Foundation (grant No. 17-14-01032)

## P-11-012
## Sorafenib, multi-kinases inhibitor, suppresses protein synthesis and enhances the translation of specifics mRNAs in HCC lines

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Protein synthesis is an essential cellular process and the main regulation step in gene expression. Its dysregulation is frequently found in several types of cancer, leading to excessive cell proliferation and the occurrence of resistance mechanisms against different treatments. Two well-studied pathways that regulate the activity of the translation machinery components are Ras-MAPK and PI3K/Akt/mTOR. Both routes, which are involved in the control of the translation capacity of the cell depending on the nutrient availability and mitogenic signals, are altered in different cancers, including the hepatocellular carcinoma (HCC). Sorafenib is the first line treatment for advanced HCC. It causes pro-


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apoptotic, anti-proliferative and anti-angiogenic effects, but the precise molecular mechanisms by which it exerts these effects remain unclear. The clinical efficacy of the Sorafenib treatment is very low because the occurrence of resistances is a very common fact. Herein, we show that Sorafenib inhibits global translation initiation in HCC cell lines by polysome profiling analysis. Moreover, we observe a reduction in the S6 ribosomal protein phosphorylation level and demonstrate that this inhibition is driven mainly through the phosphorylation of the Initiation Factor 2 (eIF2), although we do not discard the role of other pathways. Finally, we show that while the global translation is blocked, the expression of selective mRNAs is enhanced upon a Sorafenib treatment. Together, our findings indicate that protein synthesis is a target of Sorafenib. Given the essential role of translation in tumoral transformation, the study of this process in this context could lead to the identification of novel therapeutic targets of HCC.

## P-11-013
## The presence of a nested alternative open reading frame in the matryoshka gene: translation its mRNA corresponds to the mechanism underlying the ribosome scanning model

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Recently, we discovered the gene that encodes the Kunitz peptidase inhibitor-like protein (KPILP) in plants of the Solanaceae family (which includes the potato, tomato, and tobacco), and that there is little mRNA in the leaves of intact plants (unlike what is observed in the roots). However, mRNA content was sharply increased after abiotic and biotic stress. Unlike the genes that encoding the Kunitz peptidase inhibitor (KPI) in various animals, *KPILP* has no introns, which thereby excludes the possibility that its expression is regulated via alternative splicing. Here, we investigated the mechanism underlying the regulation of *KPILP* in stress conditions and verified our hypothetical model, which proposes that in an intact leaf, the ribosome "ignores" the start codon of the KPILP mRNA because it is within an unfavourable nucleotide context, and instead it prefers to translate the aORF via the mechanism of internal translation initiation. Under stress conditions, aORF translation is suppressed, and translation using the start codon of the maternal mRNA is resumed, which results in an increase in the level of mRNA content in the leaf. We tested this model on a series of vector constructs that we used to alter the nucleotide context of the start codon of the maternal mRNA, the nucleotide sequence preceding the aORF, the aORF start codon and the mRNA sequence. Analysis of the level of accumulation of mRNA encoding the mutant *KPILP* gene confirmed our proposed model, which states that the synthesis of the aORF product determines the degree of mRNA accumulation in the leaf. If the scanning ribosome "prefers" the start codon of the maternal mRNA (due to a favourable context or stress), then the aORF product will not be synthesized, and, therefore, there will be a high level of maternal mRNA in the leaf. This study was performed with the financial support of the Russian Foundation for Basic Research (project No. 17-29-08012).

## P-11-015
## Whole genome sequencing of sup45 and sup35 nonsense mutants of yeast *Saccharomyces cerevisiae*

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In the yeast *S. cerevisiae* release factors eRF1 and eRF3 are encoded by essential genes *SUP45* and *SUP35* respectively. Previously we showed that nonsense mutations in these genes (*sup45-n* and *sup35-n*) support viability in different genetic backgrounds. It was shown that the viability of strains with these mutations is increased after growth in the absence of wild-type allele. Possibly, additional mutations are selected during the first stage of selection. Thus we aimed to investigate the precise mechanism leading to readthrough of *sup45-n* and *sup35-n* mutations. To achieve this goal we have constructed a complete reference genome assembly of the strain U-1A-D1628 bearing a deletion of the *SUP45* gene and a plasmid pRS316-SUP45 which is an ancestor to the strains harboring *sup45-n* mutations obtained using the plasmid shuffling technique. To create reference genome assembly of this strain we conducted whole-genome sequencing of this strain using the Oxford Nanopore and Illumina technologies. Simultaneously we have constructed the strain U-14-D1690 having a deletion of the *SUP35* gene and a plasmid pRS316-SUP35 which is a close relative of the strain U-1A-D1628. Also now we obtained whole-genome sequencing data of the several dozen *sup45-n* and *sup35-n* mutants. Preliminary analysis of these data showed that no large-scale genomic changes occur upon substitution of the wild-type copy of the genes to its mutant version. The obtained reference genome assembly of the U-1A-D1628 and U-14-D1690 strains, as well as the whole genome sequences of corresponding nonsense mutant strains would allow us to identify the genetic changes that occur during such substitution of wild-type *SUP45*/*SUP35* alleles to the mutant ones. The authors acknowledge the RC "Biobank" of St Petersburg State University. The work was supported by the program 0112-2016-0015 (characterization of mutants) and the grant of the RSF (18-14-00050) (other parts of the work).

## P-11-016
## Aim23p interacts with the small subunit of yeast mitochondrial ribosome

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Aim23p is a yeast protein found in mitochondria. For many years, its function in the organelles remained unknown. In 2012, using a combination of bioinformatics and genetics, we have shown that Aim23p is initiation factor 3 of mitochondrial translation. However, its action in the protein biosynthesis system is somewhat non-canonical: translations does not stop in absence of Aim23p, it is just misbalanced from the point of view of


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individual proteins amount. These results forced us to think about obtaining some solid biochemical evidence of Aim23p participation in mitochondrial translation. This work presents such evidence by using two different methodological approaches. In vivo experiments with co-immunoprecipitation have shown that all Aim23p protein partners in yeast mitochondria that could be identified are the components of small mitoribosomal subunit. In vitro density gradient sedimentation assays have revealed endogenous Aim23p exclusively in small mitoribosomal subunits fraction. Interestingly, recombinant Aim23p may bind both to small and large subunits (with clear preference to the small one) and seems to be able to promote dissociation of whole mitoribosomes into subunits. Taken together, our results clearly demonstrate the interaction of Aim23p with small subunit of yeast mitochondrial ribosomes and provide further evidence of Aim23p being bona fide initiation factor 3 in the organelles.

## Single cell analysis and imaging

### P-12-001
### Separate and combined mutagenic effect of radiation and stress in the micro-nuclear test in the experiment

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The micro-nuclear test is the most well-studied, reliable method for assessing the genotoxicity of environmental factors. It is highly sensitive in studies of most mutagens, carcinogens. The aim of this experimental work was to study cytogenetic disorders of bone marrow cells caused by separate and combined effects of high doses of gamma radiation and immobilization stress. To achieve these goals were carried out 4 series of experiments on laboratory rats: intact (gr.I), irradiated (gr.II), who have undergone immobilization stress (gr.III) and those exposed to combined radiation and stress (gr.IV). Animals of group II, IV were irradiated once on the radiotherapy unit Teragam $^{60}$Co at a dose of 6 Gy. Mutagenic activity of these factors was studied in a micro-nuclear test. During the micronucleus test, 1000 polychromatophilic erythrocytes were studied in bone marrow cells of each animal. The number of these types of cells with micronuclei was taken into account. Analysis of the frequency of cells with micronuclei under irradiation at a dose of 6 Gy, the number of micronucleated polychromatophilic erythrocytes in the bone marrow was 13.1 times higher, with immobilization stress – 3.6 times higher than in the control group ($P < 0.05$). And with the combined effect of these factors, the number of micronucleated polychromatophilic erythrocytes was 15.2 times higher compared to the control ($P < 0.05$). Genotoxicity of immobilization stress may have occurred with the help of oxidative stress in this pathology. If we compare the indicators of group II, III and IV with each other, the formation of micro-nuclear cells is greater under the combined effect of immobilization stress with a high dose of ionizing radiation. Thus, in the bone marrow of experimental rats, marked increases in the genotoxic effect of the combined effect – potentiation of the effects of radiation and immobilization stress-were observed. *The authors marked with an asterisk equally contributed to the work.

### P-12-002
### Apoptosis markers of spermatozoids and polyamines of human spermoplasm

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The aim of the study was to compare the content of spermidine (Spd) and spermine (Spm) in the seminal plasma of fertile and infertile men and to identify the relationship between the level of polyamines (PA) in semen and the presence of markers of apoptosis in gametes. It was revealed that in infertile men in ejaculate 2,5 times more spermatozoa with externalization of PS and lower PA content as compared with fertile donors ($P$<0.001): the concentration of Spm is reduced almost 2 times, and the concentration of Spd is almost 7 times. However, in infertile men, a change in the ratio of Sp/Spd in the seminal fluid was detected, with a predominance of Spm over Spd 4-fold, which is the initiating factor for triggering apoptosis in gametes. This was proved by the relationship between the externalization of phosphatidylserine (PS) in gametes and the concentration of Spm in the spermoplasm in accordance with the coefficient of positive correlation ($r = 0.5$, $P < 0.01$). Thus, impaired fertility in men can be associated with a disruption of the biochemical homeostasis of the seminal plasma, and in particular, with changes in the level of PA, which are not only a necessary factor for the fertilization of spermatozoa but also a mechanism for monitoring and regulating the viability of gametes. The change in the concentration of PA in the seminal plasma of men is one of the factors controlling the apoptosis of the sex cells, which makes PA not simply an object of study, but a possible tool for application in andrology. Determination of the content of Sp and Spd in seminal plasma can be used to increase the informative value of studies of the causes of impaired fertility of the ejaculate. The study was prepared with the support of the "RUDN University Program 5-100" and "IUST University Syria".

### P-12-003
### Fluorescent β-ketoenole dye as a stain to reveal bacterial biofilm-specific amyloids

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Amyloid fibrils are high-stable protein aggregates that are associated with the pathogenesis of some human diseases. Bacteria also form cell surface-associated amyloid fibrils that increase cell adhesion and involve in biofilm formation. Such functional amyloids may enhance bacterial virulence, and pathogens spread. Since fluorescence assay is an effective tool for detection of fibrillar aggregates, there is an interest in the development of new amyloid-specific dyes for their visualization. β-Ketoenole dyes were recently shown as dyes able to increase the emission intensity in the presence of insulin and lysozyme fibrils with quantum yield up to 0.5. Here we report alkylamino β-ketoenole as a stain for imaging of functional amyloid-contained bacterial cells in biofilms in situ using a confocal laser scanning microscopy


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approach. It was shown that β-ketoenole visualized biofilms of amyloid-produced bacterial strains E. coli K12, P. fluorescence SBW25, S. aureus ATCC25923. Due to the dye specificity to fibrils, high-resolution visualization of bacterial cells and filamentous components of the matrix was obtained. The experiments on co-staining of β-ketoenole with an EtBr allowed suggesting that the dye did not bind to nuclear and extracellular DNA such as another amyloid-specific widely used stain Thioflavin T did. There was no major overlapping of ketoenole and Calcofluor-white staining in cellulose-containing biofilms which suggests no high cellulose-specific binding of the new stain. Slightly sensitivity to cellulose sites may suggest either low cellulose-specificity or amyloid-cellulose aggregations visualized by the stain. Intercellular distribution of ketoenole was also evaluated using human mesenchymal stem cells – it was shown that dye slightly stains vacuole-like organelles in the cytoplasm, and did not penetrate the nuclei. Thereby, such specificity allows to proposed β-ketoenole as a stain for visualization of amyloids in bacterial biofilms.

## P-12-004
## Ultra-soft X-ray system for imaging the early cellular responses to X-ray induced DNA damage

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The majority of the proteins involved in processing of DNA double-strand breaks (DSBs) accumulate at the damage sites. Real-time imaging and analysis of these processes, triggered by the so-called microirradiation using UV lasers or heavy particle beams, yielded valuable insights into the underlying DSB repair mechanisms. To study the temporal organization of DSB repair responses triggered by a more clinically-relevant DNA damaging agent, we developed a system coined X-ray multi-microbeam microscope (XM3), capable of simultaneous high dose-rate (micro)irradiation of large numbers of cells with ultra-soft X-rays and imaging of the ensuing cellular responses. Using this setup, we analyzed the changes in real-time kinetics of MRE11, MDC1, RNF8, RNF168 and 53BP1 – proteins involved in the signaling axis of mammalian DSB repair – in response to X-ray and UV laser-induced DNA damage, in non-cancerous and cancer cells and in the presence or absence of a photosensitizer. Our results reveal, for the first time, the kinetics of DSB signaling triggered by X-ray microirradiation and establish XM3 as a powerful platform for real-time analysis of cellular DSB repair responses.

## P-12-005
## Photocleavable oligonucleotides as convenient approach to increase efficiency of single-cell cDNA library preparation

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Photocleavable Linker (PC-linker) was incorporated in oligonucleotide chain synthesized in 5′->3′ direction on a solid surface. PC-linker was treated with mild UV for complete release of oligonucleotides. The tagging of beads with unique molecular barcode makes the whole system applicable to single-sell transcripts analysis. Considering the high demand of effective solid-phase oligonucleotide synthesis, especially in biomedical research with downstream sequencing applications, we developed a new approach increasing the efficiency of RT-PCR and offering the opportunity to analyze single-cell transcripts. The novelty of suggested approach defined by combining of 5′->3′ solid-phase organic synthesis, molecular barcoding and UV-mediated oligonucleotides cleavage for efficient cDNA library preparation in solution. We synthesized barcoded oligonucleotides on a polystyrene bead using a split-and-pool method. The applicability of the method was confirmed by sequencing of barcodes showing almost unbiased combinatorial incorporation of nucleotides. The obtained beads, being isolated with single cells in droplets of water-in-oil emulsion, serve as a vehicle for delivery of oligonucleotides assigning transcripts with unique barcode. Synthesis of DNA chain and incorporation of a PC-linker phosphoramidite were carried out on a DNA/RNA synthesizer using reverse 5′-phosphoramidites with an increased condensation time. The 'reverse' chemistry allows priming of cDNA synthesis due to formation of free 3′ OH-bound. The beads were used in reverse transcription reaction with RNA extracted from PBMCs. The beads were treated for 5 min with mild DNA-safe UV (365 nm) providing complete photolysis. The yield of RT-PCR product in reaction with PC-linker was much higher than in reaction on solid support: confirmed by agarose electrophoresis. The obtained barcoded cDNA libraries are suitable for further single cell transcriptome analysis.

## P-12-006
## Analysis of blood endothelial cells from lymphoid tissues at the single cell level

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Blood endothelial cells (BEC) are the cells which form the interior surface of the blood vessels. Their main function is the exchange of oxygen and metabolites between the blood and the tissues. They also regulate the cell trafficking thus playing a role in the immune response, as well as and tumor growth and metastasis. Multiple phenotypes of BEC found in various tissue types and organs allow for performing their specialized functions. For example lymphocyte homing into lymphoid or chronically inflamed tissues is controlled by high endothelial cells (HEC). It is well described, that during the immune challenge, the lymph node vasculature, including HEC and capillary EC (CapEC), expands. However, the origin of the new BEC in the inflamed lymph node is not well understood. We used single cell RNA sequencing (scRNA-seq) to define and characterize different BEC subsets in the mouse and human lymphoid tissues. This includes a previously unknown Capillary-Resident stem cell-like Progenitor (CRP) and "transitional" endothelial cells (TrEC) that express both capillary and high endothelial cell markers. We then applied trajectory analyses to find the developmental relationships between the defined subsets. We confirmed the main findings of the transcriptomic analyses by immunofluorescent imaging and mass cytometry (CyTOF). In summary, single cell


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analysis is a powerful method to identify the BEC subsets and their developmental pathways. Understanding those pathways may allow to identify the potential therapeutic targets for the treatment of inflammatory and neoplastic diseases. *The authors marked with an asterisk equally contributed to the work.

## P-12-007

## Multi-layer processing of cellular microscopic images based on percolation theory principles

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We present a universal approach to the multi-layer analysis of cellular microscopic imaging data. For 2D images multi-threshold analysis leads to a series of binary layers with different intensity levels. 3D structures are reconstructed from a series of 2D images obtained by the confocal laser scanning microscopy (CLSM) with the true third physical coordinate. Image segmentation and consequent object selection procedures are based on the percolation concept and follow two main strategies. The first one is to find a single-layer representation of each object that appears a posteriori the best according to certain objective criteria, e.g. shape parameter $P_s=P^2/4\pi S$ where P and S are object perimeter and area respectively. While for simple circular shape typical for cellular images $P_s=1$, it increases drastically when the object edge is fractured due to the noise influence. Thus the best selection of simple regular objects is achieved at the best threshold value $T_{opt}$ that in turn corresponds to the minimum $P_s$ value. The second strategy is to characterize the entire 3D object by its percolation coefficient $P_c=S(T_c)/S(0)$ denoting the fraction of pixels $P_c$ that should appear below the threshold $T_c$ to achieve the decomposition of the object with the base area S(0). Since $P_c$ largely depends on the object morphology, it allows to discriminate between regular smooth-edged and fractal-shaped sharp-edged objects. The proposed framework can be successfully utilized for segmentation, selection and quantitative assessment of cellular microscopic images as well as for the reconstruction and quantification of 3D structures obtained by multi-layer imaging techniques like CLSM. Moreover, the proposed 3D representation provides a flexible framework for the structural visualization of complex objects leading to a better understanding of biological structures and medical images. We thank the Ministry of Science and Higher Education for financial support (2.5475.2017/6.7).

## P-12-008

## Single-molecule Slimfield microscopy for real-time dissection of protein-protein interactions, stoichiometry and mobility directly in live eukaryotic cells

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Cells respond to changes in their microenvironmental conditions by controlling the expression of genes that are essential for survival. Gene regulation is tuned by transcription factors, which bind to target DNA sequences. In the glucose repression pathway of the yeast *Saccharomyces cerevisiae*, the transcription factor Mig1 regulates expression of genes essential for metabolism of alternative carbon sources. However, the dynamic behaviour of Mig1, the number of Mig1 molecules used in regulating a target gene and their mobility rates as well as kinetics of interactions with partner proteins remain to be elucidated. We combined traditional molecular biology approaches with the Slimfield microscopy, a powerful new technology which enables detection of biomolecules directly in living cells with millisecond sampling, also allowing simultaneous visualisation of multiple fluorescent dyes. We also applied a bespoke microfluidics system to follow Mig1 behaviour upon extracellular perturbations. We utilised image deconvolution to count the total protein copy number in separate sub-cellular compartments, one cell at a time. Our data show that Mig1 is present as monomers and oligomers in the cytoplasm and nucleus, constantly shuttling between these compartments regardless of glucose availability. Computational analysis of the yeast genome revealed > 100 potential Mig1 target promoter sequences. Mapping these sequences onto a 3D yeast chromosomes structure model allowed us to model the 3D arrangement of Mig1 in the nucleus and was consistent with Mig1 clusters, with CD spectroscopy suggesting that both molecular crowding and the phase separation of intrinsically disordered sequences may play an important role in stabilising clusters. Similar results were obtained for another transcription factor showing identical oligomeric organisation. Thus, we suggest that yeast gene regulation using transcription factor cluster may be a generic feature.

## P-12-009

## Optical coherence microscopy as a novel, non-invasive method for the 4D live imaging of early mammalian embryos

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Imaging technology based on traditional fluorescence and confocal laser scanning microscopy has significantly increased our knowledge of cell physiology and intracellular architecture. However, until now, we typically were not able to visualize most of the intracellular structures non-invasively, without fluorescent dyes or tags and sample pre-processing. Optical coherence microscopy (OCM) is a promising alternative that circumvents the technical limitations of fluorescence imaging techniques and provides unique access to fundamental aspects of cellular physiology without the requirement for sample pre-processing or labelling. In our research we utilized cells that are especially susceptible to photodamage and therefore difficult to image, i.e. mammalian oocytes and early embryos. We utilized the internal motion of cytoplasm, as well as custom scanning and signal processing protocols, to effectively reduce the speckle noise typical for standard OCM and enable high-resolution time-lapse imaging. We visualised intracellular structure (nuclei with chromatin conformation and nucleoli, spindle structure, networks of endoplasmic reticulum and mitochondria) of mouse and pig oocytes and embryos and through fertilization and the first embryonic division (4D imaging), as well as at selected stages of oogenesis and pre- and post-implantation development (3D imaging). Because all morphological and morphokinetic properties recorded by OCM are believed to be biomarkers of oocyte/embryo quality, OCM may represent not only a novel tool for basic research, but also a new chapter in imaging-based preimplantation embryo diagnostics. Currently, we test the possibility of applying OCM in oocyte/embryo quality assessment. The research has been founded by the


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Applied Research Programme grant (PBS1/A9/20/2013) from the National Centre for Research and Development (Poland) and OPUS grant (UMO-2017/27/B/NZ5/00405) from the National Science Centre (Poland).

## P-12-010
## Bioluminescence system of polychaete *Odontosyllis*: purification of luciferin and its enzymatic and non-enzymatic oxidation products

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The development of bioluminescence-based molecular instruments emerged as one of the fastest-growing fields of interest in biomedical research. Various *in vitro* and *in vivo* analytical methods based on luciferin-luciferase reactions have found applications in analysis of metabolites, investigation of protein-protein interactions, drug discovery and real-time bioimaging of live systems. High sensitivity of bioluminescent techniques in comparison with other known imaging technologies makes it more and more popular each year. *Odontosyllis undecimdonta* is a species of marine syllid polychaete that displays a spectacular bioluminescence, correlated with the lunar cycle. Previous investigations were conducted in the middle of 20th century by Harvey, Shimomura and Trainor, who have established that the *Odontosyllis* bioluminescence reaction requires only oxygen, a luciferin (substrate) and a luciferase (enzyme) to emit greenish-blue light. During the enzymatic luminescent reaction luciferin is converted into an oxyluciferin (light emitter) – a compound with fluorescence highly similar to *Odontosyllis* bioluminescence. Non-enzymatic auto-oxidation on air produces a pink-colored substance. The susceptibility of the luciferin to decomposition presented a serious challenge to investigations of the *Odontosyllis* bioluminescence system due to the difficulty of obtaining the pure substrate. We have designed the purification procedure – a consecutive series of chromatographic experiments which allowed the isolation of luciferin, oxyluciferin and luciferin non-enzymatic auto-oxidation product from lyophilized worm biomass. Together with recently discovered *Odontosyllis* luciferase our studies might be a crucial step in the development of orthogonal luminescence-based analytical methods for a variety of applications including bioimaging and pharmaceutical development. This work was supported by the Russian Foundation for Basic Research (RFBR), grant 17-03-00954.

## P-12-011
## Imaging and analysis of human blood plasma components in breast cancer patients by fluorescence spectroscopy

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Breast cancer is one of the most common cancers, affecting more than 20% of women annually worldwide in recent years. The florescence spectroscopy has become one of the most prospective cancer diagnostic methods because of its high sensitivity to alterations in the function, morphology, and microenvironment in cells and tissues. This method is very helpful in defining changes of concentration and intensity of fluorescent substances in biological material and consequently detects malignancy in clinically early stages. Biological fluids contain characteristic set of naturally fluorescent compounds. Comparison of fluorescent profiles in healthy and diseased humans determines characteristic signs usable for screening or diagnostics particular disease. Evaluation of setted cancer markers in fluorescent matrix could quality biological material at first glance even without specific identification of particular components. The main aim of this work was the fluorescence analysis of human blood plasma in breast cancer patients. The study groups considered of healthy volunteers women (n = 33), and patients at different stages of breast cancer (n = 29). Excitation-emission matrices were scanned using a Horiba Dual-FL Fluorometer and constant wavelength matrices were monitored using a Perkin Elmer Fluorescence Spectrophotometer LS55 connected to a PC with FLWinlab software. In the experimental group of patients with breast cancer, was observed a tendency to increase the total fluorescence compared to the control group. After mathematical processing of synchronous spectra was determined that the ratio of the peaks 330/350 nm were significantly reduced in patients with breast cancer compared to healthy persons. It may be due to higher NADH concentration in plasma of cancer patients. These results suggest that fluorescence spectroscopy could detect malignancy even in clinically silent stage. This project was supported by VEGA 1/0873/16.

## P-12-013
## Drug-induced alterations in cancer cell compartments studied by local stiffness measurement via Scanning Ion-Conductance Microscopy

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Scanning Ion-conductance Microscopy (SICM) is a novel method of scanning probe microscopy with nanoscale resolution, both in the lateral and vertical directions, which is allows to provide in vitro studying of very soft, native features of cells in physiologically closed conditions. Non-contact local measurement of stiffness is SICM-application, that based on elastic deformation of cell surface due to intrinsic colloidal pressure between cell membrane and nanopipette tip. This allows to simultaneously mapping topography and stiffness of non-fixed cells at nanoscale resolution. Here, we report study of drug-induced alterations in cancer cell compartments studied by local stiffness measurement, specifically, we measured human prostate cancer cell line PC3 subjected with Paclitaxel for microtubulin stabilization and Cytochalasin-D for actin depolymerization. Also, we measured fibrosarcoma cells (HT1080) transfected with Progerin, which is integrate in protein structure of nucleus membrane. Progerin was modified with GFP fluorescence dye (GFP-Progerin). In control and treated PC3 cells we measured stiffness upon the nucleus area and cytoplasm area, which are show two different values in control cells (~1.3 kPa and ~0.8 kPa, respectively). Measured


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stiffness after Paclitaxel treatment shows significantly increased stiffness value on nucleus area and cytoplasm area (~4 kPa and ~1.8 kPa), whereas Cytochalasin-D treatment reduced cell stiffness only on cytoplasm area (~0.5 kPa). Experiments with GFP-Progerin were provided in heterogeneous population of HT1080 with control and GFP-Progerin transfected cells. Control stiffness measurement shows ~1.7 kPa and ~0.7 kPa, when GFP-Progerin treated cells increased value only on nucleus area (~2 kPa). The research was carried out at the expense of the Russian Science Foundation grant (SICM supported by project No. 19-79- 30062 and biological experiments supported by project No. 17-15-01290). *The authors marked with an asterisk equally contributed to the work.

## P-12-014
## Scanning Ion Conductance Microscope as a new tool for bionanotechnology

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The ability to precisely move the nanopipette and to measure simultaneously an ion current allows an unprecedented level of nanoscale imaging of living cells – scanning ion conductance microscopy (SICM). Scanning ion conductance microscopy (SICM) – a type of non-contact scanning microscopy technique creating topographical image of sample surface by means of glass nanopipette filled with electrolyte scanning over the sample. We have introduced a novel imaging mode of SICM, referred to as the "hopping mode", which for the first time resolved topography of convoluted biological structures such as stereocilia bundles of inner ear hair cells or multi-layered neuronal networks in their full complexity at nanoscale resolution. When it comes to imaging topography of soft cells with complex morphology under physiological conditions, the capabilities of hopping mode SICM remain largely unmatched to this day. The speed of data acquisition positions this as a technology which may be suited to relatively high-speed scanning of cell membrane during various biological processes in real time. SICM can be used in combination with other techniques such as confocal and fluorescence microscopy, 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 developed semi-automated technique for spatially resolved recording of single channel and whole-terminal activity in small synaptic terminals in hippocampal neuronal culture. The technology was used to investigate the effect of subthreshold somatic potentials on the broadening of action potential recorded at the small synaptic bouton of the same neuron and also for mapping of ligand gated receptors in and sensory neurons. Acknowledgement: The research was carried out at the expense of the Russian Science Foundation grant (project No. 19-79-30062).

## P-12-015
## Novel electrochemical nanoprobes for single cell analysis

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Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells. Additionally, nanopipette probes still hold great promises as intracellular biosensors. Here we describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5–200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of melanoma cell. We applied the nanoelectrode to perform intracellular measurement of ROS and local measurements of gradient of pH in cultured melanoma cells, HEK293 and LNCap cancer cell. Upon penetration of the cells the anodic current quickly increases followed by equilibration to a level above the one measured in the cell media. A cell can withstand multiple penetrations and we measured a substantial difference between the electrochemical signal measured inside and outside the cell. We believe these results show the potential of functional nanoelectrode to probe endogenous species into cells and with further improvements they may allow the study of oxidative stress, pH and ATP under influence of different drugs and nanoparticles. Acknowledgments: This work was supported by funds of «NUST MISiS» grant K4-2017-048 (nanoparticle synthesis and characterization, ROS test). The research was carried out at the expense of the Russian Science Foundation grant (project No. 19-79- 30062) (nanoelectrode development, intracellular electrochemical measurements).

## P-12-016
## Novel rapid method for evaluating of anticancer drugs effect on tumor cells

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Reactive oxygen species (ROS) is associated with induction of apoptosis. The study of intracellular ROS levels may represent one possibility to research the effects of drugs in inflammatory


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cells. ROS are released from cells during apoptosis, play a crucial role in the development of cancer and neurodegenerative diseases. Nowadays, there is the problem of developing methods for treating cancer tumors, and quickly evaluation of the anticancer drugs efficiency is the priority. The ROS determination using nanosensors in single cells has gained increasing attention. However, traditional fluorescent dyes have a number of disadvantages. These dyes are known to be intrinsically cytotoxic and thus can significantly alter cellular metabolism. Here, we have developed an electrochemical method for determining the ROS inside the cells. Using this method, it is possible to evaluate the effect of the developed drugs on the cells. We evaluated the effect of PSMA (Prostate-Specific Membrane Antigen) – specific carrier equipped by Doxorubicin, monomethyl auristatin E or Abiraterone on cell lines with (22RV1) and without (PC-3) PSMA receptors. Our data obtained by using carbon-filled quartz nanopipettes with platinum tips showed a ROS increase using conjugates compared with native drugs. We also studied the effect of drugs based on copper complexes on the cell line MCF-7 and demonstrated that complexes with copper in various degrees of oxidation, depending on the ligands, can have different effects on tumor cells. Complexes with copper in various degrees of oxidation, depending on the ligands, can have different effects on tumor cells. In the future, this method may allow evaluating the effect of drugs in vitro and to help validate drug candidates for preclinical evaluation. The research was carried out at the expense of the Russian Science Foundation grant (project No. 19-79-30062). *The authors marked with an asterisk equally contributed to the work.

## P-12-017
## Immunohistochemistry of luciferase of fungus *Neonothopanus nambi*

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Luciferase is one of the main components in bioluminescent reaction. This protein promotes the transformation of luciferin into electronically excited oxyluciferin, which in turn releases a quantum of visible light upon relaxation to the ground state. Bioluminescence-based tools are widely used in both *in vitro* and *in vivo* applications, such as bioimaging, biosensors and various others. Both major components (luciferin and luciferase) of the first eukaryotic genetically encodable bioluminescent system from fungi were recently discovered in our laboratory. The aim of the present work was immunohistochemical investigation of *Neonothopanus nambi* luciferase (nnLuz) intracellular localization in heterologous system of mammalian cells. For this purpose, we have generated immunosorbent using purified nnLuz and were able to purify specific anti-nnLuz antibodies from the rabbit polyclonal antiserum. We cotransfected mammalian U2OS cells with vectors encoding fungal luciferase and vectors encoding fluorescent proteins with various subcellular localizations. Luciferase within all obtained genetic constructions was functionally active. We have performed immunohistochemical imaging of luciferase using monospecific anti-luciferase antibodies and native imaging of fluorescent proteins in the same experiment. We can conclude that intracellular localization of *Neonothopanus nambi* luciferase in heterologous system of mammalian cells is endoplasmic reticulum. These results may be useful for the future development of bioimaging techniques based on *N. nambi* luciferase. This work was supported by Russian Science Foundation grant 17-14-01169.

## P-12-018
## Investigation of sperm DNA damage and semen oxidative stress status in IVF failure and recurrent unexplained miscarriage

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Conventional semen parameters have not been sufficient to determine the unknown causes and risk factors for poor reproductive history. However, the increase of reactive oxygen species (ROS) causes oxidative stress (OS) damage in sperm DNA. So, the OS environment caused by ROS products may give rise to detrimental effects on sperm function, fertilization, and implantation. We aimed to investigate the contribution of sperm DNA damage and total oxidative status (TOS) to IVF failure and recurrent unexplained miscarriage. The sperm DNA damage and TOS in ejaculate were tested with comet assay and spectrophotometric analysis, respectively. Male partners of couples with a history of unexplained recurrent miscarriages and/or recurrent assisted reproductive technology (ART) failure as a study group (n = 84) and healthy fertile men as a control group (n = 22) were recruited in the study. Total comet score of sperm DNA damage analysis in control and patient groups showed 74.76 ± 22.08 AU and 171.44 ± 46.12 AU ($P$<0.001) respectively. Analysis of TOS was found 2.14 ± 0.98 μmol H2O2 Equiv./L and 4.46 ± 1.84 μmol H2O2 Equiv./L ($P$<0.05) respectively. Our data indicated that both sperm DNA damage and TOS were increased in IVF failure and recurrent miscarriage data compared to the control group. In addition, there was a positive correlation between sperm DNA damage and TOS ($r$ = 0.643; $P$<0.001). According to our results, there was a statistically significant relationship between the OS status of sperm, and implantation failure and repeated pregnancy loss. In conclusion, knowing the rate of DNA-damaged spermatozoa and TOS level in the ejaculate is important in predicting fertilization and implantation chance, and in determining the risks that the embryo may be exposed to. Keywords: Sperm DNA damage; oxidative stress; IVF failure; recurrent miscarriage.

## P-12-019
## Expression of pluripotency markers in multipotent mesenchymal stem cells derived from Wharton's jelly of the umbilical cord

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Mesenchymal stem cells (MSCs) as self-renewing, multipotent cells with great potential to differentiate are a very promising tool in regenerative medicine. *In vivo* studies confirm that MSCs exhibit chemotactic properties and after intravenous injections they migrate towards inflammatory sites. Wharton's jelly a gelatinous tissue within the umbilical cord emerged to be valuable source of MSCs. Considering the fact that MSCs isolated from perinatal tissues reveal higher potential than adult MSCs, we examined mesenchymal stem cells derived from Wharton's jelly of umbilical cord in terms of their pluripotency properties. The WJ-MSCs are commonly classified as multipotent, but they express a relatively high level of pluripotent markers characteristic for pluripotent cells. We have compared potency of WJ-MSCs


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cultured in normoxia and hypoxia conditions with reference to iPS as cells of well-defined pluripotent properties. WJ-MSCs as a perinatal stem cells exhibit high plasticity and proliferation capacity. Our studies showed that WJ-MSCs express pluripotency markers, NANOG, OCT-4, SSEA-4, but in comparison to iPS cells expression level is significantly lower. We have noticed that the expression of these genes, especially NANOG increase under hypoxic conditions that in part reflect a natural microenvironment occurring within the body. These results show that WJ-MSCs derived from fetal tissue (umbilical cord) exhibit not only mesenchymal stem cells properties, but also some features characteristic for embryonic stem cells (ESCs). The ease of isolation and expansion *in vitro*, low immunogenicity, lack of tumorigenic properties and ethical issues, give WJ-MSCs advantages in allogenic cell-based therapies. Acknowledgements This work was supported by research grant (STRATEGMED2/265761/10/NCBR/2015) from the National Center for Research and Development. *The authors marked with an asterisk equally contributed to the work.

## P-12-020
## Fluorescent probes for monitoring the relationship between metal ions and redox balance in cancer

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Small molecule responsive probes that change their fluorescence in response to the biochemical environment are key to understanding cellular chemistry. While most current efforts aim at identification of single analytes, our work focuses on monitoring several parameters simultaneously in order to better grasp the complexity of intracellular environment. Here, we report our efforts in creating multiparametric imaging tools to investigate the dependency between iron(II), hypoxia and oxidative stress in cellular models of prostate and lung cancer, as it has been implicated in cancer development and progression. Evidence for this relationship is characterised by contradictory conclusions, therefore we aim to simultaneously use several complementary probes for iron(II) and redox balance and adapt them for application in high throughput screening assays, to enable rapid multiparametric screening for biologically active compounds. We are also developing dual analyte sensors which contain two responsive motifs, enabling them to report simultaneously on pairs of analytes. These probes are highly reliable as, unlike pairs of single analyte sensors, they do not suffer from differential localisation and variable metabolism, and despite their great potential, they have been unexplored in biological research. Our molecular designs are based on derivatives of fluorophores with two points of attachment (e.g. blue coumarins, green naphthalimides, orange hemicyanines and red rhodamines), decorated with responsive groups either at different positions, sequentially, or via another fluorophore with complementary responsive moiety for a different analyte. By harnessing this wide palette of designs we hope to provide unprecedent insight into the molecular connection between labile iron(II) pool and redox balance in cells, and the role of this dependency in the development, progression and resistance of cancer.

# Calcium and ROS signaling

## P-13-001
## The effect of above average winter temperatures on the activity of superoxide dismutase and catalase in diapausing larvae of the European corn borer *Ostrinia nubilalis* Hbn

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Since larvae of the European corn borer *Ostrinia nubilalis* Hbn. overwinter by entering diapause, a state of arrested development during which they gradually become freeze-tolerant, many of the cold resistance mechanisms of this corn pest species reach their peak only after prolonged exposure to low temperatures. However, it is completely unknown how mild winters, with frequent warm periods caused by global warming, will affect the maintenance of diapause and overwintering of this species. Therefore, the goal of this study was to explore the influence of above average winter temperatures on the antioxidative defense system of diapausing larvae of *O. nubilalis* which have already developed cold hardiness. Considering the assumption that exposure to above average temperatures during the winter would increase oxidative stress levels, the activity of superoxide dismutase (SOD) and catalase (CAT) was measured spectrophotometrically in whole body homogenates of diapausing larvae and pupae. Samples were collected from November 2017 to May 2018 from two distinct experimental groups of corn stalks – one kept in field conditions (FC), exposed to subzero temperatures, and second, warm acclimated (WA), held indoors at high ambient temperatures (14–22°C). Results have shown that both SOD and CAT exhibited different changes in activity, depending on the ambient temperatures and period of diapause. The activity of SOD proved to be more responsive to changes in ambient temperature, being higher in the WA group during almost the entire duration of diapause, except in January 2018, when the field temperatures were well above the average and FC group suffered from oxidative stress, similar to the WA group. On the other hand, the activity of CAT seems to be more under the influence of the endogenous program of arrested development, since it had a similar trend in both FC and WA groups, being lower in the beginning of diapause and then gradually increasing from the February to May. *The authors marked with an asterisk equally contributed to the work.

## P-13-002
## The influence of the medium pH and hydrogen peroxide on the redox status of the *Yarrowia lipolytica* yeast

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In the last decade, reactive oxygen species (ROS) have ceased to be considered only as cell-damaging agents, and their signal functions are actively studied. The increased content of ROS in cells can lead to both an increase and a decrease in their viability, depending on the environmental conditions. In our study, we examined the effect of the culture medium pH and the addition of 10 mM and 100 mM $H_2O_2$ as the prooxidant on the redox status of the *Yarrowia lipolytica* yeast. It was found that 100 mM


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$H_2O_2$ suppressed cell division at any pH, however, hydrogen peroxide administration has no significant effect on cell survival. The 10 mM $H_2O_2$ addition initiated a significant increase in catalase activity, while at 100 mM $H_2O_2$ administration its activity remained at the control level. There was observed no significant increase in SOD activity, but the activity profile changed at different pH values. Analysis of the ratio of reduced glutathione to oxidized one ([GSH]/[GSSG]) showed that addition of hydrogen peroxide resulted in an increase in the ratio compared to the control level, and the 100 mM $H_2O_2$ administration caused a significant gap between the ratio values in the cells grown in acidic, optimal and alkaline media, namely at both pH 4.0 and pH 5.5 the ratio was four-fold more than that at pH 9.0. Using the data obtained, we could conclude that there is a non-linear dose-related dependence in the assay of the cell redox status at the prooxidant addition and the change in the medium pH. Some of the stress markers respond more evidently to low concentrations of the oxidant than to high ones.

## P-13-003
## Biophysical characterization of interaction between calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and 14-3-3 protein

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The intracellular calcium ion influx induces a conformational change of the calcium-sensing protein calmodulin (CaM), which then binds to and activates multiple target proteins, including a family of Ser/Thr protein kinases known as calcium/CaM-dependent protein kinases (CaMKs). The calcium/CaM-dependent protein kinase kinase 2 (CaMKK2), an upstream activator of CaMKII, CaMKIV and AMPK, is involved in the regulation of energy balance, glucose homeostasis, neuronal growth, inflammation and cancer. The CaMKK2 activity is regulated through phosphorylation by several protein kinases including PKA, which phosphorylates four residues in CaMKK2. The PKA-mediated phosphorylation inhibits CaMKK2 in a process involving binding to 14-3-3. 14-3-3s are a family of highly conserved proteins expressed in all eukaryotic cells where they function as scaffold and chaperone molecules that regulate the function of several hundred proteins by recognizing phosphorylated motifs. The exact role of 14-3-3 in the CaMKK2 regulation is still unclear. In this work the sedimentation velocity analytical ultracentrifugation (SV-AUC) in conjunction with the time-resolved fluorescence spectroscopy was used to investigate interaction between phosphorylated CaMKK2 and the 14-3-3 protein. Our data suggest that CaMKK2 phosphorylated by PKA interacts with 14-3-3 through two binding motifs bordering the kinase domain. Furthermore, we show that the 14-3-3 protein binding to CaMKK2 does not interfere with the calcium/CaM binding to CaMKK2. Crystallographic analysis of 14-3-3 with bound phosphopeptides containing CaMKK2 14-3-3 binding motifs suggested that the complex formation might be stabilized by small molecules. Indeed, SV-AUC measurements indicate that fusicoccin, a diterpenoid glycoside produced by the fungus *Fusicoccum amygdali*, increases the stability of the complex between 14-3-3 and phosphorylated CaMKK2. This study was supported by Czech Science Foundation (Projects 19-00121S).

## P-13-004
## Regulation of mitochondrial respiration by small calcium signals in intact cortical neurons: Roles of MCU and the mitochondrial aspartate-glutamate carrier Slc25a12

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The ability of calcium ($Ca^{2+}$) to regulate mitochondrial functions has been established for many years. In excitable cells, $Ca^{2+}$ regulates mitochondrial respiration both by consuming ATP in the cytosol and therefore increasing ATP demand, and by $Ca^{2+}$ signaling, promoting ATP production through stimulation of oxidative phosphorylation. $Ca^{2+}$, as a signaling molecule, may act via two different mechanisms: i) $Ca^{2+}$ entry into the mitochondria through the mitochondrial calcium uniporter complex (MCUc) regulates different enzymes in the matrix; and ii) Ca in the intermembrane space activates the calcium binding mitochondrial carriers (CaMCs), particularly the aspartate-glutamate carriers (AGC) represented in neurons by Aralar/AGC1/Slc25a12, a component of the malate aspartate shuttle. We studied the role of $Ca^{2+}$ signaling in the regulation of mitochondrial respiration in primary cultures of intact, mature cortical neurons exhibiting spontaneous $Ca^{2+}$ oscillations. Stimulation of acetylcholine receptors (AChRs) using carbachol (Cch) in these neurons triggered an increase in the amplitude of $Ca^{2+}$ waves in the cytosol, which also reached the mitochondrial matrix. These Cch-induced $Ca^{2+}$ signals resulted in an increase in oxygen consumption rate (OCR). To address the role of $Ca^{2+}$ entry into the mitochondrial matrix in OCR stimulation, we studied the effect of MCU silencing. Although $Ca^{2+}$ influx into mitochondria was decreased in MCU-KD neurons, the Cch stimulation of respiration was unaltered. However, neurons lacking Aralar/AGC1, showed a significant decrease in Cch-stimulated respiration. These results suggest that Aralar/AGC1-malate-aspartate shuttle (MAS), rather than MCU, is the main pathway coupling small cytosolic $Ca^{2+}$ signals to mitochondrial ATP production in neurons.

## P-13-005
## Sigma-1 receptor antagonist chlorpromazine inhibits $Ca^{2+}$ responses induced by glutoxim and molixan in peritoneal macrophages

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Sigma-1 receptors are unique ubiquitous endoplasmic reticulum-resident chaperones, which modulate a variety of physiological and pathological processes and bind multiple drugs such as antidepressants, antipsychotics, anticonvulsants, and analgesics. Sigma-1 receptors integrate many signalling pathways and in particular modulate $Ca^{2+}$ signalling 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) 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


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receptors in the effect of glutoxim and molixan on $[Ca^{2+}]_i$ in macrophages we used sigma-1 receptor antagonist phenothiazine neuroleptic chlorpromazine, widely used for treatment of schizophrenia. Using Fura-2AM microfluorimetry we have found that macrophage preincubation with 25 μg/ml chlorpromazine for 10 min before 100 μg/ml glutoxim addition leads to a significant suppression of both $Ca^{2+}$ mobilization (on average, by 58.5 ± 4.6%) and subsequent $Ca^{2+}$ entry (on average, by 59.1 ± 6.1%), induced by glutoxim. Similar results were obtained in experiments with molixan. Thus, we have demonstrated for the first time that sigma-1 receptor antagonist chlorpromazine 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 chlorpromazine in clinical practice. *The authors marked with an asterisk equally contributed to the work.

## P-13-006
## The effect of ter genes on oxidative stress response

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Tellurite resistant pathogenic bacterium *Escherichia coli* KL53 possesses *ter* gene cluster, which functions is not clear up to date. This cluster is a part of genomes, especially in pathogenic microorganisms. Due to the rare occurrence of tellurium in the environment, it's unlikely that its predestined function is tellurite resistance. We suggested that the benefit of *ter* gene cluster might be in the ability to survive in harsh stress environment of immune response cells. In our work, we focused on the measurement of antioxidative stress enzymes expression levels and other parameters present during oxidative damage of the cells. The stress conditions were induced by toxicant potassium tellurite since an enzymatic reduction of tellurite generates oxygen free radicals (ROS) as a by-product of synthesis less-toxic compounds. ROS attack all biomacromolecules, therefore, we measured the imbalance between oxidative injury and capability of antioxidative enzymes at physiological as well as DNA level. We evaluated the results of lipid peroxidation, protein carbonylation, and gene expression. Our results showed that the greatest damage was observed in control strain without *ter* genes in comparison with strains with different *ter* genes composition. The same results were obtained by qPCR where we analyzed expression levels of selected antioxidative enzymes. The conclusion is that the more complete *ter* gene cluster is, the less oxidative damage of lipids and proteins can be observed. Moreover, the *ter* gene cluster facilitates elimination of ROS resulting in a decrease of antioxidative stress enzymes expression level. We suggest that the function of *ter* gene cluster might be in facilitating the elimination of oxidative damage by unknown mechanism. *The authors marked with an asterisk equally contributed to the work.

## P-13-007
## The effect of continuous scuba diving on cardiovascular system

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Scuba diving is a special form of physical activity which due to the specific environmental conditions represents a unique stress for cardiovascular system (CVS). The aim of this study was to contribute to the understanding of changes in CVS caused by continuous scuba diving at the molecular level by monitoring the specific biochemical markers. For this purpose, 14 male divers (median age 42 years; range 17–54) performed 5 dives (30 min to 25–30 m depth) in series with a spacing of one week between the dives. Galectin-3 (Gal-3), Vascular Endothelial Growth Factor (VEGF) and Endothelin (ET-1) (ELISA, R&D), high-sensitive Troponin-I (hs-TnI) (chemiluminiscence, Beckman Coulter), N-terminal prohormone of Brain Netriuretic Pepride (NT-proBNP) (chemiluminiscence, Abbott), myoglobin and high-sensitive C-reactive protein (hs-CRP) (immunoturbidimetry, Beckman Coulter), plasma concentrations were determined in blood samples collected before and after the 1st, 3rd and 5th dive. First dive induced changes for all markers except for hs-CRP. Myoglobin, hs-TnI, NT-proBNP, VEGF, and Gal-3 significantly increased, while ET-1 decreased immediately after the first dive. Third and fifth dive caused the same sgnificant changes in all markers, including the increase of hs-CRP after the dive. There was also significant changes in all biomarkers if comparing all pre-dive values; hs-CRP, NT-proBNP and ET-1 concentrations increased significantly by every preformed dive, while myoglobin, hs-TnI, VEGF, and Gal-3 values decreased. ET-1 did not change significantly if comparing all post-dive values. This study showed that scuba diving causes the changes in specific biomarkers that reflect (patho)physiological changes in CVS after the dive. However, it is shown for the first time that contiuously performed scuba diving caused the decrease of specific biomarkers concentrations, thus highlighting possibly positive effect of diving on CVS.

## P-13-008
## Periplanetasin-4 from American cockroach triggers nitric oxide signaling in *Escherichia coli*

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The role and importance of nitric oxide (NO) have already been reported. For many microorganisms, exposure to exogenous NO exhibits high toxicity and eventually leads to cell death. In this study, periplanetasin-4 identified from Cockroach, which is highly exposed to pathogenic bacteria, is used to identify the role of endogenous NO. Periplanetasin-4 elevates superoxide anion level. NO interaction with superoxide and its byproduct induce severe intracellular damage. The role of periplanetasin-4 in *Escherichia coli* is triggering calcium-dependent NO signaling. By confirming that the intracellular damage caused by the NO signal is reduced when NO synthesis is interrupted, it has been demonstrated that NO participates in the oxidative damage of intracellular components. Damage to intracellular material occurred selectively and DNA repair proteins were also involved. The expression levels of


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RecA and LexA proteins, which are typical SOS repair proteins, were significantly changed. In the absence of *dinF* periplanetasin-4 did not induce deterioration of intracellular molecules which is followed by accumulation of superoxide and NO. In conclusion, periplanetasin-4 stimulates calcium-dependent NO signaling in bacteria and its signal is highly related to the *din* gene.

## P-13-009
## The critical role of ROS-induced calpain activation in airway and pulmonary vascular remodeling in COPD

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Chronic obstructive pulmonary disease (COPD) is a severe inflammatory lung disease which is characterized by airway and pulmonary vascular remodeling. Increased proliferation of bronchial and pulmonary arterial smooth muscle cells (BSMCs and PASMCs) and accumulation of extracellular matrix proteins such as collagen contribute to development of airflow obstruction and pulmonary hypertension (PH). Cigarette smoke (CS) induces the production of large amounts of reactive oxygen species (ROS) which play an important role in the pathogenesis of COPD. We have reported that calpain mediates collagen synthesis and PASMCs proliferation contributing to the development of PH. In this study we investigated the role of calpain in airway and pulmonary vascular remodeling in COPD. We found that treatment of BSMCs and PASMCs with cigarette smoke extract (CSE) and $H_2O_2$ increased the calpain activities, collagen synthesis and cell proliferation. Calpain activation was also elevated in bronchi and pulmonary arterioles in the lungs of COPD animal models and patients. Inhibition of calpain by selective inhibitor MDL28170 prevented the CSE- and $H_2O_2$-induced collagen synthesis and proliferation of BSMCs and PASMCs. Moreover, knockdown of calpain-2, but not calpain-1, by specific siRNA diminished increases in collagen synthesis and proliferation induced by $H_2O_2$. Exposure of mice to CS for 24 weeks increased the thickness of bronchial and pulmonary vascular walls, airway resistance and right ventricular systolic pressure. Importantly, calpain inhibition by smooth muscle-specific knockout of calpain and calpain inhibitor MDL28170 prevented the progression of PH, airway and pulmonary vascular remodeling in rodent model of CS-induced COPD. Collectively, we have shown that ROS-stimulated calpain signaling is a critical pathway in COPD which provides a novel therapeutic target for the intervention and treatment of the disease. Supported by NIH, Department of Veterans Affairs, FAMRI and AHA.

## P-13-010
## PMAP-23 triggers cell death by nitric oxide-induced redox imbalance in *Escherichia coli*

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Antibiotic resistance is a global problem and there is an urgent need to augment the arsenal against pathogenic bacteria. The emergence of different drug resistant bacteria is threatening human lives to be pushed toward the pre-antibiotic era. Antimicrobial peptides (AMPs) are a host defense component against infectious pathogens in response to innate immunity. PMAP-23, an AMP derived from porcine myeloid, possesses antibacterial activity. It is currently not clear how the antibacterial activity of PMAP-23 is manifested. In this investigation we showed that PMAP-23 generates nitric oxide (NO) in *Escherichia coli*. NO deactivated catalase and this antioxidant could not protect *E. coli* against reactive oxygen species (ROS), especially hydroxyl radical. This redox imbalance was shown to induce oxidative stress, thus leading to DNA strand breaks. Consequently, PMAP-23 treated *E. coli* cells were inhibited from growth and resulted in apoptosis-like death. In the Δ*dinF* mutant, the levels of DNA strand breaks sharply increased and the cells were more sensitive to PMAP-23 than wild type. Our data strongly indicates that PMAP-23 mediates apoptosis-like cell death by NO-induced ROS generation. Furthermore, our result demonstrates that DinF functioned in protection from oxidative DNA damage and PMAP-23-induced DNA break has surpassed the ability of DNA repair activity. The identification of PMAP-23 activity provides a promising target for antibacterial agent development.

## P-13-011
## In vitro modulation of glutamate decarboxylase activity by genistein, lycopene and zeaxanthin in normal and disturbed calcium homeostasis

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A lot of neurodegenerative and affective disorders are accompanied by disturbances of glutamate acid (Glu), gamma-aminobutyric acid (GABA) metabolism or glutamate decarboxylase (GAD) activity. Neurological diseases are also frequently characterised by impaired calcium conditions. Drugs used in treatment of diseases are not always efficient and may evoke adverse side effects. Chemicals naturally occurring in plants can provide a promising alternative target in modulating of neurotransmission through affecting enzymes involved in neurotransmitter metabolism. The aim of this study was to investigate an effect of selected phytochemicals: genistein, zeaxanthin and lycopene on activity of GAD – enzyme responsible for decarboxylation of glutamate acid to GABA. We used pseudoneuronal PC12 cell line with reduced expression of neuron specific calcium membrane pumps PMCA2 or PMCA3. Cells were differentiated with dibutyryl cyclic-AMP, incubated with investigated plant compounds in concentration 10 mM and 20 mM during 10 minutes, 20 minutes and 12 hours. Additionally, we used preincubation with vigabatrin, inhibitor of GABA transaminase. GAD activity was determined with fluorimetric methods. Our results indicate that examined phytochemicals can modulate activity of glutamate decarboxylase, and thereby change balance between Glu and GABA. Genistein decreases GAD activity in all cell lines. Cells with reduced expression of calcium membrane pumps are more


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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sensitive for its action. Zeaxanthin enhances GAD activity. However, the effect is opposite in control cell line after 12 hours incubation and in PMCA3 line after 10 minutes incubation. Lycopene inhibits GAD activity in all cell line, after 10-minutes incubation. Longer incubation evokes adverse effect. Work was funded by Medical University of Lodz Grants: 503/6-086-02/503-61-001 and 502-03/6-086-02/502-64-109. *The authors marked with an asterisk equally contributed to the work.

## P-13-012
## Ethanol metabolites induce calcium responses in pancreatic stellate cells in vitro and lead to activation of these cells in vivo

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Alcoholic pancreatitis, the most prevalent and potentially fatal disease of the pancreas, is often associated with fibrosis. This excessive deposition of collagen fibres, which may impair exo- and endocrine functions of the pancreas, is a result of pancreatic stellate cell (PSC) activation. The major inducers of alcoholic pancreatitis, non-oxidative metabolites of ethanol and fatty acids, are known to cause excessive cytosolic $Ca^{2+}$ elevations in enzyme-storing pancreatic acinar cells (PAC), triggering premature intracellular activation of trypsin and thus autodigestion of the tissue. However, pathophysiological effects of ethanol metabolites in PSC, particularly in relation to fibrosis, are yet to be elucidated. To address this, the effects of acute administration of ethanol and palmitoleic acid (POA) or palmitoleic acid ethyl ester (POAEE) were investigated in human PSC. Single cell real-time confocal measurements revealed that POA/ethanol (10 μM/10 mM to 200 μM/200 mM) not only induced global and sustained cytosolic $Ca^{2+}$ rises in PSC, leading to complete emptying of the intracellular $Ca^{2+}$ stores, but also significantly disrupted mitochondrial potential. In contrast, $Ca^{2+}$ responses induced by POAEE/ethanol (10 μM/10 mM to 200 μM/200 mM) in PSC were dependent on extracellular $Ca^{2+}$, and the effect on mitochondrial potential was much less pronounced. Further, POA/ethanol induced acute pancreatitis *in vivo*, which was characterised by mild to moderate pancreatic oedema, necrosis, immune cell infiltration and activity of pancreatic myeloperoxidase. Importantly, only in the model of alcoholic pancreatitis (but not in bile-induced pancreatitis), the marker of PSC activation α-SMA was evenly expressed across the entire tissue. Our study suggests that alcohol-induced pancreatic pathology involves not only signalling in PAC but also in PSC, likely leading to their activation *in vivo*, possibly even to a greater extent compared to bile-induced pancreatitis.

## P-13-013
## Non-thermal plasma, as a physicochemical cue to induce redox imbalance in living cells

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Reactive oxygen species (ROS) are involved in essential cellular processes and act as signalling molecules in various organisms, from bacteria to human. Depending on their concentration, ROS may promote cell proliferation and differentiation, or induce oxidative damage and cell death. Therefore, modulation of redox balance and homeostasis might be potential therapeutic opportunity in several diseases. Recent evidences suggest, that non-thermal plasma (NTP), an ionised gas, containing chemically active species, may alter the redox signalling in the living cells. A number of studies have shown the effects of NTP in many biomedical applications, from microorganism deactivation, wound healing to cancer treatment. However, the exact molecular mechanisms of NTP on cells and human remain obscure. In our study, we examined the effects of NTP on two bacterial strains *Pseudomonas aeruginosa* and *Staphylococcus aureus*. After 60 sec of exposure, we observed higher resistance of gram-positive bacteria *S. aureus* to NTP in comparison with gram-negative *P. aeruginosa*. Additionally, to assess the effect of NTP on more complex system we use three human hepatic cancer cell lines, two hepatocellular carcinomas (Huh7 and Alexander) and one blastoma (HepG2). Our results demonstrate that excessive ROS accumulation and generation induced dysfunction of mitochondria and apoptosis in Huh7 and Alexander. Additionally, NTP treatment led to p53 downregulation in Huh7 and Alexander (mutated p53 form) accompanied by downregulation of STAT1 and pSTAT1, supporting the activation of apoptosis event. On the other hand, HepG2 (wild type p53) showed greater resistance in response to oxidative stress mediated by plasma, due to overexpression of Bcl-2 protein (anti-apoptotic protein). In summary, our findings provide non-thermal plasma as interesting therapeutic approach to regulate redox-based processes in the living cells.

# Sulfur metabolism and cellular regulation

## P-14-001
## Selenoprotein MsrB1 deficiency exacerbates acetaminophen-induced hepatotoxicity via increased oxidative damage

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Acetaminophen (APAP) overdose induces acute liver damage and failure *via* reactive oxygen species production and glutathione (GSH) depletion. Methionine sulfoxide reductase B1 (MsrB1) is an antioxidant selenoenzyme that specifically catalyzes the reduction of methionine *R*-sulfoxide residues. In this study, we used *MsrB1* gene-knockout mice and primary hepatocytes to investigate the effect of MsrB1 on APAP-induced hepatotoxicity. Analyses of histological alterations and serum indicators of liver damage showed that $MsrB1^{-/-}$ mice were more susceptible to APAP-induced acute liver injury than wild-type ($MsrB1^{+/+}$)


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mice. Consistent with the *in vivo* results, primary $MsrB1^{-/-}$ hepatocytes displayed higher susceptibility to APAP-induced cytotoxicity than $MsrB1^{+/+}$ cells. MsrB1 deficiency increased hepatic oxidative stress after APAP challenge such as hydrogen peroxide production, lipid peroxidation, and protein oxidation levels. Additionally, basal and APAP-induced ratios of reduced-to-oxidized GSH (GSH/GSSG) were significantly lower in $MsrB1^{-/-}$ than in $MsrB1^{+/+}$ livers. Nrf2 nuclear accumulation and heme oxygenase-1 expression levels after APAP challenge were lower in $MsrB1^{-/-}$ than in $MsrB1^{+/+}$ livers, suggesting that MsrB1 deficiency attenuates the APAP-induced activation of Nrf2. Collectively, the results of this study suggest that selenoprotein MsrB1 plays a protective role against APAP-induced hepatotoxicity *via* its antioxidative function.

## P-14-002
## The gmhA gene is involved in the regulation of redox balance in *Escherichia coli* cells

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GmhA encodes a sedoheptulose 7-phosphate isomerase, which catalyzes the first committed step in the biosynthesis of a core component of lipopolysaccharide. The GmhA protein presents in many gram-negative bacteria, including pathogenic. Previously it was shown, that disruption of this enzyme results in hypersensitivity to different groups of antibiotics, such as novobiocin. Enhanced antibiotic sensitivity of *gmhA* mutant was purely associated with defect of bacterial wall. In this work, we have found, that inactivation of GmhA enzyme by deletion or by amino acid substitution (Thr120 = >Ile) results in dramatic increase in glutathione level, increased ROS generation and significant increase amount of dead cells in population. Sulphur-containing metabolites, such as glutathione, cysteine and hydrogen sulfide are components one of the important protection system against oxidative stress in bacterial cell. The Δ*gmhA* mutant demonstrated high sensitivity to oxidative stress induced by paraquat, but not by hydrogen peroxide. Furthermore, Δ*gmhA* mutant exhibited supersensitivity to quinolones like nalidixic acid and to rifampicin. We have found also that constitutive expression of *gmhA* under the control of strong $P_{tet}$ promoter leads to enhanced level of endogenous $H_2S$ generation, while inactivation of *gmhA* decreased $H_2S$ production in the cell. Previously we showed, that endogenous $H_2S$ suppresses oxidative stress in *E. coli* by sequestering free iron required to drive the genotoxic Fenton reaction. The data obtained imply the new important role of GmhA protein as a modulator of redox balance in bacterial cells. The contribution of GmhA to low molecular thiol metabolism and oxidative stress needs in further investigations. Acknowledgements: This work was supported by the Russian Science Foundation grant 17-74-30030.

## P-14-003
## The impact of systemic administration of cocaine on cysteine metabolism in the selected structures of the rat brain

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Cocaine, one of the most addictive substances for humans and animals, is an alkaloid found in the leaves of the South American plant Erythroxylon coca. Despite many studies attempting to explain cocaine actions, its role in the sulfur metabolism is still poorly understood. The aerobic cysteine (Cys) metabolism yields sulfates and taurine while the anaerobic route of Cys metabolism leads to synthesis of hydrogen sulfide ($H_2S$) and sulfane sulfur-containing compounds. The aim of the present study was to evaluate cocaine-induced changes in the Cys metabolism in the selected structures of the rat brain. The experiments were performed on two groups of Wistar rats treated ip with cocaine at a dose of 10 mg/kg, acutely or repeatedly for 5 consecutive days. One hour after administration of the first or last dose of cocaine, rats were killed by decapitation, and the prefrontal cortex (PFC), nucleus accumbens (NAcc), striatum (STR) and hippocampus (HIP) were dissected from the isolated brains. The whole pool of sulfane sulfur, its bound fraction as well as $H_2S$ were assayed in homogenates of brain structures as markers of anaerobic Cys metabolism while the sulfate was used as a measure of aerobic Cys metabolism. Acute treatment with cocaine increased the total pool of sulfane sulfur and $H_2S$ concentration in the NAcc and STR but decreased the sulfate content only in the NAcc. In the PFC, acute cocaine administration enhanced both the total pool of sulfane sulfur and its bound fraction, while other parameters were not affected. In the HIP only the sulfate content was decreased by acute cocaine. Chronic cocaine administration increased the levels of bound sulfane sulfur in the NAcc and HIP and $H_2S$ concentration in the STR and in the PFC. Our results show that cocaine evoked long-lasting changes in the Cys metabolism leading to the increase in $H_2S$ levels in the studied brain structures, what may have some physiological significance for brain function at some specific conditions.

## P-14-004
## Glutathione deficiency and alterations in the cysteine content during early postnatal life as potential factors initiating development of schizophrenia-like symptoms in adult rats

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There is a lot of evidence indicating that impairment of glutathione (GSH) synthesis may be a starting point initiating a cascade of events leading to the development of schizophrenia. The aim of the present study was to compare changes in the GSH and cysteine (Cys) contents in the liver, kidney and selected brain structures (prefrontal cortex, PFC; hippocampus, HIP) of 16 days old rats treated chronically with L-buthionine-(S,R)-sulfoximine (BSO, 3.8 or 7.6 mmol/kg, s.c., once daily), a specific inhibitor of GSH synthesis, and/or with a dopamine reuptake inhibitor, the compound GBR 12909 (5 mg/kg s.c., every second day). These model compounds were administered to male Sprague-Dawley pups between the postnatal days p5 and p16, alone or in combination. Four hours after administration of the last doses of BSO and GBR 12909, the rats were killed, and then the livers, kidneys and brain structures were isolated for determination of GSH and Cys. In other groups of rats treated with BSO and GBR 12909, behavioral tests, such as the social interaction test, novel object recognition test and open field test, were performed in adulthood (p90-p92). Our data showed that administration of BSO alone or jointly with GBR 12909 induced the most pronounced decreases in the GSH levels in the liver, moderate in the kidney and relatively small in the studied brain


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structures. A lower dose of BSO slightly but statistically significantly decreased GSH content only in the PFC while a higher dose both in the PFC and HIP. In contrast to GSH, BSO at the used doses increased Cys levels both in the PFC and HIP. In the peripheral tissues, only in the liver but not in the kidney, an increase in the Cys level was observed after a higher dose of BSO. Adult rats treated during development with these model compounds presented deficits in the social behaviors and cognitive functions. Acknowledgment: This study was financially supported by grant 2016/23/B/NZ7/01280 from the NCN, Poland.

## P-14-005

## The proof, that under cellular conditions Hint1 protein is involved in the release of H2S from nucleoside 5′-phosphorothioates – potential hydrogen sulfide donors in cells

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Hint1 (histidine triad nucleotide binding protein 1) is an enzyme belonging to the histidine triad (HIT) superfamily of proteins, the members of which act as hydrolases and transferases. Hint1 has been shown to have homologues in all organisms, and many diverse functions of this protein have been described. A majority of its known functions appear to be independent of its enzymatic activity, e.g. tumor suppressor activity. *In vitro*, Hint1 hydrolyses purine nucleotides containing various phosphate linkages. We have found that this enzyme is able to hydrolyze adenosine 5'-*O*-phosphorothioate (AMPS) to AMP and $H_2S$. Other (d)NMPSs are prone to desulfuration at the following order: GMPS>AMPS>dGMPS≥CMPS>UMPS>-dAMPS>>dCMPS>TMPS. We suggest that, if the above reaction occurs under cellular condition, (d)NMPSs might serve as $H_2S$ donors, to study the mechanisms of $H_2S$ signaling, and to be used in potential medicinal applications. We hypothesize that Hint1 could be the enzyme responsible for $H_2S$ production from (d)NMPS in cells. Several enzymes have been demonstrated to produce $H_2S$ in mammalian systems and the initial substrates are L-cysteine and/or L-homocysteine. In present study, we demonstrate that GMPS introduced into A549 cells was intracellularly converted to GMP and $H_2S$, which was detected by a fluorescent assay. The level of releasing $H_2S$ was correlated to the concentration of GMPS used. Moreover, we used A549 cells treated with anti-Hint1 siRNA to show that reduced levels of Hint1 protein correlate with reduced $H_2S$ production. This experiment shows that Hint1 is involved in (d)NMPS desulfuration and $H_2S$ release under cellular conditions. Our studies suggest that the GMPS and probably other (d)NMPS can serve as donors of $H_2S$ in biological systems. *The studies were supported by The National Science Centre grant no DEC-2013/09/B/ST5/03612 (to AK).

## P-14-006

## Impact of the butylparaben on the glutathione-dependent enzyme metabolism

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Glutathione-dependent enzyme metabolism is a key factor in many cellular detoxifications in xenobiotics, xenoestrogens and cellular defense mechanisms. Xenoestrogens are a group of chemicals that alter the normal function of hormones and these molecules are also known as an endocrine disruptor. Butyl p-hydroxybenzoic is one of xenoestrogen which is found in cosmetics, daily care products, food, toys, pharmaceuticals. Parabens present only in very low concentrations. Parabens are used in low concentrations as preservatives, however, we receive paraben from various ways from various daily products so that, the daily exposure dose is higher than expected. Thus, safety and toxicity of butylparaben may be an important public health problem which must be discussed. Even though several *in vivo* and *in vitro* studies showed that butylparaben have hazardous effects on reproductive system, health problems that may affect development and developmental malformations in the laboratory animal models. Butylparaben-induced oxidative stress and its relation to the tissue damage have not been widely investigated before. Therefore, we aimed to investigate the effects of butyl 4-hydroxybenzoate on the glutathione-dependent enzymes activities related with pentose phosphate pathway and glutathione-dependent metabolism cytosolic enzymes, such as glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, glutathione peroxidase and glutathione-s-transferase in the kidney, liver, brain and testis samples in our study. Rats were randomly divided into four groups and butyl paraben was orally dosed in male rats at 200, 400, 800 mg/kg/day for 14 days. We have investigated that butylparaben treatment caused imbalance in the anti-oxidant enzyme activities and tissue damage in the kidney, liver, brain and testis samples. These results are consistent with the degenerative role of butylparaben on the cellular reducing equivalent homeostasis and antioxidant defense.

## P-14-007

## Mitochondrial rhodanese activity and expression in menadione – treated cultures of normal (MCF-12A) and cancerous (MCF-7) human breast cells

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Rhodanese (thiosulfate sulfurtransferase, TST) is localized in the mitochondria. TST fulfills many biological functions, however in spite of extensive studies, its biological role is not fully elucidated. Recently, TST has been shown to produce hydrogen sulfide and polysulfides. 3-Mercaptopyruvate sulfurtransferase, evolutionarily related to mitochondrial rhodanese, functions as an antioxidant protein. It was found that behavioral abnormality in MST-KO mice is caused by an antioxidant insufficiency. To elucidate a possible function of rhodanese as antioxidant protein we have undertaken the investigation of rhodanese activity and expression changes in normal (MCF-12A: ATCC CRL-10782) and cancerous (MCF-7: ATCC HTB-22) human breast cells treated with menadione which induces the formation of reactive oxygen species and depletion of GSH. Results of our earlier studies showed large differences in rhodanese activity and glutathione levels in these two cell lines what suggests their different reaction in case of increased levels of reactive oxygen species generated by menadione in cell cultures. The 8 μM concentration of menadione, nontoxic in 24 h treatment, was established for both cell cultures. The levels of superoxide anion radical, low-molecular thiols (reduced and oxidized glutathione, cysteine and cystine) and the level of sulfane sulfur were determined. Mitochondrial fraction was isolated from both control cells and those treated with menadione and the mitochondrial membrane integrity was confirmed. The specific activity of rhodanese was determined in mitochondrial fractions. The isolation of total RNA was performed to detect the expression of rhodanese and mitochondrial


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superoxide dismutase genes in cells from menadione-free and menadione-containing cultures. Analysis of the collected results is carried out to confirm the possible role of rhodanese in the protection of mitochondria against reactive oxygen species formed in cells in the presence of menadione.

## P-14-008
## $H_2S$ role in redox-regulation of *E. coli* bacteria cells

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Hydrogen sulfide ($H_2S$) is gasotransmitter involved in many physiological processes and along with glutathione and cysteine is an important component of antioxidant defense in bacteria cells. Endogenous $H_2S$ makes bacteria more resistant to oxidative stress, but mechanism is still poorly understood. In this study the parameters characterizing cell redox status of E. coli bacteria cell the level of intracellular thiols, reactive oxygen species (ROS) and nitric oxide (NO) were characterized in the cells with altered $H_2S$ generation using flow cytometry. It was shown that deletion of the mstA gene encoding the 3-mercaptopyruvate sulfotransferase, which catalyzes the $H_2S$ production, leads to a significant increases of ROS production, but there is no change in the level of thiols, NO and the viability of cells. Simultaneous deletion of the mstA gene and the gmhA gene coding for sedoheptulose 7-phosphate isomerase, which catalyzes the first stage of biosynthesis of the lipopolysaccharide core, reduces the increase in the thiol level and percentage of dead cells caused by a single gmhA deletion. The number of E. coli mutants was created with deletions of genes encoding proteins involved in the synthesis of glutathione (gshA, gshB), synthesis of cysteine (cysE) and transcriptional regulator (cysB) which controls cysteine biosynthesis and transport. It was found that deletion of the gshA, gshB and cysE genes leads to a decrease in the level of intracellular thiols, while deletion of cysB completely eliminates the effect of an increase in thiols caused by gmhA deletion. Thus, a disturbance of the synthesis of cysteine and its transport into the cell prevents the growth of thiols in cells with gmhA deletion. The fact that disturbed $H_2S$ synthesis also leads to a less pronounced increase in thiols, observed when gmhA is deleted, indicates a significant role for $H_2S$ in the redox regulation of E. coli cells. Supported by RSF-17743003.

## P-14-009
## Unravelling the biological function of ovothiol during the embryonic development of *Paracentrotus lividus*

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Ovothiol is a marine π-methyl-5-thiohistidine showing peculiar antioxidant properties, due to the position of the thiol group (-SH) on the imidazole ring. It is produced in large amounts in sea urchin eggs to protect them from the oxidative burst at fertilisation and developing embryos from environmental cues. In this work, the temporal and spatial expression patterns of the key enzyme responsible for ovothiol biosynthesis, OvoA, were assessed during the embryonic development of the Mediterranean sea urchin *Paracentrotus lividus*. Eggs and embryos were collected and analysed through western blot (WB) and immunohistochemistry (IHC) experiments. WB analyses showed that OvoA, present in low quantities in the eggs, increased from 64-blastomeres/early blastula stage until prism stage, followed by a drop in pluteus larvae. IHC experiments revealed a cytoplasmic localisation in the early developmental stages. The expression was higher in S-phase (interphase) blastomeres compared to those ones in M-phase (mitosis). Interestingly, a restricted expression pattern was observed in PMCs (skeletogenic cells) and digestive trait in larval stages. In order to provide information on its biological role, perturbation experiments were performed by microinjection of specifically designed morpholino oligonucleotides against OvoA mRNA into sea urchin eggs. A high percentage of malformation showing an extreme phenotype, which resembled apoptotic ongoing processes, was obtained. The OvoA protein expression in specific cells and tissues suggests the involvement of ovothiol in key biological processes. While in early sea urchin developmental stages, when the cycle time is extremely short and lacks "Gap phases", ovothiol may be involved in cell cycle progression, at later stages the thiol compound could participate to defence mechanisms helping an efficient larval skeleton formation as well as gut functionality.

## P-14-010
## Therapeutic potential of marine sulfur-containing compounds

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The most abundant marine sulfur-containing compounds are probably ovothiols, histidine-derived thiols, isolated from marine invertebrates, microalgae, and proteobacteria. In sea urchin eggs ovothiols play a key role in the protection of cells towards the oxidative burst associated with fertilization by controlling the cellular redox balance and recycling oxidized glutathione. Gamma-glutamyl transpeptidase (GGT) is a cell surface enzyme involved in glutathione metabolism. High expression of GGT on membrane surface of tumor cells is associated with increase of cell proliferation and resistance to chemotherapy. Moreover, GGT is considered a diagnostic marker in several liver and renal diseases, in asthma, and reperfusion injury. GGT is generally inhibited by glutamine analogues that compete with the substrate for the gamma-glutamyl binding site. However, the glutamine analogues that have been evaluated so far in clinical trials are too toxic for use in humans. Here we report that ovothiol A, 5(N)-methyl thiohistidine, isolated from sea urchin eggs, acts as a novel potent and less toxic inhibitor of GGT activity. In detail, 5-thiohistidine compounds are uncompetitive inhibitors, binding the γ-glutamyl enzyme complex, with an intrinsic *Ki* of 26 μM. Ovothiols are 15-fold more potent than the GGT known inhibitor 6-diazo-5-oxo-L norleucine and are not toxic towards human kidney embryonal HEK293 cells. As GGT-overexpressing positive cell lines, we identified human liver cancer cells HepG2 and chronic B leukemic cells HG3. Ovothiol A treatment induced membrane-bound GGT inhibition and a cell death phenotype mediated by autophagy in HepG2 and HG3 cells. This study provides the basis for further development of 5-thiohistidines as therapeutics for GGT-positive tumors and GGT dependent pathologies, such as liver fibrosis.


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## Molecular neurobiology

### P-15-001
### Investigation of the genetic pathways in early and late stage brain regeneration using the zebrafish model

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Traumatic injuries, cancer, neurodegenerative diseases and stroke result in loss of neuronal cells, ultimately leading to visual, motor or mental damage in the central nervous system (CNS). While mammals have a very limited capacity to regenerate their brain after injury, adult zebrafish brain, by virtue of its stem/progenitor cell pool, can regenerate extensively and replenish the lost neurons with new ones. This feature makes zebrafish a useful model to understand the molecular programs underlying CNS regeneration. Although several studies have explored zebrafish brain regeneration at the cellular level using histological methods, how underlying molecular mechanisms are controlled at the whole-transcriptome level has not yet been elucidated. Here, we exploit RNA-sequencing to investigate mRNA expression profiles in brain regeneration at its early versus late stages. For this purpose, we performed stab injury into the one hemisphere of the telencephalon to trigger brain regeneration. We validated the samples by using quantitative PCR with early and mature neuronal markers and cryosectioning. We found that expression of the proliferation and glial cell markers increased at early stages that correspond to proliferative stages and decreased at later time points. On the other hand, expression levels of early and mature neuronal marker genes were similar to controls in early stages, while increasing in later stages due to the generation of new neurons. Our preliminary analysis of RNA-seq data reveal that early and late stages of brain regeneration are regulated by the different molecular mechanisms. We believe that these results will greatly extend our knowledge of molecular mechanisms involved in brain regeneration and shed light on development of new therapeutic approaches for traumatic brain injury or neurodegenerative diseases.

### P-15-002
### Oxidized cell-free DNA molecule as a potential inducer of neuronal cell survival in acute and chronic stress

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Reactive oxygen species (ROS) are generated as a response to stress signals. ROS can oxidize different molecules including DNA forming 8-oxo-dG. We have shown that the level of 8-oxo-dG content in circulating cell-free DNA (cfDNA) is elevated in schizophrenia patients. Here we investigated the concentrations of cfDNA and 8-oxo-dG during acute and chronic stress and analyzed the responses of cultivated rat neuronal cells exposed to oxidized cfDNA (oxo-cfDNA). Stress included immobilization and swimming for various time periods. After sacrificing of animals, concentration of cfDNA in plasma and content of 8-oxodG in cfDNA was determined. Granular rat neurons were obtained from the cerebellum of newborn rats. Oxidized genomic DNA was added to the neuron culture medium for 1-24 hours. The mitochondrial potential, levels of ROS, NRF2, NOX4 and SOD1 proteins concentration, the levels of NRF2 and Hmox-1 genes expression were analyzed. In rats subjected to the stress for 2 hours, 2 days and 11 days, concentration of oxo-cfDNA increased by 3.7 folds ($P < 0.01$), 7.3 folds ($P < 0.01$) and 25.9 folds ($P < 0.001$), respectively. Data demonstrated that the oxo-cfDNA penetrated neurons in vitro which was positively correlated with DNA oxidation. Exposure of rat neurons to oxo-DNA resulted in increased expression of NOX4 gene and genes of antioxidant response – NRF2 and its target Hmox-1 ($P < 0.01$). DNA oxidation positively correlated with the activation of mitochondria in cultured neurons, whereas expression of SOD1 protein and ROS were both reduced. It is suggested that the accumulation of the oxo-cfDNA in blood of patients with schizophrenia experiencing stress as well as in other oxidative stress associated diseases is an adaptive response aimed on survival of different cells including neurons. The study was supported by RFBR grant 17-29-06017 ofi_m

### P-15-003
### Astrocytes regulate density of neuronal excitatory and inhibitory receptors

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It is widely accepted that neurons and astrocytes cooperate to maintain the homeostasis of the brain. However, up to now, the molecular mechanisms of the cell-to-cell cross-talk are poorly understood. To study the neuron-astrocyte interactions monocultures of neurons and neurons-astrocytes co-cultures were used. The cells were isolated from P2 C57BL/6 mice and cultured separately for 14 days. To establish the co-culture, astrocytes were seeded at the inserts with 1 (CC1) or 3 (CC2) μm pores, placed above the well containing neurons and co-cultured for 48 hours. Cells growing on CC1 were able to communicate releasing molecules to the culture medium, while cells growing on CC2 could form physical contact with the cells in the well. The effect of astrocytes on density of neuronal receptors involved in the synaptic plasticity was investigated using immunofluorescence. Obtained results revealed significant differences in expression of the receptors between the mono- and co-cultures. In the case of the excitatory receptors AMPAs (Gria1 and Gria4), a significant effect of the direct cell-to-cell contacts was observed. The level of Gria1 isoform was strongly decreased in the co-cultures (CC1) as compared to neurons alone. The decrease was even stronger when the physical contact between neurons and astrocytes was possible (CC2). The same trend was observed for Gria4 receptors. For the most common NMDA isoforms (Grin2a and Grin2b) no significant differences were observed. In contrast to AMPA, the level of GABAA receptors (inhibition of synaptic excitability) was significantly elevated in both types of the co-cultures (CC1 and CC2) comparing to the monoculture. The results presented here demonstrate that astrocytes, interacting physically with neurons, regulate the proper strength of neuronal excitability affecting the density of AMPA and GABA receptors. They also suggest that neurons growing in the absence of glial cells exhibit a 'hyperactive' physiology.


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## P-15-004
## A method to determine the dynamic characteristics of synaptic transmission

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Synaptic transmission (ST) presents the multiphase process involving enormous number of participants. ST investigations are complicated by the fact that many characteristics of this process cannot be measured directly (so-called hidden parameters). Thus, the study of ST requires implicit methods such as mathematical modelling. In this work, we introduce the method to determine the set of ST hidden parameters based both on mathematical modelling and on experimental results. The values in question present the essential parameters of the ordinary differential equations composing the mathematical model of ST. To find the numerical values of these parameters, we use the genetic algorithm of the model fitting which aims to minimize the deviation of the modelled field excitatory postsynaptic potential (fEPSP) from fEPSP recorded in the rat hippocampal slices with the microelectrode technique. To verify model and to obtain more reliable results, we find the sets of the hidden parameters for fEPSPs that were registered under the effect of the various concentrations of inhibitors acting on the different stages of ST: cilnidipine, BAPTA-AM and 6-cyano-7-nitroquinoxaline-2,3-dione. Using bootstrap statistics, we calculate the values of the hidden parameters that allow the model to fit to all experimental conditions optimally: the density of the voltage-dependent calcium channels on the presynaptic bouton ($50.33 \pm 1.06\ \mu m^{-3}$), the maximal conductance of the single N-type voltage-gated calcium channel ($7.85 \pm 0.21$ pS), and that of P/Q-type voltage-gated calcium channel ($7.27 \pm 0.52$ pS), the rate constant of active SNARE complex dissociation ($7.36 \pm 0.33\ ms^{-1}$), the rate constant of the synaptic vesicles transition from the docked state to the inactivated state ($0.073 \pm 0.015\ ms^{-1}$).

## P-15-005
## Association between CNTNAP2, COX2 and OXTR polymorphisms and autism spectrum disorder: a pilot case-control study in Kazakhstani population

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The association of polymorphism of candidate genes with the risk of ASD in the Kazakhstani population was investigated. Buccal swabs of 27 ASD children and their 23 healthy siblings were used for DNA isolation and genotyping of rs2710102 *CNTNAP2*, rs2745557 *COX2* and rs53576 *OXTR*. The distribution of genotypes corresponded to the Hardy-Weinberg distribution. For rs2710102 *CNTNAP2*, an increased risk of developing ASD was shown for the T allele that was confirmed by the general model (for the TT genotype OR = 1.90; 95% CI = 0.42–8.67; $P$ = 0.40–0.57) and by the dominant model (for the CT + TT genotypes OR = 2.48; 95% CI = 0.21–29.23; $P$ = 0.46). For rs2745557 *COX2,* an association with ASD was established for the GA and AA genotypes (OR = 1.57; 95% CI = 0.26–9.53; $P$ = 0.82 and OR = 1.52; 95% CI = 0.13–18.03; $P$ = 0.82, respectively) and for the combination of these genotypes (OR = 1.62; 95% CI = 0.35–7.45; $P$ = 0.53). The heterozygous AG genotypes of the rs53576 *OXTR* correlated with an increased risk of ASD according to the general model (OR = 2.08; 95% CI = 0.30–14.25; $P$ = 0.7), which was also confirmed by the dominant and recessive models. Thus, the genotypes associated with an increased risk of ASD were TT/CT + TT for rs2710102 *CNTNAP2*, GA/AA for rs2745557 *COX2*, AG for rs53576 *OXTR*. Due to the small number of samples, the obtained results were not significant, however, our data are consistent to the results of other authors on the perspectives of these markers to evaluate the risk of ASD.

## P-15-006
## Agathisflavone isolated from *Schinus polygamus* (cav.) Cabrera leaves prevents scopolamine-induced memory impairment and brain oxidative stress in zebrafish (*Danio rerio*)

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Agathisflavone, a biflavonoid isolated from *Schinus polygamus* (Cav.) Cabrera leaves has been reported to promote various biological activities such as anti-inflammatory properties, promoting cognition and preventing cancer, antioxidant and antiapoptotic activities. Here, we tested the hypothesis whether anxiety, amnesia, and brain oxidative stress induced by scopolamine could be counteracted in zebrafish model by agathisflavone and tried to ascertain the underlying mechanism. Agathisflavone (1, 3 and 5 μg/l) was administered by immersion to zebrafish once daily for 8 days period. Anxiety and memory impairment was induced with scopolamine (100 μM) and measured with the novel tank diving test (NTT) and the Y-maze test. The identification of the agathisflavone was done by spectroscopy, and the structure of the compound was confirmed by (-) Electrospray Ionisation Mass Spectrometry (ESI-MS). The brain oxidative status and acetylcholinesterase (AChE) activity were also investigated. Agathisflavone from *Schinus polygamus* (Cav.) Cabrera leaves was identified. Also, we demonstrated that agathisflavone significantly reversed scopolamine-induced behavioral score alteration in the NTT and Y-maze tests. Consequently, agathisflavone promoted inhibition of AChE activity and restored the brain antioxidant status. Our results demonstrate that agathisflavone promotes brain antioxidant action and ameliorates scopolamine-induced anxiety and memory deficits in zebrafish. Acknowledgments: This project is funded by the Ministry of Research and Innovation within Program 1 – Development of the national RD system, Subprogram 1.2 – Institutional Performance – RDI excellence funding projects, Contract no. 34PFE/19.10.2018"

## P-15-007
## A Bcr-Abl inhibitor GNF-2 attenuates inflammatory activation of glia and chronic pain

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GNF-2 is an allosteric inhibitor of Bcr-Abl. It was developed as a new class of anti-cancer drug to treat resistant chronic myelogenous leukemia. Recent studies suggest that c-Abl inhibition would provide a neuroprotective effect in animal models of Parkinson's disease as well as in clinical trials. However, the role of c-Abl and effects of GNF-2 in glia-mediated neuroinflammation or pain hypersensitivity has not been investigated. Thus, in the present study, we tested the hypothesis that c-Abl inhibition


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by GNF-2 may attenuate the inflammatory activation of glia and the ensuing pain behaviors in animal models. Our results show that GNF-2 reduced lipopolysaccharide (LPS)-induced nitric oxide and pro-inflammatory cytokine production in cultured glial cells in a c-Abl-dependent manner. The small interfering ribonucleic acid (siRNA)-mediated knockdown of c-Abl attenuated LPS-induced nuclear factor kappa light chain enhancer of activated B cell (NF-κB) activation and the production of pro-inflammatory mediators in glial cell cultures. Moreover, GNF-2 administration significantly attenuated mechanical and thermal hypersensitivities in experimental models of diabetic and inflammatory pain. Together, our findings suggest the involvement of c-Abl in neuroinflammation and pain pathogenesis and that GNF-2 can be used for the management of chronic pain.

## P-15-008
## Multidirectional action of fenamates and general anesthetics on GABAA and glycine receptors: Structural explanation

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Inhibitory neurotransmission in the nervous system is mediated by GABAA and glycine receptors (GABAAR and GlyR) which are targets for numerous modulators that potentiate or inhibit their functions. We investigated the molecular mechanisms of action of two modulators etomidate (ETM) and mefenamic acid (MFA). Patch-clamp recordings showed that ETM and MFA in low concentrations potentiate GABA-activated currents in isolated Purkinje cells, but suppress glycine-activated currents in isolated rat pyramidal neurons. We found that potentiating effects of ETM and MFA are non-additive that indicates a competition of these drugs for a common binding site. To determine the structural determinants responsible for the differences in the action of these drugs we built the homologous models of α1β2γ2 GABAAR and α1β GlyR based on the α1 GlyR cryo-EM structure (3JAE) and used Monte-Carlo energy minimization for the model optimization. We used residues M286, N265 and M236, which influence the efficacy of ETM action on GABAAR, to bias the search of the MFA and ETM binding modes with minimal energy. We showed that MFA and ETM bind in the β/α interface and form H-bonds with R269, N265 (M2, β2), and van der Waals contacts with M286, F289 (M3, β2) and L232, P233, M236 (M1, α1). Our models predict that MFA and ETM stabilize the conformation of R269 side chain and the receptor open state. MFA and ETM potentiate GABA-activated currents in receptors containing β2/3, but not the β1 subunit. N265 is present only in β2/3 subunit, in β1 (GABAAR) or in α1-3 (GlyR) subunits this position is occupied by Ser. R269 forms two H-bonds with S265 in α1β1γ2 GABAAR and αβ GlyR. As a result, the side chain R269 is deeply bent inside the interface and cannot form H-bonds with Q229 and I228 and loses the ability to stabilize the receptor open state. Supported by the RFBR grants 18-015-00038 and 18-33-20087.

## P-15-009
## Chicken Cognin interactome and learning process of visual imprinting

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Chicken Cognin is a protein disulfide isomerase which resides mainly in two different sub-cellular compartments: (1) endoplasmic reticulum, from which it is suggested to escape to the cell surface after cleavage from its endoplasmic reticulum retention signal and (2) cell surface membrane fraction. We have previously shown that 24 h after training Cognin is upregulated in a learning-related manner in the chicken brain region crucially involved in the memory of visual imprinting – the intermediate and medial mesopalium (IMM). This upregulation occur in plasma membrane-mitochondrial, but not in cytoplasmic fraction. Nothing is yet known about the interactome of Cognin especially according to its subcellular localisations. Are the interacting partners in membrane-mitochondrial fractions involved in imprinting? Immunoaffinity chromatography, electrophoresis and mass-spectrometry was applied for the identification of Cognin interacting protein partners in the plasma membrane-mitochondrial, and in cytoplasmic fraction. We have revealed that protein components of Cognin interactome in plasma membrane-mitochondrial fraction includes alpha subunits of sodium-potassium ATP-ase, calcium-calmodulin protein kinase II, heterogenous nuclear ribonucleoprotein A2/B1, Dynamin-1, synaptojanin, clathrin heavy chain and mitochondrial ATP-synthase subunit b5. For most of these proteins learning related increase was shown in our previous studies. In the present experiments we have also studied changes in the amounts of alpha-3 subunit of sodium-potassium ATP-ase and mitochondrial ATP-synthase subunit b5 in the IMM and control chick brain regions after imprinting. Only in the IMM of the left hemisphere 24 h after training there was a learning-related increase in the amounts of ATP-synthase subunit b5.

## P-15-010
## Study of candidate gene polymorphisms at epilepsy patients with possibly syndromic forms

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Epilepsy is a chronic heterogeneous neurological disease characterized by recurrent convulsive seizures, which is associated with an excessively electrical activity of brain's neurons. There are more than 45 000 people suffer from epilepsy in Kazakhstan. Most of them children from the countryside – 71.3%, while the rest 28.7% are from the city. Due to polyetiology of epilepsy, doctors find it difficult to establish a diagnosis and prescribe a specific treatment. In this regard, more fundamental study of epilepsy including genetic background is demanded. The aim of our research was analysis of candidate gene polymorphisms in patients with possibly hereditary syndromic forms of epilepsy. Based on the analysis of literary data, in order to identify cause-and-effect mutations of epilepsy that is associated with hereditary syndromes, the following genes were selected: *SCN1A* (Ala1783Thr; Phe1831Ser; Gly1674Arg; Pro1657Ala), *KCNT1* (Ala934Thr; Arg928Cys), *KCNC1* (His212 = ; Arg339 = ), *KCNQ2* (Ala265Val; Tyr284Cys), *MECP2* (Thr158Met; Thr197Met; Arg306Ter). We have surveyed 108 patients (average age 21 ± 13.69, male – 64, female – 44) with various epileptic syndromes, such as SMEI, SMEB, ADNFLE, MMPSI, TLE and Dravet, that were screened with PCR-RFPL and sequencing. In this cohort there were 73.2% of Kazakhs (79 patients), Russians – 17.6% (19 patients), Uigurs – 4.6% (5 patients), Turks – 3.7% (4 patients), Koreans – 0.9% (1 patient). Study of candidate polymorphisms of the gene *SCN1A* showed the presence of mutations p. Ala1783Thr and deletions of 26 exon in 2 patients with Dravet syndrome. 3 cases of *de novo* mutation of *KCNT1* gene p.Ala934Thr were detected: 2 patients suffering from TLE,


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1 patient with residual encephalopathy. *De novo* origin of these mutations in all families was confirmed by analysis of close relatives. Thus, epileptic seizures in these patients may be associated with damages in ion channel genes. *The authors marked with an asterisk equally contributed to the work.

## P-15-011
## Scorpion toxins are highly selective ligands of voltage-gated potassium channels

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Natural venoms are a rich source of active polypeptides that can interact with voltage-gated potassium channels (Kv). Such molecules, called toxins, were successfully used in pioneer works where the structure and functions of various channel isoforms were studied. In recent years, polypeptide toxins have demonstrated an immense potential in the field of fundamental researches, drug discovery, and development of diagnostic systems. Here, we report complex study of two scorpion venoms, isolation and characterization of six novel toxins from them. All polypeptides were sequenced by Edman degradation and characterized using the voltage-clamp technique on 15 mammalian channels expressed in *Xenopus* oocytes. Among purified molecules, there are several with remarkable selectivity on specific isoforms of Kv. For example, MeKTx11-1 is a high-affinity blocker of Kv1.2 (IC50 ~0.2 nM), while its activity against Kv1.1, Kv1.3, and Kv1.6 is 10 000, 330 and 45 000 fold lower, respectively. These highly selective ligands can be further utilized as research tools in neurobiology or scaffold molecules for drug design. This work was supported by the Russian Science Foundation (grant no. 18-74-00125).

## P-15-012
## Learning and memory mechanism in phenylketonuria with BDNF pathway

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Aim: Phenylketonuria (PKU) is an autosomal recessive disease of the phenylalanine metabolism that is associated with phenylalanine hydroxylase (PAH) enzyme and is usually diagnosed in early life. Because of the toxic effects of phenylalanine and its metabolites, learning and memory mechanisms have been reported to be impaired, but their mechanism has not yet been elucidated. Recent studies have reported that the brain-derived neurotrophic factor (BDNF) signaling pathway and learning memory mechanism in hippocampus are closely related to the pathogenesis of cognitive dysfunction. We analyzed how the changes in this pathway in newborn female and male rats with phenylketonuria. Methods: Phenylalanine hydroxylase inhibitor was injected into female and male newborn rats for 20 days. After conducting behavioral tests such as locomotor activity, new object recognition and passive avoidance tests, they were sacrificed and hippocampus removed. From this tissue, the PKA-cAMP-CREB-BDNF protein levels were determined by ELISA and BDNF mRNA expressions were analyzed by RT-PCR. The blood phenylalanine levels were measured by taking blood from EDTA tube during sacrification. Results: Locomotor activities of male newborn rats with Phenylketonuria were shown to be impaired as compared to female rats. Biochemical analysis displayed that the hippocampal CREB and BDNF levels of male new born rats with phenylketonuria was decreased as compared to female rats. According to RT-PCR data, BDNF expressions of both female and male PKU rats were reported to be significantly reduced. Conclusions: These findings show that learning and memory mechanism in PKU newborn rats are impaired along with the changes in BNDF pathway. Although BDNF values decrease in both genders of PKU rats, the female rats BDNF levels are higher than those found in PKU males.

## P-15-013
## Alcohol addicted brain mapping and imaging using light-sheet fluorescence microscopy

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Addiction is a disease that affects circuits and brain areas involved in reward, stress, and self-control. Continued substance abuse can lead to dependence that is associated with a withdrawal symptom when drug availability is ceased, and increased intake upon relapse. We used a mouse model of alcohol addiction to perform whole-brain analysis of the activity during addiction-like behaviors. Mice were trained to drink ethanol in the "drinking in the dark" paradigm. Mice can drink alcohol voluntarily in a free access mode. This access is limited to a couple of hours a day. After the training, the animals were deprived of alcohol for 9 days and tested for behavior in relapse. To identify activated brain regions, we selected c-Fos as a marker of neuronal response to new stimuli (cue or access to alcohol following long withdrawal). The brains were isolated two hours after introduction of a stimulus in a form of cue directing to alcohol corner or cue alone. Whole hemisphere of each mouse brain was subjected to optical clearing. This procedure allows to turn the brain transparent and image it *in toto* in the light-sheet microscope. Brains were immunostained to detect c-Fos. Such approach enables to identify all active cells with their anatomical annotation. Z-stack of hundreds of brain mosaic pictures covering the whole hemisphere is processed as a single three-dimensional image. The c-Fos positive cells are identified as single objects or groups which, in turn, form a 3D map of brain "hot spots". These are compared between animals at particular stages of addiction. To this end, we have developed a dedicated image computational workflow which includes robust image registration techniques and cell detection algorithms resilient to imaging artifacts. The whole-organ analysis is a new, alternative high throughput pathway that could shed light to events taking place in the brain without the disturbance of its anatomical and morphological integrity.


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## P-15-014
## Mode of action and biological activity of sevanol and its analogues on acid-sensing ion channels

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Acid-sensing ion channels (ASICs), members of the family of amiloride-sensitive degenerin/epithelial $Na^+$-channels, are expressed in neurons of both the peripheral and central nervous system. Among six currently known isoforms of mammalian ASICs, two, namely ASIC1a and ASIC3 are widespread and have the largest physiological contribution such as synaptic plasticity, learning and memory as well as pain perception and inflammation development. We have previously shown that sevanol, a new lignan isolated from *Thymus armeniacus*, inhibits ASIC1a and ASIC3 currents and exerts analgesic and anti-inflammatory effects when administered intravenously. Here we present a scheme for the synthesis of sevanol, which was developed for the first time. In addition, sevanol analogues were synthesized, in which the basic core of the molecule of epiphyllic acid remained unchanged, while substituents for carboxyl groups in positions 9,10 were modified. These analogues demonstrate a clear correlation between the activity of the molecule and the number of free carboxyl groups in it. Using molecular modeling and analysis of the activity of sevanol in the presence of ASIC1a potentiator, an RF-amide peptide, we established a possible binding site for sevanol on the channel. We also showed that with intranasal administration, sevanol can have the same effective analgesic effect as with intravenous administration. Such structural and functional analysis demonstrates a correlation between the inhibitory effect value and the number of functional groups of the molecule, which may be important for the rational design of biologically active sevanol-based compounds. This study was supported by Russian Science Foundation grant No 18-14-00138.

## P-15-015
## Muscle isoform of fructose-1,6-bisphosphatase is indispensable for the induction of long-term potentiation

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Muscle fructose-1,6-bisphosphatase (FBP2) catalyzing hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate is a regulatory enzyme of gluconeogenesis. Growing body of evidence demonstrated that, except metabolic function, FBP2 is engaged in several other cellular processes, like regulation of cell cycle and protection of mitochondria against calcium stress. It was shown that FBP2 reduces the rate of calcium-induced mitochondrial swelling and interacts with mitochondrial proteins involved in energy homeostasis. Calcium ions are indispensable for induction of neuroplastic processes such as long-term potentiation (LTP) and long-term depression (LTD) in neurons. The local calcium elevation in dendritic spine during the potentiation can affect mitochondria present in the closest vicinity of calcium influx. Here, we present evidence that FBP2 co-localizes with neuronal mitochondria and the co-localization changes during LTP chemical induction. Moreover, inhibition of FBP2 activity and silencing of its expression result in a block of the expression of the LTP-related Immediate Early Genes, such as: cFos, cJun, Arc, and in a decrease of sEPSC areas in a patch-clamp records. Our data show that FBP2 interacts with CaMK2 and this interaction is indispensable for CaMK2 activation during LTP induction. Thus, we hypothesise that FBP2 plays an essential role in the induction of neuroplasticity and, because its tertiary and quaternary structure is regulated by several metabolites and ions (AMP, $NAD^+$, FBP, F6P, $Ca^{2+}$), FBP2 may be a molecular hub linking neuronal plasticity and metabolism.

## P-15-016
## Encoding and decoding of calcium ions oscillations in vivo in zebrafish brain neurons imaging

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Store-operated $Ca^{2+}$ entry (SOCE) is one of the regulatory mechanisms of $Ca^{2+}$ homeostasis in non-excitatory cells as well as in neurons (nSOCE). One of the key elements of nSOCE are stromal interacting proteins (STIMs), endoplasmic reticulum (ER) residents. These are $Ca^{2+}$ sensing proteins that have an EF-hand $Ca^{2+}$-binding domain. When $Ca^{2+}$ are depleted from the ER (a major store of $Ca^{2+}$), the decreased level of $Ca^{2+}$ is sensed by STIM proteins, which then oligomerize and interact with $Ca^{2+}$ channels at the plasma membrane. This leads to the $Ca^{2+}$ influx and refilling ER with these ions by $Ca^{2+}$-ATPase. Zebrafish larva is an exceptional prototype for whole-brain functional imaging, however, the neuronal interactions underlying the spontaneous neuronal $Ca^{2+}$ activity patterns, and their biological relevance, remain elusive. To take full advantage of zebrafish larvae as a model, we investigated the neuronal spontaneous $Ca^{2+}$ oscillations and those in response to the external stimuli. Using Lightsheet Microscopy, we performed *in vivo* imaging of transgenic fish expressing GCaMP5G (a genetically encoded $Ca^{2+}$ probe) under the neuronal promoter (*Tg(elavl3:GCaMP5*). The parameters of $Ca^{2+}$ oscillations, such as the interspike intervals (ISI) and the $Ca^{2+}$ amplitudes, were analyzed in neuronal somata of the three different regions of the brain (optic tectum (OT), cerebellum (Cereb), and inferior olive (IO)) of transgenic (TG) wild-type (WT) and *stim2b*$^{-/-}$ zebrafish lines. Using MATLAB algorithms, we quantified the differences in $Ca^{2+}$ oscillations patterns between regions in the brain neurons and showed that $Ca^{2+}$ oscillations change significantly in *stim2b*$^{-/-}$ fish. Also, ISI and $Ca^{2+}$ amplitude were more affected in *stim2b*$^{-/-}$ than in WT when fish were treated with a high concentration of $Ca^{2+}$. This suggests a role of the Stim2b protein in attenuation of $Ca^{2+}$ buffering.

## P-15-017
## Altered expression of axon guidance related genes in the FKBP5 deficient mice

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Psychological stress is a risk factor for several diseases. In particular, stressor exposure has been linked with various psychiatric disorders. Since the hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the regulation of stress responses, it has been implicated in the etiology of stress related disorders such as PTSD. The HPA axis regulate synthesis and release of glucocorticoids and its dysregulation cause abnormal response to stress. FK506-binding protein 51 (FKBP5) is a co chaperone of HSP90


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in the glucocorticoid receptor (GR) molecular complex and a key regulator of the sensitivity of GR. In this study, we profile the miRNAs in the prefrontal cortex of FKBP5 knockout mice compared to the wild type. Subsequently, we profiled target mRNAs for differentially expressed miRNAs in the FKBP5 deficient mice using several tools for sequence-based miRNA target prediction such as miRDB, DIANA tool, miRmap and TargetScan. Gene ontology analysis suggested that differentially expressed miRNAs in the brain of FKBP5 deficient mice may be involved in neuron development, cell motion, endocytosis, and cell-cell adhesion. The expression of predicted target genes was confirmed in the prefrontal cortex of FKBP5 mice using real-time PCR. As a result, we found that axon guidance related genes including *Nfasc* (neurofascin) were significantly decreased in the FKBP5 deficient mice brain. *Nfasc* is an L1 family immunoglobulin cell adhesion molecule which is important for neural cell migration and neurite outgrowth during development. It is also known that knockdown of *Nfasc* lead to reduction of synaptic transmission and impairment of synaptic plasticity, causing impairment of fear extinction. These results suggest that *Nfasc* could be a new target for understanding of the pathophysiology of PTSD.

## P-15-018
## Mutations in pore and TRP domain of noxious heat receptor TRPV1 specifically affect the channel functioning

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TRPV1 (transient receptor potential vanilloid 1) is one of the key proteins involved in noxious heat perception. Despite TRPV1 being studied extensively, treatment of pain syndromes caused by TRPV1 dysfunction remains a challenge. Hence, much is still to learn about mechanisms of activation, gating, and regulation of TRPV1. Our work highlights five novel TRPV1 residues important for thermal and capsaicin-aided activation and gating. Ion current through the pore of TRPV1 is regulated by upper and lower gates. Both static experimental structures and all-atom molecular dynamic simulations proposed Gly 643 to form a "bottleneck", the narrowest region of the upper gate. We demonstrated that substitution even to the second smallest residue alanine in this position abrogates gating. On the other side, our analysis suggested Ile 679 and Ala 680 be the "bottleneck" of the lower gate. Therefore, double I679A+A680G mutation produces an "always open" channel, probing the tightness of the TRPV1 lower gate. Next, our modeling suggested that motion of Asn 676, located in the cavity between the gates, may be sufficient to stabilize the closed and open states. Further computations proposed substitution with Ser to have the smallest impact on channel structure. However, such mutant appeared to be nonfunctional, pointing on the extreme importance of Asn 676 for channel gating. Finally, we discovered that the movements of the cytosolic TRP domain may also affect pore conductance. Our *in silico* analysis of correlated movements of pore and TRP domain revealed that residue Lys 688 in this domain forms an ionic bridge, fixing TRP-domain motions. Indeed, replacement Lys with small flexible Gly led to a sufficient increase in channel sensitivity, while rigid Pro had the opposite effect. Our results provide a better understanding of fine mechanics underlying TRPV1 functioning. This work was supported by Russian Science Foundation Grant 16-15-00167

## P-15-019
## Comparison of analgesic effects for ASIC3 inhibitors

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Acid-sensing ion channels (ASICs) are special molecular sensors on the membrane to acidification of extracellular media. Acid-sensing ion channel 3 (ASIC3) plays an important role in the perception of acidosis signals in peripheral neurons and in the development of inflammatory pain. An inhibition of ASIC3 considered being an attractive approach to relief inflammatory pain. We compared different ASIC3 inhibitors to reveal properties important for the best anti-inflammatory effect. First, we evaluated all antagonists (sevanol, peptides Ugr9-1/APETx2) on ASIC3 channels expressed in X. laevis oocytes. As was previously reported, APETx2 inhibited only transient current, whereas Ugr9-1 and sevanol decreased transient and sustained components of the current. The effect on animal pain and inflammatory response was evaluated after intramuscular injection of antagonists in the acetic acid writhing pain model and the complete Freund's adjuvant-induced thermal hyperalgesia test. All tested antagonists produced the significant analgesic effect in both tests, but these effects did not have linear dose-dependence in a certain test for each antagonist. The bell-shaped dependence of the analgesic effect was observed for sevanol and peptide Ugr9-1 in the thermal hyperalgesia test and for APETx2 in the acetic acid-induced writhing test. Nevertheless, compounds inhibiting both components of ASIC3 current produced more significant pain relief than APETx2, which is an effective inhibitor of a transient current only. Therefore, inhibition of ASIC3-sustained currents is beneficial for the promotion of acidosis-related pain relief. This work was supported by Russian Science Foundation, grant No. 18-14-00138.

## P-15-020
## Peptide derivatives of TRPA1 modulators reduce the specific response in mice to selective channel agonist

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Ion channels play a significant role in many pathological states. Therefore, the search for ion channel modulators is a promising scientific and pharmacological task. Animal venoms contain a dozen of bioactive compounds which include modulators of different ion channels. Sea anemones produce compounds that evolutionary act on orthologue ion channels and could possess therapeutic effect on human ones. During the search for modulators of TRPA1 (transient receptor potential ankyrin-repeat 1), the channel that plays a significant role in initiation and propagation of pain signals under noxious chemical, mechanical and thermal (low temperatures) conditions, from venoms of sea anemones *Urticina eques* and *Metridium senile* were isolated peptides Ueq12-1 and Ms9a-1, correspondingly. Both peptides potentiate TRPA1 activity. In electrophysiology experiments on *Xenopus laevis* oocytes expressing TRPA1 Ueq12-1 and Ms9a-1 increased agonist-induced activation of the ion channel. While injection of peptides in the mice hind paw didn't cause pain, paw edema or thermal hyperalgesia. Moreover, pretreatment of mice


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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by intravenous injection of peptides reduced nocifensive behavior and paw edema induced by TRPA1 agonist allyl isothiocyanate (AITC) as well as decreased non-specific inflammation in Freund's Complete Adjuvant test. Aiming to determine the active sites of the peptides we analyzed the effects of the N-terminal and C-terminal domains of Ueq12-1 and Ms9a-1 on TRPA1 *in vitro* and *in vivo*. We found that small peptides were able to potentiate TRPA1 less effective than natural ones, and have no effect on non-specific inflammation. However, they conserved the *in vivo* antinociceptive effect against AITC-induced pain. Hence, peptide derivatives of Ueq12-1 and Ms9a-1 possess a selective mode of action and could be considered as drug leads for treatment TRPA1-associated conditions. This work supported by Russian Science Foundation Grant 16-15-00167.

## P-15-021
## Alpha-catulin contributes to neural tube closure in the mouse embryo

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During the early development of the central nervous system, the neuroepithelial cells undergo dynamic changes in shape. Spatial and temporal changes of the cytoskeletal organization are fundamental to epithelial cell shape changes, and noncentrosomal microtubules assembled along the apicobasal axis and actin filaments and non-muscle myosin II at the apical side are central machineries of cell elongation and apical constriction, respectively. Vinculin and α-catenin are two related proteins that play crucial roles in those processes; the function of their recently characterized homologue alpha-catulin is still poorly understood. Here, using the gene trap system we unveil the function of alpha-catulin during early mouse development. Ablation of alpha-catulin causes defective neural tube closure, due to impairment of both basement membrane assembly and bending of the neural plate. Neural plate superficial cells that normally drive bending of the neural plate by apical constriction, concomitant with apical actin and P-Mlc accumulation, fail to do so in alpha-catulin KOs. Using the 3D model of MDCK cells we showed that removal of alpha-catulin from the cells alters the subcellular distribution of F-actin and apical constriction. These observations suggest that catulin is a critical determinant of the cellular architecture required for proper neurulation.

## P-15-022
## Developmental loss of serum response factor (SRF) alters maturation of hippocampal neurons

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Serum response factor (SRF) is a major transcription factor that regulates the expression of several plasticity-associated genes in the brain. Although the developmental expression of SRF in excitatory neurons was shown to be is crucial for axon development, no substantial evidence of its role in synapse formation and maturation has been provided. During brain development, dendritic spines', the locus for excitatory synapses, shapes change from thin, elongated filopodia-like structures to stable mushroom spines. This form of structural plasticity is essential for synaptic transmission and proper circuitry formation. The aim of our study was to investigate the functions of SRF in the regulation of structural plasticity during neuronal maturation *in vitro*. We found that lack of SRF in the hippocampal neurons resulted in an increased number of filopodia-like protrusions and decreased the number of mushroom spines *in vitro*. Moreover, spines of SRF depleted neurons exhibited altered morphology, highlighted by increased length and area of filopodia-like and long spines. Furthermore, SRF-depleted neurons had a lower level of surface AMPAR GluR1 and GluR2 subunits. We showed that the number of functional synapses and their activity was lower in SRF-depleted cells as shown by a reduction in the frequency and amplitude of mEPSC. These findings indicate that SRF regulates transcription program essential for synapse formation and maturation during hippocampal development. Supported by Polish National Science Center grant (SONATA BIS 2) DEC-2012/07/E/NZ3/01814.

## P-15-023
## Circadian changes of the monoamine oxidase in the pineal gland of chicken *Gallus gallus domesticus* L.

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Monoamine oxidase (MAO, EC 1.4.3.4) catalyses the oxidative deamination of a range of monoamines and the catecholamines. Two isoenzymes of MAO (MAO A and MAO B) are present in most tissues like brain or intestine. MAO A has higher affinity for the substrates as serotonin and norepinephrine, whereas MAO B has higher affinity for phenylethylamine and benzylamine. In all vertebrates special structures located in the central nervous system are responsible for the regulation of circadian rhythms. In birds, pineal gland along with suprachiasmatic nucleus and retina play the role of a rhythm generator. Pineal gland is a place of intense synthesis of melatonin and other indoles, which is immediately secreted into the bloodstream and distributes information to other organs. The presence of monoamine oxidase in the pineal gland has been known for a long time. In chicken pineal gland monoamine oxidase participate in oxidation of serotonin to 5-hydroxyindoleacetic acid and dopamine to 3,4-dihydroxyphenylacetic acid. In the chicken pineal gland individual components of the indole and catecholamine synthesis pathway show daily changes, and most of them are rhythmic in constant darkness. The studies on the rhythmicity of MAO in the pineal gland have been lacking. The fluorimetric, real-time PCR and western blot methods have been used to determinate circadian changes of activity, gene expression and protein expression of MAO A and B in domestic chicken pineal gland. Total activity MAO is rhythmic with the higher level during the night time and most of them is MAO A activity. Expression of the MAO A protein appears to be rhythmic in both LD and DD conditions. Whereas, MAO B protein expression seems to be rhythmic only in light–dark conditions. The study was supported by National Science Center No. UMO – 2016/21/B/NZ3/00364.


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## P-15-024
## Comparison of TLR-4 mediated synthesis of oxylipins by microglia and astrocytes

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Oxylipins formed from polyunsaturated fatty acids (PUFAs) are active players of inflammatory processes with pro- or anti-inflammatory roles. They are formed via cyclooxygenase, lipoxygenase, and cytochrome P450 pathways from ω-6 (arachidonic (AA)) and ω-3 (docosahexaenoic (DHA), eicosapentaenoic (EPA)) acids and also from essential fatty acids. Microglia and astrocytes are responsible for neuroinflammatory responses, but differences of oxylipin synthesis were not studied. Our aim was a comparison of microglia and astrocytes oxylipins synthesis in course of LPS activation (a TLR4 agonist). Primary astrocytes from newborn rats were cultured 12 days before experiments; microglia was isolated by "shaking method" according to the standard protocol. Western blot was used for detection of signaling pathway proteins. Cell-free culture media were taken for solid-phase lipid extraction. Oxylipins and PUFAs were analyzed by 8040 series UPLC-MS/MS (Shimadzu) with 16 standards. Astrocytes and microglia responses at 4 h and 24 h stimulations with LPS were compared. Totally we detected 43 substances upon our treatments. We obtained that microglia release significant amounts of EPA, DHA and AA in unstimulated cells that decreased under TLR stimulation and significant increase of appropriate acid derivatives via cyclooxygenase, lipoxygenase, or cytochrome P450 pathways. Astrocytes showed a weaker ability to release acids without stimulation, but activation of TLR signaling pathways resulted in release of oxylipins formed mainly via cyclooxygenase and cytochrome P450 pathways. Although most substances are common for microglia and astrocytes, such oxylipins as 9-HODE, 13-HDoHE, 16-HDoHE, PGA2, PGJ2 are formed principally by activated astrocytes. Data show new opportunities for the participation of oxylipins synthesis form microglia and astrocytes in regulation of TLR-mediated responses. Supported by RFBI 18-34-20100. *The authors marked with an asterisk equally contributed to the work.

## P-15-025
## OSKβ1, modular toxin from *Orthochirus scrobiculosus* scorpion venom

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Scorpion venoms are multicomponent mixtures of biologically active compounds, among which a large number of polypeptide neurotoxins acting on ion channels are found. One approach to search for new neurotoxins is to perform transcriptome analysis of scorpion venom glands. Using this approach we identified a putative component of *Orthochirus scrobiculosus* venom and named it OSKβ1. An important feature of this putative toxin is modular structure: OSKβ1 consists of an N-terminal linear region showing α-helical propensity, and a C-terminal cysteine-rich region that conforms to the CSα/β motif. Such structure is typical among potassium channel toxins of the β-KTx family. Using heterologous expression in bacteria and chemical synthesis, we obtained a full-sized recombinant toxin and its separate modules. All products were purified by chromatography and their identity was confirmed by mass spectrometry. The secondary structure of OSKβ1 and its modules was studied by circular dichroism spectroscopy, whereas their spatial structure in solution was investigated by NMR spectroscopy. The activity of all substances on a panel of voltage-gated potassium channels expressed in *Xenopus* oocytes was analyzed using the two-electrode voltage clamp technique. To determine the cytolytic activity, a series of assays with six strains of bacteria, oocytes and insect larvae was performed. As a result of this work, we report the structural features of the modular toxin OSKβ1, its cytolytic and insecticidal activity, and the lack of effect on potassium channels. Support from the Russian Science Foundation (grant no. 19-74-30014) is acknowledged.

## P-15-026
## Novel IIIG9 localization in cell-cell adhesion complexes during embryonic and postnatal brain development

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In the adult brain, IIIG9 is restricted to the cilia present in ependymal cells that line the ventricular layer. During embryonic development (E14), a subset of radial glia generates ependymal cells, and ependymal cell differentiation is finalized after birth (P20). We have previously determined that IIIG9 expression is high towards the apical side of radial glia, although it is not known what function they may be performing. We have characterized IIIG9 expression and localization during radial glia specification to ependymal cells in the rat brain, showing that is present in cell-cell adhesion complexes throughout ependymal development. We used embryonic rats (E13, E15, and E17) to analyze IIIG9 expression in the brain by RT-PCR and immunohistochemical analysis coupled to optical and spectral confocal microscopy. A loss-of-function effect was analyzed using adenovirus that inhibits IIIG9 *in vitro* in primary neurosphere cultures isolated from E17 cortical progenitors and *in vivo* by i.c.v. injection in neonatal rats. IIIG9 is expressed throughout the ventricular wall during embryogenesis and colocalizes with Pan-cadherin and β-catenin in the lateral membrane at the apical part of radial glial cells. IIIG9 is markedly expressed in cultured neurospheres, and *in vitro* inhibition of IIIG9 leads to disrupted neurosphere morphology and impaired attachment to the culture plate. *In vivo* IIIG9 inhibition leads to deficient radial glia differentiation, generating distinctive phenotypes along the rostrocaudal axis. IIIG9 may be involved in the maintenance of cell-cell adhesion complexes at the lateral membrane of radial glia that will generate ependymal cells in postnatal mice, suggesting a novel role for IIIG9 during embryonic CNS development.

## P-15-027
## Comparing neuronal differentiation of human induced pluripotent stem (iPS) cells from different tissue of single donor

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One of the major goals in neurobiology is to understand nervous system development and modeling of cellular interactions within the human brain. The technology of generating stem cells from


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somatic cells opened new perspectives such as cellular replacement, regenerative therapy and disease modeling. However, iPS cells' fate is guided by expression of specific genes and epigenetic modulation. The aim of this study was to elucidate whether the origin of iPS cells obtained from single donor determine their fate during neuronal differentiation. Peripheral blood mononuclear cells and keratinocytes were reprogrammed using the Sendai-virus reprogramming system. The transgene-free iPS clones were verified by analyzing expression of pluripotency markers by RT-PCR. Generated iPS clones were also screened for alkaline phosphatase expression, organoid formation capacity and ability to differentiate into the 3 germ layers *in vivo*. Interestingly, we observed that number of neuroectodermal structures were definitely higher in teratomas generated from keratinocytes-derived iPS line. The differentiation of human induced pluripotent stem cells into a large multicellular midbrain organoid-like structure showed that organoid-like structures were positive for TUJ1 and TH as shown by immunofluorescent staining. Analysis of neuronal progenitors' and neurons' markers at selected time points revealed differences in expression of LMX1A. iPS cells, generated from ectodermal origin cells, showed higher neuronal differentiation propensity. These 3D organoid models showed a potential to become a universal *in vitro* system to study human brain biology. The project was supported by the grant from the National Science Centre in Poland 2015/17/B/NZ5/00294 and K/ZDS/007055.

## P-15-028
## Effect of desipramine on ERK1/2 protein expression and differential role of alpha1-adrenergic receptor subtypes in modulation of the ERK1/2 phosphorylation

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Our previous study showed that an antidepressant drug, desipramine (DMI), similarly attenuated the noradrenaline-induced activation of all three α1-adrenergic receptor (α1-AR) subtypes assessed as inositol phosphate accumulation. The aim of the current study was to investigate if the prolonged incubation with DMI may influence the expression and phosphorylation of ERK1/2 kinases in response to receptor stimulation with noradrenaline (NA) and whether the α1-AR subtypes activity is similarly modulated by DMI. PC12 cell lines with or without expression of α1A- or α1B-AR were seeded in standard growth medium with 10 μM of DMI or vehicle. After 24 hours or 5 days, medium were replaced with serum free one for 2 hours. The cells were stimulated with 3.9 μM NA and harvested with RIPA buffer 0, 5, 10, 15, 30 and 60 minutes after stimulation. The Western Blot procedure was applied to measure the expression (tERK) and phosphorylation (pERK) of ERK kinases. The metabolic activity of cells was assessed with the resazurin reduction assay- there were not observed effect. There was no effect of NA and/or DMI on pERK and tERK in PC12 lines without expression of α1A- or α1B-AR. In cell lines bearing the receptors, stimulation with NA modulated the time course of pERK response to NA in α1-AR subtype dependent manner. The increased expression of pERK1/2 was still observed during 15 min after the α1A-AR stimulation while the sharp 5-min-peak occurred in case of the α1B-AR activation. The incubation with DMI caused the increase of tERK expression for about 50% and 60% in cells with α1A-AR and α1B-AR, respectively. Our results suggest that these two receptor subtypes are differently engaged in regulation of the ERK1/2 activity, while the DMI-induced increase of tERK1/2 expression does not depend on the α1-AR subtype. Research was funded by National Science Centre, Poland, grant No. 2015/17/B/NZ7/03018 (to IN).

## P-15-029
## Transporter clustering analysis in the neuronal membranes using three-dimensional structured illumination microscopy (3D-SIM)

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SVCT2 is a high-affinity/low-capacity ascorbic acid (AA) transporter that is widely expressed throughout the brain, especially in neurons. Although neurons actively uptake AA from the extracellular medium, SVCT2 is weakly detected in the cell membrane; however, it is clearly observed intracellularly in the cytoplasm. We propose that SVCT2 may induce clustering in the cellular membrane, decreasing the affinity for the antibody. We used lentiviral transduction and flow cytometry to generate cell lines that stably overexpress SVCT2 (N2a and HN33.11). We used three-dimensional structured illumination microscopy (3D-SIM) to evaluate the co-distribution of SVCT2 as detected using immunolocalization and overexpressed SVCT2-GFP in neurons. Our results show that SVCT2 overexpression in neurons induces a differentiated morphology with increased branching zones. Endogenous SVCT2 (immunofluorescence analysis) was detected in the cytoplasm and additionally in the plasma membrane, showing low cluster formation (red structures < 100 nm). In contrast, SVCT2-GFP was mainly observed in the plasma membrane, suggesting higher cluster formation (green structures, 100–200 nm) with little immunoreaction for anti-SVCT2. Control experiments using genetic ablation of SVCT2 with CRISPR/Cas9 technology confirmed the specificity of the observed effects. SVCT2 cluster formation at the neuronal membrane could potentiate AA uptake capacity, stimulating neuronal differentiation and branching. However, aggregation of the membrane transporter would decrease immunodetection using fluorescent antibodies against a unique and internal SVCT2 sequence. We have detected that different cells can vary the degree of clustering, changing their detection levels of SVCT2 in the cellular membrane. Supported by grants FONDECYT 1181243; PIA-CONICYT ECM-12 CMA-BIOBIO.

## P-15-030
## Spider toxin Hm-3 differently interacts with voltage-sensing domains of Nav1.4 sodium channel and blocks gating pore currents underlying periodic paralysis

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Voltage-gated $Na^+$ ($Na_V$) channels contain domains that have discrete functionalities. The central pore domain allows current flow and provides ion selectivity, whereas peripherally located


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four voltage-sensing domains (VSD-I/IV) are needed for voltage-dependent gating. Certain mutations trigger a leak current through VSDs leading to various diseases. For example, hypokalemic periodic paralysis (HypoPP) type 2 is caused by mutations in the S4 voltage-sensing segments of VSDs in the skeletal muscle channel $Na_V1.4$. The gating modifier toxin Hm-3 (crab spider *Heriaeus melloteei*) inhibits leak (gating pore) currents through such mutant channels and represents useful hit for HypoPP therapy. To investigate molecular basis of Hm-3 interaction with $Na_V1.4$ channel, we studied isolated VSD-I and VSD-II by NMR in membrane mimicking environment. Hm-3 partitions into micelles through a hydrophobic cluster formed by aromatic residues and interacts with both VSDs by the prolonged positively charged beta-hairpin. The toxin binds to different sites on the domains. On VSD-I Hm-3 interacts with the S3b helix and S3–S4 extracellular loop forming two salt bridges with conserved E208 and D211 residues, while on VSD-II the toxin binds to the S1-S2 extracellular loop interacting with E604 and D606 side chains. Nevertheless, in the both cases the allosteric changes in S4 helix conformation induced by the bound toxin block the gating pore currents. In the obtained complexes, the toxin forms lot of the stabilizing contacts with the lipids surrounding the VSDs. This suggests membrane-mediated mechanism of Hm-3/$Na_V1.4$ interaction. Work was supported by the Russian Academy of Sciences (Molecular and Cell Biology Program). NMR study was supported by the Russian Science Foundation (#19-74-30014)

## P-15-031
## Duality of presynaptic events in systems neuroscience

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Chemical synapses are key structures in the brain responsible for nerve signal transmission and its simultaneous regulation, where presynaptic nerve terminals provide neurotransmitter release/uptake. Analyzing presynaptic glutamate and GABA transport, it has been supposed that there are two main relatively independent groups of mechanisms at the presynaptic site influencing the extracellular glutamate concentration, and so it's signaling and regulation. The first one is regulated compound exocytosis of synaptic vesicles stimulated by membrane depolarization that increases significantly glutamate concentration in the synaptic cleft and initiates glutamate signaling through postsynaptic glutamate receptors. The second one is permanent glutamate turnover across the plasma membrane that occurs without stimulation, and is determined by simultaneous non-pathological transporter-mediated release of glutamate thermodynamically synchronized with uptake. Permanent glutamate turnover is responsible for maintenance of dynamic $glutamate_{in}/glutamate_{out}$ gradient resulting in the establishment of flexible extracellular level of glutamate, which can be unique for each synapse because of dependence on individual presynaptic parameters. These two mechanisms, i.e. exocytosis and transporter-mediated glutamate turnover, do not directly interfere with each other, because they have different intracellular sources of glutamate in nerve terminals for release purposes, i.e. glutamate pool of synaptic vesicles and the cytoplasm, respectively. This putative duality can set up a presynaptic base for memory consolidation and storage, maintenance of neural circuits, long-term potentiation, and plasticity. Also, it can be further developed for implementation in artificial intelligence and brain reprogramming.

## P-15-032
## ML-324, a KDM inhibitor, blocks TNF alpha-induced increase of vascular adhesion protein and neutrophil infiltration through blood–brain barrier

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Recently it has been proposed that malfunction in blood-brain barrier result in various brain diseases including Alzheimer's disease. We previously demonstrated that TNF alpha-induced increase of adhesion proteins, ICAM1 and VCAM1 is regulated by histone modification in human brain microvascular endothelial cells (HBMVEC) and that lysine demethylases (KDM)4B is responsible for demethylation of histone 3 at lysine 9. In the present study, we examined the effect of two KDM4 inhibitors, ML-324, a broad spectrum inhibitor and NSC636819, a specific inhibitor of KDM4A and KDM4B and compared the effect on ICAM1 with that on VCAM1. Our results showed that TNF alpha-induced increase of VCAM1 is blocked by the ML-324 more effectively than NSC636819. In contrast, these drugs showed no blocking effect on ICAM1. ML-324 significantly inhibited the TNF alpha-induced neutrophil adhesion and infiltration. Thus, the broad spectrum KDM4 inhibitor plays an important role in neutrophil infiltration in brain micro-vessels by modulating adhesion protein expression. KDM4 inhibitors could have the potential as therapeutics for brain diseases. The present study was supported by a research grant of the National Research Foundation of Korea (NRF2017R1A2B4002861).

# RNA turnover

## P-16-001
## Quantitative proteomic screen revealed MTRES as a factor preventing stress-induced transcription deficiency in human mitochondria

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Maintenance of mitochondrial gene expression is crucial for cellular homeostasis. Stress conditions may lead to a temporary reduction of mitochondrial genome copy number and/or affect mtDNA transcription, raising a risk of insufficient expression of mitochondrially encoded genes. Little is known how compensatory mechanisms operate to maintain proper mitochondrial transcripts levels upon disturbed transcription and which proteins are involved in. Here we applied a quantitative proteomic screen to search for the proteins that sustain expression of mtDNA under stress conditions. We found novel, poorly characterized protein, which we named MTRES, to be elevated in cells with perturbed mitochondrial gene expression. We show that MTRES functions as a protective factor to maintain proper mitochondrial RNAs level during transcription arrest. *In vivo* crosslinking and immunoprecipitation (CLIP) experiments indicated that MTRES binds RNA *in vivo*, which was confirmed by biochemical experiments using purified MTRES. Transcriptomic analysis and quantitative fluorescent microscopy showed that upregulation of MTRES prevents mitochondrial transcripts loss under perturbed mitochondrial gene expression. This function of MTRES involves


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RNA binding since mutated version of MTRES incapable of binding RNA, designed based of structural and biochemical data, does not prevent mitochondrial RNAs decrease. Functional experiments showed that MTRES acts by increasing mitochondrial transcription, without changing the stability of mitochondrial transcripts. We propose that MTRES may be an example of the protein that may have been acquired to the transcriptional machinery from the translation apparatus to protect the cell from mitochondrial RNA loss during stress.

## P-16-002
## Post-transcriptional HIF1A regulation: a new target to control the hypoxia signalling pathway

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Adaptation to hypoxia is a puzzling and tightly regulated challenge. This adaptability involves a severe gene expression rewiring, which is mainly triggered by the Hypoxia Inducible transcription Factor (HIF). HIF acts as a heterodimer composed by a ubiquitously expressed β subunit (HIF-β) that binds to the O2-sensitive α subunit (HIF-α). Canonically, the regulation of the hypoxia signalling pathway mostly relies on HIF-α protein stability, which has been described to be exquisitely regulated through the Ubiquitin Proteasome System (UPS). However, we argue that HIF1A post-transcriptional modifications might also contribute to the adaptive programme. Indeed, transcripts processing and turnover, and the rate of translation provide additional control points, which determine the amount of the protein delivered. Here, we will describe a novel and robust mechanism of HIF-α regulation that could shed light on new targets to fine tune the hypoxia signalling pathway.

## P-16-003
## Identification of novel RNA-binding proteins in human mitochondria with focus on poly(A)-binding proteins

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Binding of proteins to RNA is crucial for every step of gene expression and RNA metabolism. For instance, interaction of poly(A)-binding proteins with polyadenylated nucleus-encoded transcripts influences their stability and translation. It is known that mitochondrial mRNAs also possess stable poly(A) tails, however mitochondrial poly(A)-binding proteins remain unknown. The project's aim is to identify novel human mitochondrial RNA-binding proteins with particular focus on poly (A)-binding proteins. To accomplish this goal, mitochondrial protein extracts were subjected to affinity chromatography using poly(A) conjugated beads and purified proteins have been identified by mass spectrometry. Initial experiments showed relatively high background. A series of optimization experiments has been conducted in order to discard false positives from among identified proteins. In the improved approach the affinity chromatography has been extended to poly(U) and poly(C) conjugated beads. A group of putative mitochondrial RNA-binding proteins has been identified. This method allowed to capture known mitochondrial RNA-processing proteins and identify novel putative RNA-binding proteins. Selected identified proteins are being subjected to functional studies.

## P-16-004
## MCPIP2 – a novel regulator of RNA turnover and cell proliferation

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Monocyte chemotactic protein-induced protein 2 (MCPIP2, also known as ZC3H12B) belongs to a family of four members called the MCPIP family of proteins. Although the founder member of this family, MCPIP1 (also known as ZC3H12A or Regnase-1), is well described the information about other MCPIP proteins is very scarce. Of the whole family, MCPIP2 remains the most enigmatic one and there is no as yet published data concerning its function or expression. Our results show that MCPIP2 is able to destabilize a set of transcripts encoding inflammation related proteins including IL-6. This ability depends on the presence of a functional domain with probable RNase activity (the NYN-domain) in MCPIP2. Substitution of a single amino acid in the potential catalytic centre of MCPIP2 completely abolishes the observed regulation. The RNA destabilizing activity of MCPIP2 requires binding of the protein to a recognition motif located in the 3′UTR of the regulated mRNAs. The motif is a stem-loop structure and its removal protects the mRNA from destabilization by MCPIP2. Overexpression of MCPIP2 in HeLa cells results in lower levels of IL-6 mRNA as well as diminished production of IL-6 protein upon IL-1β stimulation. Interestingly, MCPIP2 is also an important regulator of proliferation. MCPIP2 overexpression causes proliferation inhibition of 293T cells. This action depends on the RNA destabilizing activity of MCPIP2 as no such effect is observed when the inactive D196A mutein of MCPIP2 was used. Analysis of the expression of MCPIP2 in human tissues reveals that MCPIP2 mRNA in especially abundant in brain. From the analysed human cell lines highest level of MCPIP2 transcript was found in the neuroblastoma cell line SH-SY5Y. Here, we show that MCPIP2 is a novel regulator of the turnover of inflammation-related transcripts and a negative regulator of cell proliferation. Our studies on the expression of MCPIP2 indicate that this protein may play an important role in the nervous system.

## P-16-005
## Pop2 phosphorylation at S39 is important for Pop2 to repress the expression of stress response genes, HSP12 and HSP26, upon glucose availability

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The *S. cerevisiae* Pop2 protein is an exonuclease in the Ccr4-Not complex that is a conserved regulator of gene expression. Pop2 regulates gene expression post-transcriptionally by shortening the poly(A) tail of mRNA. A previous study has shown that Pop2 is phosphorylated at threonine 97 (T97) by Yak1 protein kinase in response to glucose limitation. However, the physiological importance of Pop2 phosphorylation remains unknown. In this study, we found that Pop2 is phosphorylated at serine 39 (S39) under unstressed conditions. The dephosphorylation of S39 was occurred within 1 min after glucose depletion, and the addition of glucose to the glucose-deprived culture recovered this


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phosphorylation, suggesting that Pop2 phosphorylation at S39 is regulated by glucose. We previously reported that Pop2 takes a part in the cell wall integrity pathway by regulation of *LRG1* mRNA; however, S39 phosphorylation of Pop2 is not involved in *LRG1* expression. On the other hand, Pop2 phosphorylation at S39 is involved in the expression of *HSP12* and *HSP26*, encoding small heat shock proteins. In medium supplemented with glucose, Pop2 might be phosphorylated at S39 by Pho85 kinase to repress the expression of *HSP12* and *HSP26*. Glucose starvation inactivated Pho85, which resulted in the derepression of *HSP12* and *HSP26*. Thus, Pop2 phosphorylation at S39 is important for Pop2 to repress the expression of stress response genes, *HSP12* and *HSP26*, in the presence of glucose. Our results suggest that Pho85-dependent phosphorylation of Pop2 is a part of the glucose sensing system in yeast.

## P-16-006
## Studies on the 2-thiouridine-tRNA damage induced in the *Saccharomyces cerevisiae* yeast cells under oxidative stress conditions

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The 5-substituted 2-thiouridines (R5S2U) are present in a wobble (first) position of anticodon of specific transfer ribonucleic acids ($tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Gln}$). This thio-modification is widespread in cells of organisms belonging to all domains of life and plays an important role in proper decoding of genetic information in a process of protein biosynthesis. Our previous model studies carried out on R5S2U in oxidizing environment revealed the efficient conversion of R5S2U to its desulfured derivatives, a deprived of sulfur atom 5-substituted 4-pyrimidinone riboside (R5H2U), the main product of the reaction, and 5-substituted uridine (R5U), R5S2U → R5H2U+R5U. A similar transformation was observed at R5S2U-RNA oligonucleotide level and at natural mcm5S2U-$tRNA^{Glu}$. In the presented studies, we show that oxidative transformation of mcm5S2U-$tRNA^{Glu}$ occurs not only in a test tube but also in living cells, e.g. yeast cells. Experiments were carried out in *Saccharomyces cerevisiae,* INV Sc1 strain, cultured under oxidative stress conditions (series of concentrations of $H_2O_2$, $NaAsO_2$ or NaClO). Firstly, we monitored the presence of mcm5S2U modification in yeast tRNA via the γ-toxin assay. γ-Toxin endonuclease from *Kluyveromyces lactis* recognizes the mcm5S2U modification in the anticodon of tRNA and cleaves it in this position. We found, the amount of γ-toxin cleavage product decreased as the concentration of the oxidizing agent increased, probably due to the occurrence of oxidative damages of mcm5S2U at tRNA anticodon. The most important result was confirmation of the presence of S2U-tRNA desulfuration products (mcm5H2U and mcm5U) in the mixture of tRNA-derived nucleosides by LC-MS/MS analysis. Acknowledgements for the financial support from The National Science Centre in Poland [UMO-2016/23/B/NZ1/02316] to M.S.

## P-16-007
## Lost in transition: defining the role of exoribonucleases in the shift between exponential and stationary phases

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The transition between exponential and stationary phase is a natural phenomenon for all bacteria and requires a massive readjustment of the bacterial transcriptome. Exoribonucleases are key enzymes in the transition between the two growth phases since they can rapidly degrade the RNA that is no longer necessary. In *Escherichia coli* there are three main exoribonucleases responsible for the RNA degradation: RNase II, RNase R and PNPase. RNase II and RNase R are both hydrolytic enzymes and belong to the same family, on the other hand, PNPase is a phosphorolytic enzyme that can also act as a polymerase. In this work we used RNA-Seq experiments to analyze the transcriptomic differences between the exponential and stationary phases of WT cells and deletion mutants for the different exoribonucleases (Δ*rnb*, Δ*rnr*, Δ*pnp* and Δ*rnb*Δ*rnr*). Overall when comparing the cells from exponential phase with the cells from stationary phase more than 1000 transcripts were differentially expressed, but only 491 core transcripts were common to all strains. There were some differences in the number and transcripts affected depending on the strain, suggesting that exoribonucleases influence the transition between these two growth phases differently. We also compare the effects of the absence of the hydrolytic degradation (RNase II/RNase R double mutant) with the absence of the phosphorolytic degradation (PNPase). It seems that deletion of PNPase leads to a higher transcriptomic change than even the deletion of both RNase II and RNase R. Interestingly, we also found that the RNase II/RNase R double mutant is similar to the RNase R single mutant in exponential phase while in stationary phase it seems to be closer to the RNase II single mutant. This is the first global transcriptomic work comparing the roles of exoribonucleases in the transition between exponential and stationary phase.

## P-16-008
## New roles for ribonucleases and sRNAs

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Gene Expression involves a concerted action of RNAs, ribonucleases and RNA chaperones. Recent developments have shown that some still have unexpected properties, which will be illustrated in this talk. The almost ubiquitous bacterial YbeY protein may serve catalytic and/or RNA chaperone/Hfq-like protective functions in RNA metabolism. We have biochemically and genetically characterized the YbeY ortholog of the legume symbiont *Sinorhizobium meliloti* (SmYbeY) and we have demonstrated that SmYbeY indistinctly cleaved single- and double-stranded RNA substrates and showed that YbeY is an endoribonuclease with unprecedented catalytic features, acting as a new silencing enzyme in riboregulation (Saramago et al, NAR 2017). Transcription termination is a critical step in the control of gene expression. One of the major termination mechanisms is mediated by Rho factor that promotes termination at the end of operons, but it can also terminate transcription within leader regions. We have discovered that the *Salmonella* SraL sRNA base-pairs with the 5′-UTR of *rho* protecting this transcript against premature transcription termination by its own protein Rho. Therefore


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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SraL sRNA interaction regulates "The Terminator". (Silva et al, PNAS, 2019). An enormous progress has been made in the understanding of the way RNases and sRNAs control and regulate processes in the cell; however, the versatility of these regulators is still full of surprises.

## P-16-009
## Searching for functional interactors of XRN2 in human cells

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An immensity of different factors is involved in the regulation of the post-transcriptional life of RNA molecules in eukaryotes. Processive exoribonucleases, like enzymes belonging to the XRN family of RNases, play a major role in eukaryotic RNA processing and turnover. Human XRN2 is a nuclear exoribonuclease that degrades 5′ monophosphorylated RNA species and its best documented role is the involvement in RNA polymerase II transcription termination by a "torpedo" mechanism. XRN2 was chosen for interaction analysis because knowledge about its functions is still very fragmentary while the methodology that we use has a strong potential to identify novel regulatory pathways and functional connections between proteins. It is based on genome wide siRNA screening which reveals synthetic lethal and synthetic rescue relationships among proteins. Our preliminary results indicate that once XRN2 is dysfunctional, the depletion of RBM7 – an RNA-binding subunit of the nuclear exosome targeting complex (NEXT) – is synthetically lethal in human cells. That fact raises the possibility that the NEXT/exosome supercomplex acts redundantly with XRN2 in nuclear RNA degradation and suggest a functional interaction between the 3′-to-5′ and 5′-to-3′ RNA decay pathways in the human nucleoplasm. Our progress toward understanding the molecular basis of the observed phenomenon will be presented.

# Cytoskeleton and molecular mechanisms of motility

## P-17-001
## HAX1 impact on collective cell migration, cell adhesion and cell shape is linked to the regulation of actomyosin contractility

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HAX1 protein is involved in the regulation of apoptosis, cell motility and calcium homeostasis. Its overexpression was reported in several tumors, including breast cancer. Results presented here demonstrate that HAX1 has an impact on collective migration of the epithelial cell layer, but not on single cell migration, which highlights the role of cell-cell contacts for the HAX1-mediated effect. Accordingly, it was shown that *HAX1* knockdown affects cell-cell junctions, substrate adhesion and epithelial cell layer integrity, including cell shape. Subsequently, we have demonstrated that these effects can be attributed to the modulation of actomyosin contractility. Blebbistatin, myosin II inhibitor, was shown to restore collective migration of HAX1-deficient cells, as well as proper cell shape, indicating that *HAX1* knockdown induces excessive actomyosin contractility. These results are in line with the previous reports showing that excessive contractility is detrimental to collective migration of the monolayer. Further analysis of the mechanisms responsible for these changes revealed the role of RhoA and septin signaling in modulating contractility. Septins, filament-forming GTPases, were characterized here as a novel HAX1-interacting factors by the HAX1-targeted immunoprecipitation and subsequent mass spectrometry analysis. Additionally, the results presented here include HAX1 impact on entosis, a type of non-apoptotic cell death, driven by excessive contractility. Overall, presented results suggest that HAX1 role in breast cancer progression may include collective invasion in the early phase of metastasis. This work was supported by the Polish National Science Center grants no. 2011/01/B/NZ1/03674 and 2014/14/M/NZ1/00437.

## P-17-002
## Nitric oxide participates in cold signalling via microfilaments in Arabidopsis

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The role of cytoskeleton as one of nitric oxide (NO) downstream targets is gaining the increasing recognition because of its involvement in plenty of NO-controlled processes in plants throughout the entire life cycle – starting from seed germination to pollination as well as (a)biotic stress tolerance. It has been shown in a number of studies that low temperature leads to significant changes in the organization of cytoskeleton structures. The aim this study was to investigate the influence of low temperature itself and in combination with NO donor or scavenger on the organization of actin cytoskeleton in living plant cells using *A. thaliana* (GFP-ABD2-GFP) line. We found that SNP (NO donor) stimulated differentiation processes such as the formation of numerous germs of root hairs with active growth. After cPTIO (NO scavenger) treatment the cell size increase (swelling) in transition and elongation zones of primary roots, induction of primordial formation of root hairs were observed. The exogenous NO donor (SNP) favours to microfilaments network reorganization, while both cold and NO scavenger (cPTIO) increase its randomization and fragmentation. We have found that not only the sparseness of actin network and microfilaments polymerization/depolymerization in cells of different zones of the root apex occurs, but actin filaments orientation changes also after cold treatment and combined treatment with low temperature and exogenous NO. The obtained results testify to the existence of a functional relationship between changes in the intracellular NO content and the organization of actin filaments when exposed to cold on the plant cell. This allows us to conclude that microfilaments are important intermediaries in the realization of cold effect on the plant cell and NO is involved in the cell response to the low temperatures by signaling through these cytoskeletal structures.

## P-17-003
## Intrinsic Fgf signaling is required for primordial germ cell migration in zebrafish

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Gametes – eggs and sperm – are derived from primordial germ cells (PGCs). As one of the first cell lineages specified, PGCs migrate across the embryo toward where the gonad forms during


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development. Previous study has revealed chemokine Cxcl12a as a guidance signal for vertebrate PGCs. Here, we report that fibroblast growth factor (Fgf) signaling is required for PGC migration in zebrafish. To determine the competence of PGCs to receive Fgf signals, we isolated PGCs and detected their RNA expression. Our results demonstrated the expressions of all five Fgf receptors (*fgfr1a*, *fgfr1b*, and *fgfr2-4*) in zebrafish PGCs while the *fgfr4* transcript was the most abundant. To assess the necessity of Fgf signaling by PGCs themselves, we performed loss-of-function experiments by introducing mRNA encoding a dominant negative form of Fgf receptors (*dn-fgfr3*) with the 3' UTR of *nanos3*, which inhibited its mRNA translation in the soma. In the control embryos, representative PGCs aligned at the blastoderm margin during early gastrulation (6 h post-fertilization, hpf) and accumulated at the gonadal ridge at the end of somitogenesis (24 hpf). In those embryos whose Fgf signaling in PGCs was repressed, however, some of PGCs were centralized toward the animal pole at 6 hpf and distributed ectopically throughout the embryo at 24 hpf. Our study illustrates the participation of intrinsic Fgf signaling in PGC migration *in vivo*. To our knowledge, it is the first *in vivo* evidence to show that Fgf signaling plays a direct role in the migration of vertebrate PGCs.

## P-17-004
## SH3BGRL3 binds myosin 1c and is involved in MDA-MB-231 cell migration and invasion

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SH3BGRL3 is a gene belonging to *SH3BGR* family, it is ubiquitously expressed and encodes for a 93 AA thioredoxin-like protein evolutionarily conserved. A proteomic study reported that SH3BGRL3 binds the cytoplasmatic domain of ERBB2 receptor. On this basis we performed immuno-staining experiments in FLAG-SH3BGRL3 transfected SKBR3 cell line that showed SH3BGRL3 and ERBB2 co-localization. Nonetheless, co-immunoprecipitation (Co-IP) of ERBB2 using FLAG-SH3BGRL3 as bait and vice versa was not achievable. Therefore, to investigate SH3BGRL3 potential interactors we performed Co-IP experiments from SKBR3 lysates transfected with FLAG-SH3BGRL3 followed by mass spectrometry analysis. The results revealed myosin 1c (Myo1c) as a candidate interactor. Subsequent Co-IP experiments followed by WB analysis validated the interaction between the two proteins. To map the interaction site we performed Co-IP using SKBR3 cells co-transfected with FLAG-SH3BGRL3 and HA tagged deletion mutants of Myo1c that showed SH3BGRL3 binding to the neck region of Myo1c. We also assessed if the binding was $Ca^{2+}$ dependent and the experiments showed that SH3BGRL3 binds Myo1c in the presence of $Ca^{2+}$. Myo1c is a motor protein involved in cell membrane dynamics. Thus we investigated SH3BGRL3 involvement in cell migration and invasion using MDA-MB-231 cell line. We transfected MDA-MB-231 cells with FLAG-SH3BGRL3 and performed immuno-staining of these proteins and actin-enriched structure followed by Co-IP experiments that showed co-localization and interaction of Myo1c and SH3BGRL3. Accordingly, we performed migration and invasion assays using Boyden chambers after silencing or not SH3BGRL3 expression by means of siRNAs. The results showed a statistically significant decrease in migration and invasion capacity of silenced cells respect to controls. Our data show that SH3BGRL3 binds Myo1c neck region in a $Ca^{2+}$ dependent way and that, in our model, is involved in cell migration and invasion.

## P-17-005
## Inhibition of Nrf2 reduced cell motility and proliferation in KRAS mutant lung cancer cells

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The most commonly mutated oncogene in NSCLC is KRAS, which is found in approximately 25% of lung adenocarcinoma. So far, there is no direct or indirect targeting drug of this small GTPase to treat KRAS mutant cancer cells, even if effort has been made to identify and develop such inhibitors of KRAS. In the present study, we found that KRAS mutant lung cancer cells were more sensitive to brusatol, Nrf2 inhibitor, than KRAS wild lung cancer cells, showing decreased cell proliferation, viability and motility. Brusatol suppressed Nrf2 downstream target genes including antioxidant, anti-inflammation enzymes and RhoA-FAK axis. RhoA-FAK axis plays role in cell migration through cytoskeleton changes. To verify the suppression of RhoA-FAK axis by Nrf2 regulates cell migration and invasion, wound healing and transwell invasion assays were performed after suppression of Nrf2 by treatment of brusatol, siRNA or exogenously induced Keap1. The results showed inhibition of Nrf2 reduced motility of cancer cells. Also, Suppression of Nrf2, compared with the control, decreased formation of stress fibers (F-actin cytoskeleton structures) observed in the cytoplasm. These results suggest that Nrf2/Keap1 pathway have an impact on cell motility and stress fiber formation through dysregulation of RhoA-ROCK1 signaling pathway in KRAS mutant lung cancer cells.

## P-17-006
## Structural and functional properties of neuronal tropomyosin isoforms

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Tropomyosin (Tpm) is the major actin-binding protein in all human cells, responsible for the discrimination of actin cytoskeleton functions. In neuronal tissue Tpm involved in such processes as neurogenesis, synapses development, neurons branching by controlling actin properties. However, the details of the mechanism for Tpm ability to cause different functional characteristics in neurons have not been revealed. In the present research, we consider the structure of neuronal Tpm isoforms and the characterization of their interactions with actin filament using a multidisciplinary approach. We showed that Tpm 1.12 has the lowest affinity to actin filament among others Tpm isoforms in neurons by the cosedimentation assay. The strength of the actin complexes with different neuronal isoform measured by temperature-dependent dissociation was comparable. We also obtained data on the interaction of actin-Tpm complex with protein partners (cofilin and myosin). Experiments carried out by *in vitro* motility essay defined that Tpm 1.7, 3.1 inhibits myosin activity and whereas Tpm 1.12 can enhance it. The Tpm 1.5, 1.6, 3.7 isoforms did not show any effect in this type of experiments.


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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Investigations devoted to isoforms structure were performed by using differential scanning calorimetry. All obtained curves of heat capacity had two main melting peaks. Although temperature and enthalpies of transitions were different between isoforms. Tpm 1.6 was the most stable isoform, whereas Tpm 1.5 had the lowest stability parameters. In particular, we established a relationship between Tpm isoform stability and the Tpm interaction strength of binding to actin. These results specify the part of Tpm which involved in interaction with actin. Thus, due to different structure and characteristics, Tpm isoforms can provide neuronal cell compartments with unique functions and morphology. The work was supported by RSF grant No. 18-74-10099.

## P-17-007
## Renal carcinoma cells respond to the surface topography by coordinated changes in cell and nucleus shape

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Cell contact guidance is a critical enabler of cancer dissemination. Cancer cells movement can be determined by the chemical structure of a substrate. But could they respond to the surface topography and be guided solely by it, without biochemical signals? To answer this question, renal carcinoma cells (Caki-1) were cultivated on the grooved substrates with 1.7 and 9.6 um period and on glass for 20 h, stained with Hoechst 33342 and phalloidin-FITC and studied via confocal microscopy. Changes in cell morphology were described through linear sizes ratio – membrane aspect ratio (MAR) and nuclear aspect ratio (NAR). It was shown that Caki-1 cells behave differently depending on the substrate topography. MAR was significantly higher if the cells were grown on patterned substrate (1,4±0,4, 1,8±0,7 and 2±1 for glass, substrate with 1.7 μm period and substrate with 9.6 μm period; $P < 0{,}01$). At the same time, the rearrangement of cytoskeleton and formation of stress fibers occurred only on the patterned substrates but not on the glass. A clear positive correlation between MAR and NAR was demonstrated for all types of substrates (R=0,47 for cells grown on glass, R=0,39 for both patterned substrates; $P < 0{,}01$), thus alterations in gene transcription could be expected. Therefore, the guidance of renal cancer cells by topography is possible. For better understanding of these processes 3D systems are needed, and this work is a necessary step in studying cell contact guidance in more complex 3D-models of the tumor microenvironment. The research was supported by the Russian science foundation (grant No. 17-75-30064).

## P-17-008
## α-Catulin is essential for maintaining proper cell morphology and migration in MDA-MB-231 breast cancer cell line

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α-Catulin is an αE-catenin like gene, often upregulated in different types of cancer. To study its exact function we used the highly invasive MDA-MB-231 breast cancer cell line in which α-catulin was depleted through siRNA treatment. α-catulin KD cells exhibited profound changes in both morphology and migration patterns. Spindle shape cells encompassing up to 50% of control MDA-MB-321 cell population were almost completely absent in α-catulin KD cells. In addition, α-catulin KD exhibited significantly decreased directionality of migration but increased speed. A detailed analysis of the cytoskeleton of α-catulin-depleted cells also showed significant re-organization with almost complete loss of radial stress fibers. Further analysis revealed changes in active RhoA distribution in α-catulin KD cells, supporting the role of α-catulin as an important RhoA/cell morphology regulator. For a better mechanistic understanding of the observed processes, the function of putative α-catulin interaction partners (αE-catenin, β-catenin) was further quarried through the use of co-immunoprecipitation, siRNA gene depletion and immunofluorescence techniques. In conclusion, α-catulin emerges as master regulator of both cell morphology and cell migration properties in cancer cells, working in concert with other members of the α-catenin family. As such it may be a potential target for future drug development aiming at cancer invasion and metastasis.

## P-17-009
## The motility of lung cancer cells is affected by HMGB1 protein through RAGE signaling

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Most cancer types are characterized by increased level of RAGE and its ligands which results in chronic inflammation, epithelial cell malignant transformation stimulating proliferation, invasion and metastasis. In lung cancer cell lines, the amount of RAGE is diminished but the receptor still keeps some functional levels. It was interesting for us to examine whether HMGB1 protein through RAGE signaling affects cell motility and more precisely what is the effect on the proteins as vinculin, vimentin and beta actin that are directly responsible for the cellular architecture and adhesion. Our findings suggest that in non-small cell lung cancer (NSLC) cells nevertheless that RAGE level is decreased, the HMGB1-RAGE interaction still plays a critical role for the stimulation of cell motility that may result in increased metastatic potential of these cells. The main targets are the structural organization proteins as vinculin and vimentin. HMGB1 trough RAGE signaling causes cytoskeletal reorganization that affects the properties of vimentin and vinculin and results in disturbed cell integrity and elevated cell migration. Conclusion: Our results suggest that HMGB1/RAGE signalling should be considered as an essential process for the development of non-small cell lung cancers with great invasive potential. Key words: lung cancer, HMGB1 protein, RAGE, vimentin, vinculin Acknowledgements: This work was supported by grant DN01/10 16.12.2016 of the Bulgarian National Science Fund.

## P-17-010
## Small heat shock proteins HspB1, HspB6 interact with contractile apparatus of striated muscle

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Small heat shock proteins (sHsp) is a family of ubiquitously expressed proteins with functions inside and outside of living cells. Family members HspB1 and HspB6 are highly expressed in different muscle cells and can regulate its function. But clear molecular mechanism (or mechanisms) of action is still absent. We still don't know for sure could sHsp directly interact with thin or thick filaments, and what exactly partners do they need.


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In the current work, we investigate their ability to interact with α-skeletal tropomyosin (Tm) alone and with actin filaments armored with Tm using the Biacore device, *in vitro* motility assay and cosedimentation technics. α-Tm is one of the two main Tm isoforms of cardiac and skeletal muscles contractile apparatus. We found that HspB1, HspB6 alone doesn't interact with Tm, while phosphomimicking mutant of HspB6 (S16D) or heterocomplexes, formed by HspB1-B6 interact with Tm, according to surface plasmon resonance readings. In the motility assay we observed the unexpected effect of S16D mutation. HspB6 WT inhibited the velocity of actin filament movements in the system with high $Ca^{2+}$ concentration, whereas S16D had no effect on velocity. Finally, in the cosedimentation assay both B6 and B6 S16D slightly increased tropomyosin affinity to the F-actin. Taking together the results indicate that sHsp indeed interact with contractile apparatus of striated (cardiac) muscles. Tropomyosin plays important role in that interaction while other partners are supposed. In smooth muscles phosphorylation of HspB6 results in muscle relaxation. The same effect is not observed for striated muscles, but according to our results, sHsp phosphorylation influence the actomyosin interaction and its affinity to tropomyosin. Such effect may be important in fine tuning of muscle activity. Further investigations are needed for complete elucidation of sHsp mechanism of action in striated muscles. This research was supported by the RSF grant #19-14-00005.

## P-17-011
## KCTD5 regulates focal adhesions dynamics through a mechanism involving calcium signaling

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Focal adhesions (FAs) are multiprotein structures linking the actin cytoskeleton and extracellular matrix. They confer the necessaries adhesion and strength to move the cell body in the direction of migration. For a directional cell movement, FAs must be assembled at the leading edge and disassembled at the rear of migrating cells in a coordinated manner through several molecular mechanisms highly dependent on $Ca^{2+}$ signaling. KCTD5 is a member of Potassium Channel Tetramerization Domain (KCTD) proteins that has been proposed as a putative adaptor for Cullin3-E3 ubiquitin ligase complex. However, its physiological role remains unclear. Here, we propose to determine whether KCTD5 participates in cell migration-associated mechanisms, focusing on FAs dynamics and $Ca^{2+}$ signaling. To study this, KCTD5 expression was knocked down in B16-F10 cells using the shRNA approach. Alternatively, CRISPR/Cas9-based KCTD5-knockout B16-F10 cells were used. Cell migration was evaluated using Transwell Boyden chambers. $Ca^{2+}$ was measured using the $Ca^{2+}$-sensitive Fura-2 probe. FAs were visualized by immunofluorescence against vinculin and were tracked in living cells using the construct mCherry-paxillin. Our results showed that KCTD5 knockdown and knockout increase the migratory ability of B16-F10 cells. Accordingly, the KCTD5-silencing decreased the FAs size and altered the FAs assembly rate. Moreover, KCTD5-silencing reduced the serum-induced intracellular $Ca^{2+}$ increases, in both $Ca^{2+}$-containing and $Ca^{2+}$-free media. Finally, we observed that ionomycin-induced intracellular $Ca^{2+}$ increase reverted the KCTD5 knockdown-promoted FAs size decrease. Altogether, these data suggest that KCTD5 participates in cell migration and FAs turnover of B16-F10 cells through a mechanism involving the intracellular $Ca^{2+}$ regulation. Acknowledgments: FONDECYT 3180556 (JC, OC), FONDECYT 1160518 (OC), MiNICAD (OC)

## P-17-012
## Foretinib in combination with lapatinib or gefitinib reduces invasion of melanoma cells

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The aim of the study was to establish the impact of selected drugs combinations on invasive abilities of melanoma cells. We recently demonstrated, that combination of foretinib, an inhibitor of MET (hepatocyte growth factor receptor), with gefitinib or lapatinib, inhibitors of EGFR (epidermal growth factor receptor) has a synergistic cytotoxic effect on melanoma cells. However, cells which do not undergo apoptosis under inhibitor treatment can still invade. Thus in this study we examined the influence of these drugs on invasion and migration abilities of melanoma cells. Additionally, its effect on cells isolated from melanoma patients was analysed. Experiments were performed on primary and metastatic melanoma cell lines. To investigate cell migration and invasion Transwell inserts, and wound healing assay were used. Cells viability in 3D environment was examined by XTT method, while invadopodia formation by immunocytochemistry. Level of pSrc was verified by Western blot. Proteolytic activity of cells was analyzed using gelatin-FITC degradation assay and gelatin zymography. Combination of used inhibitors diminished movement of cells in 2D and 3D conditions. The cells covered smaller distances after drugs treatment and to a lesser extent were able to overcome the barrier of the Transwell inserts. These inhibitors induced changes in formation of invadopodia and actin cytoskeleton organization. Their application also decreased the level of kinase responsible for active invadopodia formation – pSrc. Furthermore, we revealed that used drugs may lead to reduction of proteolytic activity of examined cells. In conclusion, our data support the idea that simultaneous targeting of EGFR and MET could be a promising therapeutic strategy inhibiting not only tumor cell growth, but also its metastasis. This project was funded by National Science Centre – OPUS 8 (No. 2014/15/B/NZ5/01467) and 15 (No. 2018/29/B/NZ5/00967).

## P-17-013
## Structural studies of interaction between actin-binding proteins and septins during asymmetric cell division

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The process of asymmetric division is necessary in the early stages of organism development, also to maintain a pool of stem cells and tissue homeostasis. Cytokinesis occurs due to contraction of the actomyosin ring and the formation of a septum. Septins are collected at the site of cell division, and at later stages of mitosis are involved in the formation of the actomyosin ring, they also surround it playing the role of a barrier. HOF1 is also a major contributor to the contractile ring and septum formation. But interconnections between HOF1, septin and actin are


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not known yet. For the conducting electron microscopy experiments HOF1, Bnr1 and septins proteins were expressed in E. coli and purified with usage of nickel affinity and ion exchange chromatography. It was shown that HOF1-Bnr1 complex binds to actin filaments and inhibits actin polymerization. In addition, HOF1 can also interact directly with actin. Besides it was shown that HOF1 can interact in different ways with septin filaments: either via Bnr1 or directly. Comparing the binding of HOF1 protein to actin and septin filaments, differences were found. On actin filaments HOF1 is localized chaotically, from different sides of actin, and by different parts. On the contrary, a certain orderliness can be traced on septin filaments: HOF1, both alone and in combination with Bnr1 protein, is always located just across the septin filaments, as well as alternating with every 150 A°. Thus, septins most likely have specific sites of interaction with HOF1 protein, unlike actin filaments. Microscopy of HOF1-Bnr1 protein was also performed with actin and septin filaments at the same time and a scheme of their simultaneous interaction was proposed. The study was carried out with the financial support of the RFBR in the framework of Scientific Project No. 18-34-00347.

## P-17-014
## Biomechanical properties of glioblastoma cells studied by atomic force microscopy

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Glioblastoma is a one of most deadly cancers, thus, understanding mechanisms governing its invasion is important for the development of novel treatment approaches. Mechanical properties of living cells belong to one of essential cues shown to play an important role in glioblastoma migration and metastasis. Various studies, carried so far, have shown that the main structure responsible for biomechanics of cells is a cytoskeleton, in particular, actin filaments. In our studies, we focused on nanomechanical properties of glioblastoma cells in relation to changes induced in actin filament organization upon cytochalasin D treatment. Two cell lines with distinct morphologies were chosen, namely, U118 and U138 possessing fibroblast and keratinocyte-like characteristics, respectively. Elastic properties of cells (Young's modulus) and F-actin organization in cells were obtained by applying atomic force and fluorescence microscopes. Results identify that these glioblastoma response to cytochalasin D (5 μg/mL) in a time (dose)-dependent manner resulted in both softening and stiffening of cells. Fibroblasts-like cells (U118) increase their deformability (Young's modulus decreases) after 10 min. of cytochalasin D incubation. As Young's modulus decreases for all probed indentations, we can postulate that cytochalasin D reorganization proceeds within a whole actin filament network. Keratinocyte-like U138 cells respond differently. For the same incubation time, there was no change in elasticity while increasing the time of cytochalasin D exposure to 30 min. induced stiffening of these cells. These finding are analogous to that recently published showing pronounced effect of cytochalasin D on fibroblasts and no effect for keratinocytes. Summarizing, elastic properties of fibroblast-like U118 are governed by actin filaments while their role in deformability of U138 is less significant. TZ acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

## P-17-015
## Changes in elasticity of prostate cancer cells depend on drug–tubulin binding mechanism and are coupled with microtubules organization

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To better understand the role of biophysical clues in chemotherapy, we investigated how members of three microtubules interacting agents groups affects cancer cells biomechanics and cytoskeleton organization. Studies were conducted for prostate Du145 cancer cells, a metastasis to brain. Chosen anti-tumor drugs were vinflunine (*vinca alkaloid*), docetaxel (*taxan*) and colchicine. Each of them affects MT organization differently. Cytotoxicity of drugs was evaluated with MTT assay after 72 h of incubation while counting of cells after 24, 48 and 72 h enabled to quantify proliferation rates. Results allow to choose optimal doses of antitumor drugs for further experiments. There were 100 and 750 nM for vinflunine, 1 and 5 nM for docetaxel and 0.1 and 0.5 nM for colchicine. Proliferation of cells treated with lower doses of drugs was depleted not earlier than after 72 h. Organization of microtubules, F- actin and nuclei was visualized using fluorescence microscopy after 72 h of anti-tumor drug incubation. Atomic force microscope (AFM) was applied to evaluate mechanics of cells after 24, 48 and 72 h. Additionally, after 72 h, elasticity maps of cells clusters were acquired to determine variability of elastic properties within individual cells. Nanomechanical probing of cellular properties demonstrated significant stiffening of cells after the vinflunine treatment. For colchicine stiffening was milder while after docetaxel treatment cells becomes more deformable and microtubules aggregation was observed. Results shows that, even though microtubules interacting agents deplete proliferation of prostate cancer cells at the same level, their impact on cancer cells elasticity and microtubules organization depends on mechanism of drug binding to tubulin. These findings suggest deeper studies on the correlation between nanomechanics of treated cells and the effectiveness of antitumor drug treatment. AK acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

## P-17-016
## Mutual interactions of skin cells in wound healing and melanoma progression

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Skin, the largest human organ, mainly participates in protection against external mechanical, chemical and biological factors. Its structure is composed of distinct cells forming three main layers, i.e. epidermis, dermis and hypodermis. Epidermis and dermis are separated by basal membrane, organization of which (in adult skin) results partially from the cooperative interaction between fibroblasts and keratinocytes through production of extracellular matrix proteins. On molecular and cellular levels, wound healing and melanoma progression are strongly distinct processes. However, one common feature is the disruption of skin integrity leading to migration and/or various cooperation between skin cells.


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In our studies, mechanical properties of key cells, namely fibroblasts and keratinocytes, were evaluated aiming at the quantification of elasticity changes induced by the direct physical interaction between neighboring cells of the same and different types. In wound healing, co-cultures composed of human skin fibroblasts (FBs) and keratinocytes (HaCaT) were applied while for melanoma progression both FB and HaCaT cells were mixed with melanoma cells (WM35, from radial growth phase). Results showed that FBs appeared to be more sensitive to the presence of neighboring cells as compared to HaCaT cells. The latter cells alter their elasticity only under the influence of melanoma cells. Melanoma cells were characterized by constant level of their deformability regardless of the presence of neighboring cells.

## P-17-017
## Glutathionylation of the single cysteine in vimentin impact assembly kinetics and the physical integrity of the mature filaments

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Vimentin intermediate filaments constitute a major element of the cytoskeleton in eukaryotic cells. They participate in many dynamic processes of the cell due to their ability to rapidly assemble and disassemble. These complex structural rearrangements are controlled by posttranslational modifications such as phosphorylation. Using both hydrogen/deuterium exchange and atomic force microscopy, we investigate now the role of redox modifications of the single cysteine ($Cys^{328}$) of vimentin. We analysed how both nitrosylation and glutathionylation at $Cys^{328}$ affect the assembly kinetics of vimentin tetramers into filaments. For either modification, the lateral association of tetramers into unit-length-filaments is not affected. However, while $Cys^{328}$-nitrosylation has a moderate impact on filament elongation, $Cys^{328}$-glutathionylation essentially impedes longitudinal annealing of unit-length-filaments into extended filaments. Vice versa, glutathionylation of mature unmodified vimentin filaments causes their extensive fragmentation. Subsequent reduction of the glutathionylated vimentin filament fragments restores formation of extended filaments. Thus, we show that the modification of the single cysteine in vimentin has the potential to mobilize the subunits of vimentin intermediate filaments and to modify the kinetics of their reassembly. Hence, we discovered a potential molecular switch, operated by basic redox-controlled cellular processes, for the *in vivo* regulation of the dynamics of intermediate filament rearrangements.

## P-17-018
## Role of unconventional myosin VI in regulation of AKAP9 -PKA pathway in muscles and myogenic cells

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Myosin VI (MVI) is a unique unconventional actin-based motor involved in a panoply of cellular functions. Its diverse roles are mediated by interactions with a number of different binding partners that are believed to be tissue/cell specific. Our previous data revealed that MVI could play significant role(s) in muscle and myogenic cells by its involvement in the PKA pathway by direct interaction with AKAP9 (A-kinase anchoring protein 9). Moreover, there is an evidence that MVI globular tail domain is also directly phosphorylated by PKA kinase. That is why understanding molecular mechanisms of MVI-AKAP9-PKA interaction and its role in muscle function and myoblast differentiation seems to be an important task. We observed an increase in PKA kinase level in hindlimb muscles of P0 (newborn) mice not synthesizing MVI (Snell's waltzer, SV), natural MVI knockouts. The increase was accompanied by a decrease in the level of phosphorylated (active) PKA form. Moreover, the amounts of CREB protein and phosphoCREB, the transcription factor, which is activated by PKA phosphorylation on Ser133, were also lower in P0 SV mice. These results indicate that MVI participates in regulation of the PKA activity in muscles and therefore could be engaged in muscle function. Further studies on functional significance of the PKA-induced MVI phosphorylation as well as mechanisms of regulation by MVI of the AKAP9-PKA complex in muscles and myoblasts are in progress.

## P-17-019
## Is myosin VI involved in regulation of skeletal muscle metabolism?

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Myosin VI (MVI) is a protein which belongs to the group of unconventional myosins (*i.e.* not forming filaments). MVI plays a role in many cellular processes associated with the actin cytoskeleton such as cell migration, adhesion, maintenance of the Golgi apparatus, endocytosis, autophagy, and in gene transcription. Interestingly, this protein has been also found to be present in striated muscle. Previously, our group showed that in skeletal muscle MVI localized to the sarcoplasmic reticulum (SR), muscle nuclei and postsynaptic region of the neuromuscular junction. However, the knowledge on MVI involvement in the muscle functions is still very limited. This encouraged us to examine the role of MVI in skeletal muscle functioning. In order to achieve this goal, we used hindlimb muscle of P0 nurslings and adult mice lacking MVI (MVI-KO; *Snell's waltzer* mice). These mice have spontaneous mutation within *Myo6* gene which prevents from MVI synthesis, and therefore are considered as natural MVI knockout animals. We observed symptoms of hypertrophy, especially in gastrocnemius muscle (GM; composed predominantly of slow-type myofibers) and fibrosis. In order to examine whether MVI could be involved in regulation muscle metabolism, we performed studies on GM muscles from MVI-KO mice and wild type littermates (WT). Analysis of the levels of ATP and cAMP revealed significant changes in the content of these compounds, so crucial for cell/muscle metabolism. Also, electron microscopy analysis demonstrated the aberrations in mitochondria morphology and presence of tubular aggregates indicative of aberrations in the mitochondria and SR functions, respectively. In our opinion this data suggest that MVI could be indeed involved in the muscle metabolism and regulation of the related signal transduction pathways. This work was supported by the grant no. 2017/27/B/NZ3/01984 from National Science Centre, Poland


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## P-17-020
## Mutation in the dystrophin-encoding gene affects myoblast motility in the mdx mouse model of Duchenne muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) is the most common, inherited, X-linked recessive disorder caused by lack of dystrophin due to mutations in the DMD gene. DMD patients suffer from progressive muscle weakness and wasting leading to premature death. Dystrophin is a cytoskeletal protein, which links actin cytoskeleton with intracellular and transmembrane proteins assembled into the dystrophin-associated protein complex (DAP) and further with extracellular matrix. This complex set of interactions is important for the maintenance of a proper muscle fibre structure and function. Importantly, recently published data clearly show that DMD mutations can cause severe changes in undifferentiated muscle cells (myoblasts) and that may have an important role in the DMD pathology. In our experiments we used immortalised and primary myoblasts derived from dystrophin-positive and dystrophin deficient (mdx) mice. Using the random motility assay we found that dystrophic myoblasts move significantly slower than their wild type counterparts. Moreover, the dystrophic cells motility was dramatically slowed down under starvation conditions (decreased serum concentration in the growth medium) while dystrophin-positive myoblasts were completely insensitive to serum starvation. Finally, phosphorylated focal adhesion kinase (pFAK) levels were substantially reduced in dystrophic myoblast compared to the controls while unconventional myosin VI content was increased in mdx cells. These data indicate that the mdx DMD mutation affects cellular adhesion processes in myoblast, again contradicting the notion of abnormalities occurring in differentiated muscle cells only. Given the critical role of myoblasts in muscle regeneration and the need for these cells to migrate efficiently into the site of damage, this abnormality might affect dystrophic muscle regeneration and contribute to DMD pathology. This work was supported by the National Science Centre grant 2013/11/B/N23/01573.

## P-17-021
## Dynamics of integrin-linked kinase during the rearrangement of contractile apparatus in rat neonatal cardiomyocytes

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Integrin-linked kinase (ILK) is a ubiquitous protein-kinase linking the extracellular matrix (ECM) to intracellular signaling pathways. In the heart, ILK binds the cytoplasmic tail of $\beta_1$-integrin at the sarcomere-attached costamere complexes to transduce biomechanical signals from ECM into biochemical signals, regulating cardiac growth, contractility, and repair. Our previous data indicate the role of ECM in regulation of myofibril formation during the rearrangement of contractile apparatus in cardiomyocytes in culture. This rearrangement involves the conversion of typical myofibrils into the structures of non-muscle type due to the absence of ECM (conversion stage) followed by synthesis of ECM proteins and subsequent recovery of myofibrillar apparatus (recovery stage). This study was aimed to investigate the dynamics of ILK during the rearrangement of contractile apparatus in rat neonatal cardiomyocytes with Western-blot and immunofluorescent staining to correlate these data with ECM alteration. Here we show that, in freshly-isolated cardiomyocytes in the absence of extracellular matrix, ILK is localized in perinuclear area as individual inclusions. At the conversion stage accompanied by the extensive ECM formation, ILK is distributed uniformly within the cells. The recovery stage is characterized by the elevation of ILK level and its colocalization with sarcomeres. The results obtained suggest that the nascent ECM may stimulate formation of cell-matrix interactions with the redistribution of ILK due to its recruitment to adhesion sites. The appropriate ILK linking with integrins at focal adhesions may induce signal pathways resulting in the recovery of myofibrils and the engagement of ILK in formation of new costamere complexes, accounting for ILK elevation and sarcomere colocalization. The study is supported by the Russian Science Foundation (grant 18-74-00129).

## P-17-022
## Myosin VI interaction with nucleolar proteins

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Myosin VI is a unique actin-based motor protein moving towards the minus end of actin filaments, in the opposite direction than other known myosin. This unique molecular motor is involved in a range of cellular processes including cell migration and adhesion, endocytosis, intracellular trafficking, cytokinesis and, as it was quite recently shown, in gene expression. Myosin VI in the nucleus localizes to several nuclear compartments and interacts with numerous proteins involved in nuclear functions. Among the identified putative partners was nucleolin, a major nucleolar protein implicated in pre-rRNA transcription and ribosome assembly. Additionally, we showed that other nucleolar proteins known to localize to different subnucleolar domains also interacted with MVI. These included: FBL (fibrillarin), denoting dense fibrillar component (DFC), UBF (Upstream Binding factor) denoting fibrillar center (FC) and B23 denoting globular component (GC). The presence of MVI in all nucleolar compartments was also confirmed by the immunogold technique. To further analyze functional significance of interaction of MVI with the proteins involved in nucleolar structure and function, we used the stable cell line with MVI knockdown (MVI-KD) and control scrambled cells (scr). The cells were then cultured in the presence and absence of actinomycin D (ActD) in a concentration inhibiting the activity of RNA polymerase 1 only. Depletion of MVI caused changes in the localization pattern of all three examined proteins with respect to scr cells thus confirming functional significance of these interactions. Similar changes were observed in MVI-KD cells but not in scr cells after ActD treatment. These results indicate importance of interaction of MVI with nucleolar proteins and suggest that MVI could be involved in organization and function of nucleolus.

## P-17-023
## Insights into the role of the WH2 domains of Spire at the single-molecule level

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The actin cytoskeleton dynamics in cells is tightly regulated by the action of multiple actin-binding proteins (ABPs). Among


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them, there is a large family of proteins that are responsible for promoting the formation of the nuclei and polymerization of actin. They are known as actin nucleators and can be divided into three main subfamilies: Arp 2/3 complex, formins and WH2 domain-containing proteins. The last group is represented by Spire, a multi-domain protein having a tandem repeat of four WH2 domains. Although a significant progress has been made in the understanding of the molecular mechanism of action of Spire, its nucleation activity that accounts from the presence of WH2 domains remains elusive. Here, using a combination of single-molecule techniques (TIRF microscopy and Zero Mode Waveguides), we discover that WH2 domains of Spire alone are not sufficient for actin nucleation. In contrast to this, FH2 domains of formins that were tested in the same conditions were able to create actin nucleus. Altogether, our data provide new insights into the role of WH2 domains of Spire in terms of its actin nucleation activity.

## Rare diseases

### P-18-001
### Using yeast chorea-acanthocytosis model for drug screening

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Chorea-acanthocytosis (ChAc) is a fatal rare genetic neurodegenerative disease caused by mutations in *hVPS13A* gene, one of four *VPS13* genes in human. Mutations in *hVPS13B*, *hVPS13C* and *hVPS13D* are also implicated in human neurodegenerative disorders and effective cure for any of these diseases is lacking. *VPS13* genes are conserved from yeast to humans. Thus, yeast is a good model system to study function of Vps13 proteins, the effect of human mutations on cell physiology and to screen for suppressors of *vps13* mutations. In yeast, there is one *VPS13* gene and it is most homologous to *hVPS13A*. The deletion of *VPS13* gene (*vps13*Δ) in yeast impairs many functions such as intracellular trafficking, actin cytoskeleton organization and maintenance of mitochondrial DNA. Recently, we discovered that *vps13*Δ cells are hypersensitive to SDS. This novel and simple growth phenotype was useful for our genetic screen for multicopy suppressors of *vps13*Δ mutation. Now, we used SDS hypersensitivity phenotype for isolating chemical suppressors of *vps13*Δ. We performed a drug screen using Prestwick Chemical Library, a collection of 1280 chemical compounds, most of which is accepted for use in human. Based on the screen results and literature, we selected 7 substances, which suppress SDS hypersensitivity phenotype of *vps13*Δ, for further research. We analysed impact of these substances on other phenotypes of *vps13*Δ mutant, such as canavanine hypersensitivity, impaired Sna3 transport, mitochondrial DNA escape and impaired actin cytoskeleton organisation. Our work aims on selecting drugs with the highest therapeutic potential for studies on ChAc with use of cell lines and higher model organisms. Our findings may contribute in future to discovery of an effective therapy for ChAc and other diseases associated with *VPS13* genes. This study is funded by the National Science Centre Poland (UMO- 2015/19/B/NZ3/01515).

### P-18-002
### Determination of functionality of mutant AVPR2s by using pharmacological chaperones SR121463B and SR49059

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Protein synthesis, modification and degradation act significantly on protein homeostasis for cellular maintenance. If a newly synthesized protein has problems about proper folding due to mutations, it is mostly trapped by quality control system of the cell. Endoplasmic reticulum (ER) or Golgi apparatus are parts of this system. Therefore, mutated arginine vasopressin receptor 2 (AVPR2) proteins have been found trapped in mainly ER because of their improper folding. AVPR2 is a G protein coupled receptor (GPCR) and any mutation in this gene could be the cause of hereditary type of Diabetes insipidus (DI). DI is a rare disease which is characterized by an impaired water homeostasis because of inability to concentrate urine. Even though mutation has no effect on function of AVPR2, it could not reach to cell membrane where specifically works and trapped in ER or Golgi apparatus. From this view, it is thought that rescuing somehow of trapped receptor from control mechanism could be successful to make it functional again. Pharmacological chaperones such as SR121463B and SR49059 are cell permeable small molecules that specifically bind to target protein and help it to be correctly folded via stabilizing its native conformation. Our aim is to understand the rescue potential of SR121463B and SR49059 on some AVPR2 mutations which were introduced into literature by our group. To analyse functionality of mutant AVPR2s, we performed cAMP accumulation assay after the stimulation of cells with ligand via ALPHAScreen technology. We found that these two pharmacological chaperones have rescue potentials at different degrees according to the mutation types of AVPR2. In conclusion, determination of rescue potential of these kind of pharmacological chaperones which are specific to the ER trapped AVPR2s has many potential benefits on improvement of new treatment strategies. This study was supported by The Scientific and Technological Research Council of Turkey (SBAG Project No: 216S304). *The authors marked with an asterisk equally contributed to the work.

### P-18-003
### An isogenic cellular model for studying molecular mechanisms of carcinogenesis associated with familial MEN1 mutation

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The *MEN1*-associated carcinogenesis is observed in patients with multiple endocrine neoplasia type 1 (MEN1). It is an autosomal dominantly inherited disorder characterized as a tumor predisposition syndrome involving multiple cancerous and non-cancerous endocrine and neuroendocrine tumors with high penetrance increasing with age. Heterozygous *MEN1* gene mutations can be identified in 70–95% of patients with MEN1. The *MEN1* gene is ubiquitously expressed in endocrine and non-endocrine tissues and encodes menin protein involved in transcriptional regulation and DNA repair. Recent data demonstrates that MEN1 tumors show loss-of-heterozygosity (LOH), which leads to the complete loss of menin function. The frequency of LOH in MEN1 tumors differs in various organs. It has been suggested that biallelic


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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inactivation of *MEN1* gene is required for carcinogenesis, supporting its function as a tumor suppressor gene. However, no direct evidence has been provided. We propose two models of cancerous transformation origin in endocrine tissues with an inherited *MEN1* mutation: (i) subsequent loss of the wild-type allele is a driver of the neoplastic process, or (ii) primary transformation of the cell followed by inactivation of the second allele causes neoplastic transformation. To investigate this hypothesis we established induced pluripotent stem cell (iPSC) lines from patients with familial MEN1 syndrome. iPSCs were differentiated in endocrine and non-endocrine cell types to compare their tumorigenic potential *in vitro*. Considering the importance of genetic background in the elucidation of molecular mechanisms of carcinogenesis, isogenic control cell lines were generated using CRISPR/Cas9 genome editing. Preliminary data will be demonstrated and discussed. This work is supported by the Russian Foundation for Basic Research (grant 19-015-00209-A).

## P-18-004
## Approach towards stabilization of misfolded loss-of-function variants of human prolidase

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Prolidase is the only metalloenzyme in humans capable of hydrolyzing dipeptides with proline or hydroxyproline on its C-terminus. The catalytic activity of the enzyme is crucial for proper recycling of proline in the system and patients affected by the syndrome referred to as Prolidase Deficiency (PD) suffer due to a number of various clinical symptoms. PD is a very rare recessive hereditary disorder with an estimated occurrence less than 1 in 1,000,000 birth and no cure to PD is available so far. The cause of PD is mutation in PEPD gene coding the enzyme. Almost 30 different mutations were reported to date and eight of them leading to either substitution of deletion of single amino acids were recently characterized structurally. Three of the mutants described displayed a significant degree of structural disorder leading to almost complete loss of catalytic activity. In previous studies it was shown, that induction of Hsp70/90 expression in human fibroblasts leads to 20–40% rescue of prolidase activity depending on the nature of mutation. To investigate this effect in more details we decided to structurally characterize three partly disordered prolidase variants expressed in the absence and in the presence of Hsp70/90 homologues. In one of the variants (G278D) the side chain of the aspartate residue repulses and distorts the neighboring β-strand. If the variant was expressed in the presence of elevated levels of chaperones striking changes were observed and the resulting structure resembles the wild-type prolidase structure much more closely. Two more disordered variants were analyzed and just like in the previous studies the degree of stabilization was case dependent. Our studies show that proteins expressed in the presence of chaperonins fold more tightly and better resemble the structure of wild-type prolidase. We believe, that our findings will facilitate a new avenue leading ultimately to stabilization of misfolded loss-of-function proteins in affected patients.

## P-18-005
## Laboratory diagnosis of metachromatic leukodystrophy requires more than arylsulfatase A assay

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Metachromatic leukodystrophy (MLD) is a lysosomal glycosphingolipid storage disease caused by a deficiency of lysosomal enzyme arylsulfatase A (ASA) or its activator protein saposin B. Clinical and laboratory spectrum ranges from a severe fatal form to a milder adult onset form. The diagnosis usually made by leukocyte ASA assay. Residual enzyme activity does not correlate with the disease forms. We analyzed 29 patients suspected of childhood and adult onset forms of MLD and 40 family members using enzyme assay, sulfatide analysis, pseudodeficiency (PD) analysis, DEAE-cellulose ion-exchange chromatography, western blot analysis and DNA sequence analysis. ASA activity were found to be decreased in 20 of 29 patients. Residual enzyme activity did not correlate with the disease severity. 16 patients were diagnosed with MLD, 1 was diagnosed with Multiple Sulfatase Deficiency (MSD), 2 were diagnosed with PD, and the remaining 10 patients were diagnosed as having other leukodystrophies. Sulfatide excretion was detected in the urine of 16 MLD and 1 MSD patients but not in patients with PD mutations. DEAE cellulose chromatography distinguished MLD and MSD patients from one another. Eleven mutations were identified in ASA gene and 1 mutation in SUMF1 gene. All of the mutations caused amino acid changes in the evolutionarily conserved region of ASA protein. Western blot analysis of ASA in leukocyte homogenate of the patients revealed no protein in 4 patients. Combination of clinical and comprehensive biochemical and genetic analysis allowed for the correct diagnosis. Two MLD patients were diagnosed in the presymptomatic period. Other 2 MLD patients had bone marrow transplantation from healthy siblings. The present study contributes to the comprehensive differential diagnostic protocol of MLD and to the understanding of the biochemical and molecular basis of MLD. This information is essential for early diagnosis and the evaluation of the therapies specific for MLD.

## P-18-006
## Loss of mechanosensitivity causes skeletal muscle degeneration in LGMD2R

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Ultra-rare LGMD2R (MIM 601419) is a late onset disease with slow progression caused by a splice site mutation on desmin protein leading to interruption of lamin B binding domain. Even though mutant desmin is expressed at the same level as the control, there is skeletal muscle degeneration which could be caused by mechanical loading over years. To determine impaired mechanotransduction signaling due to loss of desmin-lamin B interaction, YAP as key regulator of skeletal muscle mechanotransduction is investigated. Since YAP translocates to nucleus under stress conditions, it was found that 37.5% of myonuclei


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were YAP positive in control skeletal muscle tissue while in patient it is reduced to 15.1%. Furthermore, cyclic stretch is applied to both patient's and control myoconverted fibroblasts. In resting state, patient's myotubes have 1.5 times higher phospho YAP which is the inactive form. After application of cyclic stretch, the amount of phospho YAP and total YAP were declined simultaneously in the patient. Moreover, the expression levels of some YAP target genes were analysed. They all responded to mechanical stretch by means of upregulation in the patient. However, the upregulation was not enough to catch up control. Taken as a whole, the data suggested reduced basal YAP activity in the patient, reduced ability of LGMD2R cells to challenge mechanical load although patient cells were still able to respond to a mechanical load. Interestingly, ankrd1 and S isoform of ankrd2 were upregulated both in static and stretch conditions in the patient. It is known that desmin and ankrd1 directly interact and mechanosensitivity caused by loss of desmin in smooth muscle might be compensated by upregulation of ankrd1 as a defense mechanism. Hence, elevation of ankrd1 and S isoform of ankrd2 may also reflect defense against loss of mechanosensitivity. The possible compensative mechanism in LGMD2R is under investigation. This study was funded by TUBITAK project no 214S174 to PD.

## P-18-007
## The role of sirtuin in the growth and encystation of *Acanthamoeba*

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*Acanthamoeba* are opportunistic human pathogens that cause granulomatous amoebic encephalitis, dermatitis, and amoebic keratitis. *Acanthamoeba* has a life cycle consisting of trophozoites and cysts. Under unfavorable conditions for proliferation, vegetative trophozoites are converted to cysts by a process called encystation, which prolongs the survival and transmission of *Acanthamoeba*. Thus, inhibition of encystation during the treatment of *Acanthamoeba* infections can lead to more favorable outcomes. During encystation of *Acanthamoeba*, the expression levels of encystation-mediating factors were upregulated. However, the regulatory mechanism in their expressions during the encystation remains unknown. Here, we identified a sirtuin-like protein (*A. castellanii* sirtuin 2, AcSir2) as a transcriptional regulator containing a highly conserved domain of the SIR2 family proteins. AcSir2 is highly expressed during encystation and is localized mainly in the nucleus of *A. castellanii*. AcSir2-overexpressing trophozoites showed increases in cell density, cell size, DNA contents, and mitochondrial membrane potential compared with those of controls. In contrast, the proliferation of *Acanthamoeba* trophozoites was suppressed by treatment with the sirtuin inhibitors, sirtinol and salermide. SIRT deacetylase activities of AcSir2 were determined in the nucleus extracts of AcSir2-overexpressing cells. In AcSir2-overexpressing cells, the rate of encystation was also significantly increased. In addition, treatment of encysting cells with sirtinol and salermide decreased mature cyst formation. The ultrastructural analysis in the encysting cells after treatment with sirtuin inhibitors revealed that sirtuin proteins are required for cyst walls construction during encystation of *Acanthamoeba*. Taken together, these results indicate that AcSir2 is a SIRT deacetylase and the regulation of AcSir2 expression itself is indeed important for growth and encystation of *Acanthamoeba*.

## P-18-008
## Functional evaluation of AVPR2 mutants causing nephrogenic diabetes insipidus

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Nephrogenic diabetes insipidus (NDI) is one of the rare kidney diseases. The disease is characterized by polydipsia and polyuria and may be inherited or acquired. Arginine vasopressin receptor type 2 (AVPR2) gene, which belongs to G protein-coupled receptor superfamily and expressed in the distal convoluted tubule and collecting ducts of the kidney, is responsible for 90% of the inherited form of the disease. In a healthy subject, AVPR2 responds to the arginine vasopressin hormone (AVP) which produced in the supraoptic and paraventricular nuclei regions of hypothalamus. After the binding of hormone to the receptor, the cyclic AMP (cAMP) volume increases and this increase leads to activation of protein kinase A (PKA). PKA promotes water intake by aquaporin 2 (AQP2) water channel. In some cases, AVPR2 gene mutations, which may lead to improper folding of the receptor proteins, may affect the water homeostasis. After all, mutant receptor protein may cause NDI. We aimed to analyses the cAMP accumulation changes after the stimulation with AVP and the functionality of several AVPR2 mutants causing NDI in Turkish families. For this purpose, G12E, V88M and R106C mutant constructs were introduced into the pLV2R, a human V2R expression plasmid, by PCR based site directed mutagenesis and restriction fragment replacement strategy and they were transiently expressed in COS-7 cells. COS-7 cells were stimulated with AVP (at different levels from 10 mM to 10 pM). Assessment of the cAMP accumulation were performed with cAMP assay via ALPHAScreen technology. According to our results, mutant AVPR2s showed reduced cAMP response at different levels. We believe that functional assessments of mutant AVPR2 proteins will ensure valuable information about understanding of molecular pathology of the disease. This study was supported by The Scientific and Technological Research Council of Turkey (SBAG Project No: 112S513 and FBA-2017-14395).

## P-18-009
## Characterization of the prevalence, functional consequences and disease associations of autoantibodies against the positive complement regulator properdin in patients with lupus nephritis

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Lupus nephritis (LN) is a common major organ manifestation of the Systemic lupus erythematosus (SLE). Complement is a key player in disease pathogenesis. It can be deregulated by autoantibodies (Abs), predisposing thus to renal injury. We screened for Abs against the components of the classical and alternative complement pathways by ELISA and identified previously unrecognized reactivity against the only positive regulator – properdin (factor P, FP) in 70.27%(52/74) of the patients with LN and


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1.33% (1/75) healthy controls. The interaction of purified IgG from these patients with FP was confirmed using surface plasmon resonance. The presence of anti-FP Abs significantly correlated with immunological markers of LN activity – ANA, anti-dsDNA and low levels of C3. Anti-FP Abs levels were significantly associated with renal histologic lesions, like endocapillary proliferation, glomerular leukocyte infiltration and glomerular sclerosis. High levels of these Abs correlated also with histological activity index. Combination of anti-FP and anti-C1q may enhance the specificity in the identification of patients with active, class IV and the most severe LN (class A according to BILAG Renal score). The clinical relevance of the anti-FP Abs could be explained by their capacity to potentiate the activity of the alternative pathway. We incubated apoptotic cells with human serum in alternative pathway condition (EGTA-Mg) +/- IgG from anti-FP positive LN patients. C3 fragments' deposits were enhanced in presence of anti-FP positive IgG, compared to IgG from healthy donors, measured by flow cytometry. This was not related to increased FP binding to the cells, but rather to a direct effect on the convertase. Therefore, anti-FP Abs have clear functional consequences, which may contribute to disease severity. *The authors marked with an asterisk equally contributed to the work.

## P-18-010
## Altered level of plasma exosomes in patients with Gaucher disease

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*In vitro* and animal models inhibition of lysosomal function was shown to increase the release of extracellular vesicles (EVs), particularly exosomes. Here we characterized plasma EVs from patients with lysosomal storage disorder, Gaucher disease (GD), coursed by mutations in the glucocerebrosidase (GBA) gene, encoding lysosomal enzyme. EVs were isolated from equal volumes (4 mL) of plasma from 7 GD patient (3 with N370S/L444P, 1 - N370S/c84dupG, 1 - N370S/G202R, 1 - N370S/W184R and 1 - N370S/N370S) and 8 controls via sequential centrifugation and evaluated by transmission electron microscopy (JEOL LEM 1011 (Jeol Ltd, Japan)). The EVs size and concentration were determined by nanoparticle tracking analysis (NTA) using the NTA NanoSight® LM10 (Malvern Instruments) analyzer. The EVs size distribution was evaluated by the method of dynamic light scattering (DLS) on a PLSS laser correlation spectrometer (INTOX MED LLC, Russia). Quantitative analysis of CD9 exosomal marker on the surface of the isolated EVs was carried out using Exo-FACS ready-to-use kit for plasma exosome analysis (Lonza, Estonia) by flow cytometry on CytoFlex instrument (Beckman Coulter, USA). Mass-spectrometry (shotgun analysis) of exosomal proteins was performed on Orbitrap Fusion Lumos MS (Thermo Scientific, USA). No difference were found between GD and controls in particle size and concentration evaluated by NTA. However, DLS showed increased proportion of exosomes (40–120 nm) in EVs fraction from GD patients compered to controls ($P < 0.001$). We also showed an increased EVs expressing exosomal marker CD9 in GD compared to controls ($P < 0.005$). Proteomic profiling of EVs proteins revealed an increase in expression levels for inflammation proteins in exosomes derived from plasma of GD patients. Lysosomal disfunction in patients with lysosomal storage disorders, particularly in patients with GD, might lead to an increase in level of plasma exosomes. Supported by RFBR 18-015-00262A.

## P-18-011
## The effect of mucopolysaccharides accumulation on the non-oxidative sulfur metabolism in mouse model of Sanfilippo syndrome type B

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Sanfilippo syndrome B is an autosomal recessive lysosomal storage disorder caused by a mutation in the gene encoding N-alpha-acetylglucosaminidase (NAGLU). The result of the *Naglu* gene mutation is the accumulation of glycosaminoglycans (GAGs), more specifically heparan sulfate. Therefore the objective of our research was to show that the accumulation of GAGs – sulfur containing compounds – affected the non-oxidative sulfur metabolism. In this project, we used the C57BL/6- mice *wild type* strain (WT) and mice with B6.129S6-Naglu$^{tm1Efn}$/J mutation in the *Naglu* gene (KO). The animals were bred, kept and sacrificed in accordance with the Polish Ethical Law, and the consent of the relevant Ethics Committees. Selected tissues (livers and kidneys) were collected from forty three animals of both sexes being at a similar age, i.e. about 3 month old. Animals were divided into four investigated groups: females (WT and KO) and males (WT and KO). In livers and kidneys the activity and expression of three sulfurtransferases: rhodanese (TST), 3-mercaptopyruvate sulfurtransferase (MPST) and cystathionine γ-lyase (CTH) were determined, as well as the level of sulfane sulfur and other low molecular sulfur-containing compounds (reduced and oxidized glutathione, cysteine and cystine). Obtained results showed that GAGs accumulation causes the increased level of cysteine in the KO livers (female and male), a decreased level of sulfane sulfur in the KO kidneys and livers of the male mice as well as sulfurtransferases activity (a decreased activity of MPST in the KO kidneys (male and female), CTH activity in the KO livers (male and female) and an increased activity of CTH in the KO kidneys (female)). The results confirm the assumed hypothesis and demonstrate that GAGs accumulation affect the non-oxidative sulfur metabolism. To date, no such observations have been described in the literature and they can contribute to a better understanding of Sanfilippo syndrome.

## P-18-012
## Clinical heterogeneity of Charcot-Marie-Tooth type 4A disease (CMT4A) corresponds with CMT genes mutation burden

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CMT4 disease is delineated as a special nosological entity due to the common genetic background i.e. mutations in *GDAP1* gene.


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In fact, CMT represents rather a wide spectrum of phenotypes beginning from early onset severe neuropathies to late onset mild mononeuropathies. In this study we have performed whole exome sequencing analysis (WES) in a group of 15 CMT affected patients, in whom previously pathogenic mutation in *GDAP1* gene was identified. Simultaneously, we have prepared an internal clinical score for analyzed patients. We have identified additional CMT rare sequence variants (average 4) in the examined patients. The patients manifesting with most wide (additional symptoms) or most severe phenotypes have been shown to carry, respectively additional sequence variants in the CMT genes. We conclude, that the combinatorial effect of rare CMT variants and main causative GDAP1 mutation is responsible for clinical heterogeneity of CMT4A disease. This study was supported by National Science Centre Poland grant no. 2016/23/B/NZ3/02035. *The authors marked with an asterisk equally contributed to the work.

## P-18-013
## A novel ARMS-PCR assay for screening MT-TL1 mutations causing mitochondrial cytopathies

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The epidemiological studies on mitochondrial disease suggest that the prevalence is at least 1 in 5000. Mitochondrial diseases can be related to mutations of genes encoded by either nuclear DNA or mitochondrial DNA. The majority of "mitochondrial cytopathies" are caused by point mutations in the tRNALeu (UUR) (*MTTL1*) gene. m.3243A>G mutation in the *MT-TL1* gene is described in 80% of MELAS patients. However, other rare mutations such as m.3256 C>T, m.3260 A>G, m.3271 T>C, m. 3291 T>C and m.3303 C>T are also identified in the same gene and associated with mitochondrial cytopathies. Within the scope of this study, a novel ARMS-PCR assay has been standardized to screen for the six described point mutations in the *MTTL1* gene. The descriptive sensitivity of this assay was aimed to be improved by combining it with HRM technology. Detection limits and the precision of this novel method is verified by using recombinant mtDNA standards, created by mutagenesis, carrying heteroplasmic mutations at different rates. Peripheral blood or skeletal muscle tissue samples of 219 patients, prediagnosed as mitochondrial cytopathies at the Departments of Neurology and Child Neurology Units of Hacettepe University Hospitals were screened using the developed method. The results of the screening procedure revealed two patients to have m.3243 A> G mutation, one patient with m.3290 T> C variation, and one patient carrying m.3303 C>T mutation. These mutations were further confirmed by DNA sequence analysis. This novel ARMS-PCR assay study and its results provide a new and technological "proof of concept" for future screening of other rarely observed mtDNA variations in patients and repositories.

## P-18-014
## Muscle-specific alterations of ionotropic and metabotropic purinergic receptors in primary myoblasts from dystrophic mdx mice

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Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutations in the DMD gene encoding dystrophin. It leads to progressive wasting of muscles and premature death. Despite intensive study pathophysiology of DMD is still elusive. Though it is commonly accepted that the dystrophin absence in myofibres causes the disease and the effects of DMD should not be observed in undifferentiated muscle cells, many data contradict this belief. Previously we have shown that immortalised myoblasts from the *mdx* mice model of DMD exhibit increased expression and activity of specific purinergic receptors, changed cellular energy metabolism, elevated store-operated $Ca^{2+}$ entry and altered $Ca^{2+}$ signalling. However, data obtained from a myoblast cell line does not allow studying potential differences between primary myoblasts isolated from specific muscles. To investigate the possibility of myoblast phenotypes being influenced by their muscle origin, we compared expression and activities of key purinergic receptors (P2X7 – ionotropic and P2Y2 – metabotropic) in myoblasts obtained from distinct limb muscles of wild type and mdx mice. We have found muscle type-dependent differences in activity, protein levels and intracellular distribution of these receptors. P2X7 content was elevated in mdx *tibialis anterior* (TA, predominantly fast-twitch muscle) but not in *soleus* (slow-twitch) while P2Y2 level was significantly increased in *soleus* but not in TA. We have also found s increased $Ca^{2+}$ fluxes in mdx myoblasts derived from distinct muscles upon stimulation with UTP (P2Y2 agonist) and ATP. These observations agree with muscle-specific effects of DMD mutation and confirm muscle-specific dystrophic abnormalities in undifferentiated muscle cells. These findings may be central to the development of effective treatments. This work was supported by the National Science Centre grant 2013/11/B/N23/01573 and the Polish Ministry of National Defense project "Kościuszko" no: 523/2017/DA.

## P-18-015
## A specific, sensitive, simplified and fast LC–MS/MS method for plasma Lyso-GB3 levels

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Fabry disease is an X linked hereditary metabolic disease that causes renal failure and many life-threatening complications as a result of alpha-galactosidase deficiency. The enzymatic deficiency causes to the lysosomal accumulation of globotriaosylceramide (Gb3), and the deacylated derivative of Gb3, globotriaosyl-sphingosine (Lyso-Gb3). Therefore, the measurement of plasma Lyso-Gb3 has become an important biomarker for diagnosis and as a therapeutic monitoring of enzyme replacement therapy. In this study we aimed to develop a simple, fast, cheap, underivatized LC-MS/MS method for measurement of plasma Lyso-GB3 levels.


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The chromatographic separation was operated on an Acquity UPLC C18 (50 mm × 2.1 mm, 1.7 μm) with gradient elution using %98 -%2 Water/Methanol containing 0.05% formic acid and methanol as the mobile phase at the flow rate of 0.4 mL/min and total run time was only 3 min. The mass quantification was carried on the multiple reaction monitoring (MRM) of the transitions of $m/z$ 786.2 → 282.1 for Lyso-GB3 and $m/z$ 624.2 → 282.1 for Lyso-GB2 (the internal standard), respectively. First of all we investigated the effect of liquid-liquid extraction solvents in terms of peak shape, peak response, matrix effect and recovery. In the scope of method validation parameters, selectivity, linearity, determination and quantitation limits, carryover effect, reproducibility, recovery, matrix effect, reference range verification and stability studies were carried out. The limit of detection was found as 0.0018 ng/mL and the limit of quantification was found as 0.0053 ng/mL. The intra-day and inter-day reproducibility studies are below 7% which is suitable for validation criteria. Recovery rate was found as 96–102% while matrix effect was found as 98–103%. At last, the validated method was successfully performed in 12 Fabry patients and 80 healthy individuals.

## P-18-016
## Identification of genomic predictive markers of lymphoma from mantle zone cells

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The mantle zone cell lymphoma (MCL) is a generalized lymphatic tumor characterized by an aggressive clinical course. MLCs comprise 3–10% of all types of Non-Hodgkin lymphoma (NHL), which corresponds to 4–7 cases per 1,000,000 population per year. Modern diagnostics of MCL is based on complex morphological, immunohistochemical, cytogenetic and molecular genetic methods. Until recently, the assessment of the prognosis for patients with MCL was based on the international prognostic index (IPI), morphological variant, level of proliferative activity, the presence of del17, etc. However, a multivariate analysis proved that only the presence of a mutation in the *TP53* gene affects the overall and non-progressive survival. In this study, exon sequencing (2–11) of the *TP53* gene was performed on 20 patients with MCL from the Russian population. For the first time in Russia, an analysis was conducted on the relationship between the presence of a mutation in the *TP53* gene and its response to therapy and the unfavorable prognosis of MCL. In 25% (5/20) cases mutations were detected in the *TP53* gene (5, 6, 7 exons). Using the FISH method (fluorescence in situ) in 3/5 patients with a mutation in the *TP53* gene, del17p (del + mut +) was simultaneously detected, and only in 2/5 the mutation (del-mut +) was detected. In one patient (1/20), del17p was detected, without a mutation in the *TP53* gene (del + mut-). Thus, there was no significant correlation between the detection of del17p and mutations in the *TP53* gene. Unlike the presence of del17p, detection of a mutation in the *TP53* gene is closely associated with a lack of response to therapy and the rapid disease progression. In the future, it is planned to identify mutations in the *TP53* gene, as a key factor in the unfavorable prognosis, for other non-Hodgkin's lymphomas (NHL). *The authors marked with an asterisk equally contributed to the work.

## P-18-017
## Heme oxygenase-1 modulates angiogenesis-related alterations in murine model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD), a debilitating genetic disease caused by the lack of dystrophin is characterized by progressive muscle weakening and degeneration. Interestingly, dystrophin is also expressed in endothelial cells (EC), suggesting that angiogenesis process might have an impact on DMD pathology. Based on our previous studies showing pro-angiogenic role of heme oxygenase-1 (HO-1) we aimed at the investigation of both angiogenesis status and the effect of HO-1 knockout in mdx mice, the murine model of DMD. Firstly, we showed that the level of several angiogenesis-related genes is changed in mdx mice in comparison to wild type (WT) counterparts, with, among others, pro-angiogenic Vegfa being strongly downregulated, as evidenced by RNA sequencing analysis. Furthermore, we noticed that Kdr mRNA expression is decreased in EC isolated from lungs of mdx vs. WT mice. Concomitantly, reduced retinal angiogenesis was observed in 6 days old mdx pups, as evinced by immunofluorescent staining of isolectin B4 suggesting impaired blood vessels formation. Moreover, by applying proteome-based angiogenesis array we showed that the level of pro- and anti-angiogenic proteins is significantly changed in mdx vs. WT mice as well as in double knockout (mdx/HO-1-/-) vs. mdx animals, implying possible role of HO-1 in angiogenesis alterations. Accordingly, functional studies utilizing murine model of hind limb ischemia (HLI) was performed and impaired neovascularization of mdx/HO-1-/- vs. mdx mice was visible. FACS analysis of different cell population 3 days after HLI revealed significant depletion of CD31+ ECs in mdx/HO-1-/- vs. mdx animals, accompanied by substantial decline in number of muscle satellite cells and severe inflammatory cells infiltration. In conclusions, we demonstrated that angiogenesis is altered in dystrophic mice and HO-1 might be proposed as a factor contributing to this process. Supported by grant No. 2016/21/B/NZ1/00293 (AL) from National Science Centre. *The authors marked with an asterisk equally contributed to the work.

## P-18-018
## Usefulness of systems medicine to identify genetic mechanisms underlying pathological phenotypes in rare disease patients

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The accurate diagnosis of rare disease patients with complex phenotypic clinical records is challenging. Despite recent advances in genomic sequencing and diagnosis, the pathological effects of many rare Copy Number Variations (CNVs) remain unresolved. In this work, principles of systems medicine are used to


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characterize the genetic and molecular bases underlying phenotypes associated with CNVs found in thousands of rare disease patients. To this end, we use heterogeneous networks made of different types of entities (CNVs, patients, phenotypes, genes and pathways) to model and study different kinds of phenotype-genotype or phenotype-phenotype (comorbidity) relationships. These approaches yield predictions of phenotype-pathway associations allowing to identify those genes, located in pathological CNVs, more likely involved in the symptoms manifested by the studied patient. The computational methodology developed in this work holds significant potential to facilitate the genetic diagnosis of novel rare clinical cases, and guiding clinicians in the search of more efficient treatments. [Our experimental work is supported by grants BIO2014-56092-R and DSF2016-78041-C2- 1-R (MINECO and FEDER) and P12-CTS-1507 and CTS-486 (Andalusian Government and FEDER) and funds from group BIO-267 (Andalusian Government). The "CIBER de Enfermedades Raras" is an initiative from the ISCIII (Spain). This communication has the support of a travel grant "Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech"].

## P-18-019
## Novel genetic variants in patients with DSD 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 susceptibility to testicular or ovarian cancer. To date there are more than 80 known genes involved in DSD and 960 candidates possibly implicated in gonadal development. The aim of the research was to identify novel DSD genetic variants using whole exome sequencing (WES). The WES was performed for 3 DSD (in one – 46XX, SRY- negative and two – 46XY, SRY+ positive) selected after clinical and cytogenetic investigation. NGS was performed on an Illumina HiSeq 4000 System. Read alignment was carried out using DRAGEN Germline Pipeline (Edico Genome). Sequencing reads were mapped to the human genome assembly GRCh37/hg19. Up to 800 variants (SNPs and indels with the population frequency <0,01) for each trio were obtained after filtering using VarSeq (Golden Helix). The remaining variants were annotated according to DSD-associated gene panel (Fan, 2017) and their implication in gonadal development inferred from published animal models, RNA-seq studies, and known pathways. Shortlisted SNPs were analyzed against PubMed, VEP, OVID, VarSome, GeneCards Human Gene Database. In UKR11 the 46XY, SRY+ patient, novel inherited from mother SNP (20:57429997-C>T) was annotated in GNAS gene, in UKR21 missense SNP maternal (X:139586222; Gly/Asp rs1184594888, no population frequency available) was annotated in SOX3, described as a likely pathogenic gene. In UKR29 46XX SRY- patient *de novo*, novel SNP (11:32413528 T/C, alternative splice site region) was annotated WT1 DSD association gene combined with new CNV (9:127253279-127265703, duplicate, *de novo*) includes NR5A1 gene controls the activity of several genes related to the development of the gonads was identified. The investigation of mutant proteins structure and functions is in progress.

## P-18-020
## Cytogenomic studies in females with gonadal dysgenesis and chromosome Y structural abnormalities

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Isodicentric chromosomes are among the structural abnormalities of the Y chromosome that are commonly identified in patients with wide range of phenotypes from males with spermatogenetic failure to mixed gonadal dysgenesis in women with signs of Turner syndrome. We investigated two cases of females with complete gonadal dysgenesis. Cytogenetic studies were performed on peripheral blood lymphocytes with further use of standard protocols of chromosomal analysis (GTG-banding, FISH- CEP, LSI). The first patient revealed a following karyotype: 47,X,idic (Y)(pter→q11.2:q11.2→pter), +mar.ishidic(Y)(q11.2)(SRY++; DYZ3++,DYZ1),+ Y(p11.1-q11.1) (DYZ3++). Opposite the second patient revealed karyotype with der(Y), one copy of the SRY signal, two copies of the Yq12 locus (DYZ1) and one copy of locus Yp11.1-q11.1 (DYZ3): 46,X,der(Y)(p11.32). ishder(Y)(p11.3-q12)(SRY+,DYZ1++, DYZ3+). Cytogenomic studies were performed on DNA extracted from peripheral blood samples by using the MicrogenChip 60K Array-CGH. We detected complex results as following: for the first patient – PAR1 duplication (Yp11.32p11.2)x2, PAR2 deletion (Yq12)x0; for the second patient – PAR1 deletion (Yp11.32p11.2)x0. We suggest that most functionally significant genes, such as *SRY, PPP2R3B, CRLF2, ZFY, RPS4Y1, TGIF2LY*, involved in duplication (first patient) and deletion (second patient) of PAR1 region can contribute to the development of DSD-phenotypes. It was shown that *SRY, ZFY* and *CRLF2* genes are strongly involved in gonadal development during embryogenesis. *PPP2R3B* and *RPS4Y1* may contribute to the development of Noonan and Turner syndromes respectively. It is also interesting to mention that *TSPY, FAM197Y, TTTY* gene families involved in revealed CNVs are transcripted in testis and involved in spermatogenesis. The further investigation of the revealed CNVs regions and implicated genes will give more comprehensive understanding of complex phenotypes for such patients.

# Signaling in brain cancer

## P-19-001
## Specific Aurora A kinase inhibitors, MK-5108 and MK-8745, induce apoptosis but not necroptosis in IMR-32 and CHP-134 neuroblastoma cells

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Aurora A kinase is highly expressed in multiple human tumors, including neuroblastoma. In this study, we investigated the cytotoxic potential of aurora A kinase, MK-5108 inhibitor in the regulation of apoptosis. MK-5108 treatment induced the expression of crucial apoptosis markers i.e. cleaved caspase-3 and cleaved-PARP proteins at 72 h in IMR-32 and CHP-134 cells. Additionally, the activities of caspase 3 and 7 were increased in MK-5108-treated cells, confirming the induction of apoptosis. To


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investigate the involvement of necroptosis, we assessed the expression and phosphorylation of key necroptosis-associated proteins i.e. RIP kinases and MLKL protein. We observed the elevated phosphorylation of RIP3 and significantly increased phosphorylation level of MLKL in IMR-32 cells. Although, in CHP-134, the phosphorylation level of RIP1 and RIP3 was markedly increased, there was no increase of MLKL phosphorylated form. To confirm the involvement of necroptosis in MK-5108-stimulated cell death, we used specific inhibitors of RIP1 and RIP3 kinases i.e. necrostatin-1 and GSK'872, respectively. No recovery of cytotoxic effect was observed in MK-5108 and necroptosis inhibitors-treated cells, as compared to MK-5108 used alone, proving a lack of contribution of necroptosis in the observed cytotoxic effect. Interestingly, inhibition of RIP1 and RIP3 kinases combined with using MK-5108 inhibitor decreased ATP level, advancing the observed cytotoxic effect, as compared to monotherapy with MK-5108 inhibitor. We report that similar results were also obtained for another aurora A kinase inhibitor, MK-8745. These studies demonstrate that pharmacological inhibition of aurora A kinase by small molecule inhibitors, MK-5108 and MK-8745, brings about cell death by apoptosis, but not necroptosis induction. The study was supported by grant no 15/2018 from Research Project Competitions for Young Researchers of the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University.

## P-19-002
## The protective effect of myricetin against oxidative stress and apoptosis induced by the fungicide Epoxiconazole in glioma cells

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Exposure to chemicals such as pesticides can lead to severe neurologic effects to humans. Due to their fat-soluble character, triazole fungicides including Epoxiconazole (EPX) can easily cross the blood-brain barrier and the placental barrier, thus the search for new strategies to reduce or even protect the toxic effects caused by these synthetic products became a necessity. The purpose of this study was to investigate the potential neuroprotective effects of Myricetin (Myr) a plant-derived flavonoid that exhibits diverse pharmacological properties on Epoxiconazole -induced apoptosis in F98 glioma cells. The cytotoxicity was monitored by cell viability, ROS generation, antioxidant enzymes activities, malondialdehyde (MDA) production, cell cycle arrest, cytoskeleton disruption and DNA fragmentation. The apoptosis was assessed through Annexin V FITC-PI, the measurement of the mitochondrial transmembrane potential ($\Delta\Psi m$) and caspases activation. We demonstrated that the pretreatment by different concentrations of Myr 2 h prior to EPX exposure significantly increased the survival of cells, inhibited the ROS generation, modulated the activities of catalase (CAT) and superoxide dismutase (SOD), reduced the MDA level, restored the arrest of cell cycle, the disruption of cytoskeleton organization and DNA fragmentation. The rate of apoptosis, the decrease of mitochondrial membrane potential, and caspases activation were also inhibited by Myr. Based on these results, Myr prevents F98 glioma cells from EPX-induced degeneration by inhibiting oxidative stress and apoptosis.

## P-19-003
## Characterization of the molecular effect of antipsychotic phenothiazines in glioma cells: Study of the cooperative potential with temozolomide

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Gliomas are tumors that derive from neuroglia stem or progenitor cells, among these glioblastoma (GBM) is the most common and aggressive primary brain tumor. Conventional therapy for GBM consists of tumor resection followed by radiotherapy and chemotherapy with temozolomide (TMZ), however, patients end up being resistant, mainly, due to a subpopulation of cells called Glioma Stem Cells (GSC). The need for more effective anti-cancer drugs has sparked a growing interest for drug repositioning, a safer and cheaper therapeutic strategy. In this line, the main objective of the present study is to evaluate the adjuvant potential of antipsychotic phenothiazines (trifluoperazine (TFP) and thioridazine (TRD)), drugs used in the treatment of schizophrenia, in GBM cell lines (U87MG, LN229 and U251). In addition, we aimed to study the role of the tumor suppressor FOXO3a in response to phenothiazines, as previous results of the research group demonstrate that these drugs inhibit FOXO3a nuclear export. First, the isobolographic analysis determined that two of the nine ratios tested (TMZ: TFP and TMZ: TRD) were always synergistic in U87MG and in U251 cells. Secondly, the clonogenic assay revealed that, the long term effect of phenothiazines was not permanent because the cancer cells did not lose the capacity to form colonies. Finally, it was clearly demonstrated that phenothiazines induced the nuclear import of FOXO3a and, consequently, its activation. These results are very promising and the basis of a clinical trial could be established.

## P-19-004
## Elucidating the potential synthetic lethality between SMARCA4 and SMARCA2 in human glioma cells

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SMARCA4/BRG1 and SMARCA2/BRM are two mutually exclusive ATPases of the SWI/SNF chromatin remodeling complex, known to be involved in transcriptional activation, as well as repression of genes. The *SMARCA4* gene is mutated in cancer and the dependence of SMARCA4-deficient cancers on SMARCA2 has been described in lung cancer cells. Inhibitors targeting a bromodomain of SMARCA2 have been developed and showed anti-tumor activity in cancer cells lacking a functional SMARCA4. The frequency of genetic alterations in these genes and their roles in gliomas are largely unknown. Gliomas are common brain tumors frequently displaying epigenetic


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alterations. Using next-generation targeted sequencing of 700-cancer related genes and epigenetic modifiers, we sequenced 202 glioma samples. We found genetic alterations in SMARCA4 in 7 glioblastomas. The newly detected mutations potentially affect SMARCA4 functions. Therefore, we sought to identify the effects of silencing of SMARCA2 and/ or SMARCA4 in human glioma cells. After determining the basal level of SMARCA4 mRNA within 8 different glioma cell lines, two established cell lines and one patient-derived glioma cell line were used for further experiments. Silencing with two different siRNA targeting different regions of SMARCA4 or SMARCA2 was performed in the cells. We tested the effects of PFI3, an inhibitor of the SMARCA family, on the survival of glioma cells transiently depleted of SMARCA4 and/ or SMARCA2. Our results show that effective knockdown gene expression *SMARCA4* and/ or *SMARCA2* (as identified by qPCR and western blotting) does not affect cell survival. Functional tests of the cells depleted of SMARCA4 or SMARCA2 were performed, including cell viability and cell proliferation assays, in the presence and absence of PFI3. In SMARCA4-deficient cells, PFI3 affected cell proliferation and slightly reduced survival. Studies were supported by the National Science Center grant Symfonia 3 2015/16/W/NZ2/00314.

## P-19-005
## The WWOX gene depletion impairs neural cell migration process and may disrupt brain development

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The loss of correct *WWOX* tumor suppressor expression is a common event associated with cancer promotion, progression and resistance to treatment. Moreover, the *WWOX* gene has been recently recognized as an important regulator of brain differentiation and maintenance. Aberrations in *WWOX* expression lead to severe developmental neuropathologies and premature death. To investigate molecular background of *WWOX* action in nerve tissue, we silenced its expression in human neural stem cell line hNSC and assessed its ability for 3D growth and differentiation. Furthermore, a global gene expression analysis was conducted with next generation sequencing (CAGE). The *WWOX* depleted cells completely lost a competence for growth in an extracellular matrix protein mixture scaffold. Interestingly, such a difference was not observed when the cells were cultured as a monolayer. Whole transcriptome sequencing revealed that *WWOX* expression in neural stem cells is associated with global changes in the regulation of a number of pivotal signaling pathways such as Notch, PI3K kinase, PDGF, Cadherin, Hedgehog and Endothelin. Our findings show that *WWOX* deprivation may lead to deficiency in cytoskeleton organization and interactions between cells and extracellular matrix proteins, which impairs correct tissue formation. *WWOX* downregulation is significantly associated with changes in the level of expression of genes involved in cell motility, adhesion, regulation of chromatin condensation and global cell signaling, what may suggests that WWOX protein is one of the master expression regulators in neural tissue and appropriate *WWOX* expression is required for hNSC maintenance and differentiation. The study was supported by the National Science Centre, Poland, grant no. 2015/17/D/NZ2/01989.

## P-19-006
## The role of tumor-derived granulocyte macrophage colony stimulating factor (GM-CSF/CSF2) in regulation of microglia-dependent invasion in gliomas

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Brain resident immune cells (microglia) and peripheral macrophages accumulate in malignant gliomas and constitute for 30–50% of the tumor mass. These immune cells are polarized by factors released by glioma and become the pro-invasive, immunosuppressive cells that support tumor progression. We have previously found that tumor-derived granulocyte macrophage colony stimulating factor (GM-CSF/CSF2) is a crucial factor controlling accumulation of microglia and macrophages in murine gliomas. The analysis of TCGA dataset revealed overexpression of the *CSF2* gene (encoding GM-CSF) in a set of mesenchymal glioblastomas (most aggressive WHO grade IV gliomas) and its association with high immune gene expression. To study the role of GM-CSF in microglia-stimulated glioma invasion, we used a co-culture system, which mimics microglia interactions with tumor cells. We silenced the expression of *CSF2* in glioma cells and found reduced microglia-dependent invasion of glioma cells. To translate those results into clinically relevant setting, we designed and tested humanized short peptides interfering with binding of GM-CSF to its receptor. Selected peptide effectively inhibited binding of GM-CSF to its receptor as demonstrated with different methods. We selected the non-cytotoxic peptides that potently blocked microglia-dependent glioma invasion in cell co-cultures. Blocking GM-CSF-receptor signaling pathway with a neutralizing antibody against a GM-CSF receptor also inhibited microglia-dependent invasion of glioma cells. Altogether, our results demonstrate that glioma-derived GM-CSF supports pro-tumorigenic polarization of microglia turning them into cells that facilitate glioma growth and shape the immune microenvironment. The study was supported by grant 2014/15/B/NZ3/04704 from The National Science Centre, Poland.

## P-19-007
## Histopathological parameters of the human glioblastoma multiforme progression are altered by vitamin C-deficiency

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Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor, with a median survival of 14.6 months. GBM is highly resistant to radio- and chemotherapy, and remains without cure; however, new strategies for treatment are sought. Vitamin C is a potent antioxidant and a cofactor for collagen synthesis; it has also been related to tumor progression and angiogenesis. Therefore, our aim was to study the effect of vitamin C deficiency on GBM progression. We generated a GBM model by the stereotactic injection of human GBM cells (U87-MG cells or C3 cells) in the subventricular zone of guinea pig brain. Then, animals received a control or a vitamin C-deficient diet for a period of 3 weeks, after which histopathological analysis and confocal and two-photon microscopy analysis were performed. We demonstrated that vitamin C-deficient condition reduced the glomeruloid vasculature (-39.5%), microglial infiltration (ILB4+, -48.0%) and collagen deposition (two-photon analysis) in U87-MG tumors. Furthermore, we demonstrated that the tumor size (-68.6%), glomeruloid vasculature (-23.0%), microglial


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infiltration (-26.0%), tumor cell proliferation (PCNA+, -70.6%) and invasion were reduced in C3 tumors carried by vitamin C-deficient guinea pigs. Therefore, here we show that the vitamin C deficiency displayed an anti-tumoral effect in both GBM models analyzed; however, this effect was different depending on the tumor cell used for the GBM induction. C3 cells, a cell line with stem cell features isolated from a human subventricular GBM, showed higher sensitivity to vitamin C-deficient condition. Grants: Fondecyt 11170959, Fondecyt 1140477, CMA-BIOBIO ECM-12.

## P-19-008
## Analysis of transcription factor motifs discovered in glioma grade-specific enhancers

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The aim of our study was to verify if there are glioma cancer grade-specific transcription factor motifs in enhancer DNA sequences. Active enhancers were defined as non-promoter regions with H3K27ac peaks in a set of Polish patients' samples representing different histopathological WHO glioma grades: *pilocytic astrocytoma* (PA) and higher-grade gliomas (HGG) consisting of *diffuse astrocytoma* and *glioblastoma*. Thus, for a given sample, its active enhancers were assigned as either PA- or HGG-specific. For all samples, RNA-seq was also performed. In grade specific enhancer sequences the transcription factor (TF) motifs were searched using known motifs from HOCOMOCO database and *de novo*. We detected 259 motifs of 227 transcription factors in PA-specific enhancers and 219 motifs of 194 TFs in HGG. Out of the aforementioned motifs 195, were common for PA and HGG. TF motifs, as given within DNA sequences, were grouped by a hierarchical clustering method what did not separate grade specific motifs common to both grades from those characterising just one grade. However, it reflected a clear separation of motifs into TF families enriched peculiarly in PA or HGG, respectively. For grade-specific enhancers where TFs were detected, the target genes were assigned and among those, the differentially expressed (DE) genes were specified. In the set of DE target genes of PA-specific enhancers there were: nine overexpressed and eleven down expressed in PA samples. In the set of DE target genes of HGG-specific enhancers there were: seven overexpressed and four down expressed in HGG samples. The majority of DE target genes were confirmed to be cancer-related. They seem to have activator or repressor functions and are regulated by TFs whose motifs do not have a specific sequence that could divide them into PA- and HGG-specific in our samples.

## P-19-009
## A2B adenosine receptor promotes invasiveness of glioblastoma stem-like cells under hypoxic conditions

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Glioblastoma (GBM) is the most common and deadly malignant brain tumour, with a patient's median survival rate from 15 to 17 months. GBM contains a cellular subpopulation known as glioblastoma stem-like cells (GSCs) that persist in hypoxic niches and are capable to infiltrate into healthy brain tissue, considered as the responsibles for GBM recurrence. Hypoxic microenvironment increases extracellular adenosine levels, activating the low affinity A2B adenosine receptor ($A_{2B}AR$) in several cellular models. Adenosine through $A_{2B}AR$ is capable to modulate the invasiveness in different cancer models, however, the role of $A_{2B}AR$ in the regulation of the migratory and invasive capacity of GSCs under hypoxic conditions is still unknown. The aim of this study is to understand the role of $A_{2B}AR$ in modulating the migratory/invasive capacity of GSCs under hypoxia. GSCs derived from U87MG (GSCs-U87MG) were cultured under normoxia (21% $O_2$) and hypoxia (0.1% $O_2$). MRS1754 was used as $A_{2B}AR$ antagonist. Protein and mRNA levels of TWIST, SNAIL, MMP9 and A2BAR were evaluated by western blot and RT-qPCR, respectively. MMPs activity of was evaluated by zymography assay. Adhesion assay was performed with fibronectin-coated plates and stained with violet-crystal. The migratory and invasive capacity of GSCs-U87MG was evaluated by transwell and transwell-Matrigel assay, respectively. Migratory and invasive capacity of GSCs increased under hypoxia conditions. $A_{2B}AR$ blockage decreased the adhesion, migratory and invasive capacity of GSCs, downregulating MMP-9 SNAIL, TWIST expression. In conclusion, extracellular adenosine through activation of $A_{2B}AR$ is able to promote the migratory and invasive capacity GSC-U87MG under hypoxic conditions. Targeting $A_{2B}AR$ can be an effective anti-invasive therapy for GBM to decrease the GSCs-dependent recurrence. Funding by FONDECYT No. 1160777 (C.Q), FONDECYT POSTDOCTORADO No. 3180621 (I.N) and CONICYT No. 21181983 (J.I.E)

## P-19-010
## Extracellular vesicles from CSF in Parkinson's disease: characterization and effect on the glioma cells proliferation

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Alpha-synuclein oligomers are believed to be the major neurotoxic agents in neurodegenerative process in Parkinson's disease (PD). The transmissivity of its neurotoxic forms by extracellular vesicles (EVs) is actively discussed. At the same time EVs depending on membrane lipid composition could accelerate alpha-synuclein aggregation. The aim of our study was to characterize the cerebrospinal fluid (CSF) derived EVs from PD patients (N=5) and to assess its effect on the human glioblastoma A172 cells proliferation. EVs were isolated from CSF by ultracentrifugation. Sizing and enumeration of EVs were evaluated by nanoparticle tracking analysis (NTA) with a NanoSight LM10 instrument (Malvern, UK) equipped with the NTA 2.3 analytical software. The presence of exosomal marker protein CD9 was estimated by flow cytometry with Exo-FACS kit (HansaBioMed, Estonia). EVs visualization and its morphology assessment were carried out on pooled samples using cryo-electron microscopy with a Titan Krios 60-300 TEM/STEM (FEI, USA)). For real-time monitoring of cells proliferation the xCELLigence system (RTCA-SP, ACEA Biosciences Inc.) was used. CSF-derived EVs of various shapes and size (100,75 $\pm$ 10,56 nm) with lipid bilayer were identified. In all cases, the presence of the exosomal marker protein CD9 on the membrane surface was shown. The influence


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of EVs on cells proliferation was not detected ($P > 0,05$). Taken together our findings indicate that CSF-derived EVs have various morphology and do not influence on glioma cells proliferation. The work was carried out with funds from the Russian Science Foundation (project No. 17-75-20159).

## P-19-011
## The equilibrative nucleoside transporters ENT1 and ENT2 modulate adenosine levels in glioblastoma stem-like cells (GSCs)

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Glioblastoma (GBM) is one of the most aggressive cancer types. This cerebral tumor has the worst prognosis, high recurrence, and low survival rates. Studies show that this is due to the presence of a cellular subpopulation with stem cell properties denominated stem cells or initiators of glioblastoma (GSCs). This subpopulation is the main responsible of GBM chemoresistance and recurrence, enhanced by increased adenosine signaling. GSCs produce higher adenosine levels and which is regulated by three different processes: production, degradation, and transport. Nucleoside transporters could be concentrative (CNTs) and/or equilibrative (ENTs), being ENT1 and ENT2 the main isoforms involved in the regulation of adenosine levels. The aim of this study is to determine the differences between CNTs, ENT1 and ENT2 expression and activity in different subpopulations of GSCs obtained from human biopsies. Primary cultures and GSCs enrichment were performed from tumor samples obtained from GBM patients. Protein and mRNA levels were measured by western blot and RT-qPCR, respectively. Transporters activity was performed by incorporation of [3H]-adenosine. Here, we identify Mes-GSCs and PN-GSCs by differentiated expression of CD44/ALDH1a3 and CD133/mir125b, respectively. Mes-GSCs present higher mRNA and protein levels of ENT1 and ENT2 in comparison to PN-GSCs. This is correlated with a higher activity of ENT1 and ENT2 in Mes-GSCs. In conclusion, transport adenosine in GSCs is mediated mainly by equilibrative nucleoside transporters. The ENT1 and ENT2 transporters modulate extracellular adenosine levels differentially between PN-GSCs and MES-GSCs. Funding: FONDECYT No. 1160777 (C.Q.M), N° 1171340 (R.S.M) and postdoctoral FONDECYT No. 3170851 (S.A)

# Synthetic biopolymers for biomedicine

## P-20-001
## Mucus enrichment with CYS domain from gel-forming mucins remodels the mucus protein matrix and hinders motile cell displacements

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Gel-forming mucins (GFMs) form the mucus protein matrix through both reversible and irreversible interactions. The CYS domain interrupts the central part of GFMs and is conserved in vertebrates. However its function remains poorly documented. Mucus from scraped colon of control wild-type (WT) and transgenic (Tg) mice expressing a recombinant molecule made of 12 consecutive CYS domains (rCYSx12) was studied. Mucus from cell cocultures at 5 ratios of HT29-MTX-E12 (MTX) cells with a MTX cell line stably transfected with an expressing vector for the rCYSx12 secretion (MTX-rCYSx12) were also used. PEG-modified particles of 200 nm and 1 μm were used to characterize the mucus matrix ex vivo. Particles were tracked and mean square displacement (MSD) curves were calculated from recorded particles trajectories in 10 fields. The obstruction scaling model was used to determine mucus mesh size from beads diffusion. The mucus permeability to 3 μm beads was determined in coculture by confocal imaging after 45 min sedimentation. Linearity and speed of motile cell displacements were determined from time-lapse microscopy movies. MSD revealed a reduced diffusivity by 60 and 47% for 1 μm and 200 nm particles, respectively in mucus from Tg and WT mouse. The averaged mucus mesh was 148 nm in mucus from WT mouse and 115 nm in mucus from Tg mouse. A significant decrease of mucus permeability to 3 μm beads was found with rCYSx12-enrichment in cell co-cultures. Motile cell trajectories were significantly more confined in a dose-dependent manner in the MTX-rCYSx12-enriched mucus. We found a significant 29 and 47% slowdown of bacterial speed in mucus enriched with rCYSx12 from mice and cell cocultures, respectively. Furthermore, more confined trajectories and a 46% slowdown of sperm cell displacements were observed in Tg mouse mucus compared to WT. Mucus enrichment with CYS domain from gel-forming mucins remodels the mucus protein matrix and hinders motile cell displacements.

## P-20-002
## Development of a polymeric nanomaterial for detection of MUC1 in biological fluids for possible applications in early diagnosis, tumor progression and treatment of cancer

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MUC1 is a glycoprotein whose overexpression has been shown to have a significant correlation with cancer cell proliferation, metabolism, invasion, metastasis, angiogenesis and chemoresistance. As such, it is not only used as a biomarker especially in breast and pancreatic cancers but also has been defined by the National Cancer Institute as the second best potential target out of 75 tumor-associated antigens for the development of cancer vaccine (cancer immunotherapy). Our research focuses on developing an advanced polymeric nanomaterial for MUC1 to be possibly used in cancer diagnosis, follow-up, and treatment. We synthesized a specific polymeric nanomaterial (p(HEMA)) with mini-emulsion polymerization technique and modified it with triethoxy-3-(2-imidazolin-1-yl)propylsilane (IMEO). Polymeric nanomaterial was characterized with several characterization methods with respect to size, shape, surface porosity, surface potential, elemental analysis, thermal characterization, crystallinity, surface area calculations. p(HEMA)-IMEO nanomaterials have 75,63 nm size, 2955,057 $m^2/g$ specific surface area, -34,76 mV surface potential at distilled water. This polymeric nanomaterial will be used as the biologically active layer of the electrochemical biosensor to determine MUC1 from biological fluids, for diagnosis, follow-up, and treatment. Surface thickness of the electrode and angle of contact were determined as 2875,16 Angström and 52,25° respectively. MUC1 recognition of the biosensor with this nanomaterial will be based on lectin affinity method. Specific lectin will be bind to p(HEMA)-IMEO as freshly and the interaction with MUC1 will be analysed with PalmSens. The performance of the biosensor will be tested comparing the results with reference MUC1 ELISA kit.


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-20-003
## Immobilized lysozyme for removal of bacterial lipopolysaccharide (endotoxin) from biological fluids

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In connection with the emergence of new antibiotic resistant strains of bacteria in the world, the problem of treatment of sepsis has become more acute. One of the key tasks in the treatment of severe sepsis is the ability to remove bacterial endotoxins from the patient's bloodstream. A high concentration of bacterial endotoxins in the human blood disrupts the normal functioning of the immune system and dramatically worsens the patient's condition. The removal of endotoxins is carried out in extracorporeal therapy procedures using sorbents. Thus, the development of new effective sorbents for the removal of endotoxin is an important task. Lysozyme is able to bind with the components of the surface of a bacterial cell, so we have suggested that a lysozyme based sorbent can potentially be able to bind bacterial lipopolysaccharides. The sorbent made by us contains 14–15 mg of a covalently immobilized lysozyme per 1 g of an insoluble polysaccharide matrix (cross-linked agarose). This sorbent shows high efficiency of endotoxin binding from solution: 50 mL of sorbent can remove up to 88% *Escherichia coli* endotoxin or 84% *Pseudomonas aeruginosa* endotoxin from a 500 mL solution with an endotoxin concentration of 500 EU / mL (50 ng / mL). The work was supported by the Federal Target Program "Research and Development in Priority Areas for the Development of the Russian Science and Technology Complex for 2014–2020", section "Development of new medical sorbents for extracorporeal treatment of sepsis with antimicrobial activity and ability to adsorb bacterial toxins" (application code 2017-14-576-0053-142; unique project ID RFMEFI57417X0181; agreement no. 14.574.21.0181).

## P-20-004
## Comparative analysis of DNA repair enzymes activity by fluorescent DNA probes

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Chemical modification of DNA leads to damages of genetic apparatus due to cytotoxic and mutagenic effect. It is known that oxidative stress inducing formation of DNA lesions causes accelerated development of degenerative processes and premature aging. The accumulation of damages in DNA during life leads to the development of cardiovascular, neurodegenerative and oncological diseases. Recognition and removal of non-bulky damaged nucleobases are mediated by the base excision repair pathway. It should be noted that a decrease in the functional activity of individual DNA repair enzymes accelerates the negative consequences. On the other hand, treatment of oncological diseases with chemo- and radiotherapy is the most important method of influencing the tumor process, by introducing damages in genomic DNA. In this case DNA repair enzymes cause a decrease in therapeutic effect due to preventing DNA damage accumulation. Therefore, functioning of enzymatic DNA repair system is one of the key factors, both in the process of tumour cell transformation and in the treatment of tumor diseases. One of the most urgent issues in this area is to create a sensitive method for determining the activity of some human DNA repair enzymes. The aim of the present work was to design high sensitive and specific DNA probes to determine the activity of several human DNA glycosylases (AAG, NEIL1, OGG1, UNG) and human AP-endonuclease APE1, which plays a key role in removing the most common lesions from genomic DNA. For this purpose, a series of fluorescent DNA probes containing various types of damaged DNA bases have been created. DNA probes were tested on purified enzymes and used to determine the activity of enzymes in lysates of human cancer cell lines. The obtained data revealed a high potential for using these fluorescent DNA probes to determine activity level of BER enzymes by analysis of blood cell extracts. The study was specifically funded by Russian Science Foundation grant 18-14-00135.

## P-20-005
## Nanostructured conjugates of DNA oligonucleotides and boron clusters as potential dual-action anti-EGFR and BNCT therapeutics

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DNA nanotechnology is a branch of technology that exploits the self-assembly ability of nucleic acids to construct nanostructures possessing specific properties. There are numerous potential applications of DNA nanostructures, including diagnostics and therapy of human disorders. Based on our previous studies on modification of nucleic acids with boron clusters, conjugates of the epidermal growth factor receptor (EGFR)-directed antisense DNA oligonucleotides with boron clusters [o-carborane, $C_2B_{10}H_{12}$; dodecacarborane, $B_{12}H_{12}^-$; and metallacarborane, [Fe $(C_2B_9H_{11})_2]^-$] were obtained and tested in antisense therapy and considered as model agents in BNCT therapy. In this communication we present the use of DNA-functionalized boron clusters (oligopods) as building blocks for construction of therapeutic nucleic acid nano-systems. Thus, tri-substituted o-carborane bis-functionalized with the EGFR-targeted sense or antisense oligonucleotides were obtained by a solid phase synthesis method. The complementary dipods self-assembled to form nano-structured complexes, which were visualized by non-denaturating polyacrylamide gel electrophoresis (PAGE), atomic force microscopy (AFM) and cryo-transmission electron microscopy (Cryo-TEM). Their EGFR-silencing activity, stability against exo- and endo-nucleases as well as melting properties were investigated. This research was financially supported by The National Science Centre in Poland [Grant number 2015/16/W/ST5/00413 for years 2015–2019].


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## P-20-006
## Synthesis and radiolabeling of PLGA encapsulated everolimus nanoparticles

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Treatment of advanced gynecologic cancers remains palliative in most of cases. Although systemic treatment has entered into the era of targeted drugs the antitumor properties of current therapies are still limited. Diagnosis of these cancer types accompanies to this. Cervix cancer (CC) and ovarian cancer (OC) are common gynecologic cancers in women. Mammalian target of rapamycin (mTOR) is a signaling pathway in mammal cells that coordinates important cell activities such as survival and proliferation of malignant cells. mTOR pathway emerges as an important therapeutic target in a wide range of tumor types, including cervical, ovarian cancers. Everolimus (EVE) is from family of mTOR inhibitor proteins and inhibits the aberrant activity of mTOR that is part of carcinogenesis in CC. It's reported that some OC tumor types shown response when treated with mTOR inhibitors including EVE. Poly(lactic-co-glycolic acid) (PLGA) have been extensively studied for a wide variety of drugs and biomedical applications due to their biocompatible and non-toxic properties. Radiolabeling of EVE may be initiative discovery of novel promising imaging agents. In current study, EVE is encapsulated with PLGA(EVE-PLGA) and radiolabeled with Technetium-99 (99mTc). Our ongoing effort is to investigate *in vitro* incorporation potentials of 99mTc labeled EVE and EVE-PLGA on CC and OC cell lines. EVE-PLGA were synthesized and characterized by Dynamic Light Scattering(DLS) and Scanning Electron Microscopy(SEM) analysis. Quality control studies of radiolabeled compounds were carried out by chromatographic methods. EVE-PLGA were synthesized approximately as 250 nm according to DLS and SEM.EVE and EVE-PLGA were radiolabeled in high yields. In this study 99mTc radiolabeled compounds synthesized as novel promising imaging agents for CC and OC diagnosis. Further studies are being planned on animal models of CC and OC. This study was supported by EU Scientific Research Projects Coordination Unit. Project No: 17-TIP-046 (ID: 109).

## P-20-007
## Fluorescent annexin A5 (ANXA5) fusions with EGFP and mScarlet for biomedical applications: construction, expression, purification, characterization

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Annexin A5 (hereinafter referred to as ANXA5) is a placental protein that supposedly downregulates blood coagulation. A major property of ANXA5 is its ability to bind to phosphatidylserine-containing cell membranes and form multimers on these surfaces. Although physiological functions of this molecule are still enigmatic, ANXA5 is widely used in biomedical research and technology as a marker of apoptosis, necrotic platelet activation and other forms of cell death. We aimed to develop a brightly fluorescent and photostable recombinant ANXA5 with a molecular weight of more than 60 kDa (to prevent kidney clearance during *in vivo* labeling). Using molecular cloning, we constructed two plasmids containing the ANXA5 protein sequence fused with EGFP protein and with mScarlet protein. ANXA5 fusion 6xHis-tagged proteins were expressed in bacterial system and purified by Ni-NTA Agarose affinity chromatography. Flow cytometry confirmed that the modified proteins rapidly (<1 min) and phosphatidylserine-specifically bound with high affinity to phospholipid vesicles and activated platelets in the presence of $Ca^{2+}$, similarly to FITC-labeled ANXA5. Absorbtion and fluorescence spectra of ANXA5-EGFP and ANXA5-mScarlet were identical to the unfused EGFP and mScarlet proteins spectra. Confocal microscopy of activated platelets confirmed exceptional brightness and photostability of these fusions. These molecules could be promising tools for *in vitro* and *in vivo* research of programmed cell death and thrombosis. This work was supported by grants 17-00-00140, 17-04-01309 and 18-34-20026 from the Russian Foundation for Basic Research.

## P-20-008
## Novel functionalized carbosilane dendrimers for applications as non-viral transfection tools

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Recently, the use of dendrimers as non-viral vectors for anticancer therapeutics has emerged. Cationic carbosilane dendrimers, are able to bind negatively charged nucleic acid molecules via electrostatic interactions with the positive charges on dendrimer periphery. These complexes, called dendriplexes, ensure not only the efficient internalization in different types of cells but also protection from degradation in systemic circulation. However, their use in the clinical practice is still limited due to the fact that transfection efficiency is sometimes low because of the inhibited release of DNA, and that they present high cytotoxicity caused by the positive charges. Herein, we present the functionalization of carbosilane dendrimers for increasing biocompatibility, decreasing cytotoxicity and dendriplex strength as well as controlling targeted delivery, which are key properties for use of these vectors as carriers of nucleic material. Cationic carbosilane dendrimers of different generations have been synthesized employing thiol-ene click chemistry and different strategies for quaternization. Functionalization was achieved using either amide covalent binding or thiol-ene click reactions. Finally, *in vitro* evaluation and comparison of the synthesized structures shed light on their biocompatibility and transfection capacity and that one showing the best therapeutic potential would be further modified to label antibodies and become a more accurate nanomedical tool. To sum up, new carbosilane structures with great potential to be used as efficient non-viral transfection tools were synthesized and characterized.


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## P-20-009
## Production of PHB from waste materials using *Bacillus megaterium* and development of electrospun composite nanomats

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Along with the increasing world population the amount and variety of organic pollutants are continually increasing. Plastic waste is one of the major problems of today's world. Bioplastics are microbially produced polymers that are deposited in the form of storage granules by microorganisms under different conditions. Biopolymers are considered as an alternative to the environmental pollution caused by plastics. Polyhydroxyalkanoates (PHAs) are biodegradable polymers of biological origin. PHAs suggested as a solution to environmental pollution and they are mechanically similar to various thermoplastics. Poly (3-hydroxybutyrate) (PHB) is the most studied and best characterized PHA derivative. PHB production from different microorganisms including *Bacillus megaterium* was reported by researchers. *Bacillus megaterium* is a gram-positive, endosporin-forming, rod-shaped bacterium which was found in the soil, has aerobic life span and considered as saprophytic. Collagen, the most abundant protein in animals, is a biopolymer that is present in all connective tissues of animals. As a result of its unique characteristics such as its biodegradability, weak antigenicity and its excellent biological compatibility, collagen is one of the most suitable biomaterial that can be used in biomedical areas. In this study *Bacillus megaterium* was screened for the production of PHB using fish industry waste materials. Fish skins and scales obtained from the local fish processing plant used for the isolation and characterization of collagen. Fatty waste material obtained from this process then used for the production of PHB. A nanofiber composite of collagen with PHB was prepared by electrospinning and characterized by FT-IR and SEM. Our results indicate that fish processing plant waste materials are excellent sources for the production of PHB. The obtained nanofiber mats displayed high potential to be used for various biomedical applications.

## P-20-010
## Apoptotic effects of folate-conjugated PLGA-PEG nanoparticles loaded with dual agents on HepG2 cells

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The use of nanoparticle (NP) as a drug delivery system to enhance the *in vivo* efficiency was shown by pharmaceutical and clinical researches. One of the properties of nanoparticles is their longevity in the blood allowing for NP accumulation in pathological sites. Folic acid as a targeting ligand, is broadly used to immobilize on the surface of nanosized polymeric carriers to deliver these NP into cells via receptor-mediated endocytosis. Poly (lactide-co-glycolide) block-poly(ethyleneglycol) – folate (PLGA-PEG-Folate, NP), loaded with the dual agents – ε-viniferine (EV) and vincristine sulfate (VS), was prepared by the nanoprecipitation method, as described previously. DSC analysis was performed on pure components, the final NP and on the physical mixture of the pure components and the folic acid. Pure components and the physical mixture exhibited a broad endothermal effect, ranging between 220 and 300 °C, corresponding to their dehydration. The dehydration endotherm disappeared in the thermogram of the NP, while the degradation peak appeared at 290 °C. The observed effect indicated decomposition of folate-conjugated PLGAbPEG NP. NP loaded with 10 μM EV1 μM VS inhibited cell viability (62%) compared to the free drug system treated cells (88,9%) (MTT test). Apoptosis was evaluated by FACS analysis, with Annexin V staining. After treatment of cells with combination of drugs used in the same concentration, the number of the early apoptotic cells increased up to 31% versus 15,3% viability of cells treated with the free drugs system. Consequently, using Folate-conjugated PLGAb-PEG NP, loaded with the drugs, enhanced the efficacy of cytotoxic and apoptotic effects in HepG2 cells as compared to the effects induced by the free drug system. Therefore, *in vitro* studies with these nanoparticles are already carried out in our laboratory using HepG2 cancer cells (Project No. 1406S313).

## P-20-011
## Opioid-like peptides alter the mechanics and electrostatics of biomimetic membranes

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The increasing application of peptides in the prevention and treatment of various diseases and conditions requires the detailed characterization of the peptide interaction mechanisms at cellular and biomembrane level. Here we study the influence of valorphins on the elasticity and electrical properties of biomimetic lipid membranes. These endogenous hemorphin-related peptides are released from sequentially hydrolyzed hemoglobin. With affinity for opioid receptors valorphins exhibit morphinomimetic properties. Revealing the governing principles involved in signaling and nerve pulse propagation is closely related to the investigation of the electrical properties of cell membranes. Furthermore, the stability and deformability of biomembranes believed to play a key role in many processes at cellular level, is related to the membrane rigidity, which is characterized by its bending elasticity. This physical property of biomembranes is in close relation to the physical mechanisms of biological processes in cells and living organisms. Flicker spectroscopy measurements on unilamellar lipid vesicles were performed in order to deduce the membrane bending rigidity of valorphin-containing samples. For probing the effect of valorphins on the electrical properties of lipid bilayers we measured their specific capacitance at 10ˆ(-4) mol/mol peptide-to-lipid ratio. Our results showed changes in the membrane capacitance in the presence of valorphins, thus providing evidences for possible alterations in the dielectric permittivity of the bilayer. The measurement of the lipid bilayer capacitance permits to evaluate the charging time scale of membranes as well as to better control the membrane-field interactions. Acknowledgements: Financial support of the National Science Fund of Bulgaria (Grant DN08-7/2016) is acknowledged.


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## P-20-012
## Peptide-protein conjugation in the context of targeted therapy: modeling and experiment

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Bioconjugation techniques are widely used in the area of imaging, drug delivery, mapping of protein interactions, etc. One particularly intriguing line of research is development of irreversible inhibitors for use in targeted therapy, including covalent peptidic ligands. In this context, it should be useful to develop a modeling technique to assess the feasibility of covalent binding and to predict possible changes in target protein structure in response to ligand binding. To develop such computational technique, we have chosen the model system reported by Yu et al. which consists of N-terminal SH3 domain from adapter protein Grb2 and its Sos1-derived peptide ligand containing non-native C-terminal residue (chloroacetyl lysine, X'). Using NMR spectroscopy, mass-spectrometry and other techniques, we have shown that the modified peptide initially binds to its target non-covalently before reacting with the proximal cysteine C32 at the binding site. We profiled the pH dependence of this reaction, demonstrating that it proceeds via the thiolate anion. Based on these experimental observations, we have designed an MD-based protocol to model the conjugation of Sos1X' with Grb2 N-SH3. Specifically, we have derived a set of force-field parameters necessary to model the non-native reactive amino acid, chloroacetyl lysine, as well as the resulting thioether linkage. We have also devised a procedure to model the formation of covalent bond while avoiding any significant perturbations to the macroscopic MD parameters. A series of MD simulations with the net length of 10 μs led to high-quality atomic models of Grb2 N-SH3 / Sos1X' conjugates. These models have been successfully validated using the experimental NMR chemical shift data. We believe that the new methodology presented in this report will help to develop new covalent peptide inhibitors targeted toward cell-surface receptors implicated in human diseases. This research was supported by RSF grant 15-14-20038. *The authors marked with an asterisk equally contributed to the work.

## P-20-013
## New chromogenic and fluorogenic substrates of glutamine specific peptidases

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Proteins with high content of Gln residues are often resistant to hydrolysis. For instance, complete hydrolysis of prolamins – major proteins of cereal seeds – is a problem due to repeated sequences with Pro- and Gln-rich motifs. Hydrolysis of prolamines in the human stomach "stops" at the step of large peptides. Some of these peptides are toxic to people predisposed to autoimmune disease of the gastrointestinal tract – celiac disease. Currently no drugs are available against this disease. Patients are prescribed to life-long refusal of food containing wheat, rye and barley. Treatment of celiac disease may be achieved by enzyme therapy using postglutamine-cleaving peptidases (PGCP). PGCP may also be used in processing of food raw materials for gluten-free foods. However, most of the known enzymes including human and mammalian digestive enzymes do not cleave Gln-rich peptides. The search for new PGCP is difficult because they are rare, but the key problem is the lack of reliable and sensitive compounds suitable for screening and detection of PGCP. The aim of this work was to create selective and effective Gln-containing substrates for different PGCP, including cysteine peptidases of the papain C1 family. General formula of synthesized substrates is Glp-Phe-Gln↓B, where B = pNA, AMC. Synthesis of the substrates was performed by fragment condensation. Hydrolysis of the obtained substrates was studied by plant enzymes papain, bromelain, ficin, lysosomal mammalian cathepsins B and L, and digestive cathepsin L of an insect stored product pest Tribolium castaneum. All tested substrates were cleaved by all cysteine peptidases. The important advantage of the proposed substrates to the commercially available Arg-containing substrates is the selectivity for the cysteine peptidases, and the ability to differentially test the activity of cysteine peptidases in complex multi-component natural mixtures. This work was supported by RFBR grants 17-54-61008 Egypt_a and 18-04-01221_a.

## P-20-014
## Human adipose-derived stem cells behaviour and cytoskeleton development in contact with electrospun fibrous gelatin materials enriched with magnetic nanoparticles

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Magnetic nanoparticles (MNPs) are currently used as bioactive cues for therapy in nanotechnology and medicine. Recently, incorporating MNPs in different polymeric materials designed for tissue engineering (TE) generated better biocompatibility and positive cell response in contact with the composites. Here, we tested electrospun fibrous biocomposites, based on fish gelatin, loaded with 1–2% MNPs exposed or not to a static magnetic field (FG_MNPs) for biocompatibility and potential to support cell adhesion, namely focal adhesions formation. Fibers were crosslinked with ethanolic glutaraldehyde to prevent dissolution in culture media. Fibers morphology and the homogenous distribution of MNPs within the fibers was shown in electron microscopy. After seeding human adipose-derived stem cells (hASCs) on FG_MNPs substrates, MTT and LDH assays were performed, together with confocal microscopy to visualize live and dead cell population. Additionally, immunolabeling for β-tubulin and paxillin, together with phalloidin-FITC staining were used to study in depth cell attachment to the substrates. No significant cytotoxic effect was reported for all tested compositions. Better cell viability and proliferation were found in contact with FG_MNPs 2%, as compared to FG control and composites with lower MNPs-content. Cell attachment was enhanced in the presence of MNPs, suggested by better developed F-actin


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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cytoskeleton. Interestingly, nucleus shape and cell morphology were found more elongated after 24 h of contact with magnetized FG_MNPs, suggesting a distinct cell behavior under magnetic field conditions. Moreover, β-tubulin network and paxillin contacts exhibited different arrangement in magnetized conditions, as compared to their distribution in cells grown in contact with non-magnetized FG_MNPs. In conclusion, FG_MNPs 2% good biocompatibility and properties for further magnetic field-related TE applications. This work was supported by UEFISCDI-PNIII-P1-1.2-PCCDI-2017-0782/REGMED. *The authors marked with an asterisk equally contributed to the work.

## P-20-015
## Bisphenol A, which has systemic effects as an endocrine disruptor, measurement optimization using chromatographic methods in the biological tissues and fluids

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Bisphenol A (BPA) is a high-production-volume industrial chemical mainly used in the production of polycarbonates and epoxy resins utilized in the manufacture of containers, bottles, toys, and medical devices. Toxicological studies on BPA indicate its negative health effects including endocrine-disrupting properties, even at low doses. The low BPA concentration is difficult to determine. Trace amounts of BPA can be present in solvents, laboratory vessels, and plastic equipment. Therefore, it is difficult to develop an analytical methodology characterized by high sensitivity and good reproducibility of results. Most commonly used for the analysis of bisphenols are High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) and Liquid Chromatography with electrospray tandem Mass Spectrometry (HPLC-MS/MS). However, there were not many studies, which simultaneously evaluated the two methods in real analyses. We modified a BFA measurement method on HPLC and HPLC-MS/MS devices to use in our study which we planned to show the effect of BPA on diabetes-related DNA methylation in zebrafish. In the study with HPLC-FLD, we used an excitation wavelength of 280 nm and an emission wavelength of 310 nm. We used acetonitrile and water as the mobile phase with a gradient method by reverse phase C18 column. Work with HPLC-MS/MS was performed by reverse phase liquid chromatography using gradient elution with acetonitrile-water-formic acid. And positive/negative Ions modes were used. The calibration curve for BFA in HPLC-FLD method was between 1–100 ng/mL concentration range, linear and R2 0.999. In HPLC-MS/MS method, in contrast to the literature, ions were displayed in positive mode. Due to this difference, the method development study is continuing. We believe that we can overcome the measurement challenge in the BFA by using two methods simultaneously and develop an effective measurement.

## P-20-016
## Novel thermoplastic polyurethane foams enriched with ZnO nanoparticles exhibit antibacterial effect for wound healing applications

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The development of biomaterials with low cytotoxicity and antimicrobial effect is a continuous concern in tissue engineering. Lately, zinc oxide (ZnO) antibacterial properties started to be of interest for designing biomaterials with intrinsic antiseptic effect for wound healing applications. In this study, we evaluated if the addition of different ZnO nanofiller concentrations (1, 2, 5 and 10 wt.%) improved the properties of novel thermoplastic polyurethane (TPU) materials in terms of biocompatibility and antibacterial effect. Human adipose-derived stem cells (hASCs) were cultivated in contact with TPU/ZnO nanocomposites for 7 days. During biocompatibility assessment, MTT assay was used for cell viability and proliferation evaluation, while nanocomposites' cytotoxic potential was tested by LDH assay, compared to a plastic control (TCPS) and a pure TPU foam. The ratio between live and dead cells in the constructs was visualized using Live/Dead staining. TPU/ZnO antimicrobial activity was evaluated against two gram-positive strains (*E. faecalis, S. aureus*) and two gram-negative bacteria (*E. coli, P. aeruginosa*). Overall, TPU/ZnO foams displayed low cytotoxic potential (varied proportionally to the ZnO content) and good biocompatibility. Highest cell proliferation was registered for TPU materials enriched with 2 and 5 wt.% ZnO, as compared to the controls. All TPU/ZnO foams were found to be active against all four tested biofilms, with the most statistically significant difference in antibacterial activity reported for TPU/ZnO 10 wt.%. However, the addition of 10% nanofiller inhibited cell development, suggesting that the appropriate ZnO dose to exert its antibacterial effect without negatively affecting cell growth and proliferation, should be carefully selected. In conclusion, flexible TPU/ZnO nanocomposite foams can be considered as potential active wound dressings for biomedical applications. This work was supported by UEFISCDI-PNIII-P1-1.2-PCCDI-2017-0782/REGMED. *The authors marked with an asterisk equally contributed to the work.

## P-20-017
## 3D organoid model to study midbrain-associated neurodegenerative diseases

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Recent progress in 3D cell culture systems enables generation of midbrain organoids derived from human induced pluripotent stem cells (iPS). The main goal of this study was to overcome variations in differentiation capacity and necrosis inside growing


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organoid during prolonged cultures. These serious problems in the production of organoids were omitted using carbon and poly (lactide-co-glycolide) microfilaments. Organoids were grown in three ways: without a scaffold, so-called wild type (WT) and on scaffolds, which were selected on the basis of physicochemical tests from several different biocompatible materials. Carbon fiber modified with carbon nanotubes (SR7) and surgical threads with PLGA, Polysorb 5-0 (PLGA) proved to be the best materials for the scaffolds. We compared the influence of these scaffolds on organoid growth and differentiation. We found that carbon fibers accelerate the growth of midbrain organoids and changes their architecture in comparison to PLGA. Furthermore, microfilament-engineered midbrain organoids display similar structure as observed in human substantia nigra pars compacta. Thus, this organoids model may provide a reproducible *in vitro* system to study the human midbrain and its related diseases. Project was supported by National Scientific Center in Poland K/PBO/000376.

## P-20-019
## Hydroxyapatite-based coatings on Mg-1.4CaO-1Zn enhance osteogenic potential of murine pre-osteoblasts

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Recently, magnesium-based biodegradable metallic implants have received great interest in the field of regenerative medicine due to their excellent mechanical properties, similar to those of natural bone. However, their low corrosion resistance and high degradability in physiological environments limits their biomedical applications. In this context, the present study approaches *in vitro* behavior of the new magnesium (Mg) alloy, Mg-1.4CaO-1Zn, synthesized by rapid sinter pressing and coated with hydroxyapatite (HA) and (HA)/polylactic acid (PLA) films. The *in vitro* performance of bare and coated Mg-based materials was explored by indirect contact assays using MC3T3-E1 pre-osteoblast cell line. The cell behavior was assessed in terms of cell viability/proliferation and osteogenic differentiation (alkaline phosphatase activity, osteopontin and osteocalcin protein expression, collagen synthesis and extracellular matrix mineralization). The results obtained emphasize that all samples support good cell viability and proliferation. Furthermore, the coated samples showed a higher efficacy in inducing MC3T3-E1 cell osteogenic differentiation when compared to the bare Mg alloy. In addition, HA/PLA coated samples elicited the most remarkable and significant effects on pre-osteoblast differentiation. Taken together, our results demonstrate that both coatings, although to a different extent, offer a promising approach to enhance osseointegration ability of Mg-based materials. Acknowledgements: This work has been supported through project ERANET-MANUNET II-BioImplantMag-2017.

## P-20-020
## Synthesis and characterization of 4-VP/SPMA cryogels

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Tissue engineering is an interdisciplinary area to use of a combination of cells, materials, and biochemical factors to improve or replace biological functions in an effort to improve for the repair of damaged tissues and organs. The scaffold is the most important component that have been engineered to cause attractive cellular interactions to contribute to the formation of new functional tissues. Cells are seeded into these structures capable of supporting three-dimensional tissue formation. Scaffolds mimic the extracellular matrix of the native tissue, repeat the *in vivo* environment. Also, they serve to allow cell attachment and migration, deliver and retain cells and biochemical factors, facilitate diffusion of vital cell nutrients and expressed products, implement mechanical and biological influences to modify behavior of the cell phase. The cryogels are most popular scaffold materials which have three-dimensional hydrophilic structures with highly interconnected macroporous networks which are synthesized at subzero temperature. In this study, we reported that the synthesis and characterization of 4-vinyl pyridine and sulfopropyl methacrylate-based cryogels by cross-linking in the presence of N, N′-methylenebisacrylamide (MBA) as a cross-linking agent, ammonium persulfate (APS) as an initiator, N,N,N′,N′-Tetramethylethylenediamine (TEMED) as an accelerator agent. We investigated the effect of MBA, TEMED, and APS cryogel formation using different amounts of the related substances. Monomer quantities were also changed to obtain materials in varying hydrophilicity and swelling characteristics. The cryogels were characterized both morphologically by SEM and structurally by FTIR. The swelling test was applied to the cryogels in order to evaluate their swelling and water retention capacity depending on the cryogel structure. At last, the cell viability test was applied to the materials to estimate any detrimental effect of the cryogels. *The authors marked with an asterisk equally contributed to the work.

## P-20-021
## The cytotoxicity studies of polycationic polymers with star topology in polyplexes with nucleic acids

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For medicine applications, a use of nanostructured polymers every year brings more and more possibilities. The creation of new polymers and the study of their biocompatibility is very important finding better and safer vectors for gene therapy. In this work we synthesized, characterized and tested for cytotoxicity new star polymers using HT-1080 cells as a study model. Star copolymers of *N,N*-dimethylaminoethyl methacrylate (DMAEMA) and oligo(ethylene glycol) methacrylate (OEGMA) were synthesized via atom transfer radical polymerization using hyperbranched poly(arylene oxindole) as the macroinitiator. Stars with molar masses from 100000 g/mol to 257000 g/mol and with varying amounts of OEGMA groups in the arms were prepared. These polymers were tested as carriers of nucleic acids that could be administered into a body to achieve a specific therapeutic effect. The results indicated that the amount of OEGMA groups in the arms of the star polymers but not the ratio of polymer amino-groups to phosphate residues (N/P) was the most important parameter for obtaining satisfactory effect on the cell viability. Funding: This work was supported by the Polish National Science Center contract no. UMO-2015/17/B/ST5/01095. Keywords: star polymers; cytotoxicity; human fibrosarcoma.


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## P-20-022
## Fluorescence and circular dichroism spectroscopy studies of interactions between interferon α2b and yeast oligoribonucleotides

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RNA-based drugs can bind epigenetic regulators and transcriptional proteins and affect their activity. Using fluorescence quenching we determined dissociation constants for interactions of yeast oligoribonucleotides (ORNs), their $Na^+$ salts (ORNsNa), and complex with D-mannitol (ORNs-D-M) with Interferon α2b – a key protein of the antiviral cell defense mechanism. Using ORD CD we analyzed conformational changes of the protein. Mass spectrometric analysis showed that the dominant fraction consisted of RNA oligomers of 4–7 nucleotides in length. The analysis of IFN secondary structure changes was made by external service (Bestsel). For INF/ORNs-D-M the dissociation constant Kd=6,96±1.019 μM was found, while those for IFN/ORNs and INF/ORNsNa Kd=5.38±0.423 μM and Kd=9,64 ±2.259 μM, respectively, were noted. The analysis of IFN secondary structure showed 27.2% of α-helix (α-h), 26.9% of antiparallel β-stand (aβ-s), 0% of parallel β-stand (pβ-s), 9% of β-turn and 39.9% of random coil (r.c.) Upon addition of ORNs we observed 0% α-h, 33.3% aβ-s, 0% pβ-s, 21.2% β-turn and 45.5%, r.c., so one can note an increase in β-turn content compared to the native protein. Addition of ORNs-D-M gave 0.8% α-h, 54.4% aβ-s, 30.4% pβ-s, 5.6% β-turn and 8.9% r.c., so increase in aβ-s contents was found. On the other hand, addition of ORNsNa led to 1.5% α-h, 44.4% aβ-s, 42.8% pβ-s, 0% β-turn and 11.3% r.c. (increase in aβ-s and pβ-s content). CONCLUSIONS: ORNs and ORNs-D-M interact with IFN more strongly than ORNsNa. The observed effects of different forms of ORNs on the secondary structure of INF can be explained by involvement of various binding sites.

# Integrative approaches to structural and synthetic biology

## P-21-001
## Validation of a new gas chromatography-mass spectrometry (GC-MS) method to evaluate aromatase activity

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Estrone ($E_1$) and Estradiol ($E_2$) are steroidal molecules with regulatory functions in normal breast and in estrogen-receptor positive ($ER^+$) breast cancers. These estrogens are converted from the androgens, androstenedione (A) and testosterone (T), through an aromatization process catalysed by the enzyme aromatase. Thus, aromatase inhibitors (AIs) are considered the first-line treatment for $ER^+$ breast cancers. Currently, the screening of new potent AIs is performed trough a radiometric assay, in human placental microsomes, that relies on the quantification of tritiated water resultant from the conversion of a radiolabelled substrate, [1β-$^3$H] androstenedione. As this method is expensive and not environmental-friendly, we aimed to develop a new simple and fast GC-MS method to quantify A and $E_1$, and consequently, determine aromatase activity. Method performance was evaluated through linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. The method showed good linearity with a coefficient of determination ($r^2$) of 0.9884 and 0.9974, for A and $E_1$, respectively. LOD were 3 nM and 1.5 nM while LOQs were 10 nM and 5 nM, for A and $E_1$, respectively. The relative standard deviation (%RSD) of the intra- and inter-day precision were lower than 18% and the extraction efficiency was higher than 65%, for both hormones. Moreover, the dose-responses curves for Exemestane, Anastrozole and Letrozole, the AIs used in clinic, were compared to the ones obtained by the radiometric assay, being the rate of inhibition similar in both methods. Therefore, this new GC-MS method is sensitive and precise for the simultaneous quantification of androgens and estrogens and suitable to estimate aromatase activity. Tiago Augusto thanks Fundação para a Ciência e Tecnologia (FCT) for PhD grant (BD/128333/2017) funded by FCT PhD i3DU Programme. Sara C. Cunha acknowledges FCT for the IF/01616/2015 contract. *The authors marked with an asterisk equally contributed to the work.

## P-21-002
## Investigation of dipeptides binding to double-stranded DNA using molecular modeling and docking

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Peptides are promising drug candidates due to its high chemical diversity, selectivity, broad range of targets, low toxicity and accumulation in tissues than that of small drug like organic compounds. It is known that DNA is a target for many ligands of different chemical nature, including globular proteins, low molecular weight organic compounds and metal ions. However, there are only a few crystal structures of DNA complexes with short peptides in PDB. This is particularly surprising given large number of known interactions of short peptides with globular proteins used by living cell in the course of evolution. In this work we systematically analyzed ability of dipeptides (all possible combinations of the 20 standard amino acids) to bind with all possible combinations of tetra-nucleotides in central part of double-stranded DNA in classic B-form using molecular docking (ICM-Pro, Molsoft LLC) and molecular dynamics (AMBER, University of California). Totally 108800 DNA complexes of dipeptides with free and blocked N- and C-termini were analyzed. Our results indicate that vast majority of dipeptides showed no abilities of DNA binding. As can be expected the most preferable DNA binders are positively charged peptides containing Arg+ and Lys+, although with very low selectivity. However, we also were able to identify dipeptides capable of selective binding with only one or two DNA sequence motifs. These results were confirmed by MD simulations of the low energy complexes in a periodic water box. We present complete maps of dipeptide-DNA interactions including structural features essential for DNA binding. It was found that N- and C-terminal blocking groups significantly increase selectivity of the dipeptide binding to DNA indicating potentially much higher DNA binding selectivity of tri- and tetra-peptides. These findings may improve our understanding of mechanisms of interactions of short peptides with DNA that may have both theoretical and practical significance.


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## P-21-003
## Deep functional profiling of microbiomes

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Analyzing complex microbial communities is the milestone of modern microbiology, calling for "deep functional profiling" techniques. While next generation sequencing revolutionized our understanding of microbiota communities, we still lack high-throughput technologies to precisely determine their functionality. Hence, the development of the next generation ultrahigh-throughput screening (uHTS) techniques is of particular interest as it allows direct detection of biological activity of even minor components of specific microbiota communities. Moreover, uHTS techniques allow investigating the dynamics of microbiota community that is very important in complex mixtures of different species with highly distributed growth parameters and sensitivity to the external influence of environmental conditions. We have shown that cultivation of individual microorganisms encapsulated inside droplets of microfluidic double water-in-oil-in-water emulsion followed by FACS enable isolation of clones with a desired activity. This approach was successfully applied for quantitative probing of enzymatic activity on a single-cell level. uHTS of oral microbiome of Siberian brown bear for the presence of bacteria displaying antagonistic properties against pathogenic bacteria allowed us to isolate the potent antibiotic producer with a distinct mechanism of self-resistance that we specified on molecular level. Finally, we repurposed this methodology for personalized determination of antibiotic activity/resistance spectrum. Application of deep functional profiling techniques enabled us to assess microbiota composition and antibiotic efficiency on the level of entire microbiomes simultaneously. We believe that the outcome of this methodology could be effectively transferred to numerous applications in biotechnology, microbiology and synthetic biology. This work was supported by grants 19-34-70021 and 18-29-08054 from the Russian Foundation for Basic Research.

## P-21-004
## Alternative splicing in GPCR signalling diversity

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G protein-coupled receptors (GPCRs) are key regulators of cell physiology, as well as one of the most relevant drug target classes to date. And still, the molecular basis of differential receptor signalling in response to particular ligands or in diverse cellular contexts is not completely understood. In this work, we explore this question by considering the role of splice variants in differential receptor signalling. In order to comprehensively explore the overall impact of alternative splicing on receptor function, we have combined transcript-level expression information from 30 different human tissues present in the Genotype-Tissue Expression (GTEx) project database with structural and pharmacological information from all non-olfactory GPCRs. Our analysis reveals the extent of GPCR variation resulting from alternative splicing in humans and the effects this variation can have on receptor integrity and signalling. In particular, along with variants with truncated synthesis and export signals, we find receptor isoforms with altered sites for ligand binding, coupling to intracellular transducers, dimerization or sensitivity to post-translational modifications. These variants also differ in their tissue distribution, an observation that could help explain past discrepancies in pharmacological measures of receptor signalling. Taken together, our analysis reveals the extent to which GPCRs exploit tissue-specific alternative splicing to generate functionally diverse receptor variants, and points to previously uncharacterised receptor isoforms that could clarify past observations on system-specific receptor signalling, as well as represent an additional source of selectivity for the development of new GPCR drugs.

## P-21-005
## Interaction of pentapeptide repeat proteins with *Escherichia coli* DNA gyrase

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The Pentapeptide Repeat Proteins [PRPs] are diverse family of found in prokaryotes and eukaryotes which fold into a right-handed β-helix with a quadrilateral cross section. PRPs include a number of examples of postulated DNA mimicking proteins which are thought to approximate both surface charge and size of dsDNA. These PRPs act on DNA topoisomerases, in particular on bacterial DNA gyrase and protect it against different gyrase-targeting toxins and drugs, including clinically important fluoroquinolones. Presently there are at least three hypothesized models of how these topoisomerase-acting PRPs [TA-PRPs] work. However, the exact mechanism remains unknown. In order to understand which model is correct, we have studied the ability of several PRPs to protect DNA gyrase against fluoroquinolones or natural product toxins such as albicidin and microcin B17. We produced proteins incorporating unnatural photocrosslinkable amino acids which allowed us to map PRP-gyrase interactions *in vitro* and *in vivo*. This, combined with structure-guided mutagenesis has revealed the importance of certain residues of the PRPs for the interaction with *E. coli* DNA gyrase and for the protective effect. Additional *in vivo* and in *vitro* experiments have shed further light on details of the mechanism of PRP action and these collected data allows us to begin to build a new model for the mechanism of protection by TA-PRPs.

## P-21-006
## Centre of molecular structure in BIOCEV – current status and improvements

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The Centre of Molecular Structure (CMS) operates already more than 3 years in BIOCEV as a part of the Institute of Biotechnology, AS CR. It represents a complex of core facilities specialized in the structure solution and interactions of biomolecules. The CMS includes facilities for crystallization, X-ray diffraction and scattering, physicochemical characterization of biomolecules, advanced mass spectrometry analysis, and infrared and fluorescence spectroscopy. As a part of the Czech Infrastructure for Integrative Structural Biology and Instruct, the CMS provides a comprehensive service and consultation for researchers from BIOCEV, and for other Czech or foreign researchers. The CMS mainly relies on 15 T Solarix XR FT-ICR mass spectrometer, D8 Venture single-crystal diffractometer with Excillum MetalJet


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source, Formulatrix RI1000 crystallization hotel, Prometheus NT.48 for differential scanning fluorimetry and Monolith NT instruments for microscale thermophoresis. During the last year, the number of instruments and techniques provided to researchers in the CMS had increased: SAXS Point 2.0 equipped with Excillum MetalJet source for small angle X-ray scattering studies of biomolecules in solution, MALDI TOF mass spectrometer, and the newly equipped room for spectroscopic techniques including a Fourier-transformed Infrared (FTIR) spectrometer and a FLS1000 spectrofluorometer. The Centre of Molecular Structure is supported by: MEYS CR (LM2015043 CIISB); project Czech Infrastructure for Integrative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; project Structural dynamics of biomolecular systems (CZ.02.1.01/0.0/0.0/15_003/0000447) from the ERDF.

## P-21-007
## Tumor-targeting peptides as a platform for the development of targeted drugs

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Glioblastoma is the most aggressive and widespread brain tumor. Standard treatment, including surgical resection, radiation and chemotherapy, allows extending the average lifespan after the start of treatment to 14 months. The greatest challenge in handling glioblastoma will be to identify a tumor specific target that is suitable for the targeted therapy of glioblastoma patients. The goal of this study to identify glioblastoma specific peptides carried out from phage peptide libraries using glioblastoma cultured cells *in vitro* and animal models *in vivo*. The selected glioma-specific peptides can be used for conjugation with different antitumor drugs as well as for targeting liposomes or nanocontainers to glioma cells. We have used a screening technology for phage display combinatorial libraries for peptide selection, which specifically bind to the surface of human glioblastoma cells (U-87 MG). Using Ph.D.12 phage display library, we screened for peptides binding to U-87 MG cell surface. After 5 rounds of enrichment, 80 phages were randomly selected, amplified and tested for presence of library insert sequence. A unique peptide, [SWTFGVQFALQH], was presented in 24,3% cases. Also we screened Ph.D.7 phage peptide library for U-87 MG tumor in xenograft model for selecting tumor targeting peptides. After 3 rounds of enrichment, 40 phages were randomly selected, amplified and tested for presence of library insert sequence: two clusters of peptides were obtained. Unique peptides [HPSSGSA] and [PVSNKMS] were presented in 25,9% and 22,2% in case, respectively. The binding efficiency of tumor-targeting phage particles into glioblastoma cells *in vitro* was estimated using immunocytochemistry and flow cytometry. Further work is planned on the purification and characterization of peptides for subsequent conjugation with drugs. The present study is supported by the Russian Science Foundation project No. 19-44-02006. *The authors marked with an asterisk equally contributed to the work.

## P-21-008
## Structural basis of ADP-dependent glucokinase catalysis and inhibition

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ADP-dependent glucokinase (ADPGK) is an alternative glucose phosphorylating enzyme. In contrast to classical ATP-dependent hexokinases, ADPGK utilizes ADP as a phosphoryl group donor. In humans, ADPGK's involvement in modified bioenergetics of activated T cells has been postulated and elevated ADPGK expression has been reported in various cancer tissues. However, the physiological role of ADPGK is still poorly understood and effective ADPGK inhibitors still await discovery. In the first part of our work we show that 8-bromo substituted adenosine nucleotide inhibits human ADPGK. By solving the crystal structure of archaeal ADPGK in complex with 8-bromoadenosine phosphate (8-Br-AMP) at 1.81 A resolution we identified the mechanism of inhibition. We observed that 8-Br-AMP is a competitive inhibitor of ADPGK and that the bromine substitution induces marked structural changes within the protein's active site by engaging crucial catalytic residues. In the second part, we present a crystal structure of archaeal ADPGK from *Methanocaldococcus jannaschii* in complex with an inhibitor, 5-iodotubercidin, d-glucose, inorganic phosphate, and a magnesium ion. The crystal structure shows how the phosphate ion, while mimicking a β-phosphate group, is positioned in the proximity of the glucose moiety. In addition, we demonstrate that 5-iodotubercidin inhibits human ADPGK-dependent T cell activation-induced reactive oxygen species (ROS) release and downstream gene expression. The structural insights presented herein provide a critical basis for rational development of novel ADPGK inhibitors.

## P-21-009
## Isolation and characterization of UPEC bacteriophages

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Urinary tract infections (UTI) are the most common type of infectious diseases worldwide and more than 80% UTI cases are caused by uropathogenic *E. coli* (UPEC). Despite effective antibiotic therapy, these infections tend to recur. The prevalence of UTIs caused by antibiotic-resistant *E. coli* is steadily increasing. Bacteriophages belong to perspective alternative therapeutic agents for control of these infections. The aim of this work was isolation of multiple UPEC-infecting phages. Eight bacteriophages were isolated from waste water on unique strains and characterized by genome sequencing. Six phages belonged to different branches of *Tevenvirinae* family, two others were members of *Autographivirinae* and *Tunavirinae* groups. The host specificity of phages was determined on panel of 41 UPEC strains possessing different MLST and CH types. The phages infected 1–17 strains and 38 strains was lysed at least by one phage. The highly prevalent strains belonging to CH types 40–30 and 40–41 (ST-131) were infected by five and four phages. The vKMB40 phage was the most efficient one infecting 17 different strains. Obtained results showed that isolated phages have a promising potential for phage therapy of UTIs.


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## P-21-010
## Quantification of the amount of mobile components in intact stratum corneum with natural-abundance 13C solid-state NMR

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The outermost layer of the skin is stratum corneum (SC) in which the major fraction of both lipids and proteins form solid structures at ambient condition. The minor amount of mobile lipids and proteins, on the other hand, is crucial for macroscopic material properties of the SC, including skin barrier function. Conventional quantitative direct polarization (DP) NMR experiment is not applicable to quantify this minor mobile amount since its signals overlap with those of the solid fraction. This study describes an NMR method based on INEPT polarization transfer scheme, denoted as Q-INEPT, to selectively observe the mobile components and quantify the molar ratio of mobile components in $^{13}$C natural abundance sample. Simultaneously, quantitative information on scalar couplings and dynamics including different relaxation rates are obtained. Utilizing Q-INEPT together with a reference chemical, we can, for the first time, quantify the amount of the lipids and proteins in intact SC that are mobile at the molecular level, a first step toward a quantitative understanding of SC macroscopic properties. By melting all the lipids at high temperature, we can further quantify the total amount of lipids in SC which has only been approached by lipid extraction. The Q-INEPT method was validated using different model systems containing lipids and small molecules with different hydrophobicity and dynamics. It is capable of producing results that are comparable to the traditional quantitative DP NMR experiment, when DP is applicable and comparisons can be made. There would be potential applications of this method in many different systems with coexisting amount of rigid and mobile components, e.g., solid and fluid domains in the bilayers, membrane proteins with rigid transmembrane and mobile extramembrane domains, protein fibrils with rigid core and mobile flanking domains, or equilibrium between bound and free states in systems containing ligand-protein and membrane-protein complexes.

## P-21-011

Abstract withdrawn

## P-21-012
## In solution structural characterization of human histone deacetylase 6

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Histone deacetylases (HDACs) play a critical role in a plethora of cellular processes by removing ac(et)yl groups from lysine side chains of target proteins. HDAC6 is a zinc-dependent deacetylase mainly localized to the cytoplasm that acts on many non-histone targets including tubulin, Hsp90, and cortactin. HDAC6 is a multidomain protein comprised of five domains a molecular weight of 140 kDa. Given the structural complexity of the full-length HDAC6 protein it is a challenging target for X-ray crystallography. To glean more structural information of HDAC6 we focused our efforts on creating an integrative structural model of the full-length HDAC6 by combining several approaches including small-angle X-ray scattering, analytical ultracentrifugation, chemical crosslinking, H/D exchange, and molecular modeling. Combined, these data allowed us to put forward a solution model of full-length human HDAC6 including the description of its concentration dependent oligomerization.

## P-21-013
## New genetically encoded photosensitizers

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Fluorescent protein KillerRed is a genetically encoded photosensitizer, which under light irradiation produces active forms of oxygen. This feature is a great advantage of KillerRed, because it allows to use it in many areas of biology as a tool for the precise inactivation of cellular proteins. Nevertheless, this protein is much inferior to chemical photosensitizers in the efficiency of formation of active forms of oxygen and phototoxicity. To date, numerous attempts to increase the phototoxicity of KillerRed by using rational design have not been successful. The using of directed evolution is limited by the difficulty in selecting mutant proteins: cells expressing more phototoxic protein variants die and cannot be selected by standard methods. In our work, we propose a method that will solve the problem of selecting the phototoxic mutants of the KillerRed protein. This novel method allows to study the genotype-to-phenotype connections in phototoxic fluorescent protein and to create its more phototoxic variants. In addition, our approach will be applicable to the selection of libraries of other toxic molecules, for example, in the development of antiviral and anticancer drugs based on small interfering RNAs, as well in the development of new antibacterial agents.
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FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-21-014
## PRobe Incorporation Mediated by Enzymes methodology adopted for evaluation of protein stability index

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Nowadays there is a wide diversity of methods for protein visualization aiming for identification of cellular networks and protein-protein interactions. Nonetheless, current techniques have a number of deficiencies and limitations such as cellular toxicity or substrate impermeability, a large size of enzymes, and low substrate specificity. Here we showed that previously reported PRIME-based methodology (PRobe Incorporation Mediated by Enzymes) may be applied for monitoring of protein degradation in mammalian cells utilizing in-gel fluorescence, microscopy and flow cytometry. Proposed technique is functioning in a "pulse-chase" mode, which means absence of any substances, that may interfere with protein synthesis, e.g. cycloheximide; isolation of protein of interest from cell lysates prior analysis is not required, and, finally, site-specific fluorophore labeling only slightly changes functionality of the targeted polypeptide and may be applied in the real-time mode. PRIME-based methodology is a direct method, that does not require any sample preparation stages, e.g. enzymatic treatment, widely used in proteomic mass-spectrometry-based studies. According to our data PRIME-based technique in comparison with cycloheximide chase tends to slightly increase estimated half-life of short-living proteins and provides more accurate values for the stable proteins. Using PRIME we successfully determined stability and accumulation index in of the major component of the ubiquitin-proteasome system – ubiquitin itself, being incorporated into the variously linked polyubiquitin chains. The reported study was supported by the Russian Science Foundation project #19-14-00262.

## P-21-015
## Improvement of *Aspergillus awamori* glucoamylase thermostability by combined methods of molecular modeling and directional evolution

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Glucoamylase (α-1,4-glucan-glucohydrolase, EC 3.2.1.3, GA), a glycoprotein of the GH15 glycoside hydrolase family, catalyses hydrolysis of α-1,4- and α-1,6-glycoside bonds in starch, glycogen and other oligosaccharides. GA from *Aspergillus awamori* has an optimal temperature of action of 50–60 °C. At higher temperatures, GA is rapidly and irreversibly inactivated. This protein is used in the starch-processing industry together with α-amylase to convert starch into glucose. A more thermostable GA is desirable for industry, not only because less cooling would be needed, but also because a higher reaction temperature would yield higher GA activity. Based on the data of small-angle neutron scattering, a GA model was constructed using homologous modeling and molecular dynamics methods. Using molecular dynamics methods in an explicit solvent we simulated the wild-type glucoamylase heat-inactivation with molecular dynamics methods and searched for unstable regions in the protein. The obtained data allowed us to carry out random and site-saturating mutagenesis of gene regions encoding variable unstable glucoamylase regions. Such an approach made it possible to significantly narrow the size of DNA libraries and reduce the complexity of the experiment of directed evolution. Using this technique a number of variants of glucoamylase with increased thermostability were obtained. The resulting amino acid substitutions were used to create thermostable chimeric GA variant, designated 6B9. Free energy of thermoinactivation ($\Delta\Delta G$) increases at 2.88, 3.88 and 5.00 kJ/mol compared to the wild type protein at 70, 75 and 80 °C, respectively. Also variant 6B9 GA has catalytic activity at 50 °C similar to that of wild-type GA The reported study was funded by RFBR according to the research project 18-34-01003. *The authors marked with an asterisk equally contributed to the work.

# Induced pluripotent cells

## P-22-001
## AMP-activated kinase (AMPK) up regulation inhibits hepatocellular carcinoma pluripotent stem-like cells properties

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Hepatocellular carcinoma (HCC) remains one of the most common and lethal malignancies worldwide despite the development of various therapeutic strategies. HCC treatment is hindered by the frequent appearance of chemoresistance to the multi-kinase inhibitor Sorafenib which has been related to the presence of cancer stem cells (CSC) that often scape from therapy. The key metabolic sensor AMP activated kinase (AMPK) has recently been recognized as a tumor growth regulator. In this study, we aimed to elucidate the role of AMPK in the development of stem cell phenotype of HCC cells. To that end, the sorafenib-resistant cell line was developed by culturing HepG2 and Huh7 cells in increasing sorafenib concentrations for 6 months. Analysis of AMPK revealed that sorafenib-resistant cells had stem cell features as well as lower levels of total and phosphorylated AMPK as well as its downstream substrate ACC compared with their parental cells. Interestingly, AMPK knock down with siRNA increased the expression of stem-cell markers in parental cells and blocked sorafenib induced cell death. Conversely, upregulation of AMPK either by transfection or by pharmacological activation with A769662 in sorafenib-resistant cells decreased the expression of the stem cell markers and restored sensibility to sorafenib.


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## P-22-002
## Generation of patient-specific iPSC disease model of neonatal diabetes with insulin mutation

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One of the causes of diabetes in infants is the defect of the insulin gene (*INS*). The mutations that lead to hypoinsulinemia are usually heterozygous and lead to the absolute deficiency of insulin clinically manifested before 6 months of age as permanent neonatal diabetes mellitus (PNMD). It is suggested that the mutation can lead to proinsulin misfolding that can induce beta-cell apoptosis. However, in humans the mechanism underlying such beta-cell failure in patients with heterozygous mutations remains unclear. Recent studies had enabled the *in vitro* generation of insulin producing beta cells from human pluripotent stem cells (hPSC). Genome editing technologies provided an opportunity for correction of the mutation in the *INS* gene and therefor generating an isogenic system: a pair of insulin-producing cell lines with the presence and the absence of a mutation in the *INS* gene. In our study iPS cell lines were generated from primary fibroblasts by non-integrative method of reprogramming from the patient with an intronic mutation in the *INS* gene. The mutation, that caused an ectopic splice site within the *INS* gene, was edited by CRISPR-Cas9 system. IPSC lines with mutant *INS* and with edited *INS* gene were *in vitro* differentiated into the pancreatic beta cells. The editing led to the restoration of the RNA sequence of *INS* gene. Such isogenic system will enable to confirm the restored function in the edited cell line and to study the pathogenesis at the molecular and cellular level.

## P-22-003
## Generation of functional parathyroid cells from patient-specific iPS cells with a mutation in the CASR gene

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Neonatal severe hyperparathyroidism (NSHPT) is a rare disorder caused by inactivating calcium-sensing receptor (CASR) gene mutations that results in life-threatening hypercalcemia and metabolic bone disease. NSHPT mostly cured with total parathyroidectomy as medical treatment is not effective. Hypoparathyroidism develops after parathyroidectomy so patients need constant therapy with alfacalcidol and calcium. Substitution therapy with recombinant teriparatide showed good results [Winer KK et al.], but still it has lack of long term clinical evidence in children. Autologous transplantation of parathyroid tissue has been carried out quite successfully, but for patients with inactivated mutations in CASR gene, it led to the recurrence of hyperparathyroidism due to the mutation in transplanted tissue. The technologies of reprogramming of somatic cells made it possible to generate patient-specific induced pluripotent stem (iPS) cells that can be further differentiated to PTH-producing cells. Genome editing technologies provided an opportunity for correction of the mutation and therefore generating patient-derived tissue without the mutation in CASR. In our study, we generated iPS cell lines from primary fibroblasts from the patient with a heterozygous compound mutation in the CASR gene. Both mutations lead to the stop codon formation. IPS cell lines were verified for the presence of the mutation that was found in the patient. They were also characterized for the presence of pluripotent markers by immunocytochemistry: cells were positive for OCT4, SOX2, TRA-1-60, SSEA-4 and functional pluripotency was demonstrated by embryoid bodies formation. Next step we plan to establish the robust protocol of iPS cells differentiation into PTH-producing cells. This study was supported by grant from the Russian Foundation for Basic Research (project No. 19-015-00327\19).

## P-22-004
## The role of the methyl-DNA binding protein in establishment of epigenetic landscape in mammalian cells

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One of the key steps in the reprogramming of somatic cells is the establishment of a DNA methylation profile similar to the undifferentiated state. Main readers and interpreters of methylated DNA are methyl-DNA binding proteins, transcriptional regulators that provide a link between DNA methylation and histone modifications. The Kaiso transcription factor is one of the protein interpreters of methylated DNA. By binding to methylated DNA, Kaiso attracts corepression complexes that affect chromatin structure. Previously, we found that Kaiso knockout MEFs have increased reprogramming efficiency. Here, we have shown that already in MEFs prior to reprogramming process Kaiso deficiency lead to a reduced level of Oct4 promoter methylation comparing to wild type cells. Along with, we detected increased cell proliferation activity. We found that Kaiso deficiency not only increases the number of formed iPSCs-like colonies but also reduces the time required for their formation, compared to iPSCs from wild-type cells. Using different cellular model we were able to show that Kaiso influences the establishment of DNA methylation in dependence of its posttranslational modification, SUMOylation. Here we determined, that Kaiso not only modified by SUMO but also can interact with SUMO noncovalently. The significance of this interaction remains to be explored. This work was supported by the Russian Science Foundation (19-74-30026, interplay of Kaiso and SUMO) and the Ministry of Science and Higher Education of the Russian Federation (grant 01201371085, role of Kaiso in reprogramming).

## P-22-005
## Modeling of cardiomyopathy in Duchenne muscular dystrophy with a use of isogenic hiPSC-derived cardiomyocytes

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Duchenne muscular dystrophy (DMD) is an X-linked genetic disease caused by mutations in the largest known human gene encoding dystrophin (DMD) – component of dystrophin-associated glycoprotein complex present in muscle cells. Lack of this protein leads to progressive muscle weakness followed by respiratory failure and cardiomyopathy, which is a major cause of death of DMD patients. Mechanisms of cardiomyopathy are not yet fully known, therefore patient-specific human induced pluripotent


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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stem cells (hiPSCs) and hiPSC-derived cardiomyocytes (hiPSC-CMs) were proposed as a tool for *in vitro* modelling of this disease. In our studies, we utilize DMD hiPSCs lines derived from fibroblasts of two patients with DMD. Moreover, for more reliable and efficient modeling and to exclude the influence of genetic background, isogenic cell lines were established by deletion of exon 50 in *DMD* gene in control hiPSCs using CRISPR-Cas9 method. Preliminary patch-clamp experiments evaluating differences in electrophysiology of control and DMD hiPSC-CMs demonstrated a longer duration of hyperpolarization in DMD cardiomyocytes. Additionally, voltage-clamp analysis revealed that a negative and positive current are increased in these cells in comparison to their control counterparts and both of these parameters indicate an impaired activity of potassium and calcium channels. Moreover, as hiPSC-CMs resemble more fetal phenotype and 2D cell culture does not reflect sufficiently physiological conditions and architecture of original tissue, 3D cell culture system was established by differentiating the cells in a form of spheres. Gene expression analysis performed after differentiation of hiPSCs to cardiomyocytes in 2D and 3D system suggest more mature characteristic of cells cultured in 3D system, therefore such model can be also used for better understanding of mechanisms of cardiomyopathy in DMD patients. Supported by 2014/14/M/NZ1/00010 and 2012/06/A/NZ1/0004. *The authors marked with an asterisk equally contributed to the work.

## P-22-006
## The influence of extracellular matrix on human pluripotent cell stemness and differentiation

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Many studies have demonstrated the important role of extracellular matrix (ECM) in pluripotent stem cells fate. For example, ECM can ensure their active proliferation with maintaining of pluripotency or stimulate directed differentiation. The purpose of this work was to study the influence of ECM components on maintenance of induced human pluripotent stem cells (iPSCs) pluripotent state and differentiation. In this work we analyzed the following ECM compounds: collagens I, III and IV, fibronectin, vitronectin, laminin. Matrigel was used as a control. Collagens I and III did not provided iPSCs adhesion. Collagen IV promoted an adhesion of single iPSCs colonies and ensured their growth up to 9 days. Fibronectin was more effective both in adhesion and in maintaining the growth of colonies. Vitronectin and laminin demonstrated the similar to control results both in adhesion and growth rate and maintained the expression of pluripotency markers Oct4 and Sox2. We investigated the ability of the ECM molecules to promote the epidermal differentiation of iPSCs in presence of BMP4 and retinoic acid. All the ECM components downregulate Sox2 expression and upregulate epidermal differentiation markers: keratin 14, keratin 18 and p63. Laminin and fibronectin maintain the growth rate of epidermal cells, while collagens I, III and IV significantly suppress the proliferation. Eventually, we can assume that laminin is important for maintain stemness and proliferation of iPSCs. Laminin and fibronectin support the early stages of epidermal differentiation. In further research we are going to analyze the influence of ECM components on neural differentiation of iPSCs. The work was performed according to IDB RAS Basic Researcher Program No. 0108-2019-0004.

## P-22-007
## Neurotransmitter systems in cultures of human neurons derived from IPSCs

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Therapy with neural cells obtained from dedifferentiated human cells is one of the promising approaches in the field of regenerative neurology. The aim of our investigation was to explore the neurotransmitter diversity in the developing cultures of human neural precursors derived from induced pluripotent stem cells (iPSC) *in vitro*. The IPSC-KYOU-DXR0109B cell line were purchased from the ATCC cell bank. Using the DUAL SMAD inhibition, we obtained neural stem cells that were then differentiated into neurons. To observe neuronal excitation signals, with "Sleeping Beauty" transgenesis system we obtained a transgenic cell line of IPSC with a constitutive expression of GCamp6s fluorescent calcium indicator. Analysis of transcriptomes of eight separate neuron cultures suggested that a number of genes related to the synthesis and reception of various neurotransmitters (glutamate, GABA, glycine, acetylcholine, serotonin, dopamine, nitric oxide) were expressed already in 3 weeks old cultures. Immunocytochemical study allowed to characterize the distribution of the revealed neurotransmitter phenotypes in the cell cultures. Our data confirms the presence of various neurotransmitter phenotypes in neurons differentiated from the IPSC-KYOU-DXR0109B cell line. We suggest that our approach will allow to investigate the role of early neurotransmitter signals in the development of human functional neuronal ensembles *in vitro*. This study was funded by the Presidium of the Russian academy of sciences, Program No. 42 «Fundamental research for the development of medical technologies», Government basic research program, No. 0108-2018-00015. *The authors marked with an asterisk equally contributed to the work.

# Long noncoding RNA

## P-23-001
## Search and functional analysis of long non-coding RNAs that stimulate CCDC6-RET oncogene formation

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Chromosomal rearrangements play important role in the development of most types of cancer. However, the mechanisms that direct the formation of specific chromosomal aberrations remain unclear. Recently it was shown that formation of such mutations in humans can be directed by long non-coding RNAs (lncRNAs). These RNAs contain sequences complementary to two chromosomal regions. In this recent study it was shown that in prostate cancer cell line a deletion leading to the formation of cancer oncogene TMPRSS2-ERG may arise from the increased expression of AZI1 lncRNA that fulfills the above mentioned conditions. Based on the approach described in this study, we have developed a method for searching for lncRNAs, potentially capable of stimulating the formation of certain chromosomal


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mutations. According to this method, we conducted a search for lncRNAs expressed in the cells of the tissues of which this mutation is characteristic. LncRNAs that are potentially capable of binding to regions of chromosomal junction were selected among them. Using the described approach, among the lncRNAs synthesized in the thyroid cells, we selected 5 potentially capable of stimulating the fusion of the *CCDC6* and *RET* genes. According to our preliminary data, the expression some of these lncRNAs led to an increase in the frequency of the oncogene formation. This work was carried out according to the scheme specified in the application for the grant 19-74-10083 from Russian Science Foundation. *The authors marked with an asterisk equally contributed to the work.

## P-23-002
## Roles of long non-coding RNAs in regulating expression of yeast extracellular proteins

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Eukaryotic genomes encode numerous transcripts that are not translated into proteins and are therefore referred to as long non-coding RNAs (lncRNAs). In yeast, this class of RNAs includes stable unannotated transcripts (SUTs), cryptic unstable transcripts (CUTs), meiotic unannotated transcripts (MUTs) and Xrn1-sensitive unstable transcripts (XUTs). Several classes of lncRNAs are targeted by the conserved 5′-3′ exoribonuclease Rrp6. This enzyme is associated with the exosome, a multi-subunit complex important for RNA processing and stability. Some lncRNAs are known to be important in various cellular processes such as gene expression, nuclear organization and chromatin structure, however roles of most of these transcripts are still unexplored. High-throughput RNA-sequencing methods discovered a plethora of intergenic transcripts which are transcribed across genes encoding extracellular proteins, such as genes related to cell wall biogenesis and phosphate metabolism. The cell surface is a dynamic environment, which has to rapidly adapt to environmental changes, as well as cell cycle and life cycle phases and therefore expression of these genes is highly regulated. Our preliminary results suggest roles for lncRNAs in this process, acting *via* modulation of chromatin structure, promoter interference and/or overlapping sense/antisense pairs. The implications of these observations for the expression and stability for lncRNAs, mRNAs and proteins will be discussed.

## P-23-003

This poster has been moved to a Short Talk

## P-23-004
## SNPs in MEG3 lncRNA affect its tumor suppressive function via increased proliferative capacity and resistance to apoptosis

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The maternally-encoded long noncoding RNA (lncRNA) MEG3 is a tumor suppressor expressed in normal cell lines and tissues but downregulated in cancer cell lines and cancers of various tissue origins. Interestingly, over 110 single nucleotide polymorphisms (SNPs) of MEG3 are recorded in the NCBI SNP Database. Due to the immense number of existing MEG3 variants, it is hypothesized that these SNPs play a role in the susceptibility of an individual to cancer. Specifically, changes in the secondary structure of the lncRNA induced by the presence of SNPs are suspected to alter the capacity of MEG3 as a tumor suppressor. To examine the role of SNPs in MEG3 function, three MEG3 SNPs as well as wild-type MEG3 were selected. Secondary structural changes in the MEG3 due to SNPs were determined *in silico*. Afterwards, wild-type MEG3 and the SNPs were cloned into pTARGET™ expression vector and used for the transfection of HCT116 colorectal cancer cells for subsequent phenotypic characterization. Changes in proliferation rate, resistance to apoptosis, and migration rate were investigated. For the proliferation cancer hallmark, BrdU cellular proliferation assay was conducted. Results showed increased rate of proliferation in cells transfected with MEG3 SNPs suggesting that the MEG3 tumor suppressive function may have been abrogated. Similar observations were obtained for the apoptosis assay which was done through staining with annexin V and propidium iodide. A lower percentage of cells transfected with the MEG3 SNPs underwent apoptosis. Finally, migration rates were determined through scratch wound healing assay. Initial findings suggest that cells transfected with MEG3 constructs containing SNPs migrate faster compared to wild-type. Overall, results obtained from functional assays hint at the loss of function of MEG3 due to the presence of SNPs. Proliferation assay by flow cytometry and Caspase-GLO 3/7 assay are currently underway to validate preliminary results.

# Neurodegeneration

## P-24-001
## miR-302 protects against mutant huntingtin-induced neurotoxicity through restoration of insulin signaling

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Huntington's disease (HD) is autosomal-dominant neurodegenerative disease, resulting in motor, cognitive, psychiatric impairments and eventually death. HD is caused by CAG triplet repeat expansion in the exon 1 coding region of huntingtin (Htt) protein. This mutation encodes the mutant Htt (mHtt) which accumulates within cells as abnormal aggregates or inclusions, thereby causing cellular function impairments that ultimately lead to neuronal death. Interestingly, some epidemiologic studies have revealed that defective insulin signaling may be associated with HD patients. This indicates the blockade of brain insulin signaling may initiate or exacerbate mHTT-induced neurotoxicity. In this study, we used an *in vitro* HD model to investigate whether the microRNA miR-302 is involved in mHTT-mediated neurotoxicity. Our results demonstrated that mHTT overexpression significantly downregulates miR-302 intracellular expression levels and causes apoptosis. However, restoration of miR-302 markedly upregulates insulin sensitivity and enhances cell viability. In addition, miR-302 also stimulated autophagy through AMPK activation, which attenuates the accumulation of mHTT aggregates within neuronal cells. In conclusion, our results suggested that miR-302 can reduce mHTT-induced neurotoxicity through restoration of insulin signaling. Accordingly, attenuation neurodegeneration by regulating miR-302 may lead to novel diagnostic or therapeutic methods against HD in future.


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## P-24-002
## Peptide (60-76) from RAGE and its analogue protect spatial memory in transgenic 5xFAD mice and induce calcium signaling in primary culture via activation of RAGE

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Receptor for advanced glycation end products (RAGE) is expressed in many cells of the body and involved in Alzheimer's disease. RAGE is associated with the beta-amyloid induced cell death. Previously we have revealed one synthetic peptide corresponding to the amino acid sequence (60-76) of the RAGE which was able to restore the disturbed spatial memory of olfactory bulbectomized mice developing Alzheimer type neurodegeneration, and to inhibit amyloid mediated caspase-3 activation. Here we investigate the ability of peptide (60-76) and its analogue Ac-(60-76)-$NH_2$ to protect spatial memory in transgenic 5xFAD mice. We also study the effect of peptide (60-76) on calcium signal in primary culture of neurons and astrocytes. Protective activity of the peptides was studied in 5xFAD mice. Animals were intranasally administrated with peptides (60-76) or Ac-(60-76)-$NH_2$ daily, for 70 days. Then the animals were trained in the Morris water maze and their memory was tested. We have revealed that both peptides protected mouse memory from impairment but the analogue had a long-lasting effect. Application of peptide (60-76) to the primary culture of neurons and astrocytes derived from hippocampus and cortex of rat brain induced peak-like oscillations of calcium in the cells. We found that the presence of FPS-ZM1, a high affinity antagonist of the RAGE, which binds with the V-domain of the receptor, abolished the $Ca^{2+}$ signal in response to the RAGE peptide (60-76). Thus, both RAGE peptides – (60-76) and its analogue, protect memory of transgenic 5xFAD mice, but the analogue has long-lasting effect. The possible mechanism of the neuroprotective properties of the RAGE peptide involve activation of RAGE leading to calcium signaling. Peptide synthesis and processing of the data were performed with support of RSF grant 19-14-00009. The experiments on animals and cell culture were supported by RFBR 19-04-00624 and 19-015-00064.

## P-24-003
## Hippocampal proteomics of mouse prion disease: unravelling disease networks and understanding the mechanism of disease-modifying ligands

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Studies from our lab have shown that targeting the M1 subtype of muscarinic ACh receptors (M1 mAChR) with BQCA, a selective M1 mAChR positive allosteric modulator (PAM) restores cognitive function and improves survival in mouse prion disease, a condition that show many of the hallmarks of human Alzheimer's disease (AD) such as progressive terminal neurodegeneration and memory deficits. The current study applied proteomics to unravel the protein signatures of mouse prion disease and to understand the mechanisms of the disease-modifying effects of BQCA. Tg37 hemizygous mice were inoculated by intracerebral injection with 1% of prion-infected brain homogenate according to the Rocky Mountain Laboratory (RML) process prion while control mice received 1% of normal brain homogenate (NBH). Seven weeks after inoculation, the mice were given daily intraperitoneal injection of 15 mg/Kg BQCA or 5% glucose (vehicle) for 2 weeks. The hippocampus was then removed and processed for mass spectrometry. Analysis of the data revealed that a number of proteins were significantly upregulated in prion disease compared to the control (NBH) mice (calponin-3: 2.5-fold; vimentin: 3-fold, GFAP: 4-fold, SERPINA3: 2.5-fold; and APOE: 2-fold). Interestingly, these proteins were significantly downregulated in similar fold changes in BQCA-treated prion mice compared to vehicle-treated prion mice. The identified proteins have a wide range of functions in the CNS including maintenance of blood brain barrier (BBB) integrity, inhibition of enzymes that are involved in clearance of prion protein, markers of neuroinflammation/neuronal death and regulation of synaptic plasticity. This study presents an important list of key signature proteins of prion-induced neurodegeneration and serves as a significant step to understanding mechanisms of the disease-modifying action of M1 AChR PAMs. This data also open up the possibility for M1 PAMs as novel therapeutic tool for neurodegenerative disorders such as AD.

## P-24-004
## *In vitro* aggregation of the tau protein and its inhibition

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder which affects brain and is defined by a progressive memory loss, impaired cognitive function, behaviour changes and disruption of integrity of the individual, hallucinations, impaired self-control and loss of decline of spoken and written speech. AD is based on the degenerative changes associated with neuronal death and formation of characteristic histopathological changes. The treatment of this disease is still unknown, but various hypotheses (e.g. neurotoxic effect of Aβ1-42 aggregates, hyperphosphorylation of τ protein, disrupted circulation of acetylcholine, oxidative stress, etc) are examined in order to understand the mechanism of formation of disease and by that to find effective therapy [1]. The disease process is associated with formation of extracellular amyloid-peptide-containing neuritic plaques and intracellular neurofibrillary tangles formed in brain. The tau-hypothesis suggests that the disease cascade initiates tau microtubule-associated protein abnormalities. It is a soluble protein, but its insoluble aggregates are produced during the formation of neurofibrillary tangles (inside nerve cell bodies) which disrupt the structure and function of neuron. The tau in neurofibrillary tangles is hyperphosphorylated. Neurofibrillary tangles are aggregates most commonly known as a marker of AD [2] and tau-cascade is one of a promising target for development of drug candidates for AD treatment [3, 4]. This work was focused on the study of tau protein self aggregation process *in vitro* followed by a detection of the influence of potential newly synthetized inhibitors (A-D) in comparison with standard inhibitor myricetin.


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## P-24-005
## The effect of chronic treatment with antidepressants and L-DOPA on binding of the labelled ligands to monoamine transporters DAT and SERT in a rat model of advanced Parkinson's disease

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Administration of antidepressants is recommended for treatment of depression in Parkinson's disease but their role in the modulation of L-DOPA-induced neurochemical markers is poorly explored. The aim of the study was to compare the impact of chronic treatment with selective serotonin reuptake inhibitor fluoxetine (FLU) and classical tricyclic antidepressant amitriptyline (AMI) on binding of [3H]GBR12,935 and [3H]citalopram to dopamine (DAT) and serotonin (SERT) transporters, respectively, in the striatum (STR) of unilaterally 6-OHDA-lesioned rats. Wistar Han rats received unilaterally 16 μg/4 μl of 6-OHDA into the medial forebrain bundle (MFB). Two weeks later, rats exhibiting at least 100 contralateral turns/1 h in the apomorphine test were treated with FLU (5 mg/kg) or AMI (10 mg/kg) alone or jointly with L-DOPA (12 mg/kg) once daily for 3 weeks. Bindings of [3H]GBR12,935 to DAT and [3H]citalopram to SERT were analyzed autoradiographically on brain sections. Injection of 6-OHDA into the MFB resulted in a dramatic decline in [3H]GBR12,935 binding to DAT in the ipsilateral STR while on the contralateral side up-regulation of this binding was observed. Such treatment with 6-OHDA also induced up-regulation of SERT expression in the STR. Chronic administration of FLU+L-DOPA significantly decreased DAT expression in the contralateral STR while AMI+L-DOPA maintained it at the control level. Both FLU and AMI administered alone or in combination with L-DOPA, markedly decreased [3H]citalopram binding to SERT on both sides. The effects mediated by FLU were more distinct than that induced by AMI. The obtained results indicate that FLU and AMI differently affect [3H] GBR12,935 binding to DAT in the contralateral STR. The impact of chronic administration of FLU+L-DOPA indicates that such treatment can extend the duration of L-DOPA-derived DA in the synapses on contralateral side, prolonging its action, what may have a more beneficial effect on motor functions than L-DOPA alone.

## P-24-006
## L-type calcium channels hyperactivity in 5xFAD mouse hippocampal neurons

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Pathology of familial Alzheimer's disease (FAD) often includes calcium signaling abnormalities such as changes in activity of calcium channels on plasma membrane (PM) and endoplasmic reticulum (ER), $Ca^{2+}$-binding proteins and $Ca^{2+}$-ATPases. Shift in calcium signaling leads to change in metabolism, gene transcription and cell survival. Early it has been shown that human neuroblastoma cells and mouse hippocampal neurons bearing PS1 M146V mutant (linked with FAD) had the overload of internal calcium stores. ER calcium overload affects activity of $Ca^{2+}$-binding molecules including ER calcium sensor STIM1. STIM1 sensor has $Ca^{2+}$-binding domain in ER lumen. Depletion of ER calcium stores leads to STIM1 translocation to PM and its interaction with calcium channels. STIM1 is able to activate store-operated calcium channels and also inhibit activity of L- and T-type voltage-gated calcium channels. There were numerous reports that aging and Alzheimer's disease neurons had spontaneous activity and hyperactivity of voltage-gated channels. Using calcium imaging and patch-clamp techniques similar L-type voltage-gated channels hyperactivity have been revealed in mouse hippocampal neurons infected with PS1 M146V constructs and in neurons of 5xFAD transgenic mice. 5xFAD mice are a transgenic mouse model of Alzheimer's disease with 5 mutations in APP and PS1 genes including PS1 M146L mutation. STIM1 knockdown and ER calcium store depletion were used to address the role of STIM1 in hyperactivity of voltage-gated calcium channels in this FAD models. This study for the first time links hyperactivity of L-type calcium channels and overload of internal calcium stores in 5xFAD mouse hippocampal neurons. The data obtained allow considering store-operated and voltage-gated channels as therapeutic targets for future drug development. This work was supported by the RFBR No. 17-54-80006 BRICS and program of Presidium of RAS No. 19 «Modern problems of hi-tech medicine».

## P-24-007
## Antisense (C4G2)n RNA transcripts from C9orf72 gene mutation sequester RNA binding proteins

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Mutation in *C9orf72* gene is the most common genetic cause of two incurable, progressive neurodegenerative diseases – amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), which share clinical, neuropathological and genetic characteristics. The mutation occurs in non-coding part of *C9orf72* gene in the form of increased number of polymorphic hexanucleotide repeats of GGGGCC sequence. In healthy person number of repeats is up to 23 repeats, while in patients the number of repeats equals several hundred to several thousand. In my project, I am focused on studying the RNA toxicity of hexanucleotide repeats. The repeats can be transcribed to RNA in both sense and antisense direction producing $(G_4C_2)_n$ and $(C_4G_2)_n$ RNA molecules, respectively. The resulting RNA molecules can form stable secondary RNA structures which form RNA foci in the nuclei of neurons in spinal cord and brain of C9 ALS/FTD patients. It is predicted that this structures can bind different RNA binding proteins and, therefore, sequester them from their normal cellular functions, this is named RNA toxicity. The whole set of proteins interacting with the antisense RNA transcripts has not been determined so far. We have set up an RNA-pull down assay using *in vitro* transcribed long, biologically relevant RNA constructs ($(C_4G_2)_{32}$), containing S1 m aptamer on one side. This enabled us binding of the constructs to streptavidin magnetic beads and preforming pull-down on mouse and human brain lysates. The pull-down eluates were analysed by mass spectroscopy, results validated by western blot and in cells by RNA fluorescent *in situ* hybridization (RNA-FISH) in combination with immunofluorescence for detection of RNA-protein co-localization. I will present our most recent findings and hypothesis on antisense $C_4G_2$ RNA transcripts and their binding proteins in regard to disease development and progression.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-24-008

## Endogenous metabolites rescue a *C. elegans* model of Alzheimer's disease by triggering a heat shock response

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Age-related changes in cellular metabolism can affect brain health, creating the conditions for the onset and progression of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Although the roles of metabolites have been studied with regard to cellular signaling pathways, their effects on protein aggregation remain relatively unexplored. Here, we identified two endogenous metabolites, carnosine and kynurenic acid, that inhibit the aggregation of the amyloid beta peptide and rescue a C. elegans model of Alzheimer's disease by triggering a heat shock response through the transcription factor HSF-1. These results help rationalize previous observations that both these metabolites have previously been reported for their anti-ageing benefits, but not having a clear mechanism of action reported. Taken together, our results provide a link between metabolite homeostasis and protein homeostasis that could potentially be exploited for therapeutic interventions against neurodegenerative disorders.

## P-24-009

## S100A8 or S100A9 increase SH-SY5Y cell apoptosis and enhance neurodegeneration in a rat model of Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra pars compacta. S100 proteins are EF-hand calcium-binding proteins with various intracellular functions that include cell proliferation, differentiation, migration, and apoptosis. In SH-SY5Y cells, we found S100A8 or S100A9 increased MPP+ (1-methyl-4-phenylpyridinium) induced apoptosis by activating MAPKs including p38 MAPK and JNK. When cells were treated with S100A8 or S100A9, caspase 9 and 3 were activated, BCL-2 and MCL-1 expressions were decreased, and BAX expression was increased. Furthermore, RAGE (receptor for advanced glycation end products) expression was decreased, but that of Toll-like receptor 4 (TLR4) was unchanged. Animals treated with 6-OHDA plus S100A8 or S100A9 significantly showed more tyrosine hydroxylase (TH)-positive cell loss and a greater increase in Iba-1-positive microglial cells than animals treated with 6-OHDA. Taken together, these results indicate that S100A8 and S100A9 might enhance neurodegenerative effects in Parkinson's disease.

## P-24-011

## Establishment of the novel zebrafish model system for physiological and pathophysiological functions of HTRA2 and the therapeutics of neurodegenerative disorders

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Here, we identify zebrafish *htra2* as an ortholog of human *HTRA2*, and demonstrate its essential functions acting as a serine protease and important cell death modulator, which are highly conserved in zebrafish and mammals. These results were confirmed by in silico sequence analysis of genomic DNA and molecular biological techniques: 58.6% and 90% amino acid identity in the entire protein sequence and serine protease domain of HTRA2, respectively. In detail, we have demonstrated that the mature cytosolic Htra2 induces atypical cell death in a serine protease-dependent and caspase-independent manner, similar to mammalian HTRA2. Based on the previous reports that *HTRA2*-knock-out and the inactive mouse models represent characteristics of brain and muscle injury, we generate the *htra2*-knock-down (KD) model by using the splice-blocking morpholino, which exploits the advantages of zebrafish, such as *in vitro* fertilization and transparent in the embryonic development. The *htra2*-KD model exhibited brain and muscle damages as shown the *HTRA2* mouse models. Overall, our study demonstrates a high degree of functional conservation between zebrafish Htra2 and mammalian HTRA2, thus establishing a novel *htra2*-zebrafish model to fully explore the *in vivo* molecular mechanisms of cell death control in neurodegenerative disorders. *The authors marked with an asterisk equally contributed to the work.

## P-24-012

## Evaluation of astrocytes homeostasis in an experimental *in vitro* model of hypocobalaminemia: an approach to explaining the pathomechanism of neurodegeneration from vitamin B12 deficiency

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Vitamin B12 deficiency is common in both developing and developed countries, particularly among the elderly people. The major non-hematological symptoms of cobalamin deficiency are neurological and neuropsychiatric disorders. There are also numerous reports available which show that cobalamin deficiency may be a risk factor of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease, however the cellular and molecular mechanisms underlying this phenomenon remain unknown. It is becoming increasingly evident that disturbance in astrocytes homeostasis may be crucial for the neurodegeneration process. The aim of this study was to develop an *in vitro* model of hypocobalaminemia, by optimizing the conditions of astrocytes culture in the presence of vitamin B12 antagonist –


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hydroxycobalamin (*c*-lactam), and then, the model was used to examine the effect of cobalamin deficiency on astrocytes homeostasis. The cells were examined using immunoenzymatic, spectrophotometric, and fluorescence assays as well as image cytometry. Our results indicate that cobalamin deficiency causes inhibition of astrocytes proliferation, elevation of intracellular reactive oxygen species level and the increase of caspases activity. Interestingly, neither phosphatidylserine externalization nor DNA fragmentation has been detected in cobalamin-deficient astrocytes, which excludes apoptosis in analysed cells. The study provided a significant evidence for astrocytes homeostasis disturbance under hypocobalaminemia, thus indicating an important element of the molecular mechanism of nervous system disorders and the neurodegeneration process initiated and/or promoted by vitamin B12 deficiency. This research was funded by National Science Centre, Poland (PRELUDIUM 13, project 2017/25/N/NZ7/00978) and by Medical University of Silesia in Katowice (KNW-1-O85/N/8/O).

## P-24-013
## The cAMP/PKA-mediated Tau phosphorylation through GSKIP/GSK3β axis in SH-SY5Y and Alzheimer's disease with induced pluripotent stem cells

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In this study, our data showed that GSKIP-WT overexpression in SH-SY5Y cells increased phosphorylation of Tau S409 site over that of PKA- and GSK3β binding-defective mutants (V41/L45 and L130) under forskolin challenge, indicating that both PKA and GSK3β bindings may be associated to phosphorylate Tau at S409 site. In addition, overexpressed kinase-dead GSK3β K85R (retains capacity to bind GSKIP) in SH-SY5Y cells, but not K85M (loss of capacity to bind GSKIP), has a higher Tau S409 phosphorylation, ensures that GSK3β acts solely as an anchor binding protein rather than its kinase activity in this signaling axis. We next conducted *In vitro* kinase assay and observed two results: (i) PKA played a phosphorylation role on Ser214 Ser262 and Ser409 residues of Tau. (ii) GSK3β provided a conformational shelter in PKA/GSKIP/GSK3β/Tau complex to harbor Tau Ser409 residue so that PKA is failed to phosphorylate Tau Ser409 residue. To extend the clinical applications of phosphorylated tau, the phosphorylation status of tau in neurological disorders (NAD) and mild cognitive impairment (MCI) with their Cerebrospinal Fluid (CSF) had also been evaluated. Significantly, AD group had shown the higher total Tau, PKA phosphorylation site (Ser214, Ser262 and Ser409) levels comparing with normal, NAD and MCI group. Moreover, using CRISPR/Cas9 system to produce amyloid precursor protein (APP)-WT/D678H, APP-WT/WT and APP-D678H/D678H induced pluripotent stem cells (iPSC), the results of analysis possessed intense phosphorylation of all phosphorylation sites by PKA (Tau Ser214, Ser262 and Tau Ser409) in (APP)-WT/D678H. Taken together, we provide compelling evidence to implicate that both GSKIP and GSK3β function as anchoring proteins to enhance cAMP/PKA/Tau axis signaling during AD pathogenesis. Especially, suggesting a good feasibility of a standard criterion of total tau and PKA phospho-tau sites (Ser214, Ser262 and Ser409) in CSF for the prediction of AD.

## P-24-014
## Pore-forming activity of α-synuclein is modulated by polyphenols

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α-Synuclein is a presynaptic membrane-active neuronal protein that is linked genetically and neuropathologically to Parkinson's disease. Using model lipid membranes and electrophysiological registration of transmembrane currents we evaluated the effects of 20 μM polyphenol additions on pore-forming activity of α-synuclein in palmitoleoylphosphocholine bilayers at -50 mV. α-Synuclein was added to aqueous solutions (0.1 M KCl, pH 7.4) at one side of the bilayer to obtain final concentrations of 1–3 μM. We found that additions of phloretin, butein, and isoliquiritigenin to the membrane-bathing solutions produced an increase in the protein-induced multichannel current by 5, 1.5 and 2 times, respectively. Cardamonin, 4′-hydroxychalcone, and resveratrol did not practically affect the membrane channel-forming activity of α-synuclein. Taking into account the results of our earlier investigation of polyphenol's effects on pore-forming activity of fragments 25–35 of β-amyloid peptide in lipid bilayers we can now suggest that the flavonoids hydroxylated in the 7-position of A-cycle and in the 4′-position of B-cycle with open propane fragment between the A- and B-rings can specifically interact with the amyloid oligomers. The observed enhancing of pore-forming activity of amyloidogenic polypeptides exerted by polyphenols combined with their neuroprotective action allows for speculations on the role of amyloid-induced pores in the pathogenesis of neurodegenerative diseases. The study was supported by RFS (#17-74-10137). S. Efimova was awarded by scholarship SP-484.2018.4.

## P-24-015
## Experimental mutations in SOD1 associated with familial amyotrophic lateral sclerosis provide insight into its folding mechanism

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Amyotrophic lateral sclerosis (ALS) is a motor neuron disease characterized by degeneration of motor neurons in the brain, brainstem, and spine. Its symptoms include twitching, cramping, or stiffness of muscles and difficulty in swallowing. Mutations in the superoxide dismutase 1 (SOD1) gene have been found in 20% cases of familial ALS. Six mutations have been recognized at glycine 93 of SOD1, and G93A has been studied intensively in transgenic mice, in which protein aggregates were detected. In the present study, we generated 19 mutations of SOD1 glycine 93 fused with a GFP tag and examined their ability to form aggregates in neuronal cells. Aggregation was assessed using fluorescence microscopy, and the mobility of wild-type and mutant SOD1 proteins in the cytosol was examined by fluorescence loss in photobleaching. Neuronal toxicity was observed in cells with mutant SOD1 proteins including G93A, G93P, G93T or G93Y. Our results demonstrated that the loop flexibility of glycine seems to play a critical role in the folding of SOD1 protein. This finding may provide novel insights into protein aggregation.


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## P-24-016
## Early candidate urine biomarkers before amyloid-plaque deposition in Alzheimer's disease transgenic mouse model

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Biomarkers are measurable changes associated with disease. Unlike blood, which is stable because of homeostasis mechanisms, urine can accumulate many kinds of changes; some of these changes are associated with disease and will become biomarkers. In addition, urine is a resource for biomarker discovery that can be easily and non-invasively collected. As a sensitive biomarker source, urine therefore might reflect pathological changes, especially in the early stages of disease. In this study, an APP (swe)/PSEN1dE9 transgenic mouse model was used to identify candidate biomarkers for early AD. Urine samples were collected from 4-, 6-, and 8-month-old transgenic mouse models, and the urinary proteomes were profiled using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The levels of 29 proteins differed significantly between wild type and 4-month-old mice, which had not started to accumulate amyloid- plaques. Among these proteins, 13 have been associated with the mechanisms of AD, while 9 have been suggested as AD biomarkers. Our results indicated that urine proteins enable detection of AD before amyloid- plaque deposition, which may present an opportunity for intervention. *The authors marked with an asterisk equally contributed to the work.

## P-24-017
## GDAP1 deficiency causes a dysregulation of basal autophagy

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Ganglioside-Induced Differentiation-Associated Protein 1 (GDAP1) is a glutathione S-transferase located in both the outer mitochondrial membrane and the mitochondria-associated membranes (MAM). MAM are specialized subdomains of the endoplasmic reticulum (ER) with a particular lipid and protein composition that participates in the cross-talk between the ER and mitochondria. These contact sites allow the direct interaction between ER proteins and lipids with outer mitochondrial membrane (OMM) to modulate different cellular signaling pathways. MAM dysfunction is associated with neurodegeneration, and mutations in some MAM genes are related to Mendelian neurological diseases. One of these genes is *GDAP1* that causes Charcot-Marie-Tooth neuropathy (CMT). The pathophysiology of *GDAP1*-related neuropathies includes defects in the mitochondrial network and ER, $Ca^{2+}$ homeostasis and oxidative stress. In addition, GDAP1 depletion reduces the juxtaposition between ER and mitochondria, possibly affecting the functionality of the MAM, which includes the regulation of autophagy and autophagosome formation. Recent findings of our group reported an increment of autophagic vesicles in *Gdap1*$^{-/-}$ mouse embryonic motor neurons by electronic microscopy, thus suggesting the autophagy involvement in the pathophysiology of CMT caused by GDAP1 mutations. Here, we found that GDAP1 deficiency produces a dysregulation of basal autophagy without affecting the autophagosome-lysosome fusion. PLA and co-IP experiments showed the constitutive interaction of GDAP1 with Syntaxin 17 and LC3, proteins that are essential for autophagosome biogenesis and for a wide variety of membrane trafficking processes. In addition, we observed accumulation of enlarged perinuclear lysosomes with an abnormal distribution. Altogether these results assign a role for GDAP1 in both the autophagy pathway and the lysosome function, highlighting new candidate cellular and molecular therapeutic targets.

## P-24-018
## Study of the effect of intranasal administration of YB-1 protein on estradiol (E2) levels in animals with a model of Alzheimer's disease

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It is known that women are more at risk for Alzheimer's disease (AD). A higher prevalence of AD in women is associated with a decrease in estrogen levels. The 5XFAD (TG6799) transgenic mouse line is an *in vivo* AD model. Since it is shown that intranasal administration of YB-1 protein protects memory from deterioration, and also lowers the level of cerebral amyloid beta in transgenic 5XFAD mice, we investigated whether intranasal administration of YB-1 protein affects estradiol (E2) levels. The studies were performed on 8–9 monthly transgenic female 5XFAD mice; the age of ones corresponds to the premenopausal period in women. Determining the level of E2 in the peripheral blood of female mice was carried out by enzyme-linked immunosorbent assay. It was found that in the groups of transgenic females, the level of E2 at all stages of the study was significantly lower than in the groups of non-transgenic females of the same age. In the case of transgenic females, it was found that intranasal administration of a solution of YB-1 protein leads to an increase in the concentration of E2. Two weeks after the start of the administration YB-1, the concentration of E2 was $40.29 \pm 14.29$ pg/mL, and by the end of the fifth week: $82.10 \pm 4.24$ pg/mL. It was established that in 3 weeks the level of E2 in the blood of transgenic females increased almost 2 times. Thus, for the first time, it was found that daily intranasal administration of a solution of YB-1 protein leads to an increase in the concentration of estradiol of 5XFAD female mice. This effect should be considered as positive, since the hormones estrogens have a pronounced protective effect on the state of hippocampal cells, stimulate the formation of dendritic spines on its neurons, synthesizing acetylcholine, and also inhibit beta-amyloid aggregation and reduce its neurotoxicity. The work is supported by the Russian Scientific Foundation (No. 18-15-00392). *The authors marked with an asterisk equally contributed to the work.

## P-24-019
## Development of novel DYRK1A inhibitors for the treatment of Alzheimer's disease

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Alzheimer's disease (AD) is a chronic, irreversible neurodegenerative disorder where patients develop dementia, cognitive disorders and mood changes. The drugs currently used for AD are limited due to lack of efficacy and only slow deterioration. Therefore, it is urgent to find new targets for developing drugs to treat AD patients. Amyloid plaques, an aggregation of beta amyloid protein (Abeta), are one of the two brain abnormalities that define AD. The other hallmark is neurofibrillary tangles (NFT), which


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are aggregates of hyperphosphorylated tau protein. Previous studies have shown that DYRK1A (Dual specificity tyrosine-phosphorylation-regulated kinase 1A) are highly expressed in the hippocampus of AD patients and involved in the pathogenesis of plaques and NFT formation. Thus, inhibition of DYRK1A is a potential target to treat AD. By using computational modeling analysis, our research team has filtered five potential DYRK1A inhibitors. Three specific aims will be executed in 3 years: (i) Based on these five structures, we will optimize and synthesize potent DYRK1A inhibitors. (ii) To validate the inhibitory effect of DYRK1A inhibitors on reduction of Abeta formation and Tau phosphorylation using neuronal cells. (iii) To select the most potent compound as our candidate DYRK1A inhibitor and evaluate its effect on learning and memory enhancement in both aging mice, scopolamine induced memory impairment mice and transgenic mice. Our preliminary result showed that one of our five candidates, 711-D-001, inhibit only DYRK1 family out of 97 human kinase families. Further validation experiment showed that 711-D-001 inhibited only DYRK1A but not DYRK1B indicating that this is a highly selective inhibitor against DYRK1A. From this project, a new category of drugs will be developed for the treatment of AD.

## P-24-020
## Dendrimer functionalization with neurotrophin 4: controlled release of protein into vitreous in response to retinal pigment epithelium damage

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NT-4 has been regarded as a promising therapeutic protein for treatment of damaged retinal pigment epithelium cells. Here, we studied physicochemical parameters of NT-4-PAMAM electrostatic complex, which can provide a sustained concentration of protein in intraocular space over an extended time after delivery. We determined kinetics of neurotrophin 4 desorption in PBS buffer, vitreous and damaged retina. Adsorption/desorption of NT-4 molecules to/from positively charged PAMAM dendrimers have been precisely determined to control the bounded/unbounded protein molecule, diffusion coefficient and size of a protein-laden dendrimer structure. Initially, adsorption of NT-4 molecules at PAMAM dendrimers was studied in PBS buffer using the dynamic light scattering, electrophoresis, solution depletion techniques, enzyme-linked immunosorbent assay and atomic force microscopy. This allowed us to precisely determine desorption of neurotrophin 4 from nanoparticles under *in situ* conditions. The maximum coverage of irreversibly adsorbed neurotrophin 4 determined by the ELISA assay allowed us to devise a robust procedure for preparing stable and well-controlled coverage of NT-4 at PAMAM nanoparticles. Thereafter, we tested nanospheres containing NT-4 molecules by injecting them into vitreous cavity of mice exposed to intravenous injection of sodium iodate and evaluated their intraocular desorption kinetics from drug carriers *in vivo*. Our measurements revealed the NT-4-dendrimer nanoparticles can be used for continuous neurotrophic factor delivery enhancing its distribution into mouse vitreous as well as damaged retina over 28 days of post-injury observation. Understanding of polyvalent neurotrophins interactions with dendrimer nanoparticles might be useful to obtain well-ordered protein layer, targeting future development of drug delivery systems especially for neuroprotection of damaged retinal neurons.

## P-24-021
## Activation of pro-survival signaling inhibits alpha-synuclein aggregation in dopaminergic neurons

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Parkinson's disease (PD) is an incurable neurodegenerative disorder, primarily characterized by progressive death of dopaminergic neurons. The brains of both sporadic and familial PD patients contain Lewy bodies – intraneuronal inclusions containing aggregated and phosphorylated (at serine 129; pS129) alpha-synuclein as a main component. Accumulation of pSer129-alpha-synuclein-positive neuronal aggregates can lead to synaptic and mitochondrial dysfunction, endoplasmic reticulum (ER) stress and deregulation of autophagy and lysosomal pathways, compromising neuronal functions already at early stages of PD. The treatments targeting and attenuating alpha-synuclein aggregation may thus slow down or even stop neurodegeneration before the onset of major motor symptoms. We and others have previously shown that activation of pro-survival cellular signaling is neuroprotective in several mouse PD models. To study the effect of specific pro-survival pathway activation on alpha-synuclein aggregation, we utilized lentiviral vector to express constitutively active molecule stimulating specific signaling pathway in primary dopaminergic and hippocampal neurons treated with alpha-synuclein preformed fibrils (PFFs). We show that this activation promoted neuronal survival after thapsigargin-induced ER stress and strongly reduced accumulation of pS129-positive alpha-synuclein aggregates in dopaminergic and hippocampal neurons. Furthermore, our data suggest that activation of specific pro-survival signaling pathway stimulates lysosomal degradation of misfolded alpha-synuclein, preventing PFF-induced alpha-synuclein aggregation. We also study the effect *in vivo* in PFF-induced alpha-synuclein aggregation mouse model. These proof-of-principle results establish a basis for the use of lentiviral vectors to develop neuroprotective gene therapy strategies for dopaminergic neurons in PD. *The authors marked with an asterisk equally contributed to the work.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-24-022
## Oxidative stress and inflammation gene expression changes in cognitive impairment

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Cognitive impairment is closely related to oxidative stress and inflammation processes that play a key role in the pathophysiology of neurodegenerative diseases. Moreover, age-related disorders seem to be influenced by multiple environmental factors. In this study we investigated how the expression levels of a panel of genes related to oxidative stress and inflammation correlated with cognition, and how environmental factors could influence the cognitive decline. The expression levels of 168 genes were evaluated in whole blood from 83 individuals. The following features have been collected: age, sex, BMI, education, diabetes, smoking, toxicity exposure, childhood environment, pre-diagnostic decade environment and Mini-Mental State Examination (MMSE) score. Thirty-four out of the 83 individuals presented symptoms of cognitive impairment (MMSE below 26). No difference was observed comparing individuals with cognitive impairment and the normal-ones except for education and age. We found that the expression of 19 genes correlated with MMSE score in the entire cohort. Among these, 17 correlated also with age, whereas the expression of two peroxiredoxin genes negatively correlated exclusively with MMSE in an age-independent way. The correlation was stronger in the case of PRDX1 when considering only individuals with MMSE below 26. However, when controlling the correlations by education, the significance for both PRDX1 and PRDX3 was lost. Interestingly, even controlling for education, SOD1 expression significantly decreased with MMSE from 30 to 26 and increased when individuals showed cognitive impairment (MMSE from 25 to 4) in an age-independent manner. Our results emphasize that the levels of some antioxidant genes in blood correlated with cognitive function decline. The observed activation of endogenous antioxidant mechanisms in blood from patients with cognitive impairment might be an adaptive mechanism aimed at counteracting the chronic low grade oxidative stress.

## P-24-023
## Study of the antioxidant effects of coffee metabolites in C6 glioma cells exposed to diesel exhaust particles

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The attention on neurodegenerative diseases is growing due to the increase of the life expectancy and the consequent increase of average age. A contributory cause of neurodegeneration is the chronical exposure to ambient air pollution that can affect the SNC inducing neuroinflammation and oxidative stress. In this scenario, nutrition, through the assumption of essential micronutrients, might modify the oxidative impacts of air pollution on health. Recently, it has been shown that the use of phenolic acids such as caffeic, chlorogenic and ferulic, can modulate oxidative stress. Once introduced into the body through the diet, these substances undergo a process of metabolization by the microbiota and liver leading to different metabolites generation. To investigate the possible antioxidant action of these molecules, C6 glioma cells were pretreated with coffee metabolites and subsequently treated with diesel exhaust particles (DEP). ROS levels and cell viability were assessed. Preliminary results showed that cell vitality reduction and significant ROS level increase obtained after DEP treatment, are decreased after coffee metabolites administration, bringing back vitality and ROS levels comparable to those of the controls. Therefore, this data suggest an antioxidant effect that might counteract the oxidative stress induced by DEP. The results obtained may potentially be applicable in the future for the production of functionalized foods containing coffee metabolites, in order to mitigate the harmful effects of atmospheric pollution

## P-24-024
## Ischemic conditions affect neutral lipid reshaping in blood–brain barrier cells

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Ischemic/reperfusion (I/R) injury contribution in Alzheimer's disease (AD) onset has definitely been endorsed in the last decades. Oxygen and nutrient deprivation occurring during I/R induced a signaling pathways remodeling with alteration in oxidative stress, inflammatory response initiation, protein and lipid homeostasis that can lead to blood brain barrier (BBB) breakdown. Moreover starvation occurring during I/R is a condition leading to autophagy activation. Autophagy allows proteins and lipids degradation for abnormal material clearance or for amino acids and fatty acids recycling in proteins and lipids essential for survival. In the present study we monitored the lipid reshaping and peroxidation as well signaling pathway linked to those occurring under oxygen and glucose deprivation (OGD, an experimental condition mimicking cerebral ischemia), focusing on the autophagy involvement, in order to evaluate their contribute in BBB alteration/adaptation to the starvation. Rat brain endothelial (RBE4) were subjected to OGD treatment for 3 h. After restoration of standard conditions for 1 or 24 h (R1 h and R24 h respectively), lipids and proteins analyses were performed. Data obtained demonstrated that I/R injury induced a reshaping in triglycerides


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and in CE, along to lipid droplets (LD) biogenesis leading to excessive lipid storage. In parallel, the increase of LC3-II/LC3-I ratio and LC3 co-localization with LD suggest that a specific autophagy, *lipophagy*, is activated to counteract the cell engulfment. Moreover, LD seemed to move towards plasma membrane following exocytosis pathways related or not to exophagy, a secretory path activated to eliminate misfolded proteins in neurons subjected to OGD [Lonati et al., J. Mol. Cell. 2018]. Nevertheless, unconventional spreading of abnormal material, activated against I/R harmful effects, might leads to neurodegeneration.

## P-24-025
## Modification in blood BDNF, bFGF and S100B levels following exercise to volitional exhaustion in sedentary individuals exposed to acute normobaric hypoxia

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The CNS can be modified by neuropeptides which are synthesized and released by neurons and glial cells. The best-known environmental factors affecting the CNS are physical exercises and/or exposure to hypoxic conditions, but the question is how these conditions modify the secretion of neuropeptides from neurons and astrocytes? The impact of these factors can be determined indirectly in humans by measuring serum concentration of neuropeptides which are synthesized in aforementioned cells and can cross the blood-brain barrier. The study aims to investigate whether (i) maximal exercise affects peripheral levels of brain-derived neurotrophic factor (BDNF),basic fibroblast growth factor (bFGF) and S100 calcium-binding protein B (S100B), which are secreted by neurons, neurons and astrocytes, and astrocytes, respectively, and (ii) whether exposure to acute normobaric hypoxia (ANH) modifies this effect. For this purpose, seven sedentary young men performed cycle ergometer test to volitional exhaustion (EVE) under normoxic (N) and hypoxic conditions, equivalent to 2,000 m altitude (H2; FiO2=16.6%) and 3,000 m altitude (H3; FiO2=14.7%). Increases were observed in serum BDNF levels after EVE in N ($P < 0.01$) and H2 ($P < 0.01$), but there were no differences after EVE between the examined conditions. There was a tendency ($P < 0.08$) for EVE to increase bFGF levels in N. EVE did not affect serum concentration of S100B, but baseline level was higher in H2 than N ($P < 0.05$) and higher after EVE in H3 compared to N ($P < 0.01$). The results suggest that EVE on cycle ergometer in normoxic condition can positively affect activation of neurons, but there were no effect on astrocytes in young sedentary men. ANH can activate astrocytes, but there is no additional effect of EVE on this process. Considering that BDNF and bFGF promote the survival of neurons, and S100B is among others marker of brain injury, it can be assumed that EVE in both normoxia and hypoxia is safe for the CSN. Grant: 2013/09/B/NZ7/00726

## P-24-026
## Protective effect of currant polyphenolic compounds against pathogenic functions of apolipoprotein E4 associated with Alzheimer's disease

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Natural polyphenolic compounds in currants (Vitis vinifera L.) such as quercetin (QUE), kaempferol (KAE), resveratrol (RES) and epigallocatechin gallate (EGCG) have been shown to display neuroprotective effects against Alzheimer's disease (AD), although the exact mechanisms of action are not known. Furthermore, it has not been examined whether these compounds could affect the AD-related pathogenetic functions of apolipoprotein (apo) E4, the strongest genetic risk factor for late-onset AD. We have shown before that a rare apoE4 mutant, the apoE4[L28P], that burdens carriers with an added risk for AD and an apoE4 C-terminal truncated fragment, the apoE4-165, similar to apoE4 fragments found in AD patients brain, promote amyloid-peptide b42 (Ab42) accumulation in neurons and induce oxidative stress. Here, we examined the effect of QUE, KAE, RES and EGCG on functional properties of apoE4[L28P] and apoE4-165 associated with AD pathogenesis. By using confocal and traditional fluorescence microscopy, we examined the effect of QUE, KAE, RES and EGCG on the capacity of apoE4[L28P] and apoE4-165 to promote Ab42 cellular uptake and to induce intracellular reactive oxygen species (ROS) production by human neuroblastoma SK-N-SH cells. Our analyses showed that all polyphenolic compounds prevented cellular ROS formation which is dependent on cellular Ab42 uptake promoted by the lipid-free apoE4 variants. Furthermore, by using a MTT cell viability assay, we showed that the polyphenolic compounds averted the capacity of lipoproteins containing apoE4[L28P] to reduce cell viability and increase ROS burden in SK-N-SH cells, independently of Ab42. These findings demonstrate a protective action of polyphenolic compounds in currants against the AD-related pathogenic functions of apoE4 variants, and provide motivation to test whether the consumption of currants, which has been already shown to improve cognition in healthy older adults, could be a promising approach for the treatment of AD.

## P-24-027
## Glycogen phosphorylase inhibition – new anti-aging approach?

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Inhibition of inhibition of glycogen phosphorylase (Pyg) activity has been shown to block memory formation and disrupt the Long Term Potentiation (LTP) formation in the brain of young animals. Unexpectedly, inhibition of Pyg activity significantly improved the LTP induction in hippocampal slices isolated from old animals. This suggested that blocking of Pyg activity may be used for the improvement of age-associated deficits of memory formation. To test this hypothesis, young (1 month) and old


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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(18 months) mice were treated for 2 weeks with BAY (BAY U6751; Pyg low molecular inhibitor) by daily injections. Behavioral and metabolic parameters were monitored. The most important, the two novel object recognition test revealed that 2 weeks BAY administration significantly improved learning potential in old animals as compared to the control old group ($P = 0.046$). Moreover, behavioral changes were reflected by an alteration in hippocampal dendritic spine morphology: in old animals, BAY treatment was associated with statistically significant elongation and thinning of dendritic spines while in the young animals the spines became shorter and thicker. Additionally, we found differences in metabolic enzymes expression profiles in BAY treated vs control in primary cell cultures as well in hippocampal tissue slices. To obtain an insight into the genetic mechanism responsible for the observed changes we analyzed the genomic DNA modifications. Our data revealed that BAY decreased the level of DNA methylation in old animals which correlated with a reduction of an oxidative stress-induced 8-oxo-guanine level. In contrast to the old animals, both 5-methylcytosine and the 8-oxo-guanine level was increased in young ones treated with BAY. Taking together, the results presented here demonstrate that inhibition of Pyg results in changes in the global transcriptional capability which manifests on a protein and morphological level and which leads to behavioral changes.

## P-24-028
## Mice with selective loss of noradrenergic neurons as a potential novel model to study presymptomatic phase of Parkinson's disease

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Parkinson's disease (PD) symptomatology is directly correlated with diminished level of dopamine produced by dopaminergic cells within the region of SN/VTA. However, clinical data show that degeneration of noradrenergic neurons of locus coeruleus (LC) is also associated with PD and may precede the loss of dopaminergic cells. The aim of this study is to conclude whether selective loss of LC noradrenergic neurons in progressive manner, may negatively influence the dopaminergic system. To elucidate this issue, we created two models of mice with progressive degeneration of noradrenergic system, based on deletion of the gene encoding transcription factor TIF-IA. We applied the conditional inactivation of the *Rrn3* by the Cre-loxP system expressing Cre recombinase under dopamine beta-hydroxylase (DBH) promoter. TIF-IADBHCre mice showed signs of noradrenergic innervations failure associated with enhanced expression of various neurodegeneration markers within dopaminergic system (not targeted by the mutation): upregulation of micro- and astroglial markers, enhanced level of oxidative stress, pro-inflammatory proteins and changes in gene expression related to PD. To eliminate the problems with targeting the peripheral noradrenergic cells in the TIF-IADBHCre mice, in a second model a Cre-dependent lentiviral vector carrying the *Rrn3* deletion created by the CRISPR/Cas9 system was directly delivered to LC in DBHCre mice (study in progress). Worth to emphasize, majority of animal PD models have been created with the administration of neurotoxins that immediately cause the death of dopamine neurons. In our transgenic mice, neural degeneration is progressively delayed in time, enabling better mimicking the human nature of PD. If we provide additional evidences that prolonged, selective noradrenergic degeneration impairs dopaminergic system functioning, mice with ongoing neurodegeneration of LC neurons may became a valuable tool for study the presymptomatic phase of PD.

## P-24-029
## Disturbances in calcium signaling in iPSCs-based models of polyglutamine neurodegenerative diseases

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Reprogramming patient-specific somatic cells to induced pluripotent stem cells (iPSCs) represents a promising tool for investigation a number of different pathologies including neurodegenerative diseases. Here we studied abnormalities in calcium signaling in three iPSCs-based neuronal models specific for Huntington's disease (HD) and Spinocerebellar ataxias type 1 (SCA1) and 17 (SCA17). Using a patch-clamp technique and fluorescent calcium imaging approach we showed that such a ubiquitous and important mechanism of calcium uptake as a store-operated calcium entry (SOCE) was pathologically enhanced in HD and SCA17 which is also known as HD-like pathology 4. At the same time SOCE was significantly decreased in SCA1. Further we demonstrated a pathological upregulation of voltage-gated calcium channels in HD and SCA17 but not in SCA1 neurons. To study all 3 pathologies we used GABA-ergic striatal medium spiny neurons which are known to be the most affected in HD. Thus, the abnormal increasing in SOCE may underlay a selective vulnerability of these neurons in HD pathogenesis. Detailed studying of different patient-specific HD models allowed us to conclude that severity of SOCE increasing does not depend on length of polyglutamine tract in mutant huntingtin: SOCE was 2-fold higher in both low-repeat (40-45Q) and juvenile (76Q) HD models compared to wild type neurons. Preliminary western blotting results indicated increasing in expression levels of huntingtin and STIM2 proteins in HD neurons compared to wild type neurons. It should be noted that previously published data obtained in collaboration with Prof. Kuznicki suggested that increased level of calcium sensor STIM2 may drive a constitutive activity of SOC channels in neuronal HD model overexpressed huntingtin-associated protein 1A. The study was supported by the RSF grant No. 17-74-20068 and the Grant from the President of RF.

## P-24-030
## Spheroid cell culture as an innovative model for studies of prions and prion infection

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Prion diseases represent a group of fatal neurodegenerative disorders affecting mammals including humans. Prion infection is associated with a conformational transformation of the cellular


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prion protein ($PrP^C$) to its abnormal, partially proteolytically resistant $PrP^{TSE}$ variant. To investigate the physiological role as well as the pathogenesis of prions, exponentially growing tissue cultures are widely used in addition to classical animal studies. However, their benefit is limited because of the absence of toxic $PrP^{TSE}$ accumulation and different physiology of cells grown in the 2D monolayer compared to tissues in the living organism. The aim of this study is to develop a new 3D model of differentiated cells that will better simulate common conditions in the nerve tissue. For this purpose, a mouse neuronal CAD5 cell line was selected based on its ability to be infected with prions and also *in vitro* stimulated to differentiate by change of culture medium and serum withdrawal. Furthermore, the control CAD5KO cell line with a deleted gene encoding the cellular prion protein was created as a part of this project using CRISPR/Cas9 genome editing. Cultivation of CAD5 cell suspension under constant rocking resulted in the formation of spherical multicellular aggregates, so-called spheroids. The elimination of serum from the cell culture medium allowed the long-term cultivation of spheroids for at least 6 weeks. Concurrent incubation of cells with an infectious brain homogenate enabled subsequent immunohistochemical detection of a prion infection on spheroid cross sections. Our data indicate that CAD5-derived spheroids represent a valid model that could complement other tools generally used across the prion research field. The project was supported by GAUK 530217, SVV 260369 and Progress Q26/LF1.

## P-24-031

## The neuroprotective effect of conditioned medium from neuronal and glial progenitor cells in $H_2O_2$-induced oxidative stress model

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Oxidative stress plays an important role in pathogenesis of neurodegenerative diseases. We investigated the secretory activity of neuronal and glial progenitor cells, as well as the neuroprotective effect of their conditioned medium in $H_2O_2$-induced oxidative stress model of human neuroblastoma cells SH-SY5Y. IPS cells were cultured in DMEM/F12, 1% N2, 200 nM LDN193189, 10 μM SB431542 and 2 μM dorsomorphin to obtain neural stem cells (NSC). NSC were cultured in DMEM/F12, 2% B27, 20 ng/mL FGF2, 2 μM purmorphamine for receiving neuronal progenitor cells (NPC) and in DMEM/F12, 1% N2, 10 ng/mL FGF2, 20 ng/mL EGF and 20 ng/mL CNTF for obtaining glial progenitor cells (GPC). Quantitative analysis of neurotrophin expression was performed by real-time PCR and ELISA. We incubated SH-SY5Y cells with 200 μM $H_2O_2$ for 24 h for initiation of the oxidative stress. NPC and GPC were cultured for 48 h to obtain the conditioned medium (CM). The samples of CM were added 24 h before oxidative stress, and cell viability was measured after 24 h by MTT assay. Results demonstrated the high level of GDNF, BDNF, NGF and NT-3 expression in cultures of neuronal and glial progenitor cells. The highest level of neurotrophin expression was observed in glial progenitor cells. The CM from the neuronal and glial progenitor cells increased the viability of the SH-SY5Y cells under oxidative stress by 14 ± 2%. In this study the neuroprotective effect of CM from neuronal and glial progenitor cells was demonstrated. This work was supported by the Ministry of Education and Science of the Russian Federation (project No. 14.604.21.0184 RFMEFI60417X0184) and RFBR state assignment for RCMG.

## P-24-032

## Assessment of alterations in taste sensitivity and its impact in Parkinson's disease

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Parkinson's disease (PD), in addition to the characteristic motor symptoms, may present a variety of non-motor symptoms. It is now known the role played by an altered sense of smell, but more recent, studies want to investigate whether there is a similar role in taste sensitivity and what are its implications. The purpose of the present study was to better investigate the presence of an alteration in taste sensitivity in PD patients with respect to controls, by studying the response to the administration of different tastant substances (citric acid, sucrose, quinine hydrochloride, natrium chloride, rapeseed oil, deionized water). We also tried to establish whether such alterations were compared to a prevalence of gender and/or duration of the disease. 33 patients (18 men and 15 women, aged between 34 and 86 years) affected by idiopathic PD were enrolled at the Neurorehabilitation Clinic of the "Ospedali Riuniti" University Hospital of Ancona. The taste test is based on filter paper strips soaked with four substances (sodium chloride, citric acid, sucrose, quinine hydrochloride), evoking the 4 basic taste qualities (salty, acid, sweet, bitter), each presented at 4 different concentrations; pure rapeseed oil and water were administered, evoking fat taste and neutral taste. The stimuli were applied to the left and right side of the protruded tongue, just posterior of the anterior third, with filter paper strips soaked in the different solutions. Patients were asked to fulfill a self-assessment lifestyle test and an eating habits questionnaire. The data collected confirmed the presence of an alteration of the overall taste perception in affected subjects compared to the control group. The reduction in taste sensitivity is significantly higher in male subjects in relation to the salty, sour and bitter tastes. This alteration must be carefully investigated in Patient's clinical interview, as it may be associated with a reduced intake of food and consequent malnutrition.

## P-24-033

## Effects of cotinine and 6-hydroxy-L-nicotine on memory impairment in Aβ25-35 peptide-induced an Alzheimer's disease rat model

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Alzheimer's disease (AD) is the most typical form of dementia characterized by memory and cognitive loss. One hallmark pathology of AD is the loss of forebrain cholinergic neurons. Nicotinic acetylcholine receptors (nAChRs) are present in the cholinergic neurons indicating their involvement in cognitive functions such as learning and memory. Nicotine is a well-known agonist of nAChRs and was reported to improve attention, learning, and memory. The cardiovascular and addictive side-effects of nicotine have limited its therapeutic use in AD. Thereby, here we focus on two structural related nicotine metabolites, cotinine and 6-hydroxy-L-nicotine (6HLN) that showed promising cognitive-improving effects without exhibiting the nicotine's side-effects. Using a series of behavioral tests we evaluated the effects of cotinine and 6HLN on memory impairment in an AD rat model induced by intracerebroventricular infusion of $A\beta_{25\text{-}35}$.


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Using *in-silico* docking experiments, we attempted to relate behavioral results with the calculated binding potential of the two nicotinic compounds into two different α4β2 nAChRs (PDB ID 6CNK) allosteric binding sites. Also, we are currently evaluating the impact of the two nicotine derivatives on the oxidative status of this animal model. We have shown that 6HLN and cotinine attenuate the spatial, recognition, working and reference memory deficit induced by $A\beta_{25\text{-}35}$ to a greater extent than nicotine. Moreover, we demonstrated that cotinine and 6HLN indeed bind to α4β2 nAChRs with similar or even higher energy than nicotine and that the binding site at the α4-β2 interface is preferred over the binding site at the α4-α4 interface. These findings suggest that cotinine and 6HLN might improve memory by modulating the nAChRs and thus might represent new therapeutic agents in AD. This work was supported by CNCSIS-UEFISCSU, project number PN-III-P1-1.1-TE-2016-0367. *The authors marked with an asterisk equally contributed to the work.

## P-24-034
## The occurrence of unique G-rich DNA sequences in human genes related to neurodegeneration

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G-rich DNA sequences often give rise to so-called G-quadruplex structures, which are non-B DNA structures stabilized under certain cellular conditions by specialized proteins. G-quadruplexes participate in many biological processes including replication, transcription, translation, genome integrity maintenance, as well as many pathogenic processes like neurodegeneration or cancer development. Our bioinformatics studies have found unique G-quadruplex forming sequences composed of $(G_6N)_3G_6$, where N is any non G base. These particular types of sequences are incredibly rare in the human genome, but almost all of them occur in regions which are associated with neurodegenerative disease development and progression, particularly Alzheimer's. The best example is the presence of this sequence within the intron of Amyloid beta precursor protein (APP), the precursor molecule to generate beta amyloid, which can form amyloid plaques in Alzheimer's. Another is found in the promoter region of the gene coding for AE binding protein 1, which is implicated in Alzheimer's disease pathology. Using the 1000 genomes project we have investigated the presence of global minor allele frequencies. Only 6 percent of the world population have preserved $(G_6C)_3G_6$ putative quadruplex loci in APP gene, however there are quite big differences between nations (based on the 1000 Genomes data). Indel (insertion/deletion events) for this sequence are highest in the Finnish population, which correlates with this nation also having the highest rate of death by Alzheimer's disease. We believe that this work will help to discovery and subsequent characterization of novel therapeutic targets and speed-up research in this field remarkably. *The authors marked with an asterisk equally contributed to the work.

## P-24-035
## Photocontrolled slicing of amyloid beta fibrils as a potential therapeutic strategy against Alzheimer's disease

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According to the common view, amyloids are correlated with many currently incurable aging-related conditions such as Alzheimer's disease (AD), which is associated with deposition of neurotoxic amyloid beta (Abeta) fibril plaques in brain. Abeta fibrils are formed by normally soluble proteins, which aggregate into highly stable and insoluble assemblies under diverse conditions. Despite huge research efforts there is still no cure for AD while conventional diagnostics methods are not conclusive, but invasive and very expensive. Considering that, a question arises: where else shall we look for new and effective theranostic strategies against AD? We believe that the answer may lie in a small but portentous molecule, azobenzene (azo), which achieves reversible changes in geometric structure (*cis*/*trans* isomerism), triggered by light. For that reason, azos have been widely used to build photo-mechanical responsive systems. We have designed and investigated azo-derivatives able to bind and disrupt the structure of Abeta aggregates, which allow for a nonrandom dissociation of the fibrils into neuroprotective monomers. For this reason, we have established a simulation protocol that integrates several levels of theory for reliable investigations of Abeta's dynamics and its interactions with potentially dissociating them azo-ligands. We would like to present the successfully designed family of azo-derivatives, whose abilities to dissociate Abeta fibrils in a controlled manner have been tested and proven experimentally, including *in vitro* studies, which have also confirmed their non-toxicity. Presented azo-ligands when labelled with a short-lived $^{68}$Ga radionuclide can become a safe, well-tolerated and cyclotron-independent Abeta-tracer for PET diagnostics. Besides Alzheimer's disease theranostics, these azo-derivatives may also contribute to the future development of medicine, research, and technology dealing with other pathological amyloids (*e.g.* bacterial biofilms). *The authors marked with an asterisk equally contributed to the work.

## P-24-036
## Biochemical changes in the visual system in mice models of Parkinson's disease

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Parkinson's disease (PD), characterized by loss of nigrostriatal dopaminergic neurons, is diagnosed by the appearance of motor disorders many years after the onset of neurodegeneration, which explains low efficiency of treatment. The only hope to improve the treatment is the development of preclinical diagnosis, based on searching biomarkers as changes in premotor functions. Since impaired visual function is among PD manifestations, this study aimed to search for biochemical changes in the eye of mice when modeling preclinical (presymptomatic) and clinical (symptomatic) stages of PD using neurotoxin MPTP. The mRNA level and protein content of tyrosine hydroxylase (TH) were assessed in the retina using real-time PCR and Western blot. In addition, the


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concentration of catecholamines was measured in the retina and iridociliary complex by HPLC. Presymptomatic mice show the increased levels of dopamine and metabolites in retina. Apparently, this is due to a compensatory increase in TH activity, since the TH protein content remains at the control level. Symptomatic mice show an abrupt decrease in the TH protein content, whereas the retinal dopamine content remains at the control level. This observation is also a manifestation of a compensatory reaction. The above data suggest that compensatory changes observed in the retina are similar to those previously found in the substantia nigra in these animal models. In the iridociliary complex, a decrease in the norepinephrine concentration was found in symptomatic mice, which may be due to a degradation of noradrenergic neurons innervating the iris and ciliary body. Changes found in parkinsonian animals correlate well with clinical data that the reaction of the pupil is slowed and the incidence of glaucoma is increased in PD patients. Thus in presymptomatic mice, pathological processes extend to the eye, followed by changes in the tear fluid and intraocular pressure, which can serve as preclinical diagnostic markers. *The authors marked with an asterisk equally contributed to the work.

## P-24-037
## Compensatory mechanisms under the degeneration of dopaminergic neurons of the arcuate nucleus: dopamine synthesis by non-dopaminergic neurons

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Degeneration of dopaminergic neurons of the arcuate nucleus, which provide inhibitory control of prolactin secretion and proliferation of lactotrophs, causes the development of hyperprolactinemia. Although the plasma prolactin concentration returns to the control level over time, compensatory mechanisms remain uncertain. Therefore, this study was aimed to determine the mechanisms for restoring lactotroph functioning. For this, we evaluated the functioning of the arcuate nucleus dopamine-producing system and pituitary lactotrophs in rats on days 14, 45 after intraventricular administration of 6-hydroxydopamine or saline. On day 14 after 6-hydroxydopamine injection, the plasma prolactin concentration doubled, which is associated with the degeneration of two thirds of dopaminergic neurons and a 4-fold loss of dopamine in the arcuate nucleus. The latter is due to a decrease in dopamine production not only in dopaminergic neurons, but also in non-dopaminergic neurons expressing single enzymes of dopamine synthesis (monoenzymatic neurons). Attenuation of the dopamine inhibiting control of prolactin secretion is enhanced by a decrease in the expression of the D2 receptor gene in lactotrophs. On day 45 after 6-hydroxydopamine administration, the plasma prolactin concentration returns to the control level, mainly due to an increase of dopamine synthesis in monoenzymatic neurons, which increased in number. Simultaneous elevation in expression of the D2 receptor gene in lactotrophs is considered as an additional compensatory mechanism. Thus, increased prolactin secretion caused by degeneration of dopaminergic neurons returns to the control level over time due to an increase in dopamine synthesis, mainly in non-dopaminergic neurons and expression of the D2 receptor gene in lactotrophs. *The authors marked with an asterisk equally contributed to the work.

## P-24-038
## FUS(1-359) mouse model of ALS: pathophysiological and molecular aspects of the proteinopathy

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Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease, which leads to the imminent death of the patients due to progressive decay of motor neurons and skeletal muscle paralysis. Recent studies of ALS pathology have pointed out the significance of dysfunction to two related RNA-binding proteins: FUS and TDP43. Mutations of these genes lead to the disruption of RNA processing and the formation of intracellular protein inclusions. However, it still not clear what is the root cause of the motor neurons failure during ALS either a disturbance of the RNA metabolism or the accumulation of protein aggregates? Herein, we performed a comprehensive analysis of the *in vivo* model of FUS-mediated proteinopathy (FUS1-359 mice) with the goal to address these issues. Despite the absence of clinical symptoms, aggregation of mutant FUS protein in motor neurons becomes detectable in 90 days after birth. Observed aggregation is accompanied with events in alternative splicing, such as intron retention of the transcripts encoding the ribosomal proteins. Just a month later, the gene expression profile exhibits dramatic fluctuations that apparently underlined the malfunction of acetylcholinergic neurons, the disruption of cholesterol biosynthesis as well as developing of the pronounced proinflammatory phenotype of microglial cells in the spinal cord of FUS(1-359) mice. Overall, the accumulated data showed that FUS-mediated proteinopathy is virtually asymptomatic in terms of clinical symptoms until it reaches the terminal stage of the disease progression (120 days). From this time point, the disease develops very rapidly resulting in the cluttering of neuronal cells with FUS-positive inclusions, transcriptional "burst" and massive death of motor neurons. Further study of cellular adaptation systems associated with ALS progression, such as chaperone system will allow to uncover molecular mechanisms of proteinopathy-associated ALS cases.

# Cell therapy and regenerative medicine

## P-25-001
## Human muscle cells as a model to study the expression and physiological role of the NRE enzyme

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The objective of our study was to investigate neuropathy target esterase-related enzyme (NRE; PNPLA7) expression and its functional characteristics in human muscle cells as a model organism


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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for insulin-targeted tissues. More specifically, our previous preliminary studies confirmed the presence of this unexplored enzyme in both myoblasts and myotubes and indicated a role for NRE in the energy metabolism. The human NRE enzyme is predicted to be around 146 kDa. Though the crystal structure of NRE has not yet been defined, gene sequence analysis and homology modelling predict the presence of N-terminal single-pass transmembrane domain, three cyclic nucleotide binding sites, possible glycosylation sites and C-terminal patatin-like catalytic domain. Furthermore, analysis also predicts the existence of several isomers some of which do not have a catalytic domain or in other words, possibly lack esterase/lipase functions. Sequence alignment reveals that NRE is conserved through rat, mice and human species. High NTE homology with more investigated enzyme NTE or PNPLA6 suggests that NRE might also be a target of organophosphorus compounds (OP) which implies NRE involvement in OP caused pathological conditions including poorly defined intermediate-myopathy syndrome. Therefore, by following changes in NRE mRNA, the protein and activity level in cells exposed to different stimuli mimicking real life conditions, we tried to define NRE's physiological role and its potential to be used as a new therapeutic target in OP poisoning or in wider research. The enzyme was cloned, expressed and studied as the esterase in kinetic experiments *in vitro* following interactions with potential substrate and inhibitor. In this way, we also evaluated its enzymatic properties. Since little is known about this enzyme's physiological role and biological relevance, any findings would most certainly contribute to the understanding of the importance of NRE, which still calls for a detailed clarification.

## P-25-002
## Time-dependent cytotoxicity of pyridinium antidotes

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The better selection of a lead candidate for preclinical drug development has come into strong demand in recent years. In that sense, early, rapid and robust results, that unambiguously rank compounds for their desirable and undesirable effects, put cell-based *in vitro* toxicology in major focus. In the preclinical selection of the most efficient antidotes for organophosphorus pesticide and nerve warfare agents poisoning, we evaluated the time-dependent cytotoxicity of the leading oxime compounds. We evaluated a structurally dependent series of oximes bearing up to two chlorine atoms that showed a promising antidote potential in the previous studies. The cytotoxic effect was evaluated on several cell lines in a wide oxime concentration range and at least three time points. The results were compared to the oxime HI-6, which is used today as an antidote in medical practice. As results indicate, time-dependent toxicity of tested oximes was strongly related to their structure and the ones having both a but-2(E)-en-1,4-diyl linker and chlorine atoms were the most toxic to all cells tested. The $IC_{50}$ determined by the MTS assay was in the μM range. This effect was also confirmed by additionally testing the ATP status of the cells. Though the mechanism behind this toxicity needs to be confirmed, such a result indicates that these oximes have limitations if considered for further antidote development. Acknowledgment: This work was supported in part by the Croatian Science Foundation under the project UIP-2017-05-7260.

## P-25-003
## The effects of peripheral mononuclear stem cell and differentiated Langerhans cells application on wound models in experimentally diabetic rats

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Diabetic wound healing is a costly treatment that is difficult and reduces the patient's quality of life. Recently, cellular products have taken place in the treatment of clinical use. Peripheral Blood Mononuclear Cell (PBMC) is a type of stem cell that can be easily obtained from the peripheral circulation. The effects of Langerhans cells (dLC) differing from PBMC and PBMC in wound healing treatment are not known. The aim of this study was to investigate the effect of healing of PBMC and dLC on experimental diabetic graft wound model. PBMC isolation, proliferation in culture and differentiation of dLC were achieved in blood samples obtained from rats. Four full-thickness skin grafts of 1x1 cm were performed on the back of adult Wistar albino rats. Diabetic and non-diabetic rats were divided into 10 groups as control, sham, PKMH, fLH and PKMH + fLH. 1x106 cells were administered to each wound for 7 days. Samples taken from 3rd and 7th days of grafts were examined. Histochemical (HC) staining was performed as H-E, Masson trichrome (MT) and PAS. Immunohistochemical staining included e-NOS and i-NOS for oxidative stress, PCNA as a marker of proliferation, VEGF as a marker of vascularity, IL-1 as a marker of inflammation, and TUNEL for apoptosis. Results were evaluated statistically. It was observed that recovery was rapid and between 2 and 4 days for NDG and between 4 and 7 days for DG the wound was closed. On day 3, histopathological improvements were observed in H-E, MT and PAS staining which became more evident and effective for DG. e-NOS, i-NOS and PCNA was not significantly increased, VEGF increased, IL-1 decreased in IHC staining and apoptosis was significantly decreased. It was found that this new cellular therapy would improve graft efficiency and success in diabetic wound healing. It was thought that researching the advanced mechanisms of this study would contribute to science and would improve the quality of life by decreasing costs in clinical practice.

## P-25-004
## Progenitors from several origins with chondrogenic potential in 3D hydrogels *in vitro*

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Ear cartilage malformations, resulting from congenital anomalies, trauma or cancer, are commonly encountered problems in reconstructive surgery since cartilage has low or no self-regenerating capacity. Malformations that impose a psychological and social burden on one's life, are currently treated using ear prosthesis, synthetic implants or autologous flaps derived from rib cartilage. These approaches were challenging, not only they do request high surgical expertise, but also because they face progressive resorption, lack of flexibility and severe donor-site morbidity. Through the last decade, a new approach to tissue engineering has gained attention. It aims at replacing, regenerating human tissues or organs in order to restore or establish normal function.


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This technique consists of three main elements, cells, growth factors, regulating cellular activity and above all, a scaffold that support cells and guide their behavior. Several studies have investigated different scaffolds prepared from both synthetic and natural materials, and their effects on cellular differentiation and behavior. In this study, we investigated natural scaffold (alginate), a three-dimensional (3D) hydrogel where it was seeded with progenitors from different origins such as bone marrow, perichondrium, dental pulp. In contact with the scaffold, these cells remained viable and were able to differentiate into chondrogenic lineage when cultured *in vitro*. Quantitative and qualitative results show the presence of different chondrogenic markers, upon different conditions of culture. We also investigated the presence of elastic markers for the purpose of ear cartilage. Our results provide some updates for cartilage engineering and regeneration for future clinical applications.

## P-25-005
## Epigenetic mechanisms of mesenchymal stem cells adaptation to low oxygen concentration *in vitro*

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It is known that low oxygen concentrations (5% $O_2$), the so-called physiological hypoxia, in the cell growth medium have a positive effect on the vital processes, including proliferative activity, viability and maintenance of multipotency, of the mesenchymal stem cells (MSCs) *in vitro*. It has been revealed that one of the main regulators of adaptation reactions in MSCs during hypoxia are the transcription factor HIF1 and the porphyrin/heme transporter protein ABCG2. Considering this, the purpose of this research was to study the effect of hypoxic cultivation conditions on the level of DNA methylation of the promoter regions of genes of such proteins as oxygen-dependent α subunit of the transcription factor HIF1 and ABCG2 in human adipose tissue-derived MSCs. It was confirmed that at 5% $O_2$ in the growth medium, compared with 21% $O_2$, in the presence of 10% fetal bovine serum, the viability and proliferative activity of MSCs *in vitro* increased while maintaining the differentiation potential as well as synthetic processes involving heme and porphyrins are activated. At the same time, the expression of the *Hif1α* and *Abcg2* genes in cells increases in 1.9 and 2.3 times ($P < 0.05$), respectively. It's important that, adipose tissue-derived MSCs DNA sequencing after its bisulfite modification showed a demethylation of the promoter regions (CpG islands) of the HIF1α and ABCG2 proteins genes, which correlates with their expression. The obtained results demonstrate the involvement of the epigenetic mechanisms of regulation in the adaptation processes of human adipose tissue-derived MSCs to changes of the oxygen conditions of cell cultivation *in vitro*.

## P-25-006
## Condition of lipid peroxidation and antioxidant protection in the organs of the immune system against effects of phytopreparations

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The effects of natural components of medicinal raw materials on the metabolic processes of intact animals in the conducted experiment were studied. Two types of tinctures from different compositions of medicinal plants were used as herbal preparations. The first infusion, conditionally called "Composition-1" consisted of components such as creeping thyme, birch dangling, elecampane high, while second solution, called "Composition-2" included next: peppermint, plantain medium, elecampane high in a ratio of 1: 2: 2 respectively. Experimental animals were orally administered tinctures "Composition 1 and 2" at a dose of 2.5 mL/kg for 14 days. The levels of malondialdehyde (MDA), the activity of glutathione reductase (GR), glutathione peroxidase (GPO) and catalase were determined in the liver, spleen and thymus of all experimental animals. Decrease in the activity of GR and GPO and an increase in the activity of catalase were determined in the healthy body under the influence of the tinctures "Composition-1" and "Composition-2". Activation of lipid peroxidation was manifested in the liver by the actions of the Composition-2, while it was detected in the spleen under the influence of both Composition-1 and Composition-2, whereas in the thymus both of tinctures did not significantly affect the peroxidation processes.

## P-25-007
## Exosomes derived from dermal papilla cells regulate hair cycling in mice and improve the hair-inductivity of three-dimensionally cultured human dermal papilla cells

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Exosomes are important for the transfer of mRNAs, microRNAs, and proteins to target cells, thereby altering the gene and protein levels of recipient cells to regulate their function. Dermal papillae (DP) play key roles in hair growth and regeneration. In this study, we investigated whether DP-derived Exos, especially those from three-dimensional (3D)-cultured DP cells, affect hair cycling and regeneration. Exosome isolation was performed using the Total Exosome Isolation Kit and sizes were measured by a nanoparticle tracking analysis. For the anagen prolongation study, 5-week-old female C57BL/6 mice in the anagen stage of the hair cycle were shaved and 50 μg of exosome was injected for 4 days. Mice were killed and the treated region of the dorsal skin was stained with hematoxylin and eosin. For the anagen induction study, 7-week-old female mice in the telogen stage of the hair cycle were shaved and injected with 50 μg of exosome in 100 μl of PBS 3 times per week for 26 days. For hair reconstitution assay, a total of 100 exosome-treated DP spheres or control-treated DP spheres were re-suspended in 100 μl of PBS and co-implanted subcutaneously with fresh isolated epidermal cells into 7-week-old female BALB/c nude mice. Mice were sacrificed 17 days after cell implantation, and the implantation sites were excised and the formed hair follicles were counted by visual observation. Local injections of Exos derived from 3D DP induced anagen from telogen and also prolonged anagen in mice. Moreover, Exosome treatment in human DP spheres augmented new hair follicle formation. Collectively, our findings have implications for the development of therapeutic strategies for hair loss using DP exosomes.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-25-008
## Establishing a model of epidermolysis bullosa simplex via CRISPR/Cas9 editing in HaCaT cells

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Epidermolysis bullosa simplex (EBS) is a rare inherited genodermatosis caused by mutations mainly in KRT5 and KRT14 genes, which disrupt basal epidermis structure and lead to extreme skin fragility. HaCaT is a spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin. Due to its close similarity to freshly isolated human keratinocytes, it could become a useful tool for testing different approaches to cure EBS. We have used Cas9 and designed several sgRNAs to obtain indels in exon 7 of KRT5 gene. Mutant HaCaT cells were examined by immunocytochemistry and live cell imaging methods. We investigated differences in cytokeratin network structure (keratin aggregates) and migration rate (measured by wound healing assay). Structure of cytokeratin network under different conditions (caffeine addition) and the influence of stress factors (hypo-osmotic shock; heat shock) was evaluated. Under heat shock and hypo-osmotic shock, keratin aggregates were observed in cells that previously show normal cytokeratin network structure. Caffeine addition leads to thicker keratin 5 filaments and dislocation of aggregates from perinuclear region to periphery. In the presented work, we have created the model of EBS in HaCaT cells using the CRISPR/Cas9 system. HaCaT cells that carry EBS-like mutations show similar characteristics to keratinocytes obtained from EBS patients. Therefore, this EBS model can be used for testing experimental therapies for EBS like pharmacological drugs and gene therapy. The research was performed according to the IDB RAS Government basic research program. *The authors marked with an asterisk equally contributed to the work.

## P-25-009
## The role of autophagy in salivary gland stem cell self-renewal

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Stem cells are by definition cells that have the capacity of both endless self-renewal and differentiation into mature cells, which results in regeneration of specific cell types and tissues, in embryos and adults (H. Clevers 2015). This ability of self-renewal is a key property for tissue maintenance. Autophagy, an evolutionarily conserved, tightly regulated molecular pathway responsible for cell homeostasis is also playing a central role in cell development and differentiation, through still largely unclear mechanisms (Boya, Reggiori, and Codogno 2013; Rodolfo, Di Bartolomeo, and Cecconi 2016). With the recent development of new culturing technologies in 3D organoid systems, there is a new and poorly explored field that unprecedentedly permits to study the role of autophagy in tissue maintenance and renewal. In our research, we aim to establish the role of autophagy during self-renewal of salivary gland stem cells (SGSCs) in a 3D organoid system. SGSCs have been shown to have a high regenerative capacity (Burford-Mason et al. 1993). In our organoid model, we have monitored autophagy activity using LC3 lipidation and p62 turnover as readouts, and modulated this pathway by siRNA-based silencing and isolation of salivary glands from autophagy knockout mice, We have observed that autophagy is necessary for self-renewal of SGSCs in culture and that the rate of autophagy is slower in the stem cells within the salivary gland tissue compared to the ones in culture.

## P-25-010
## Fetal nervous tissue grafts injected in gelatin hydrogel conduits promote peripheral nerve regeneration

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The regeneration of the peripheral nerves after injuries is still a challenging fundamental and clinical problem. In this study, we analyzed the development of the fetal neocortex solid grafts injected into the gelatin hydrogel conduits and their effects on nerve regeneration after cut injury. The study was performed on C57Bl/6 and transgenic heterozygous C57BL/6-Tg(ACTB-EGFP) 1Osb/J mice. Frontal neocortex tissue was obtained from E19.5 fetuses harvested from transgenic EGFP mice. The grafts were injected into the hydrogel conduits which were connected the nerve stumps after cut injury. The recovery of motor function was estimated with walking track analysis at 2, 5, and 8 weeks after surgery. Then immunohistochemical study was performed. Eight weeks after surgery, the histological examination showed that fetal neocortex solid graft cells had survived after implantation. Immunostaining revealed that some of the transplanted cells expressed neural markers such as neurofilament protein and NeuN, which is normal for mouse brain tissue at stage E19.5. But the cells mostly differentiated in glial lineage, which was confirmed with immunostaining for GFAP and S100β. Additionally we didn't find any DCX- and MBP-positive cells. To estimate the motor function recovery the walking-track analysis was performed. There were no differences between the control and experimental groups until 8 weeks after surgery, at which point the neocortex group differed significantly from the control ($P < 0.05$). The sham group was significantly different from the other groups throughout the experiment ($P < 0.0001$). We revealed that the hydrogel conduit is suitable for nerve re-growth and that the fetal neocortex grafted cells can survive, differentiate, and stimulate functional recovery after the nerve injury. This research was funded by the IDB RAS government program of basic research "Development of a new biomedical technology for the treatment of peripheral nerve injury".

## P-25-011
## Differentiation of human amniotic stem cells into endothelial and muscle progenitor cells for blood vessels engineering

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The general goal of tissue engineering is to generate living tissue constructs *in vitro* that are morphologically and functionally similar to native tissue. The aim of this study was to obtain endothelial (EPC) and muscle progenitor cells (SMC) from amniotic fluid stem cells (AFSC) and to investigate the biocompatibility with a hyaluronic acid-based hydrogel in a blood vessel model. AFSC were cultured in endothelial and smooth muscle cell media and characterized by qRT-PCR and flow cytometry. The blood vessel model was designed in BioCad (RegenHu, Switzerland) and


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bioprinted using a bioink formed by mixing the EPC and SMC with hyaluronic acid-based hydrogel. The biocompatibility was assessed using cell tracker Red CMTPX and MTT assay. The results showed that AFSC differentiated in EPC and SMC after 3 weeks cultivation. Gene expression assay showed that AFSC start express endothelial and muscle markers such as ICAM-1, PECAM-1, vWF, VEGFR2, smooth muscle actin, α-tropomyosin, caldesmon, and Ca(v)1.2 calcium channel α-subunit. Flow cytometry assay showed that AFSC expressed endothelial markers such as CD31, CD105, CD133, CD144 and VEGFR2. MTT assay and fluorescent microscopy images showed that the viability of EPC and SMC is maintained up to 21 days of culture in hyaluronic acid hydrogel. Furthermore, histological sections of blood vessel model showed that cells have a relatively uniform distribution, crosslink with each other forming a network. In conclusion, hyaluronic acid hydrogel enables growth of EPC and SMC and is suitable for bioprinting three-dimensional scaffolds that mimics blood vessel. Acknowledgements. This work was supported from the Romanian UEFISCDI Agency, Project PN-III-P1-1.1-PD-2016-1660, No. 19/2018, BIOPRINT.

## P-25-012
## Identification of direct target genes of canonical BMP pathway in hair follicle stem cell regulation

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Hair follicle (HF) is a dynamic miniorgan which undergoes cyclic phases of growth, degradation and quiescence. Recently, we discovered molecular mechanism of Bone Morphogenetic Protein (BMP) signaling governs the homeostasis of hair follicle Stem Cells (hfSCs). Smad1, 5 and 8/9 are signal transducers and transcriptional modulators activated by phosphorylation by BMP type 1 receptor kinase (Bmpr1). Here we show the crucial role of Smad1 and 5 in maintaining the quiescence of hfSCs. Moreover, phosphorylated Smad proteins (pSmads) play nonoverlapping function in hfSCs regulation and hair morphogenesis. For this reason, we focused further on identifying the key interactions of Smads in canonical BMP signaling in hfSC regulation. Using our inducible constitutively active Bmpr1a transgenic murine system, we were able to perform ChIP-seq analysis of phosphorylated form of Smad proteins. Comparison between differentially expressed genes in hfSCs after inhibition of BMP signaling to genome-wide pSmads interactome received from our Chip-Seq predicts the direct connection between the pSmad binding sites in regulatory regions and the change of specific gene expression. This gives significant insight into the transcriptional regulation and maintenance of hfSCs quiescence.

## P-25-013
## Deciphering molecular mechanism of Wnt7 ligands action in activation of hair follicle stem cells during hair regeneration

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The hair follicle in the sense of a mini-organ is an excellent model for studying molecular mechanisms that governance the transition between quiescence and cyclic activation of the hair follicle stem cells (hfSCs) during the hair growth. Our previous finding, revealed that competitive balance between two well known molecular pathways, wingless/integrated pathway (WNT) and bone morphogenetic protein pathway (BMP) is crucial for hfSCs homeostasis *in vivo*, thus their activation and quiescence. Our data propose a mechanism of hfSC regulation whereby BMP inhibition regulates ligand–receptor-dependent canonical Wnt activation. We found that hfSCs with suppressed BMP signaling show expression up-regulation of several WNT pathway activators, including the Wnt7a and Wnt7b ligands. Those ligand were identified as a potential targets of BMP signaling in hfSCs *in vivo,* moreover we revealed that loss of Wnt7b function during hair cycle result in delayed of hfSCs activation. However, how Wnt7b works at the molecular level on hfSCs activation and self-renewal was not addressed until now. To answer this question, we created a unique genetic mouse model to simultaneously label and specifically inactivate the Wnt7b pathways within the hfSCs. We isolated living eYFP-marked, Wnt7b knockout hfSCs, using fluorescence activated cell sorting (FACS). Total RNAs from isolated stem cells was subjected to perform RNA-seq analyses and gene expression profiling of hfSCs after inactivation of Wnt7b and thus characterize target genes relevant for Wnt7b signaling. Moreover, to investigate mechanism of action of Wnt7a ligand alone and also answer the question regarding redundancy and possible partial compensation between WNT7b and WNT7a ligands during hfSCs activation, we will use CRISPER/Cas9 system. This approach allows us to inactivate both genes within the hfSCs *in vitro* at the same time and then further investigate their biological functions in *in vivo* model, using reconstitution assay.

## P-25-014
## T cells redirected toward malignant TCR for leukemia and lymphoma therapy

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The T-cell Leukemia/Lymphoma comprises 15 to 25 percent of the Malignant Lymphoid diseases by incidence and represents 22 clinicopathologic entities in the most recent classification. In recent years, as our understanding of the systematic and molecular basis for malignancy advances, precision therapy has made tremendous progress for a variety of B cell malignancy. The intelligent application of chimeric antigen receptor T-cell (CAR-T) immunotherapy in relapsed or refractory B acute lymphoblastic leukemia has generated consistent benefits and improvements in overall survival and progression-free survival, albeit accompanied by certain side effects. The success of cell therapy in B cell leukemia and lymphoma provided a paradigm, which may lead to potentially better treatments for other cancers. T-cell malignancy, in general, has highly variable courses, typically aggressive and less responsive to conventional chemotherapy than their B-cell counterparts. Lately, researchers have started to investigate the approaches using CAR-


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T cells to treat T-cell Leukemia/Lymphoma by targeting common antigens such as CD4, CD5, TRBC1/2, and the chemokine receptor CCR4. However, the CD4, CD5, CCR4 antigens are not only expressed in the malignant T-lineage, but also in normal T cells, as well as platelets, thymocyte, B-1 cells, which leads to serious concerns of side effects for being used as targets. Nevertheless, due to the limited number of associated antigens to discriminate malignant from healthy T cells, the pathogenesis has remained elusive, and it is difficult to devise more efficacious therapies for most of the T-cell malignancies. Therefore, it is critical to identify and validate new targets for CAR-recognition for achieving improved efficacy and reduced toxicity. The project is supported by the Russian Scientific Foundation 17-74-30019.

## P-25-015
## Chrysin complexed with cyclodextrins as a novel regenerative therapy for liver fibrosis

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Liver fibrosis is a pathology that affects tissue integrity and can progress to irreversible stages. Treatment often targets one key event, the hepatic stellate cells (HSCs) activation. Chrysin (Chr) is a natural anti-inflammatory and hepatoprotective compound, recommending it for liver fibrosis treatment. However, its low bioavailability requires complexation with cyclodextrins (CDs). The aim of this study was to investigate the potential of a new drug delivery system based on Chr to reverse liver fibrosis. HSCs activated using TGFβ (aHSCs) and murine hepatocytes were treated with CD-Chr complexes, namely RAMEB and HPBCD for 24 h, using 1–100 μM range of concentrations. To confirm HSCs activation, desmin and phalloidin-FITC were immunostained and Oil red O staining showed reduced lipid droplets. To assess CD-Chr biocompatibility, cell viability was evaluated by MTT and Live/Dead tests, while LDH assay showed CD-Chr cytotoxicity. To investigate CD-Chr antifibrotic potential, treated aHSCs were tested for fibrosis markers expression, such as collagen type I (col I) and smooth muscle actin a (a-sma) both at gene and protein levels. a-SMA overexpression confirmed HSCs conversion towards a myofibroblastic-like phenotype. In normal hepatocytes, the CDs induced higher cytotoxicity correlated with lower cell viability and proliferation rate than CD-Chr in all tested concentrations. In contrast, CD-Chr induced a lower aHSCs viability than CDs, suggesting their positive effect. Cell viability was significantly reduced compared to control for >30 μM in both cell types. a-sma and col I gene and protein expression profiles were significantly decreased 24 h after treatment, with more efficient results for HPBCD-Chr complex, suggesting its antifibrotic potential. Both complexes are biocompatible and exert antifibrotic effect in 30–60 μM. Chr biodisponibility to the aHSCs was improved by complexation with HPBCD. This work was supported by UEFISCDI-PNIII-P1-1.2-PCCDI-2017-0782/REGMED. *The authors marked with an asterisk equally contributed to the work.

## P-25-016
## Graphene oxide actively supports bone regeneration in biocompatible 3D scaffolds based on chitosan and gelatin

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Graphene oxide (GO) is currently one of the most explored compounds during material synthesis for regenerative purposes in bone tissue engineering (BTE). Because of its outstanding physicochemical properties, GO influences cell survival, growth, proliferation and activation of molecular pathways involved in osteogenic differentiation. In this study, an original scaffold based on chitosan and gelatin, reticulated by genipin and enriched with 0.5–3 wt.% GO (CHT/Gel/Gen/GO) was tested for its biocompatibility and potential to promote osteogenic differentiation. Murine pre-osteoblasts were put in contact with the composites, generating 3D cell-scaffold systems and their biocompatibility was determined by MTT and LDH assays and Live/Dead staining. In order to evaluate CHT/Gel/Gen/GO potential to support the osteogenic differentiation during 28 days, qPCR, histological staining and immunostaining for bone markers were performed. Overall, a low rate of cell death was found for these composites, showing no significant level of cytotoxicity. CHT/Gel/Gen/GO 3 wt.% displayed best biocompatibility, in terms of cell viability and proliferation. One of the major components of mineralized bone extracellular matrix (ECM)- osteopontin (Opn)- gene expression was found to be significantly higher in contact with CHT/Gel/Gen/GO 3 wt. % as compared to control. Moreover, a correlation between Opn profiles of gene and protein expression demonstrated a more efficient osteogenic differentiation depending on increasing GO concentration. Alizarin Red S histological staining showed significant mineralized ECM deposits in CHT/Gel/Gen/GO 3 wt.% compared to composites with lower GO-content. In conclusion, CHT/Gel/Gen/GO 3wt. % has supported most efficiently *in vitro* osteogenic differentiation, therefore confirming that GO is beneficial for bone development and could be further used for applications in regenerative medicine. This work was supported by UEFISCDI-PNIII-P1-1.2-PCCDI-2017-0782/REGMED. *The authors marked with an asterisk equally contributed to the work.

## P-25-017
## Identifying the cause of the waved alopecia (wal) mutant mice phenotype

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The *waved alopecia* (*wal)* gene was mapped on mouse Chromosome 14 (qE2.1), its locus spans 10 Mb. Homozygous *wal* mice are characterized by a wave coat followed by alopecia. The protein of *wal* gene is unknown. Of all the genes mapped in this region, we proposed Pcdh 9 as a candidate for the mutant *wal* gene, but its exon sequencing has not revealed any changes in the exon sequences of Pcdh9 gene. Therefore, we performed whole genome


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sequencing of mutant mouse. In the mapped region of chr14 no variants were found that could lead to a change in the sequences of protein products of encompassed genes (missense, nonsense, deletions and insertions in coding regions of genes, mutations in canonical splice sites, mutations in start and stop codons). According to the results of the analysis of variations in the number of copies (CNV), duplication of the chr14 region was revealed: 85042892-85052937 in a likely homozygous state. This interval does not affect the coding sequences of genes and is located between the Pcdh17 genes (about 0.5 Mb downstream of the gene) and Diaph3 (about 1.6 Mb upstream of the gene). According to the UCSC Genome Browser (track ORegAnno, OREG1428524), in this region there is a binding site of the transcription factor Cdx1, related to the Pcdh17 gene, with coordinates chr14: 85046284-85046534. By the totality of information, duplication should be regarded as a variation of the number of copies with an uncertain biological significance, which deserves further research. *Waved alopecia* (*wal*) mutant mice can serve as a valuable model for studying the alopecia development as well as functioning of the epidermis and hair follicles. The reported study was funded by RFBR according to the research project No. 18-34-01022.

## P-25-018
## Dissecting the regulatory function of Id2 in hair follicle stem cell homeostasis

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Differential gene expression orchestrates stem cells homeostasis and is controlled by genomic *cis*-regulatory elements (CRE) regulated by distinct signaling pathways e.g. BMP (Bone Morphogenetic Protein) or WNT (wingless/integrated). CREs serve as platforms for binding of transcription factors (TFs) and transcriptional regulators (TRs), facilitating specific spatiotemporal gene expression. Previously, we revealed how the competitive balance between BMP and WNT signaling pathways governs the homeostasis of hair follicle stem cells (hfSCs) *in vivo*, orchestrating regenerative hair cycle behavior. We focus on testing role of *Id2*, a helix-loop-helix (HLH) TR binding to other bHLH TFs inhibiting their ability to bind DNA suppressing their activity. Id2 is one of the downregulated target genes in hfSCs after inhibition of BMP pathway (Bmpr1a-KO). To test the function of *Id2* in hfSCs *in vivo*, we use Id2 gain of function approach (GoF) by generating transgenic mouse line. We show that Id2 overexpression in hfSCs results in prolonged telogen and a delay in anagen activation, maintaining stem cells quiescence. Performing RNA-seq on FACS-isolated Id2-GoF hfSCs at first postnatal hair cycle allowed comparison with common signature genes in quiescent bulge. Almost 10% (41 of 426) genes is affected by Id2 overexpression out of which half (22 of 41) are also BMP-dependent. Furthermore, the intersection of genes affected by Id2-GoF and Bmpr1a-KO reveals that one third of the second group (405 of 1249) is affected by overexpression of Id2 in hfSCs. Interestingly, 83% of common genes (338 of 405) is discordantly regulated in Id2-GoF and Bmpr1a-KO indicating synergistic BMP-Id2 interplay. Altogether, Id2 is the direct target and the effector of BMP pathway in hfSCs *in vivo*, affecting only 32% of BMP dependent genes. This indicates that the other two thirds of BMP regulated genes are independent from Id2 and are controlled by other mechanism in quiescent bulge yet to be discovered. *The authors marked with an asterisk equally contributed to the work.

## P-25-019
## The influence of hypoxia on the selected properties of the mesenchymal stem cells derived from Wharton's jelly

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Mesenchymal stem cells (MSCs) are classified as an adherent cells with the ability to self-renew and multipotent differentiation. Given abilities enable to create the possibility to incorporate them in regenerative medicine. The latest studies demonstrate that proliferation, self-renewal and differentiation capabilities of MSCs are highly dependent on the environment. In these terms the key parameter to consider is the oxygen concentration. The aim of the work was to evaluate the impact of low oxygen concentrations on the selected properties of MSCs isolated from Wharton's jelly. The research tasks were: the evaluation of the selected adhesive molecules; the examination of adhesion mechanism between endothelial cells and MSCs in different oxygen concentrations; the evaluation of growth kinetics and survival assays the evaluation of the response of MSCs to a given oxygen concentration at the protein level and mRNA. The results of this work showed that hypoxia does not change the survival rate of the monitored cells and does have a beneficial effect on the cell proliferation. The presented work also demonstrated that there is a response of MSCs to the low oxygen concentration at the HIF-1α protein level and PFKFB4 target gene. This work also showed that the expression of genes such as BNIP-3, VEGF or AM does not depend on the oxygen concentration. No significant differences in expression of the investigated adhesion markers were observed for the cells cultured in hypoxia and normoxia. Furthermore the research demonstrated lack of impact on cell adhesion to the endothelium observed in hypoxia. The response of MSC as well as their high proliferation potential, lack of pro-apoptotic effect and no impact on cell adhesion in a low oxygen environment gives the new perspectives for the use of these cells in regenerative medicine. The work was financially supported by The National Centre for Research and Development (grant number STRATEGMED 2/265761/10/NCBR/2015).

## P-25-020
## The effects of antioxidant molecules on the oxidative stress induced by hydrogen peroxide in the mesenchymal stem cells and endothelial precursor cells

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Multipotent mesenchymal stem cells obtained from Wharton's jelly around the umbilical cord have been shown to be superior to the stem cells obtained from adult bone marrow due to their immunosuppressive functions and not to express the proteins that cause tissue rejection. The aim of this study was to determine the effect of oxidative stress response of the cells and also to evaluate the effects of different antioxidant molecules on cell viability and


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the antioxidant enzyme activity of the mesenchymal stem cells and endothelial precursor cells. Wharton's jelly mesenchymal stem cells were obtained from postpartum human umbilical cord by using explant method. The levels of CD44, CD90, CD 105 gene and the protein expressions were determined for RT-PCR, immunohistochemistry and flow cytometry for stem cell characterization. Stem cells were differentiated into endothelium-like cells by ten-day induction. The effects of N-acetyl cysteine, ascorbic acid and Na-selenite on the cell viability were evaluated by MTT reduction assay and the microscopic evaluation. The changes in the antioxidant enzyme activity levels were investigated by the fluorometric and photometric methods. The viability and antioxidant enzyme levels were significantly decreased by the 3-h H2O2 treatment (100 μM). In the endothelial differentiation process, catalase, glutathione peroxidase enzyme activities of the cells significantly increased. N-acetyl cysteine, ascorbic acid and Na-selenite protected the stem cells against to oxidative stress. These treatments increased the viability and proliferation of the cells. In conclusion increased production of reactive oxygen species has been implicated in the pathogenesis of cardiovascular diseases. The data and protocols obtained by our study are thought to be the basic step for the projects which can be planned later in the scope of the regenerative medicine applications for the cardiovascular diseases. *The authors marked with an asterisk equally contributed to the work.

## P-25-021
## Regenerative potential of Wharton's jelly mesenchymal stem cells for treating hindlimb ischemia

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Mesenchymal stem cells (MSCs) hold great promise for treating cardiovascular diseases, such as myocardial infarction and limb ischemia. MSCs isolated from Wharton's jelly (WJ-MSCs) may be utilized in both cell-based therapy and vascular graft engineering to restore vascular function, thereby providing therapeutic benefits to patients. In the present study, we focus on the therapeutic properties of human WJ-MSCs and we investigated the role of WJ-MSCs in promoting angiogenesis and relieving hindlimb ischemia. The murine model of hindlimb ischemia was designed to evaluate properties and *in vivo* regenerative capacity of WJ-MSCs. The femoral artery ligation was performed on NOD-SCID mice. WJ-MSCs were isolated from the human umbilical cord and characterized using flow cytometry technique. The expression level of specific proangiogenic factors was evaluated by Real-Time PCR method and Elisa Multiplex-Luminex technique. Vascular and functional outcomes were measured using laser Speckle perfusion imaging and the point evaluation of animal behavior. Muscle histology analysis was also performed. During 21 days lasting experiment the blood flow and functional outcomes were improved in WJMSCs-treated mice compared with controls. We have observed no deterioration in the health of mice. We also did not notice visible changes in the site of the cells implantation. The histological analysis of ischemic muscles also showed positive changes in their structure that indicate their regeneration. Local WJ-MSCs transplantation induces a neovascular response resulting in a significant increase in blood flow in the ischemic limbs. WJ-MSCs are also capable of spontaneously regenerating the muscular tissues. The results of our study have confirmed the high therapeutic potential of WJ-MSCs and their proangiogenic properties that promote angiogenesis. This work was supported by research grant (STRATEGMED2/265761/10/NCBR/2015) from the National Center for Research and Development.

## P-25-022
## The impact of the mesenchymal stromal cells origin on their tenogenic potential

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Cells based therapy is considered in patients with musculoskeletal disorders. Researchers working on MSC-based therapies stand before a choice the use of undifferentiated cells, or suitably modified in the desired direction. Regardless of the direction chosen the influence of the cells' origin on the expression of key genes may be significant. The aim of the study was to compare basal expression of genes and proteins related to tenogenesis in human bone marrow- (hBM-MSCs) and adipose tissue (hASCs) derived mesenchymal stromal cells. Cells were isolated from the bone marrow (n=8) and adipose tissue (n=8). MSCs were identified using flow cytometry and multilineage differentiation assay. The expression of *MKX, SCX, COL14A1, TGF-β2, SMAD3* genes was evaluated using Real Time PCR method. The secretion of TGF-β2 was quantitatively assessed in cell culture supernatants using ELISA assay. Four other factors were tested for protein expression by western blot. The isolated cells displayed typical MSCs characteristics. hBM-MSCs showed a statistically significant higher expression of *SCX* (fold change 1.94), *COL14A1* (fold change 8.31), *TGF-β2* (fold change 6.73), *SMAD3* (fold change 1.77) than hASCs. The expression of *MKX* did not differ significantly between groups, however the trend of higher expression in BM-MSCs than hASCs was maintained. The analysis of protein expression confirmed gene expression data. Conclusions: Based on the obtained results it can be concluded that the basal gene and protein expression of factors related to tenogenesis differ significantly between hBM-MSCs and hASCs in *in vitro* conditions. BM-MSCs can be more effective in tendinopathies therapy without differentiation of cells. In addition, our results constitute a reference point for studies involving the differentiation of cells with potentially tenogenic agents before transplantation.

## P-25-023
## Dental pulp cells promote recovery after stroke in OGD model for organotypic hippocampal cultures

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Mesenchymal stromal cells (MSCs) are population of cells that have been extensively studied for their use in regenerative medicine. Recently attention has been paid to the paracrine effects exerted by MSCs. Due to them MSCs stimulate endogenous


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repair processes in tissues without differentiation. The aim of the study was to assess the whether mesenchymal stromal cells derived from dental pulp might regenerate damaged nerve tissue by means of paracrine effects. MSCs were isolated from rat incisors by enzymatic digestion of dental pulp. Oxygen-glucose deprivation (OGD) of hippocampal organotypic cultures was applied to model stroke conditions. Hippocampus were isolated from 6–7 day rat pups and cut into 350 μm thick slices with use of tissue chopper. They were then grown in the inserts for 7 days. OGD was obtained by placing organotypic cultures in the medium without glucose in the hypoxic chamber with gas mixture of 95% nitrogen and 5% of carbon dioxide for 40 min. After OGD inserts with hippocampal slices were put into cell culture plates with mesenchymal stromal cells and kept in coculture for 24 h. The viability of the hippocampal slices was assessed by spectrophotometric measurement of NO and LDH secretion. Analysis of fluorescent images of slices stained with propidium iodide showed that upon OGD model significantly increase in number of dead cells is observed in hippocampal slices. Necrotic area was mainly focused in CA1 region of hippocamp. Moreover after OGD significant increase in HIF-1α protein expression was observed. Coculture of hippocampal slices after OGD result in increased viability of hippocampal cells, what was confirmed by both NO and LDH release decrease. To sum up this study shows that in organotypic model of stroke MSCs derived from dental pulp promote regeneration and survival after OGD without differentiation but rather due to their paracrine activity. N.B. acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

## P-25-024
## Cytoprotective and pro-regenerative potential of human induced pluripotent stem cells-derived extracellular vesicles – implications for heart repair

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Cardiovascular diseases constitute one of the major health problems in the world, with the highest rate of mortality and morbidity, despite available modalities. Thus, there is an unmet need to develop novel therapies for cardiac disorders. Here, we investigated utility of extracellular vesicles (EVs) released by human induced pluripotent stem cells (hiPSCs) under various environmental conditions in cytoprotection and promotion of cardiomyogenesis of human cardiac stromal cells (hCSCs). Integration-free hiPSCs were cultured in xeno-free, serum-free and feeder-free conditions to collect conditioned media. EVs were isolated by sequential ultracentrifugation at 100 000 x g and were subsequently analyzed by numerous assays, including: nanoparticle tracking analysis (NanoSight), RT-qPCR, high resolution flow cytometry (Apogee) and Western blot. hCSCs were treated with 1 μg EVs/$10^3$ cells and were either treated with 1 μg/mL staurosporine for apoptosis analysis or subjected to cardiac differentiation. EVs obtained from cultures of hiPSCs constituted heterogenous population of small vesicles with average size of 140 nm in dimeter. Importantly, our data revealed that hiPSCs-EVs were rich in mRNA, miRNA and proteins originated from parental cells, including transcripts and proteins related to pluripotency (OCT4, SOX2, NANOG). Bioactive EV cargo was transferred to hCSCs and enhanced their proliferation, metabolism and differentiation ability towards cardiomyocytes. Moreover, treatment with hiPS-EVs exerted cytoprotective effect on hCSCs, by reducing apoptosis induced by staurosporine. We postulate that hiPSC-EVs are effective tools for transferring iPSC-derived bioactive molecules to target cells, which influences target cell fate. Thus, hiPSC-EV-mediated horizontal transfer of RNAs and proteins to injured tissues may be considered as a novel strategy for therapeutic tissue repair, including heart regeneration.

## P-25-025
## Generation and characterization of human induced pluripotent stem cells (hiPSC) and hiPSC-derived cardiomyocytes (hiPSC-CM) lacking microRNA-378a

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Human induced pluripotent stem cells (hiPSC) demonstrate potential for self-renewal and differentiation into any cell type of adult organism including cardiomyocytes (hiPSC-CM). However, currently used protocols for hiPSC-CM generation derive cells with immature phenotype, which limits their potential in cardiac disease modelling. microRNA-378a (miR-378a) is a small, non-coding RNA located in the first intron of *PPARGC1B* gene which regulates many processes including metabolism. The expression of miR-378a is substantially increased in maturating hiPSC-CM however its precise role in human cardiomyocytes has not been investigated. Thus, the aim of our study was to characterize miR-378a-lacking hiPSC-CM (hiPSC-CM-miR-378aKO). First, miR-378a coding sequence was removed from control hiPSC using CRISP/Cas9 method and five isogenic hiPSC-miR-378aKO cell lines were derived for further studies. Deletion of miR-378a did not affect expression of pluripotency markers and *PPARGC1B* as well as differentiation potential into three germ layers. hiPSC-CM-miR-378aKO were derived using small molecule-mediated regulation of WNT pathway which allowed for generation of approximately 90% cardiac-specific troponin T-positive, contracting cells. After a change of energetic source from glucose to lactate, increased expression of LDHA and mitochondrial membrane activity was observed in hiPSC-CM-miR-378aKO in comparison to control line. Additionally, increased expression of mitochondrial complex I and II subunits and PGC1β as well as reduced phosphorylation of Erk was observed in hiPSC-CM-miR-378aKO. Of note, culture of control hiPSC-CM in palmitate acid -containing culture medium increased expression of miR-378a. In conclusion, lack of miR-378a does not influence pluripotent phenotype of hiPSCs, however, it can be involved in regulation of hiPSC-CM metabolism and thus maturation. This study was supported by HARMONIA grant (2014/14/M/NZ1/00010) funded by National Science Centre. *The authors marked with an asterisk equally contributed to the work.


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## P-25-026
## Maturity of human cardiomyocytes derived from induced pluripotent stem cells lacking heme oxygenase-1

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Human induced pluripotent stem cells (hiPSCs) can be generated from easily accessible somatic cells and differentiated into virtually any cell type found in adult organism, including cardiomyocytes (CMs). Thus, hiPSCs serve as invaluable tool for cardiac disease modeling and drug toxicity testing. However hiPSCs-derived CMs (hiPSC-CMs) are characterized by immature phenotype and further studies regarding their maturation are required. There is evidence for important role of heme oxygenase-1 (HO-1), a cytoprotective enzyme, in differentiation of CMs in murine model. However it has not been assessed in human CMs so far. Thus the aim of this study was to evaluate the role of HO-1 in hiPSC-CMs maturity. hiPSCs lacking HO-1 expression (HO-1 KO) were derived using CRISPR/Cas9 method and further differentiated into CMs by small molecule-mediated regulation of WNT pathway. Importantly, HO-1 KO hiPSC-CMs demonstrated less mature phenotype than control cells, basing on shortened action potential and lower expression of ion channels. Additionally, by employing ImageStream technique, we were able to detect terminally differentiated, binucleated hiPSC-CMs. However, in long-term culture (60d), lack of HO-1 didn't affect percentage of binucleated CMs. One of the obstacles in studying binucleation of hiPSCs-CMs, however, might be the culture conditions. Medium rich in glucose does not reflect physiological environment after birth and thus does not promote proper maturation. To partially mimic *in vivo* process of CM maturation, change of energy source from glucose to fatty acids is essential. Indeed, switch from glucose to palmitate increased mitochondrial activity, which is linked with maturation of CMs. Thus, maturation of hiPSCs-CMs in presence of palmitic acid might be crucial for proper studying of binucleation. Nevertheless, our studies revealed potential role of HO-1 in maturation of CMs, basing on electrophysiological properties of HO-1 KO CMs.

## P-25-027
## Impact of MSC transplantation on mouse skeletal muscle regeneration

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Skeletal muscles possess a remarkable ability to regenerate thanks to the pool of satellite cells, i.e. muscle specific stem cells. After injury inflammation starts: the damaged tissue is removed through phagocytosis and satellite cells get activated what triggers their proliferation and differentiation into skeletal myoblasts. Newly-formed myoblasts fuse into multinucleated myofibers and subsequently replace damaged tissue. Unfortunately, in some cases as a result of extended inflammation the functional muscle fibers are replaced by non-functional connective tissue what leads to impaired function.

Mesenchymal stem cells (MSCs) were previously shown to have immunomodulatory effects: they are able to interact with immune cells by blocking their activation and proliferation through direct interactions or secretion of immunomodulatory cytokines. For this reason they could reduce inflammation and scarring of skeletal muscles. In the presented project we studied how allogeneic or syngeneic MSC transplantation influence skeletal muscle inflammation and regeneration after cardiotoxin-induced injury. Detailed analyses examined how injection with MSCs affected muscle structure, fibrosis, as well as infiltration of immune cells. We also studied the profile of pro-inflammatory and anti-inflammatory cytokines secreted by MSCs in treated muscles. By comparing how these treatments influences muscle regeneration we were able to determine which strategy is the most beneficial for skeletal muscle regeneration and reduction of fibrosis. This study was funded by National Science Centre; decision number: UMO-2016/23/N/NZ6/00565

# Small noncoding RNA

## P-26-001
## Riboswitch-ligand structure determination using an integrative structural biology approach

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Riboswitches are regulatory 5' untranslated regions of mRNA molecules that specifically bind small molecules, resulting in conformational changes that regulate the production of proteins encoded by the mRNA. In recent years, riboswitches came under the spotlight as they are promising new targets in biomedicine. The *yjdf* riboswitch, present in many bacteria, regulates the gene expression of the Yjdf protein-coding gene. The function of the Yjdf protein is unknown, although it is possible that its function will be related to detoxification for the ligand that regulates the gene expression. Although the *yjdf* riboswitch is known to bind a variety of compounds, its natural ligand remains unknown. Therefore, to understand how the *yjdf* riboswitch regulates gene expression in bacteria, we decided to determine and validate its structure through an integrative approach involving X-ray crystallography, SAXS, chemical probing, gene reporter assays, and associated experimental and computational analyses. I will present the structure of the *yjdf* riboswitch in complex with one of its ligands. The ligand-bound structure is stabilized through long-range tertiary interactions, differing only mildly from the apo structure. The computational modeling approach used in this project was independently validated in the RNA puzzles experiment, where *yjdf* was also one of the targets. This structure unveils the ligand-binding site of the *yjdf* riboswitch, providing the first insight into the ligand binding mode of its ligands, which are yet to be found. *The authors marked with an asterisk equally contributed to the work.

## P-26-002
## Pioneering structural and functional studies of miRNA G-quadruplexes

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miRNAs are short non-protein coding RNAs, being though crucial regulators of gene expression of up to 90% of human genes. These tiny RNA molecules tune cell growth, tissue


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differentiation, cell proliferation, embryonic development. Even slight shift in miRNA level could lead to significant changes of transcriptome, and in a result of cell phenotype. In the last decade, over 2500 human mature miRNA sequences were deposited in miRBase. The function of many of them have been found and anti-miRNA, as potential therapy tools have been designed. Despite of an enormous data of miRNA, there are still many questions concerning miRNA function to be solved. The generally accepted model of the miRNA-guided RNA down-regulation suggests that mature miRNA targets mRNA in a nucleotide sequence-specific manner. Recently, we analyzed the nucleotide content of human mature miRNAs and showed that the most abundant nucleotide in miRNAs is guanosine. We identified guanosine-rich miRNAs and found that the level of G in some miRNAs is even over 90%. We noticed that some miRNAs (179 of 2565 human ones) have quadruplex specific motif GGN(1-7) GGN(1-7)GGN(1-7)GG. Furthermore, we found that at least 50% of miRNAs may adopt tertiary structure. Using specific nucleases, NMR, UV/Vis and CD spectroscopies, small-angle X-ray solution scattering (SAXS) as well as and molecular dynamics, as the first ones, we gave evidences that some miRNAs may adopt quadruplexes, e.g. miR-3620, 4507, hairpin and/or homoduplex structures, e.g. miR-21, miR-93 and miR-296. We also showed that the structure of miRNA has functional consequences and suggested that miRNAs may function also beyond RISC and are even more sophisticated regulators, that it was previously expected. We think that the knowledge of the miRNA structure and its dynamic may give a new insight into miRNA-dependent gene regulation mechanism and be a step forward in the understanding their function and involvement in cancerogenesis.

## P-26-003
## Bacterial 6S RNAs under stress conditions

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6S RNA is a highly abundant small non-coding RNA found in diverse classes of prokaryotes, including bacteria with an extremely small genome, that indicates an important role of 6S RNA in the cell. 6S RNA has a unique secondary structure which mimics DNA promoter, thus 6S RNA is able to bind RNA polymerase (RNAP) holoenzyme and inhibit transcription in case of an unfavorable environment. Moreover, DNA-dependent RNAP can use 6S RNA as a template for synthesis of short pRNAs, when it needs to release from 6S RNA and proceed transcription of genes. Under normal conditions 6S RNA knockout does not affect *E. coli* growth, while it becomes essential for cell adaptivity to starvation and alkaline stress. Some other bacteria express 6S RNA at highest levels under standard cultivation conditions. These facts raise a number of questions, like (i) what is the mechanism of 6S RNA-dependent transcription inhibition (and its retraction) in these bacteria, (ii) whether 6S RNA is involved in some stress responses, and (3) what type of genes are affected. In the present work we characterized properties and functions of 6S RNAs from *B.* subtilis and *R.* sphaeroides in comparison to *E. coli*. It was shown that in B. subtilis 6S RNA knockouts leads to widespread changes in cellular transcriptome and proteome and results into high cell viability under alkaline conditions. For the first time, *R.* sphaeroides 6S RNA was characterized and osmotic and oxidative phenotypes of cells with deletion of 6S RNA gene were found. Surprisingly, decreased viability of *E. coli* 6S RNA knockout strain was observed in case of response to one of the stress, and some genes affected by the lack of 6S RNA under these conditions were revealed by qRT-PCR. Thus, for the first time we experimentally proved a great variety of 6S RNA functions in different bacteria and its involvement into diverse cellular stress responses. This work was supported by the Russian Foundation for Basic Research (project No. 19-04-00791).

## P-26-004
## MIR3648 and MIR3867 target heterochromatin

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Non coding RNAs (ncRNAs) participate in various biological processes from regulating enzymatic activities to sustaining spatial organization of the genome. MicroRNAs, whose canonical function is suppressing mRNA translation in the cytoplasm via RNA interference, occur in the nucleus as well where these miRNAs may pair with other ncRNAs localized in certain genome regions and trigger repression or activation of these regions. Here we studied RNA-DNA interactome of the human erythroleukemia cell line K562 by a new method, which is based on adapter-mediated RNA-DNA ligation in cross-linked nuclei followed by sequencing. We identified several hundreds of ncRNAs enriched in active or repressed chromatin. Of particular interest are MIR3648 and MIR3867. We found that these miRNAs establish contacts genome-wide and are ranked among the first in localization to repressed chromatin and the inactive spatial chromatin compartment among all ncRNAs identified in the study. MIR3648 and MIR3867 favor bulk heterochromatin over Polycomb repressed chromatin. They associate with regions of late replication and lamina associated domains, are depleted from the bodies of transcribed genes and enriched in gene deserts. The frequency of contacts of MIR3648 and MIR3867 with gene poor chromosome 18 is about 2 times higher than genome average. Interestingly, MIR3648 and MIR3867 genes are hosted within the 5' external transcribed spacer of the 45S rRNA operon. Importantly, the rRNA itself does not show preferences for active or repressed chromatin. Based on the above observations we speculate that MIR3648 and MIR3867 play a role in heterochromatin formation at the genome scale. Of note, previous studies have revealed multiple examples of miRNA involvement in transcriptional silencing of individual genes. However, MIR3648 and MIR3867 represent the first example of miRNAs associated with inactive chromatin genome-wide. This work was supported by the Russian Science Foundation (grant 18-14-00011).

## P-26-005
## Inhibition of SIRT1/p53 pathway by miR-211 and induction of cell death in breast cancer cells

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Sirtuin1 (SIRT1), a class III histone deacetylase enzyme, can deacetylate various proteins including p53 and regulate diverse physiological and pathological processes. SIRT1 has been suggested as a promoting factor in tumor development and


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progression via deacetylation and suppression of several tumor suppressors. MicroRNAs are among the regulators of SIRT1 and their expression is deranged in many different types of cancers. The aim of this study was to evaluate whether inhibition of SIRT1 expression and function by miR-211 can influence survival of breast cancer cells. Methods: MCF-7 and MDA-MB-231 breast cancer cells were transfected with miR-211 mimic, inhibitor and their negative controls. SIRT1 levels were assessed by real -time PCR and western blotting. SIRT1 activity was measured by a fluorometric method. Cell survival and apoptosis were evaluated by WST-1 test and flow cytometry, respectively. The binding of miR-211 to SIRT1 3'-UTR was assessed by bioinformatics tools and confirmed by luciferase reporter assay using a vector containing the relevant 3'-UTR. Results: SIRT1 levels were higher in MCF-7 and MDA-MB-231 cell lines compared to MCF-10A cells. SIRT1 3'-UTR was found to be directly targeted by miR-211. Up-regulation of miR-211 significantly reduced SIRT1 expression, both at the mRNA and protein levels. SIRT1 activity was also decreased by miR-211 leading to a significant decrease in the cell viability and induction of apoptosis. The acetylation of p53 was increased in response to miR-211, especially in MCF-7 cells that harbor wild type p53 protein. The antisense oligonucleotide of miR-211, used as negative control, led to increased SIRT1 expression and activity as well as inhibition of apoptosis, presumable by sequestering and downregulating the endogenous miR-211. Conclusion: Targeting of SIRT1 expression by miR-211 and the subsequent decline in cell survival may be considered a novel therapeutic strategy for breast cancer management. *The authors marked with an asterisk equally contributed to the work.

## P-26-006
## *Arabidopsis thaliana* mRNA adenosine methylase (MTA) is a new player in miRNA biogenesis regulatory pathway

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Methylation of adenosine at N6 position ($m^6A$) is one of the most abundant mRNA modifications. In this study we uncovered the role of MTA and $m^6A$ methylation in plant (*Arabidopsis thaliana*) miRNA biogenesis. We used NGS to show that miRNA levels are downregulated in *mta* hypomorphic mutant whereas pri-miRNA levels are upregulated in such plants. We then identified a set of 11 pri-miRNAs that are $m^6A$ methylated using $m^6A$-IP seq. Furthermore, RNA-IP using MTA-GFP tagged Arabidopsis plants showed enrichment of pri-miRNAs in the MTA-GFP line (including 8 pri-miRNAs found in m6A IP), further proving that pri-miRNAs are also substrates for $m^6A$ methylation by MTA. We also report that MTA interacts with RNA Pol II and TGH (known miRNA biogenesis related player) indicating that MTA acts in early stages of miRNA biogenesis. Lastly, we show that MTA modulates auxin response in plants via methylation of pri-miR393b. Our data indicate that MTA is an important player in the biogenesis of a set of Arabidopsis miRNAs.

## P-26-007
## MicroRNA content in human milk exosomes: isolation, detection, normalization

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Human milk is a unique biological fluid that provides nutrition for the child, contributes to its development and protection from pathogens. Milk contains various proteins, lipids, sugars, nucleic acids, and its complexes. Exosomes are 40–100 nm diameter extracellular vesicles found in various biological fluids. It is proposed that milk exosomes may deliver molecular signals to recipient cells in the newborn's intestines. Human milk exosomes are currently much less studied than exosomes of blood or cell culture fluid. Analysis of miRNA of human milk is of particular interest. According to the literature, milk may contain more than 700 different miRNAs and significantly more different mRNA molecules. These RNAs, in particular, regulate the expression of genes associated with the development of the newborn's immune system. Here we show the content of several miRNA, obtained from exosomes on the different stages of exosome isolation procedure. MicroRNA was isolated from aliquots of exosome preparations by several methods. The microRNA content was analyzed with qPCR with stem-loop primers and polyA polymerase method. Several mRNA and small RNA targets were used as normalizers of expression. The results of miRNA analysis do not entirely coincide with those previously described in the literature. And the content of miRNA significantly depends on the degree of exosome purification. The study was funded by the Russian Scientific Foundation (research project 18-74-10055 to S. Sedykh) and by the Program of Fundamental Research of Government Academia (0309-2019-0003 to Prof. G. Nevinsky). *The authors marked with an asterisk equally contributed to the work.

## P-26-008
## Analysis of primary miRNA processing in normal and malignant B-cells

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MiRNAs are transcribed as primary miRNAs (pri-miRNAs) that are processed in two steps, first by the Drosha-DGCR8 complex into the precursor miRNA and then by Dicer to the ~22-nt mature miRNA. The miRNA biogenesis is regulated at the transcriptional and posttranscriptional level. In this study, we aim to identify miRNAs that undergo regulated processing. To gain global insight in the number of miRNAs that are potentially regulated, we analyzed pri-miRNA and mature miRNA levels in 12 normal B cells and B-cell lymphoma samples. Since a significant subset of the pri-miRNAs is not well characterized, we designed a custom gene expression array containing up to 6 probes for each pri-miRNA that cover the stem-loop region and sequences flanking the stem-loop regions. We analyzed 974 miRNAs that were present on both the mature and the pri-miRNA microarrays. Comparison of the pri-miRNA and mature miRNA levels in 12 samples revealed a positive Pearson correlation for 160 miRNAs and inversed correlation for 134 miRNAs. For vast


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majority of the analyzed miRNAs, i.e. 629 (65%), we only detected the pri-miRNA transcripts and not the mature miRNAs. Introduction of shRNA against DGCR8 in L1236 Hodgkin lymphoma cells enhanced levels for a limited subset (n=53) of the pri-miRNA transcripts. There was a striking overlap, i.e. 33 of the 53 (62%), between the miRNAs that showed enhanced pri-miRNA levels upon DGCR8 inhibition and the miRNAs that showed a positive correlation between the mature and primary transcript levels. Together, our results suggest that most of the primary transcripts are expressed, but not processed to mature miRNAs in B cells or B-cell lymphoma. The limited processing indicates that regulation of miRNA biogenesis may be a much more general phenomenon than currently anticipated. Funding: This work was supported by grants from National Science Centre, Poland (2015/19/D/NZ1/03443 to ISP) and (2015/19/B/ST7/02984 to PB).

## P-26-009
## DrrS and Mcr11, small RNAs of *Mycobacterium tuberculosis,* are involved in bacterial adaptation to persistence in the host organism

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Bacterial small RNAs participate in regulation of transcription and translation by affecting the level of gene expression and mRNA stability. Mostly, sRNAs are expressed in response to the external factors; helping bacteria to adaptively react to the changing environmental conditions and regulate the key stages of pathogenesis. DrrS and Mcr11, sRNAs of *Mycobacterium tuberculosis*, are highly conservative, and are found only in pathogenic mycobacteria, that indicates their involvement in bacterial adaptation to persistence in the host organism. The expression of these sRNAs increases during the transition to the stationary growth phase, in the dormant state, also in mouse models of infection during the formation of adaptive immune response. The synthesis of both sRNAs occurs in a similar way, a long precursor is processed into a shorter, very stable isoform. Despite the fact that these sRNAs were described almost 10 years ago, the functions and mechanisms of their action are still very poorly understood. We created *M. tuberculosis* strains, overexpressing DrrS and Mcr11, as well as the deletion mutant strains. A comparative analysis of the transcriptomes of these strains revealed significant functional differences in the sRNAs under study. DrrS activates a number of molecular mechanisms necessary for survival within macrophages (changes in the pattern of transcription factors, switch to anaerobic respiration, down-regulation of the amino acids biosynthesis, up-regulation of proteins involved in protecting against oxidative stress), and also participates in modulating the host immune system due to synthesis of proteins of the PE/PPE family. Increased expression of Mcr11 contributes to the adaptation of mycobacteria through the activation of the synthesis of alternative ribosomal proteins in Zur-regulon. The work was supported by Russian Science Foundation grant No. 18-15-00332

## P-26-010
## Small RNA fragments as putative components of RNA–protein regulatory networks

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There is ample evidence that small RNA fragments derived from all RNA classes accumulate in various cells under both physiological and pathological conditions. Despite extensive studies, little is known about the functions that RNA fragments fulfil in maintaining and/or disturbing cellular homeostasis. To better understand this issue, we identified proteins that directly or indirectly interact with the selected set of RNA fragments originating from tRNA, snoRNA and snRNA. To this end, we employed RNA pull-down followed by mass spectrometry and interactome network modeling. We found that RNA fragments interacted with different proteins than their parental full length RNAs. Interestingly, RNA fragments not only captured RNA-binding proteins, but also proteins devoid of known RNA-binding domains, including those involved in DNA recombination/replication and mitochondrial metabolism. In total, 14 proteins were identified as candidate unconventional RNA binders. Based on our data, RNA fragments emerged as putative endogenous aptamer-like molecules and potential players in the recently revealed RNA-protein regulatory networks. Detailed examination of interaction network formed by RNA fragments and their proteins partners pointed towards two hypothetical functions of these small RNAs: (i) scaffolds organizing larger ribonucleoprotein complexes and (ii) direct regulators of metabolic enzymes. Taken together, our results revealed new areas of the functional engagement of RNA fragments and pointed to this pool of molecules as to a rich collection of potential novel regulators.

## P-26-011
## RNA-seq transcriptomic profiling of Dicer-deficient human cells

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Endoribonuclease Dicer is recognized as one of the most important protein factors involved in the biogenesis of small interfering RNAs (siRNAs) and microRNAs (miRNAs). However, over the last years Dicer has been shown to serve a number of functions outside of the miRNA/siRNA pathways, including processing of diverse RNA classes, such as tRNA and snoRNA, and detoxification of repeat-element-derived RNAs. Given the essential role of Dicer in the RNA silencing pathways, RNA profiling of Dicer-deficient cells has been typically limited to RNA shorter than 30 nt, in particular to miRNA. In this study, we performed RNA sequencing to characterize global RNA composition of wild-type and Dicer-knockout HEK293T cells. We characterized the pool of small RNAs (15–82 nt long), including stable RNA fragments (RFs), and determined changes in gene expression upon *DICER1* knock-out. Additionally, we performed the enrichment analysis of processes and molecular functions associated with differentially expressed genes. We found that both wild-type and Dicer-deficient cells, apart from canonical types of small RNAs, contained a wide spectrum of RFs derived from virtually all RNA classes. Lack of Dicer resulted in both qualitative and quantitative changes in the expression profiles of several small RNA classes and their derivatives. Among them, as expected, miRNAs were found to be the most markedly depleted group. The analysis of differential gene expression associated with


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*DICER1* knock-out indicated profound changes in cardinal cellular and metabolic processes, which is consistent with the phenotype of the mutated cells, and presumably results from the miRNA deficiency. Altogether our data reinforce the notion that Dicer is involved in diverse aspects of RNA metabolism and functioning. However, the mechanistic basics of the observed changes require further studies.

## P-26-012
## Precursors of particular miRNAs are present in the phloem of *Cucurbita maxima*

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Plant development and responses to environmental cues largely depend on mobile signals including microRNAs (miRNAs) required for post-transcriptional silencing of specific genes. Short-range cell-to-cell transport of miRNA in developing tissues and organs is involved in transferring positional information essential for determining cell fate. Among other RNA species, miRNAs are found in the phloem sap. Long-distance transport of miRNA via the phloem takes a part in regulation of physiological responses to changing environmental conditions. As shown for regulation of inorganic phosphorus and sulfate homeostasis, mature miRNAs rather than miRNAs precursors are transported in the phloem as signaling molecules. Bioinformatics analysis of transcriptomic data for *Cucurbita maxima* long phloem RNAs was carried out to elucidate whether miRNA precursors could also be present in the phloem. 120 annotated *Cucumis melo* miRNA precursor sequences were used as queries for BLAST searches in *C. maxima* phloem transcriptome assembly. Obtained sequences were filtered by estimated sequencing coverage. Results contained several pre-miRNA sequences such as pre-miR159, 166, 319, 390, 393 and others. In addition, we demonstrated that the phloem transcriptome contained a subset of *C. maxima* pri-miRNAs that differed from a subset of pri-miRNAs in leaf mesophyll transcriptome identified using the same procedure. Differential accumulation of pri-miRNA was confirmed by PCR analysis of *C. maxima* phloem and leaf RNA samples. Therefore, this data indicate that a number of *C. maxima* pri-miRNAs are selectively recruited to the phloem translocation pathway. This work was supported by the Russian Science Foundation (grant 17-14-01032).

## P-26-013
## Investigation of the microRNA complement of the newly sequenced genome of hazelnut cv Tombul and implications for crop improvement

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European hazelnut (*Corylus avellana* L.) is an important crop tree due to its nuts, which are widely consumed directly and as the main ingredient of butter, chocolate products, and various pastes. The Black Sea region of Turkey produces around 70% of the global hazelnut supply and hazelnut is Turkey's most valuable agricultural export. In the present study, we show the first draft genome of newly sequenced Turkish hazelnut cultivar "Tombul" and the identification of microRNA genes at the chromosome level. We isolated DNA from leaves of a Tombul individual obtained commercially and cultivated at Sabanci University using optimized methods, and Illumina sequencing was performed to generate a draft genome assembly. Then, we screened important microRNA families, their targets and representations (copy number of microRNA genes) in the hazel genome. We also compared our new results with other close relatives including the "Jefferson" cultivar developed in the US, *Betula nana*, *Betula pendula*, *Juglans nigra*, *Populus trichocarpa* and *Prunus persica*. According to our results, we could identify 57 unique miRNA families in the Tombul genome and the highest number of unique miRNA families (16) was identified on chromosome 2, which is one of the largest chromosomes. The highest number of common miRNA families were found between Tombul and Jefferson as expected, and 9 miRNA families (miR11109, miR11111, miR1438, miR1511, miR2619, miR3436, miR6260, miR7486, miR7804 and miR837) were found as unique to Tombul, whereas the least similarity of miRNA families was between Tombul and Populus. Since plant miRNAs are critical regulators in processes ranging from development to a/biotic stress tolerance in plants, our findings will provide insights into understanding the newly sequenced hazel genome structural elements and the potential role of miRNAs as targets for crop improvement.

## P-26-014
## Analysis of non-coding RNA in chronic dialysis patients

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Chronic kidney disease is an irreversible disease that has been developing for several years. It is a morbid condition with heterogeneous manifestations when there is a disturbance of the constituents in the organism. High morbidity and mortality of patients during the first 3 months of dialysis is caused by the absence of adequate dialysis treatment. More advanced stages of the disease share a similar clinical course characterized by linear decrease in glomerular filtration, the development of non-selective proteinuria and the accumulation of waste matter in the internal environment. Early diagnosis and proper patient monitoring can delay or prevent disease progression, which can lead to renal insufficiency. Small non-coding RNAs causing post-transcriptional regulation of gene expression, miRNA serve as non-invasive markers of many disease processes. Micro RNA can be a possible biomarker that responds to changes occurring during pathological processes that occur in the kidneys. The aim of the present work was to analyse the effects of intradialytic resistance training on circulatory miRNA profile in haemodialysis patients. The experimental training group consisted of patients (n=73) with diagnosed renal failure and control group without training program consisted (n=26) with diagnosed renal failure. The obtained plasma material was used for isolation of miRNA and next transcription into cDNA. The quantification of the changes in expression levels of selected miRNA-206 and miRNA-23a were detected by qRT-PCR. In the experimental group, was monitored change of expression level of both, miRNA-206 and miRNA-23a, of the patients in experimental group in comparison with patients in control group. The changes in expression of specific miRNA have been correlated with clinical status of individual patients too. Research was supported by: APVV-2016-0490 and VEGA 1/0372/17


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## P-26-015

### A transcriptome-wide approach to identifying RNA targets of the Prader-Willi locus snoRNAs

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Prader-Willi syndrome (PWS) is the most common known genetic cause of life-threatening obesity in children. It is a complex genetic disorder with implications for the metabolic, endocrine, neurologic and behavioral impairments. The PWS results from lack of expression of paternally expressed genes from 15q11.2-q13 genomic region. In a close up, a deletion of paternally expressed SNORD116 gene cluster (a group of C/D-box snoRNAs located in PWS region) was shown to be the primary genetic determinant of the PWS phenotype. Since molecular mechanism instigating PWS still remain unknown, there is an urgent need to study their identification. SNORD116 (HBII-85) family consists of 29 homologues snoRNAs (small nucleolar RNAs), which display no apparent sequence complementary to canonical RNA targets and are considered as orphan snoRNAs with yet unknown function. Still, ectopic overexpression of SNORD116 in HEK293T cell line, endogenously not expressing this gene, resulted in the changed expression of more than 200 protein coding genes. This implies on SNORD116 cluster to play a regulatory role in mRNA stability. Whether the SNORD116 RNAs play a direct role in that or indirect by regulating transacting factors remains to be resolved. Thus we study the function of SNORD116 snoRNAs by primarily identifying its interacting RNA targets. In our SNORD116 overexpressing cell models, the combination of transient interaction capture via psoralen cross-linking and subsequent enrichment of cross-linked RNAs, followed by high-throughput sequencing are allowing us to efficiently detect interacting events. We are using the PARIS protocol with our own modifications that support targeted approach. NTERA-2 cell line, with high endogenous expression of SNORD116, was used for hybrid library construction and for identification of SNORD116-RNA hybrids.

## Proteins: structure, disorder and dynamics

## P-27-001

### Amino acid substitutions in essential domain of eRF3 are lethal in combination with [PSI+] prion in yeast *Saccharomyces cerevisiae*

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The essential *SUP35* gene of yeast *S. cerevisiae* encodes the translation termination factor eRF3 (Sup35p). The Sup35p consists of three domains: C-domain is essential for translation termination and possess GTF- and eRF1-binding sites. N-domain is responsible for Sup35p aggregation and [$PSI^+$] prion formation. M-linker is involved in the maintenance of [$PSI^+$]. Both *sup35* mutations and [$PSI^+$] cause reduction of the translation termination fidelity and lead to the nonsense suppression. It was shown that *sup35* mutations affecting the N-domain have an influence on [$PSI^+$] appearance and maintaining. In this work, we have studied three *sup35-m* (missense) mutations located inside the C-domain of Sup35p and affected its GTF-binding motifs. We have shown using semi-denaturing detergent agarose gel electrophoresis and fluorescence microscopy that [$PSI^+$] aggregates disappear after replacement of wild type *SUP35* by mutant allele. To check whether mutant Sup35p is able to aggregate we purified corresponding proteins from *E. coli* and investigate their amyloidogenicity *in vitro*. Products of *sup35-m* alleles form high-molecular weight aggregates. We assume that investigated substitutions extremely affect structure and aggregation speed, and due to this, cells cannot maintain aggregates of corresponding proteins. Significant defects in the translation termination process may be another reason of incompatibility of prion and *sup35-m*. Both [$PSI^+$] and *sup35-m* are strong suppressors, which working together can severely reduce cell viability. We concluded that the incompatibility of mutations that lead to amino acid substitutions in the Sup35p nonpyrogenic C-domain is associated with the alterations in [$PSI^+$] structure or/and translation termination process. The work was supported by RSF grant 18-14-00050. Part of experimental work was done in the resource centre of SPBU "Centre for Molecular and Cell Technologies".

## P-27-002

### The contribution of N- and C-terminal regions to chaperone function and oligomerization of small heat shock protein IbpA from *Acholeplasma laidlawii*

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Small heat shock proteins (sHSPs) are ubiquitous molecular chaperones that prevent the irreversible denaturation of proteins under the heat shock conditions. Well studied sHSPs IbpA and IbpB from *Escherichia coli* work in strong cooperation and are necessary for bacterial survival at high temperatures. *Acholeplasma laidlawii*, the only one free-living bacterium of Mollicutes, carries only one gene encoding the sHSP protein IbpA (*Al*IbpA). Here we report the role of the N- and C-termini of *A. laidlawii* IbpA for its oligomerization and chaperone function. Independently on the temperature, a protein forms heterogeneous mixture of globular and fibrillar structures with ratio of 1:6, while the removal of either 12 or 25 N-terminal amino acids lead to the formation of only fibrillar structures. Since in *E. coli* the IbpB blocks fibrils formation by IbpA, we suggest that the N-terminus of *Al*IbpA carries inhibitory motif which complements the lack of IbpB and is responsible for globular structure formation. By contrast, the ΔN12 and ΔN25 *Al*IbpA retained the chaperone functions on the insulin, leaving the question regarding the functional role of N-terminus opened. The deletion of the C-terminal conserved LEL motif, which is shown to be required for oligomerization of *E. coli* IbpA, or its substitution to SEP, impaired the temperature stability of *Al*IbpA and abrogated the chaperone function, while the protein remained presumably in globular state. By contrast, only N- and C-terminally truncated protein containing pure α-crystalline domain was unable to interact with substrates while formed a huge oligomeric conglomerates, probably, because of α-crystalline self-oligomerization. These data suggest non-trivial properties of *Al*IbpA, which seems to combine functions of both IbpA and IbpB-like proteins and recognizes the substrate proteins via both N- and C-termini. This research was funded by the Russian Science Foundation (project No. 17-74-20065).


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## P-27-003
## 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-27-004
## DNA damage triggers the interaction of cytochrome c with the disordered region of ANP32B

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Cytochrome c (Cc) has recently emerged as an inhibitor of the histone chaperones SET/TAF-1β in mammals and NRP1 in plants, upon DNA damage. It has been posed Cc inhibits their activity in early stages of the DNA damage response to increase the time available for the repair machinery. Likewise, Cc targets other histone chaperones, such as Acidic leucine-rich Nuclear Phosphoprotein 32 family member B (ANP32B). Whereas ANP32B modulates mRNA nucleo-cytoplasmic trafficking upon its Thr244 phosphorylation, another ANP family member, ANP32A, acts as a Protein Phosphatase 2A (PP2A) inhibitor. Both ANP forms comprise four Leucine-Rich Repeats (LRR) at its N-end, along with an unstructured C-end Low-Complexity Acidic Region (LCAR). We used immunofluorescence and pull-down assays to confirm ANP32B/Cc interaction under DNA damage. In addition, Isothermal Titration Calorimetry (ITC) and Nuclear Magnetic Resonance (NMR) assays revealed that ANP32B and its phosphomimetic T244E mutant interacted with Cc, whereas the LRR domain did not. The LCAR contains the nuclear localization signal (NLS) close to Thr244. Hence, we tested two peptides containing LCAR residues 232–251, WT and T244E mutant. ITC and NMR titrations showed that both peptides bound Cc similarly as the full-length ANP32B; albeit the T244E mutant peptide had a higher affinity towards Cc. Besides, in silico simulations using distance restraints derived from 2D 1H NMR spectra exhibited that both peptides acquired different spatial layouts on their NLS-containing side chains. This finding explains the molecular mechanism of the shuttling that ANP32B undergoes upon phosphorylation. Surprisingly enough, our pull-down assays evince that both ANP32B WT and T244E species bound to PP2A, thereby inhibiting PP2A activity. Further sequester of ANP32B species by adding Cc to these assays resulted in recovering PP2A activity. Altogether, our data reveal that LCAR phosphorylation and/or binding to Cc tightly regulate ANP32B function.

## P-27-005
## Intrinsic disorder of the C-terminus of nucleobindin-2 from *Gallus gallus*

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Nucleobindin-2 (Nucb2) is a multidomain protein, composed of six putative domains: signal peptide, Leu/Ile rich region, DNA binding domain (DBD), two EF hand domains, acidic amino acid residues rich region and leucine zipper motif. Nucb2 is implicated in variety of cellular processes for instance calcium homeostasis, cancerogenesis and food intake inhibition. It has been demonstrated that Nucb2 can be proteolytically converted to three peptides: nesfatin-1, -2 and -3. Nucb2 from *Gallus gallus*, the object of this study, is probably involved in biomineralization of the eggshell. The multifunctionality of Nucb2 indicates that it may belong to family of inherently disorder proteins (IDPs). The structural characterization of Nucb2 is essential to further physiological research. In this study, we utilized homogenous recombinant Nucb2 from *Gallus gallus* expressed in BL21(DE3)pLysS *Escherichia coli* cells. The hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) experiments revealed that Nucb2 has characteristics of a partially disordered protein. The analysis of results showed that the carboxyl terminus of protein (corresponding to nesfatin-3) is completely unstructured. Sedimentation velocity analytical ultracentrifugation (SV-AUC) results also confirmed an elongated shape and a partially unstructured conformation of the Nucb2 molecule in solution which is characteristic for inherently disordered proteins. We also observed that Nucb2 exhibits a propensity for oligomerization. Furthermore, SV-AUC analysis suggested that the conformation of Nucb2 changes upon binding of the calcium ions leading to the putative compaction of its disordered regions. These findings may explain the functional flexibility and adaptability of Nucb2, which might be crucial for proteins participating in many physiological processes.


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## P-27-006
## Biophysical and physiological studies of Nha1 antiporter:14-3-3 complex

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The importance of stable homeostasis is essential for every type of cell within the species: from bacteria to higher eukaryotes. Regulation of cellular pH, cytoplasmic metal cation concentration and cell volume are crucial aspects for normal metabolic activity. In applied sciences the research is focused on specific antiporters as interesting therapeutic targets due their role in pathophysiological processes. The Na+/H+ antiporters are the main transporter systems that ensure the proper homeostatic processes and were found in the cytoplasmic and organelle membranes of almost all the species. The Na+/H+ antiporter (Nha1) was identified to play an important role in these processes. The direct interaction between Nha1 and yeast isoforms of 14-3-3 proteins (Bmh) increases the chance of survival cells under stress conditions such as high cation concentrations and pH changes. We confirmed the interaction between disordered C-terminus of Nha1 with Bmh2 and stoichiometry 1:2 *in vitro*. We used circular dichroism spectrometry and small angle X-ray scattering (SAXS) to confirm the fully structured Nha1 C-terminus upon complex formation. Moreover we crystallized the Nha1 peptide containing the pSer481 with Bmh1. Our results *in vivo* (salinity tolerance test and cation efflux rate measurement) show that Ser481 is crucial for Nha1 interaction with Bmh proteins. This work was supported by the Czech Science Foundation (project 17-01953S).

## P-27-007
## Structure-activity relationship of optimised allosteric effectors of cathepsins K and S

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Cathepsins K and S are enzymes which belong to the family of papain-like proteases. Their fundamental role is in the degradation of extracellular and intracellular proteins in (endo)lysosomal compartments. In the human body their activity must be carefully regulated since their dysregulation can lead to numerous diseases, such as osteoporosis in the case of cathepsin K and rheumatoid arthritis in the case of both cathepsins K and S. Therefore, both enzymes represent potential targets for drug development. It is already known that allosteric regulation is one of the processes by which cathepsin K can be regulated, in particular by its natural effectors glycosaminoglycans and by two synthetic compounds NSC13345 and NSC94914. Furthermore, we have recently synthesized and characterized a novel effector of cathepsin K and showed that it binds to the same allosteric site as the aforementioned effectors. We have also confirmed it partially inhibits not only cathepsin K but also cathepsin S. This mechanism of action is consistent with allosteric regulation which is the reason we hypothesise the novel effector binds to the same allosteric site on cathepsin S as well. Using this effector as a scaffold we have prepared compound libraries with three different sites of diversification. By analysis of structure-activity relationship we have identified groups on different sites contributing to the optimized affinities and selectivities for cathepsins K and S and to their mechanism of inhibition. By site-directed mutagenesis we have also identified amino acid residues critical for the binding of the optimised effectors to the allosteric site of cathepsin K. The obtained results will be used for further optimization of cathepsin K effectors. Furthermore, we will combine different groups on different sites of diversification contributing to the optimised affinities and selectivities for cathepsin S to further optimise effectors with potential research applications.

## P-27-008
## Otolin-1 – the expression and purification of the calcium carbonate protein-scaffold of fish otoliths and vertebrate otoconia

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Biomineralization is a process in which living organisms create precisely shaped minerals with significant role in individual's function. These minerals embedded in hard tissues fulfill their physiological role as a component of a skeleton, shell or otoconia. The key role in biologically controlled mineralization is played by proteins which modulate crystal growth. They have both an influence on the shape of biominerals as well as supporting the growing structure, providing an organic scaffold. One of these molecules known for supporting the structure of calcium carbonate crystals in vertebrate otoconia and fish otoliths is otolin-1, a collagenous protein containing C- terminal C1q globular domain. In this work we present the expression and purification protocol for recombinant otolin-1 from *Danio rerio*. The expression system was composed of *Escherichia coli* cells transformed with a plasmid containing otolin-1 cDNA optimized for prokaryotic organisms. Immobilized metal affinity chromatography and size exclusion chromatography techniques were applied for the process of purification. The protein is used for preliminary studies of the calcification and interactions between organic components in otoliths.

## P-27-009
## Crystal structure of plant inorganic pyrophosphatase with unusual autoproteolytic activity

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Inorganic pyrophosphatases (EC 3.6.1.1) are enzymes that hydrolyze inorganic pyrophosphate to phosphate in presence of divalent metal cations. These enzymes play an integral part of the pathway involved in maintaining phosphorus homeostasis in the cells. There are several structures of procaryotic and eucaryotic inorganic pyrophosphatases already deposited in PDB but there was no structure of a plant inorganic pyrophosphatases so far. DNA coding inorganic pyrophosphatases from *A. thaliana* (*At*PPA1) and *M. truncatula* (*Mt*PPA1) were cloned into a bacterial expression vector and the proteins were produced in *E. coli* cells and crystallized. In terms of their subunit fold, *At*PPA1 and *Mt*PPA1 are reminiscent of other members of Family I soluble pyrophosphatases from bacteria and yeast. Similar to their


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bacterial orthologs, both plant PPases form hexamers, as confirmed by multi-angle light scattering and size-exclusion chromatography. This is in contrast to the fungal counterparts, which are dimeric. Unexpectedly, the crystallized *At*PPA1 and *Mt*PPA1 proteins lack ~30 amino acid residues at their N-termini, as independently confirmed by chemical sequencing. *In vitro*, self-cleavage of the recombinant proteins is observed after prolonged storage or during crystallization. The cleaved fragment corresponds to a putative signal peptide of mitochondrial targeting, with a predicted cleavage site at Val31. Site-directed mutagenesis shows that mutations of the key active site Asp residues dramatically reduce the cleavage rate, which suggests an unusual proteolytic activity. Atomic coordinates and structure factors for the crystal structures of inorganic pyrophosphatases from *Arabidopsis thaliana* (*At*), and *Medicago truncatula* (*Mt*) have been deposited with the Protein Data Bank (PDB) under the accession codes 4lug (*At*PPA1-Na), 5ls0 (*At*PPA1-Mg), 6mt1 (*Mt*PPA1-R3), and 6mt2 (*Mt*PPA1-I23).

## P-27-010
## Mechanistic insights of the attenuation of quorum-sensing-dependent virulence factors of *Pseudomonas aeruginosa*: Molecular modeling of the interaction of taxifolin with transcriptional regulator LasR

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*Pseudomonas aeruginosa* is one of the most dangerous superbugs and is responsible for both acute and chronic infection. Current therapies are not effective because of biofilms that increase antibiotic resistance. Bacterial virulence and biofilm formation are regulated through a system called quorum sensing, which includes transcriptional regulators LasR and RhIR. These regulators are activated by their own natural autoinducers. Targeting this system is a promising strategy to combat bacterial pathogenicity. Flavonoids are very well known for their antimicrobial activity and taxifolin is one of them. It is also known that flavonoids inhibit Pseudomonas aeruginosa biofilm formation, but the mechanism of action is unknown. In the present study, we tried to analyse the mode of interactions of LasR with taxifolin. We used a combination of molecular docking, molecular dynamics simulations and machine learning techniques, which includes principal component and cluster analysis to study the interaction of the LasR protein with taxifolin. We show that taxifolin has two binding modes. One binding mode is the interaction with ligand binding domain. The second mode is the interaction with the "bridge", which is a cryptic binding site. It involves conserved amino acid interactions from multiple domains. Biochemical studies show hydroxyl group of ring A in flavonoids is necessary for inhibition. In our model the hydroxyl group ensures the formation of many hydrogen bonds during the second binding mode. Microsecond simulations also show the stability of the formed complex. This study may offer insights on how taxifolin inhibits LasR and the quorum sensing circuitry.

## P-27-011
## Dynamics of glucose transporter GluT1 brings new insights into the impact of point mutations on the glucose transfer

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Human glucose transporter GluT1 is the uniporter protein responsible for the glucose uptake into erythrocytes and endothelial cells of the blood-brain barrier. GluT1 deficiency and inactivating mutations are associated with the severe central nervous system dysfunction. In our study we aim to investigate the impact of the GluT1 point mutations on the protein dynamics and glucose transport with help of molecular dynamics (MD) simulations. We used the available X-ray structure of the GluT1 transporter (PDB ID: 4PYP) to build a model of GluT1 wild type and performed long MD simulations in the membrane environment. We observed the protein conformational transition and stabilization in new outward-facing state with protein cavity open towards the extracellular medium. Starting from this conformation, we have obtained a complete unbiased trajectory of the glucose transport through GluT1. According to our simulations, glucose transfer can occur without any prominent transition of the overall GluT1 conformation. It is actually driven by the side chain translocation and minor but concerted rearrangement of helical segments. Thus, our results suggest an alternative to the generally accepted alternate access mechanism of the solute transfer implying obligatory high amplitude conformational change. Finally, we performed extensive analysis of the protein energy network (PEN) and identified hotspot residues playing a crucial role in GluT1 functioning. Most of the identified residues are located at the protein cavity and thus directly participate in the ligand binding. Besides these key residues, the analysis of GluT1 PEN revealed long-range interactions involving residues distant from the binding sites. Interestingly, mutations of these residues were previously reported to impair the GluT1 activity. Accordingly, we performed MD simulations for the corresponding mutant models and identified the particular changes in GluT1 dynamics.

## P-27-012
## Interactions of carbohydrate and aromatic amino acid in protein binding sites

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Interactions of saccharides with receptors belong to the most important ones in cell recognition, growth or differentiation, as well as in many pathological processes. These interactions are mediated by so-called glycocode – saccharide code which is read by many proteins. There are several ways how saccharides interact with proteins; hydrogen bridges, metal-ion mediated interaction, hydrophobic interaction etc. CH-π stacking, dispersion driven interaction has been underestimated for a long time. However, we found out that it is a highly important interaction in carbohydrate-protein complexes [1]. In our computational structure-based study we examined structures stored in Protein Data Bank (PDB) database with focus on the complexes with


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carbohydrates in close proximity of aromatic amino acid (tryptophan, tyrosine, phenylalanine, and histidine). We detected CH-π stacking interaction being involved in sugar binding in 61% of these complexes. Each aromatic amino acid showed a unique CH-π stacking pattern, demonstrated by a characteristic orientation, bond distances, and bond angles between the carbohydrate and a particular amino acid. Besides CH-π stacking interaction, we detected also hydrogen bridges and compare the frequencies of these two types of carbohydrate-protein interaction. These results provide insight into the importance of CH-π stacking in carbohydrate-protein interactions and may help in drug development, receptor studies or protein engineering. *This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).* [1] WIMMEROVÁ, Michaela, Stanislav KOZMON, Ivona NEČASOVÁ, Sushil Kumar MISHRA, Jan KOMÁREK a Jaroslav KOČA. Stacking Interactions between Carbohydrate and Protein Quantified by Combination of Theoretical and Experimental Methods. *Plos One*, SAN FRANCISCO: PUBLIC LIBRARY SCIENCE, 2012, roč. 7, č. 10. ISSN 1932-6203. https://doi.org/10.1371/journal.pone.0046032.

## P-27-013
## Mass spectrometric analysis of extracellular proteasome post-translational modifications

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The ubiquitin-proteasome system is one of the major protein degradation pathways and abnormal function of this system has been observed in cancer and neurological diseases. The presence of proteasomes in the extracellular space has been found in recent studies. In patients suffering from cancer, liver diseases, and autoimmune diseases the concentration of extracellular proteasomes (exPS) has been found to be elevated and depended upon the disease state being of prognostic significance. However, functions of exPS and mechanisms of their exit from cells remain unknown. In order to better understand these two issues, we have set to explore post-translational modifications (PTMs) of exPS in comparison to cellular proteasomes (PS). The PS and exPS were affinity purified and separated by SDS-PAGE for subsequent trypsinization and MALDI-FTR-MS-analysis. In total, we could identify 91 and 75 PTM sites for exPSs and PSs, respectively, including phosphorylation, ubiquitination, acetylation, and succinylation. We observed newfound phosphosites S216 of α4-subunit, S181 of β3, Y125 of β5 to be potentially specific for exPS. Interestingly, succinylation of β6 at K228 residue seems not to be present in exPS in comparison to PSs, whereas both exPS and PS may also be acetylated at this site. The same situation takes place in β3 subunit K201 residue where ubiquitination is seemingly specific for PS. Moreover, crosstalk between acetylation, ubiquitination, and succinylation has been observed in α3 subunit of both proteasome populations. Also, exPSs might have specific acetylation at K237, 238 of β2 and K192 of β3. Hence, we report the first study of exPS PTMs. These data will further serve as a basis for CRISPR/Cas9 genome-editing system, aimed at dissection of the roles of exPS-specific PTMs in transport of these proteasomes into extracellular space.

## P-27-014
## Novel immune checkpoint targeting molecules

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Protein–protein interactions have a key role in most biological processes and offer attractive opportunities for therapeutic approaches. The immune checkpoint proteins like programmed cell death-1 (PD-1) and its ligand (PD-L1) has been recognized as a promising therapeutic target in cancer immunotherapy. The evasion of tumors from the immune surveillance is attributed to the protein-protein interaction between PD-1 protein expressed on T lymphocytes and PD-L1 protein expressed by cancer cells. In context of normal cells, PD-1 with its ligand PD-L1 interaction prevent prolonged inflammation, but overexpression and activity on tumor cells promote its survival. The interaction of PD-L1 with T cell PD-1 receptor leads to the inactivation and death of T cells resulting in immunosuppression and undisturbed tumor development. Accordingly, there is considerable interest in the biology and mechanism of the immune checkpoints and their interaction with potential inhibitors. In our work, we are focused on immune checkpoint proteins interactions, their function and breaking down immunosuppression by PD-1/PD-L1 blockade. Moreover, we are concerned of development new small molecules that could act as PD-1/PD-L1 association inhibitors. Those molecules can be also considered as a potential alternative to recognition and active-targeting of the antigen on cell surface. Thus, these molecules may serve as guiding points for discovery and optimization of more potent, selective small-molecule inhibitors of PD-1/PD-L1. *The authors marked with an asterisk equally contributed to the work.

## P-27-015
## Structural and biochemical insights of new atypical Fpg/Nei DNA-glycosylases

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DNA glycosylases are enzymes that maintain genome integrity as a key element of base excision DNA repair. In particular, Fpg and Nei are two homologous bacterial glycosylases removing oxidized purine and pyrimidine bases, respectively, and their eukaryotic homologs NEIL1, NEIL2, NEIL3, and MMH also act on oxidized bases. All Fpg/Nei superfamily proteins share structural features important for damage recognition and catalysis: the (P/V)E *N*-terminal catalytic dyad, the helix–two-turn–helix DNA-binding motif, and the zinc- or zincless-finger motif. Recently, whole-genome sequencing revealed new bacterial Fpg/Nei-like sequences that diverge from known Fpg, Nei, and NEIL proteins, forming four separate clusters. In this work, we investigate structural and biochemical aspects of two new Fpg/Nei-like proteins (Flp) from two such clusters. We have used total gene synthesis and an optimized overexpression protocol to obtain Flps from *Streptomyces coelicolor* (Flp1) and *Bacteroides thetaiotaomicron* (Flp3b). We have characterized biochemical properties of these enzymes such as affinity and substrate specificity; both were able to cleave abasic sites in DNA opposite all four canonical nucleobases. The crystal structure of free *B. thetaiotaomicron* Flp3b was solved to 2.0 A resolution and was found to exist in a "closed" state similar to Fpg but different from Nei. Fundings: RSF (17-14-01190), RFBR (17-04-01761-a), Russian Ministry of Higher Education and Science (VI.62.1.5, 0309-2019-0003).


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## P-27-016
## Structural studies of transmembrane proteins in lipid environment by synchrotron small-angle X-ray scattering

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Transmembrane proteins present a challenging object to study as they form unspecific aggregates in water solutions. At the same time they play an important role in molecular medicine. Due to the non-solubility of such proteins, they should be incorporated into micelles, amphiphilic polymers, bicelles, nanodiscs or liposomes for structural investigations. In the present study the liposomes or micelles prepared respectively from the lipids and detergent molecules were employed to solubilize the proteins of interest. Small-angle X-ray scattering (SAXS) technique has been employed to structural investigation of complex systems involving two transmembrane proteins in their lipidic environment (micelles/liposomes). The first protein component is Aβ-peptide from amyloid precursor protein (APP) yielding the formation of amyloid fibrils. The peptide is considered as the key mediator of Alzheimer disease. The second one is alkali-sensing insulin receptor-related receptor tyrosine kinase (IRR), involved in the regulation of acid–base balance. Here we applied two novel, developed by us, methods for structural characterization of liposomes and micelles by small angle scattering. The methods are based on quasi-atomistic approach and electron density profile evaluation. Thus, dodecylphosphocholine micelles in free state and loaded with Aβ-peptide were structurally characterized, and a hybrid low-resolution model of protein-micelle complex was built. New approaches for SAXS data interpretation of liposome-protein interactions were also implemented to demonstrate a physical model, which describes the specificity of the interaction of IRR protein with liposomes. Additionally, molecular tectonics was used to reveal quaternary structure of the IRR ectodomain (ectoIRR) in solution. The work has been partly supported by Russian Foundation for Basic Research (projects 18-54-74001, 17-00-00487, 17-00-00488 and 18-54-00019).

## P-27-017
## Structural study of Influenza virus hemagglutinin C-terminal region

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Influenza virus belongs to *Orthomyxoviridae* family of enveloped viruses entering a host cell via endocytosis. Dynamic interplay between transmembrane glycoprotein hemagglutinin (HA), membrane associated M1 matrix protein and lipid membrane at acid vs. neutral pH underlie virus life cycle. The 3D-structures of HA ectodomain and (partially) transmembrane domain are resolved. A low resolution structure of the full-length M1 was recently resolved in solution by synchrotron small-angle X-ray scattering (SAXS) analysis. In contrast, no structural data for the HA C-terminal region are available. We now applied bioinformatics predictions and circular dichroism (CD) spectra analysis to reveal the HA C-terminal region secondary structure. Based on ab-initio PepFOLD 3.5 modeling we hypothesized there is a beta-hairpin with a beta-turn around a glycine within $NH_2$-FWMCSNGSLQCRICI-COOH peptide (FI15) corresponding to residues 552–566 of A/Puerto Rico/34(H1N1) virus HA. In accordance with our predictions, beta-structure elements were clearly detected by CD measurements in synthetic peptides FI15 and WI14, and their portion was higher at pH 5.0 compared to pH 7.4 that is consistent with a model of fusion pore dilation. The 3D-structure homology modeling based on human m-calpain form II (PDB ID 1kfu) was performed for a sequence 1-CSNGSLQC-8 where a loop SNGSLQ is fixed between two cysteines post-translationally modified with long fatty acids in native HA. There are two surfaces in that model: a hydrophobic one composed of residues C1, L6, C8 potentially interacting with viral lipid membrane and a hydrophilic one composed of residues S2, N3, G4, S5, and Q7 that might interact with M1. Structural study of liposomes built from viral lipids, the same liposomes containing the HA C-terminal anchoring peptides before and after their incubation with isolated M1 using SAXS analysis is in progress. The work was supported by RFBR grants 18-54-00019, KOMFI 17-00-00487 and BRFFR grant B18R-113.

## P-27-018
## High-resolution NMR studies of antibiotics in cell membranes

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The alarming rise of antimicrobial resistance requires novel antibiotics with unexploited mechanisms. Ideal templates could be antibiotics that target Lipid II, known as the Achilles' heel of bacteria, at an irreplaceable pyrophosphate group. Such antibiotics, like nisin [1], plectasin [2], or teixobactin [3] would kill the most refractory pathogens without causing antimicrobial resistance. However, due to the challenge of studying small membrane-embedded drug-receptor complexes in native conditions, the structural correlates of the pharmaceutically relevant binding modes are unknown. Here, using state-of-the-art solid-state NMR methods that enable atomic-resolution studies directly in native bacterial cell membranes, we report on the pharmaceutically relevant binding modes of Lipid II–peptide complexes.[4] We show on the example of nisin and plectasin, which are two of the preeminent Lipid II binding peptides, that previously published structures do not report on physiologically relevant drug binding modes, which we further validate with other biophysical data and extensive mutational studies in combination with activity tests. We do report comprehensive high-resolution data of the relevant physiological lipid II - peptide complexes, and we show how the intriguing complexity of bacterial membranes critically modulates the binding mode. Altogether, our high-resolution approach provides critical insights into the physiologically and pharmacologically relevant binding modes for a promising class of antibiotics. [1] Breukink et al, Science 1999 [2] Schneider et al., Science 2010, [3] Ji et al., Nature 2015 [4] Medeiros-Silva et al., Nature Comm. 2018


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## P-27-019
## The pH-dependent conformational changes of the riboflavin-binding protein

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Riboflavin-binding protein (RfBP) belongs to the folate receptor domain-containing proteins. We have performed structural (X-ray crystallography) and spectroscopic (circular dichroism) studies to characterize pH-dependent conformational changes of RfBP in order to determine whether this protein undergoes the same mechanism of conformational rearrangements as recently revealed for human folate receptor, a close structural homolog of RfBP. The crystal structures of "apo" and "holo" forms of chicken RfBP at pH 8.0–8.5 have been solved. The structures of both forms are almost identical but some changes can be detected in the binding pocket. As the protein lacks a tight hydrogen bond pattern provided by the α-helices and β-sheets, the S-S bridges act as a "clips" crucial for RfBP stabilization. RfBP also possesses a highly disordered phosphorylated motif that is only partially visible on the electron density maps. Attempts to crystallize RfBP at other pH have failed so far but using the circular dichroism we have identified several pH-dependent structural changes in RfBP, indicating that at pH 2–3, protein adopts significantly different conformation. Such changes may originate either from the ordering of phosphorylated motif or might indicate structural changes around the ligand binding site. This last hypothesis is supported by the observation that at low pH, RfBP has conformation that facilitates ligand releasing, however, such structural reorganization does not itself lead to riboflavin dissociation. The complete removal of the ligand requires protein adsorption on chromatographic resin and systematic washing-out by the low-pH buffer. Using available structural and spectroscopic data we proposed the pH-dependent mechanism of ligand binding and releasing, which seems to be strongly associated with conformational rearrangements of RfBP. *The authors marked with an asterisk equally contributed to the work.

## P-27-021
## The role of transient protein–protein interactions in base excision repair

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Base excision repair (BER) is critical for preventing mutagenesis and cell death from accumulation of small non-bulky lesions in the genome. During BER, damaged DNA is channeled between several enzymes: one of several DNA glycosylases removes the damaged base, an AP endonuclease cleaves the resulting abasic site, a DNA polymerase inserts undamaged nucleotides, and a DNA ligase seals the nick. Rather than forming a single complex, the BER enzymes work by sequentially replacing each other at the site of the lesion. We have studied DNA-mediated interactions of BER proteins in the repair of several lesions including abasic sites, 8-oxoguanine, and uracil. Human AP endonuclease APEX1 stimulated 8-oxoguanine-DNA glycosylase OGG1, mismatched adenine glycosylase MUTYH, and oxidized pyrimidine glycosylase NEIL1. The stimulation of OGG1 was due to the interactions with APEX1 5′ to the bound glycosylase molecule, while the stimulation of NEIL1 required interactions 3′ to the glycosylase. Molecular docking revealed possible APEX1/OGG1 and APEX1/NEIL1 interaction interfaces, and coevolutionary analysis of APEX1 and DNA glycosylases suggested that some residues in these pairs change to compensate for mutations in the interacting partner. Using concatemeric substrates we have shown that, in all cases, the displacement of the glycosylase molecule caused its sliding off the repair intermediate along DNA rather than dissociation of the glycosylase/DNA complex. Using the system with two different damaged DNA substrates, we have demonstrated that many DNA polymerases, including DNA polymerase β, the main BER polymerase in human cells, may displace tightly bound protein molecules from DNA repair intermediates. Even very tight model complexes such as those formed by RNA-targeted Cas9 nuclease can be disrupted in this manner. Moreover, considerable stimulation of DNA glycosylases can be achieved by proteins non-specifically sliding along the DNA contour. Supported by RSF (17-14-01190).

## P-27-022
## Structural insight for production of catechol derivatives at acidic pH by tyrosinase from *Burkholderia thailandensis*

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Tyrosinases (TYRs) catalyze two-step consecutive oxidation reactions of monophenolic compounds. Since known TYRs have optimal pH at neutral to somewhat basic pH, they have limitations to be used for production of catechol derivatives. In this study, we identified TYR from Burkholderia thailandensis (BtTYR), which exhibited high tyrosinase activity at low pH. We determined the crystal structure of BtTYR and provided the structural basis for the regulation of its activity in response to pH change. At high pH, BtTYR is inactivated due to the tight binding of its TYR and CAP domains, although it is stable in monomer form; at low pH, however, the protein is activated by the typical opening of the CAP domain, and the formation of tetramers maintains the stability of the protein. Such unique tyrosinase activity of BtTYR at acidic pH was successfully applied to highly efficient production of catechol derivatives and fabrication of an adhesive hydrogel.

## P-27-023
## Structure and inhibition properties of archaeon citrate synthase from *Metallosphaera sedula*

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*Metallosphaera sedula* is a thermoacidophilic archaeon and has an incomplete TCA/glyoxylate cycle that is used for production of biosynthetic precursors of essential metabolites. Citrate synthase from *M. sedula* (MsCS) is an enzyme involved in the first step of the incomplete TCA/glyoxylate cycle by converting oxaloacetate and acetyl-CoA into citrate and coenzyme A. To elucidate the inhibition properties of MsCS, we determined its crystal structure at 1.7 A resolution. Like other Type-I CS, MsCS functions as a dimer and each monomer consists of two distinct domains, a large domain and a small domain. The oxaloacetate binding site locates at the cleft between the two domains, and the active site was more closed upon binding of the oxaloacetate substrate than binding of the citrate product.


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Interestingly, the inhibition kinetic analysis showed that, unlike other Type-I CSs, MsCS is noncompetitively inhibited by NADH. Finally, amino acids and structural comparison of MsCS with other Type-II CSs, which were reported to be non-competitively inhibited by NADH, revealed that MsCS has quite unique NADH binding mode for non-competitive inhibition.

## P-27-024
## Conformational change upon binding AMP in an LOG of *Pseudomonas aeruginosa* PAO1

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Cytokinin activating enzyme, called LOG, is a phosphoribohydrolase that hydrolyzes nucleotide into nucleobase and phosphoribose. This reaction is a fascinating target for regulation of cellular active-form of cytokinins in plants. However, misannotation of LOG as a possible lysine decarboxylase and the lack of detailed catalytic and substrate-binding mechanisms in biochemistry community have prevented studies of LOG at a protein-level. Moreover, the fact that homologues of LOG are highly conserved in a variety of bacterial lineages implies an interaction between bacteria and higher organisms via cytokinins or a novel signaling pathway. Here, we report the crystal structure of *PA*4923 from *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. The overall structure of *PA*4923 resembles those of type-I LOGs, which are identical to the plant cytokinin activating LOGs, and it exhibited phosphoribohydrolase activity against AMP. These observations indicated that *PA*4923 functions as a LOG. We also determined the *Pa*LOG structure in complex with AMP and elucidated the detailed binding mode of LOG against the AMP substrate. Interestingly, *Pa*LOG undergoes an open/closed conformational change upon binding AMP, during which the Glu74 residue located on the β3-β4 connecting loop flips 180° and moves 13 A toward the AMP molecule. Structural and amino acid sequence comparisons of LOGs suggest that this conformational change upon substrate binding might be a common phenomenon in type-I LOGs.

## P-27-025
## Structural and functional characterization of the GKAP post-synaptic density scaffold protein

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Scaffold proteins participating in the formation and maintaining the spatial organization of the post-synaptic density (PSD) actively take part in the regulation and modulation of signaling through glutamate receptors. GKAP (aka DLGAP), an essential member of the PSD, dynamically connects several proteins through its highly flexible disordered regions, which are not yet fully characterized. On the N terminal, the GK-binding region (GKAP-GBR) consisting of five 14 amino acid long repeats associates with the GK region of the well-known PSD-95 protein, while the DYNLL binding region (GKAP-DLC) binds to the light chain of the dynein motor protein. Using *E. coli* bacteria, recombinant proteins corresponding to rat GKAP-GBR and GKAP-DLC were produced. The GKAP-DLC construct was mainly expressed in inclusion bodies, therefore a denaturing-renaturing immobilized metal affinity chromatography protocol was applied to purify it using Ni-charged columns and the 6x His-tag attached to the protein. Preliminary NMR and ECD spectroscopic measurements confirmed that that GKAP-DLC is intrinsically unstructured. The dynein-binding activity of GKAP-DLC was confirmed in several experiments with in-house produced dynein light chain protein (DYNLL2), like pull-down assay and ITC measurements. Production of $^{15}N$ and $^{13}C$ labeled form of these constructs is under way to enable detailed NMR characterization of the segments and their interactions.

## P-27-026
## Structural study on FlhDC, the master transcriptional regulator of the bacterial flagellum

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The flagellum plays a key role in the motility and pathogenicity of bacteria. The bacterial flagellum is a complex organelle that is composed of at least 30 different proteins. To form the functional flagellum, meticulous transcriptional control of various flagellar genes is required. FlhDC is the master transcription regulator of flagellar expression in γ-proteobacteria. To structurally characterize FlhDC and reveal the flagellar gene regulation mechanism used by FlhDC, we performed expression, purification, and crystallization screens for diverse FlhDC orthologs and determined the crystal structure of *Cupriavidus necator* FlhDC (cnFlhDC). The cnFlhDC structure presents a hexameric ring architecture that consists of two FlhC chains and four FlhD chains. One FlhC subunit binds one FlhD dimer using an extensive interaction network, and the trimeric FlhC-$FlhD_2$ complex homodimerizes into a hexameric $FlhC_2$-$FlhD_4$ complex. Based on our biophysicochemical study on the interaction of FlhDC with the operator DNA of the *fliA* gene, we propose an FlhDC-mediated transcriptional regulatory mechanism.

## P-27-027
## Crystallization in lipid mesophases affects pigment composition of photosynthetic reaction center

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Structural studies of membrane proteins are often challenging. One of the popular techniques for this purpose is crystallization using lipid mesophases. It has several advantages over other approaches but we have shown that it has a serious flaw. It turned out that crystal growth in lipid mesophase can affect pigment composition in the resulting x-ray structure. In purple bacteria, the primary process of photosynthesis occurs in a membrane pigment-protein complex termed a reaction center (RC). To obtain structure with the highest resolution, we tried different crystallization techniques. Three specimens of RC crystals were obtained: (i) from detergent conditions without lipids; (ii) from lipid cubic phase (LCP) conditions; (iii) from lipid sponge phase (LSP) conditions. Crystal structures were solved and refined with final resolution of 2.3 A for "detergent


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crystals", 2.0 A for "LSP crystals", and 2.1 A for "LCP crystals". Comparative analysis of RC structures obtained in lipid and detergent conditions showed several differences. First major difference is the absence of carotenoid spheroidene in "lipid" structures. In this structures carotenoid-binding pocket possess an electron density that does not correspond to a carotenoid molecule. Second major difference is the presence of an unfamiliar molecule in the secondary ubiquinone binding site. Both electron densities most likely correspond to 2-monoolein molecules. 2-monoolein is a monoacylglyceride (MAG) and an isomer of 1-monoolein, the main mesophase matrix lipid. MAGs can undergo spontaneous isomerization over time, therefore, the presence of 2-monoolein in the crystallization mix is highly probable. We assume that during the crystallization process alien molecules can displace natural carotenoid and ubiquinone. This study was supported by the program of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology and Postgenomic Technologies" and the RFBR (grants 18-02-40008 and 17-44-500828).

## P-27-028

## Modeling the intrinsically unstructured regions of the post-synaptic density scaffold protein GKAP

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The Guanylate Kinase-Associated Protein (GKAP) is part of the Postsynaptic Density (PSD), an intricate network of proteins involved in signal transmission between neurons of the brain. Adaptor and scaffold proteins are important part of this network. The GKAP protein is one of them, taking part in the regulation of the trafficking and activity of some glutamate receptors, thereby being part of the regulation the activity and shape of the synapse. The protein is predicted to be intrinsically disordered except for its C-terminal GH1 domain. The intrinsically disordered parts contains five copies of a 14-residue repeat involved in the binding of GK domains of MAGUK proteins as well as a region interacting with the DYNLL (dynein light chain) protein. Our aim is to build a structural ensemble of the protein that can later be used for refinement with experimental, NMR-derived parameters when these become available in our laboratory. In order to build a structural model of parts of this protein, a pipeline has been developed, inspired by previously published methods. The aim is to get and ensemble that properly reflects the local structural preferences of the disordered segments. We have assembled an amino acid diamide conformers library from experimentally determined protein structures and use this to generate initial models of disordered segments by taking into account local sequence neighborhood. We evaluate the ensembles by assessing their structural diversity and context-specific Ramachandran maps.

## P-27-029

## SmAP from *Halobacterium salinarum* has two different forms with the same structure but a different RNA-binding affinity

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Lsm (Sm-like) proteins are found in representatives of all the three domains of life. The functions of bacterial and eukaryotic Lsm proteins have been studied well. Eukaryotic Sm/Lsm are part of various RNP particles and are involved in RNA processing. Bacterial Lsm protein Hfq exhibits RNA-chaperone activity, facilitate the interaction of regulatory rRNA with mRNA, thus regulating gene expression. Nowadays, there is a little information about the functions of the archaeal Lsm proteins SmAP, some data shows that they appear to be involved in the processing of some RNA (such as tRNA). Our work concerns structural and functional studies of SmAP from *Halobacterium salinarum*. The protein has no unstructured N- and C-terminus or extended loops between secondary structure elements thus representing a minimal Lsm core. However, in the genomic data we have found a variant of the protein with nine amino acids length N-terminal extension. Both variants of the SmAP from *H. salinarum* have been isolated and their affinity to oligo(A) and oligo(U) RNA have been measured. A difference in the affinity of the alternatives to the RNA has been found. Besides, the proteins were crystallized and X-ray diffraction data have been collected at ERSF in Grenoble. SmAP from *H. salinarum* has the characteristic for the Lsm proteins doughnut-shape form with seven monomers organized into a torus. The work is supported by RFBR grant #18-04-00222

## P-27-030

Abstract withdrawn


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-27-031

## X-ray structure of a novel ketose 3-epimerase from *Shinella zoogloeods* which is capable of D-allulose production

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D-Allulose (also known as D-psicose) is one of rare sugars of which physiological functions are focused for human healthcare, such as moderating a blood glucose level and fat accumulation. So far, various enzymes for the production of D-allulose have been reported, D-tagatose 3-epimerases from *Pseudomonas cichorii*, *Rhodobacter sphaeroides*, and *Sinorhizobium* sp., D-allulose 3-epimerases from *Agrobacterium tumefaciens*, *Agrobacterium* sp., *Clostridium bolteae*, *Clostridium cellulolyticum*, *Clostridium scindens*, *Clostridium* sp., *Desmospora* sp., *Dorea* sp., *Flavonifractor plautii*, *Ruminococcus* sp., and *Treponema primitia*, and ketose 3-epimerase from *Arthrobacter globiformis*. However only several crystal structures of them were reported. The available structures of the potential enzymes for D-allulose production from abundant sugar D-fructose, are *P. cichorii* D-tagatose 3-epimerase (PcDTE, homo dimer), D-allulose 3-epimerases from *A. tumefaciens* (AtDAE, homo tetramer) and *C. cellulolyticum* (CcDAE, homo tetramer), and *A. globiformis* ketose 3-epimerase (homo tetramer). The ketose 3-epimerase from *A. globiformis* has been originally reported as D-allulose 3-epimerase (AgDAE) due to its similar substrate specificity to the known D-allulose 3-epimerases, but is currently known to show the highest activity towards L-ribulose, and its structure was rather similar to that of L-ribulose 3-epimerase from *Mesorhizobium loti* (MlLRE, homo tetramer), though MlLRE shows little activity for D-allulose. In this study, we determined the X-ray structure of a novel ketose 3-epimerase from *Shinella zoogloeods* (SzK3E). The overall structure of the His-tagged recombinant SzK3E shows homo tetramer, and its structure is similar to those of MlLRE and AgDAE. Although the recombinant SzK3E would be classified as L-ribulose 3-epimerase, it shows the enzymatic activities not only for L-ribulose but also for D-allulose.

## P-27-032

## Functional and structural studies of tolloid-like 1 mutants associated with atrial-septal defect 6

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Inactive mammalian tolloid-like 1 (tll1) and mutations detected in tolloid-like 1 (TLL1) have been linked to the lack of the heart septa formation in mice and to a similar human inborn condition called atrial-septal defect 6 (ASD6; OMIM 613087, formerly ASD II). Previously, we reported four point mutations in TLL1 found in approximately 20% of ASD6 patients. Three mutations in the coding sequence were M182L, V238A, and I629V. In this work, we present the effects of these mutations on TLL1 function. Three recombinant cDNA constructs carrying the mutations and one wild-type construct were prepared and then expressed in HT-1080 cells. Corresponding recombinant proteins were analyzed for their metalloendopeptidase activity using a native substrate, chordin. The results of these assays demonstrated that in comparison with the native TLL1, mutants cleaved chordin and procollagen I at significantly lower rates. CD analyses revealed significant structural differences between the higher order structure of wild-type and mutant variants. Moreover, biosensor-based assays of binding interactions between TLL1 variants and chordin demonstrated a significant decrease in the binding affinities of the mutated variants. The results from this work indicate that mutations detected in TLL1 of ASD6 patients altered its metalloendopeptidase activity, structure, and substrate-binding properties, thereby suggesting a possible pathomechanism of ASD6. This work was supported by the Institutional Grants [KNW-1-010/P/1/0, KNW-1-007/P/2/0, KNW-1-041/K/3/0 (to A.L.S.)]; and the Young Scientist Institutional Grant Award KNW-2-043/D/3/K (to L.S.)].

## P-27-033

## Structural basis for the interaction of 14-3-3 proteins with phosphopeptides of STARD1 revealed by the chimeric app

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14-3-3 proteins are involved in numerous protein-proteins interactions often mediated by intrinsically disordered protein regions (IDPRs) and partner protein phosphorylation. Hundreds of known partner proteins notwithstanding, 14-3-3 complexes are structurally underexplored, which is challenged by IDPRs and transient interactions. To fill the gap, we recently proposed utilization of 14-3-3 chimeras with fragments of their partners and demonstrated its application to solve crystal structures of 14-3-3 with phosphopeptides of the small heat shock protein HSPB6. Here, we describe successful application of this approach to characterize the interaction of 14-3-3 with phosphopeptides of steroidogenic acute regulatory protein (STARD1), which is the crucial cholesterol transport factor at the first stage of steroidogenesis in mammals. Previously, two potential 14-3-3-binding sites in STARD1 centered at pSer57 (in the mitochondrial peptide) and pSer195 (in the β5-β6 loop of the STAR domain) were described. We cloned the corresponding STARD1 sequences, LRRSS57LLGSR and LRRGS195TCVLA, after the human 14-3-3 sigma sequence and produced the chimeric constructions in E. coli strains bearing also a plasmid for the expression of the catalytically active protein kinase A subunit. The 14-3-3 sigma sequence was engineered to remove the flexible C-terminal tail and to reduce surface entropy thereby enhancing chances for crystallization. Bacterial co-expression of the chimeras with PKA and their convenient isolation by subtractive IMAC and gel-filtration allowed obtaining of homogeneous phosphoproteins in preparative amounts. The chimeras readily crystallized in many conditions, and both crystal structures were solved to 1.95 and 2.62 A resolution, respectively, providing the evidence that both sites may contribute to the 14-3-3/STARD1 binding and allowing further modulation of the interaction by small molecules. Partially supported by RFBR (18-34-00428 mol_a).


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## P-27-034
## New evidence supporting the ping-pong mechanism of catalysis by L-asparaginases

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Asparaginases (E.C. 3.5.1.1) catalyze hydrolysis of L-Asn to L-Asp. This chemically simple reaction forms a basis of anti-cancer therapy, since type II *E. coli* L-asparaginase has been successfully used in treatment of childhood lymphoblastic leukemia for over 40 years. Various properties, including structures, of this enzyme and its homologues from other bacteria have been studied even longer. Currently, there are almost 100 structures of different L-asparaginases and their mutants deposited in the Protein Data Bank. Despite of such extensive information and a relative simplicity of the catalytic reaction, it is surprising that the mechanism of catalysis is still a matter of ongoing debate. The main disagreement is whether catalysis follows the single-displacement or double-displacement mechanism. Another poorly understood peculiarity of most of L-asparaginase is that they act as homotetramers. The third important yet unresolved question is the molecular basis of their substrate specificity, as most of L-asparaginases can also hydrolyze L-Gln, although less efficiently. These are not mere academic questions, since such knowledge is needed to address number of deficiencies associated with the anti-cancer therapy utilizing these enzymes (i.e. side effects or plasma stability) by designing an improved next generation of asparaginase-based therapeutics. In this report, we discuss in detail structural and functional evidence strongly supporting a double-displacement mechanism for the catalytic reaction. Our evidence includes crystals structures of all intermediates of the catalytic process. Our current description of the mechanism is in full agreement with all available evidence, including that published previously by supporters of the single-displacement mode of catalysis.

## P-27-035
## A molecular dynamics modeling study on Grb2 SH2 dimer interacting with peptides

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As an adaptor protein, Grb2 plays a role in regulating cell growth, differentiation, migration, and death. Grb2 SH2 domain recognizes peptides carrying a phosphorylated tyrosine. There are two types of Grb2 SH2-pYVN complexes available. One is a monomeric SH2 bound with a pYVN peptide; another is a dimeric SH2 bound with two pYVN peptides where the two SH2 domains are intertwined with their extended αB helixes to form domain swapping. Thermodynamic experiment conducted by Benfield et al. suggested that the monomer Grb2 SH2 associating pYVN with a higher affinity ($K_a = 6.1\times10^5$) while the dimer possesses $K_a = 1.5\times10^5$ for the first binding and $K_a = 4.7\times10^4$ for the second binding, indicating negative cooperativity. Herein, we conducted a series of molecular dynamics simulations on apo and pYVN-bound Grb2 SH2 systems and aimed to explain the structural and dynamic asymmetry caused by the first pYVN binding onto the dimeric SH2.

## P-27-036
## Novel inhibitors of deubiquitinases – early biochemical and structural studies

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Protein ubiquitination is one of the most pivotal posttranslational modifications being a vital regulator of a number of cellular processes. It may take a form a simple monoubiquitination event as well as more complex patterns of polyubiquitinations, which together create a versatile ubiquitin code. Deubiquitinases (DUBs) are cysteine proteases capable of reversing ubiquitin signalling via selective cleavage of ubiquitin. Accumulating evidence proved that DUB deregulation contributes to tumorigenesis at multiple levels. On account of that, DUBs are considered as attractive drug targets. However, until recently the development of selective DUB inhibitors has been limited by insufficient understanding of DUB function on the structural level. Recently we design several selective inhibitors of DUBs involved in malignant transformation. Since only a few structures of complexes DUB-inhibitors are known our recent goal is to unravel the molecular mechanism of inhibition. We selected several proteins, mostly from USP family, cloned them to appropriate expression vectors and optimise their expression in E. coli. Constructs composed of the catalytic domain either of USP7 and USP15 were expressed, purified and subject to crystallization screening in order to find the best condition for co-crystallise with inhibitors Moreover, we plan to use ligand-based NMR methods including STD and Water-LOGSY to determine ligand-protein affinity and figure out the fragments of ligand structure which interact with proteins. On the other hand, we found that neither native USP1/UAF1 complex nor its mutant are expressed in a bacterial system. Hence, we switch to eukaryotic expression system choosing LEXSY, based on Leishmania cells and we are optimizing protein expression in this system. A better understanding of inhibition mechanism has not only biological relevance but also allows designing more potent and selective inhibitors which may find applications in targeted anti-cancer therapies.

## P-27-037
## Scop3P: the bridge between human phosphosites, protein structure and proteomics data

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Protein phosphorylation is one of the key post-translational modifications (PTMs) due to its importance in various cellular processes. Phosphorylation can alter the physico-chemical properties, conformation, stability and flexibility of proteins. The accuracy of identification, annotation and functional analysis of these phosphosites is therefore crucial for understanding the regulatory roles of protein phosphorylation. However, available resources on phosphorylation usually contain sequence and phosphosite information, but only rarely structural information. Yet such structural information is particularly relevant in a crucial task: to differentiate between functional and non-functional phosphosites.


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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We therefore developed Scop3P: a database of human phosphosites derived from re-processing public proteomics data that are annotated with detailed, residue-level structural annotation based on state-of-the-art prediction tools. Moreover, these phosphosites are also directly mapped onto 3D protein structures when available in PDB. Human phosphosites information were obtained from UniProtKB/Swiss-Prot and by re-processing public phosphoproteomics data from PRIDE. For each human protein – even when no structure is available – backbone dynamics, disordered propensity and early folding properties were predicted using DynaMine, DisOmine and EFoldMine respectively. Each Phosphosite is annotated with valuable structural information, including structural propensity, solvent accessibility, probability of being a disordered region, backbone dynamics, early folding properties and the frequency of observation of this phosphosite in different PRIDE projects. Scop3P uses a relational database as data repository. Scop3P will thus be a valuable resource as it establishes the link between phosphorylation status information, sequence and structural level information and data retrieved from re-processing proteomics experiments. *The authors marked with an asterisk equally contributed to the work.

## P-27-038
## Solution structure of DNA-binding domain of forkhead transcription factor FOXO1

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Transcription factor FOXO1 belongs to the FOXO subfamily of forkhead family transcription factors. FOXO subfamily consists of four members – FOXO1, FOXO3, FOXO4 and FOXO6 playing important roles in numerous cellular processes. FOXO proteins consist of four domains/regions: the highly conserved DNA-binding domain (DBD), the nuclear localization signal (NLS), the nuclear export sequence (NES), and the C-terminal transactivation domain. Despite their highly conserved DBDs FOXO proteins display different functions including cell-cycle regulation, homeostasis, oxidative stress response, differentiation and apoptosis. FOXO-DBD is a compact domain containing three alpha-helices (H1-3), three beta-strands (S1-3) and two wing-like loops (W1,2) with the overall topology H1-S1-H2-H3-S2-W1-S3-W2. It has been suggested that less conserved regions, such as H2-H3 turn and both W1 and W2 wings are responsible for fine-tuning of DNA recognition and functional variability among FOXO proteins. While crystal structures of FOXO-DBD: DNA complexes were reported for all FOXO members, solution structures of apo-DBDs were reported only for FOXO3 and FOXO4. To enable detailed structural comparison of FOXO-DBDs and to obtain insight into the FOXO DNA-binding specificity, we solved the solution structure of FOXO1-DBD. Comprehensive backbone and side-chain resonance assignments for the 13C,15N-labelled FOXO1-DBD were obtained using established double and triple-resonance experiments. Comparison of obtained FOXO1-DBD structure with solution and crystal structures of other FOXO proteins revealed substantial differences mainly in regions involved in DNA binding: the turn between helices H2 and H3, and the loop between H3 and wing W1. This work was supported by the Czech Science Foundation (project 17-33854L).

## P-27-039
## Size-exclusion chromatography small-angle X-ray scattering of insulin receptor-related receptor ectodomain expressed in mammalian cell culture

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The Insulin receptor-related receptor (IRR) was originally discovered due to its high homology to the other family members: insulin receptor and insulin-like growth factor 1 receptor. We determined that IRR can be activated by alkaline extracellular media and has typical features of the ligand-receptor interaction, including its specificity, dose dependence and reversible. Because pH-sensitive properties of IRR are determined by ectodomain we chose as option study the soluble an extracellular portion IRR. Based on the CHO-K1 cell line an effective system of constitutive heterologous expression was developed. Purification protocol that was developed, allows to obtain IRR ectodomain with a purity of more than 99%. The identity of the protein was confirmed by the Western blot analysis and MALDI mass spectrometry. Synchrotron SEC-SAXS measurements were performed at the European Molecular Biology Laboratory on the EMBL-P12 BioSAXS beamline at the PETRAIII storage ring (DESY, Hamburg). The scattering curves of the IRR ectodomain measured at pH 7 and 9, have insignificant differences. The shape of the scattering curves is characteristic for monodisperse protein solutions. The structural parameters Rg (radius of gyration), Dmax (maximum particle dimension), Vp (excluded& Porod volume), Rh (hydrodynamic radius), Mw (calculated molecular mass from SEC-MALS uses multi-angle light scattering) extracted from the SAXS, DLS, MALS data are reported. pH 7: Rg=5.7 nm, Dmax= 19 nm, Vp=651 nm3, Rh=7.1 nm, Mw=231 kDa. pH 8: Rg=5.8 nm, Dmax= 19.4 nm, Vp=663 nm3, Rh=7.2 nm, Mw=233 kDa. The main structural characteristics of the protein at different pH showed poor differences. From the data obtained it can be concluded that the protein exists in solution as a dimer with molecular mass close to that calculated for an amino acid sequence with the contribution of glycosylation. This work was financially supported by Russian Foundation for Basic Researches (project No. 17-00-00489 KOMFI).


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## P-27-040
## Glyoxylate/hydroxypyruvate reductases – structural, biochemical, and evolutionary characterizations

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The d-2-hydroxyacid dehydrogenase (2HADH) family illustrates a complex evolutionary history with multiple lateral gene transfers and gene duplications and losses. Using a combination of biochemical, structural, and bioinformatics approaches, we show that the previously delineated glyoxylate/hydroxypyruvate reductase (GHPR) subfamily consists of two evolutionary separated GHRA and GHRB subfamilies [1]. We chose two enzymes from Sinorhizobium meliloti, whose genome encodes 9 GHPR members. The kinetic results for SmGhrA and SmGhrB show that both reduce several 2-ketocarboxylic acids with overlapping, but not equivalent, substrate preference. In particular, we determined for both a series of crystal structure, among which SmGhrB with 2-keto-d-gluconate is the biggest substrate cocrystallized with a 2HADH member. The research revealed significant differences between SmGhrA and SmGhrB, both in the overall structure and within the substrate-binding pocket, offering insight into the molecular basis for the observed substrate preferences and subfamily differences. At our latest research, we focused [2] on a detailed evolutionary characterization of the whole 2HADH family (http://2hadh.bioreproducibility.org/). We will present our latest results and plans regarding the use of a versatile enzyme system to obtain optically pure synthons, building blocks for the synthesis of the pharmaceuticals. 1. J. Kutner, I. G. Shabalin, D. Matelska, K. B. Handing, O. Gasiorowska, P. Sroka, M. W. Gorna, K. Ginalski, K. Wozniak, W. Minor Biochemistry (2018) 57 (6), pp 963–977 2. D. Matelska, I. G. Shabalin, J. Jabłońska, M. J. Domagalski, J. Kutner, K. Ginalski, W. Minor BMC Evol Biol. (2018) 18:199 The "Core facility for crystallographic and biophysical research to support the development of medicinal products" project is carried out within the TEAM-TECH Core Facility programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. *The authors marked with an asterisk equally contributed to the work.

## P-27-041
## 14-3-3 protein chimera with its full-length phosphorylated partner protein HSPB6: design, solution structure and dynamics

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14-3-3 proteins are the regulatory factors of many vital processes acting via protein-protein interactions (PPIs). Phosphorylation of partner proteins necessary for the 14-3-3 binding mostly occurs within their intrinsically disordered protein regions (IDPRs), which challenge structural studies of 14-3-3 complexes because of the high structural flexibility and, rather often, a transient character of the association. As the result, out of hundreds of known 14-3-3 partners, only a few 14-3-3 complexes were characterized structurally (e.g., with AANAT, HSPB6, Nth1, ExoS/T), limiting the utilization of the 14-3-3 PPIs for the discovery of new drugs against physiological conditions affected by 14-3-3 regulation. To avoid traditional mixing of 14-3-3 and their *in vitro* phosphorylated partners, often leading to non-natural stoichiometries and partial occupancies, the chimeric approach has recently been proposed based on fusion of the 14-3-3 core with a partner phosphopeptide or a longer fragment and bacterial co-expression of the resulting constructs in E. coli strains together with the specific protein kinase. Here, we developed this approach further and engineered a chimera of human 14-3-3 sigma connected to the almost full-length partner HSPB6 via a short linker allowing the correct placement of the crucial phosphoserine of HSPB6, pSer16, in the binding site on 14-3-3 and obtaining of an assembly with the fixed stoichiometry. The chimera could be readily purified to homogeneity in mg amounts for the characterization by analytical SEC and SAXS. Phosphorylation of Ser16 was confirmed by mass-spectrometry and the expected 2:2 stoichiometry of the assembly was confirmed by SEC-SAXS. Molecular modeling against the SAXS data indicated the dynamic character of the assembly, consistent with the presence of long IDPRs in the N-terminus of HSPB6. The approach may be helpful for other applications. Partially supported by RFBR (18-34-00428 mol_a).

## P-27-042
## Structural modeling of tobacco mosaic virus and its coat protein according small angle X-ray scattering data

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Tobacco mosaic virus (TMV) has been extensively studied in the past using crystallographic and electron microscopy approaches. In the present work structural characterization and modeling of TMV and virus-like particles (VLP) formed by the virus coat


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protein (CP) was performed using synchrotron small-angle X-ray scattering (SAXS). Scattering profiles from the samples in solution were collected at the P12 BioSAXS beamline at EMBL Hamburg. As a structural template for the virus modeling the EM structure (2OM3.PDB) containing a fragment with helical symmetry has been chosen. The reconstructed rod-like model reasonably fits the experimental SAXS patterns. TMV CP self-assembles in solution forming VLP. Structural modeling of these VLPs was performed using a fragment of crystallographic model of the four-layer aggregate (1EI7.PDB). The resulting VLP's model was represented by hollow cylinder yielding a good fit to the experimental SAXS data. Thus, for the first time, low resolution structures of TMV and virus-like particles were determined in solution, i.e. under conditions close to natural. However, solution structure of the individual TMV coat protein is still undefined due to its higher tendency to aggregation. One of the ways to solve the problem is to use dissociation by surfactants. In the present work highly stable partially unfolded intermediates (at low ionic strength and 42 °C) were investigated by SAXS and complementary spectral methods. It was found that these intermediates retain about half of the initial content of the secondary and tertiary structure. Further dissociation was obtained by incubation at 42 °C with 3.5 mM sodium dodecyl sulfate (SDS) without changes in tertiary structure, while incubation with 17.3 mM SDS resulted in a complete loss of the tertiary structure leading to a formation of the well-known "necklace and bead" organization of CP in solution. The work was supported by RFBR grants 18-04-00525 and 17-00-00487.

## P-27-043
## Structural studies on *Trichomonas vaginalis* virulent proteins cyclophilin 1 and cyclophilin 2 and its association with myb3 transcription factor

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Trichomoniasis, is the most common sexually transmitted protozoan disease in humans caused by *Trichomonas vaginalis* and its emergence of drug resistant has serious public health concern especially in HIV and HPV positive patients. It shown that in *T. vaginalis, Tv* Cyclophilin 1 (*Tv*Cyp1) regulates the nuclear translocation of Myb proteins (Myb1 and Myb3), which regulate the expression of malic enzyme for cytoadherence to human vaginal epithelial cells. Here we unravel the structural features of *T. vaginalis*, *Tv* Cyclophilin 2 (*Tv*CyP2) having extended N-terminus, which is similar with (*Tv*Cyp1) of our previous structural studies. The Crystal structure of *Tv*CyP2 shows a monomer conformation, where its extended N-terminus loop mimics as a cyclosporine substrate and localizes in the catalytic and auxiliary pocket of neighboring monomer. Interestingly, we notice that N-terminus residue Valine 9 accommodates in hydrophobic core of its active site, which is consistent with cyclosporine's binding mode. Our Analytical Ultra Centrifugation (AUC) and size exclusion chromatography results also support the monomeric nature of *Tv*Cyp2 in solution. With aid of Fluorescence Polarization and Isothermal Titration Calorimetry (ITC) studies, we also mapped the *Tv*CyP1 and *Tv*CyP2-binding sequence of Myb3 (Myb3 50–87) as the minimum binding motif in Myb3 for its activity. Furthermore, NMR titration experiments revealed that residues 50–87 of Myb3 binds to the active site of *Tv*Cyp1 and *Tv*CyP2. Since cytoadherence is one of the early steps in pathogenesis; we anticipate that *Tv*CyP1 and *Tv*Cyp2 might be a potent drug targets for designing a specific cyclosporine analogue inhibitors to prevent disease progression and pathogenesis.

## P-27-044
## Characteristic features of proteins involved in postsynaptic density

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The postsynaptic density is a disk-shaped electrodense entity beneath the postsynaptic membrane, responsible for modulating signal transduction and orchestrating a number of processes linked to learning and memory. Despite our growing knowledge on specific proteins and their interactions, atomic-level details of their full three-dimensional structure and their rearrangements are still largely elusive. In this work we investigated the structural characteristics of postsynaptic proteins using numerous bioinformatic tools. We also analyzed whether disorder plays a role in the organizing of PSD proteins and the dynamic rearrangement of PSD. Our approach revealed that the postsynaptic proteome has markedly different features compared to those of other proteins included in the human proteome. Our results also suggest that the intrinsically disordered segments might contribute to the versatility of the interactions between PSD proteins, a key factor allowing dynamic reorganization of the protein network.

## P-27-045
## Solving the structure of Dps protein by multiple-crystal macromolecular crystallography methods

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The increasing brightness of X-ray beamline for macromolecular crystallography has made it possible to obtained appropriative diffraction data for further procession by standard crystallographic software from very small crystals. But for obtaining bright enough diffraction spots we should deliver more radiation dose to the crystal, it leads to the critical radiation damage before we able to collect enough data to solve the crystal structure. But if a few degrees of oscillation data per crystal are available, diffraction images can be processed by standard crystallographic software, and when the resulting partial datasets were checked for high level of isomorphism, them could be merged to produce the final complete data set. In present work the process of diffraction data collection from E. coli bacteria Dps crystals 1–5 μm sized was described. The study of influence on final data set of various data collection parameters such as exposure time and diffraction wedge wideness per one crystal were carried out. There were also a comparison between three ways of monocrystal diffraction data isomorphism determining: Hierarchical analysis based on a calculation of correlation coefficient for each pair of monocrystal diffraction data implemented by ccCluster; The multiple-crystal diffraction data set R-factor minimization by genetic algorithm; The selection method of monocrystal diffraction data with low R-factor on low resolution shells calculated by XSCALE. Final diffraction data set consist of 256 monocrystal diffraction data, overall 450 monocrystals diffraction data were processed. The highest resolution of obtained structure is 2.2 A. Structure was deposited in PDB with code 6QVX. The research was carried out using supercomputers


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at Joint Supercomputer Center of the Russian Academy of Sciences. This work was supported within frameworks of the state task for ICP RAS 0082-2014-0001 (state registration #AAAA-A17-117040610310-6)

## P-27-046
## Atomic-level interaction model of the third PDZ domain of PSD-95, represented by structural ensembles

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Postsynaptic density protein 95 (PSD-95), the most abundant scaffold protein of the postsynaptic density of excitatory synapses is a fundamental component of signal transduction and synaptic plasticity. The third PDZ domain of PSD-95 has an additional helix aside from the conserved PDZ fold, which effects peptide binding, despite not being in direct contact with the binding site. Atomic-level details of this intermolecular allosteric mechanism, involving conformational entropy, are not yet elucidated in detail. Molecular dynamics simulations of bound and unbound, as well as full and truncated PDZ3 structures were performed incorporating NMR-derived backbone and side-chain mobility data, available in the literature, as restraints. The resulting structural ensembles correspond well to the experimental parameters. Detailed analysis of the obtained ensembles highlight the role of internal dynamics in ligand binding and provide hints at the nature of the motional changes underlying the measured entropy-change differences between the full and truncated forms upon ligand binding.

## P-27-047
## Dimerization of transmembrane domain of insulin receptor: structure and possible role in activation

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Insulin receptor (IR) family is represented by three membrane proteins participating in organism development, growth, and vital activity. Modulation of the functioning of these receptors by external agents looks very perspective from a pharmaceutical point of view. Although IR is well studied, little is known about the role of its transmembrane (TM) domain in receptor activity Nowadays, the major model of signal transfer by these receptors describes ligand-triggered conformational changes in the extracellular domain, bringing together TM domains that dimerize. This allows trans-autophosphorylation of intracellular domains followed by activation of secondary messengers. However, the conformation of the TM dimeric state is still unknown. Here, we studied in silico dimerization of TM segments of two closest members of the family: IR and IGF-1R. As a result, TM dimeric structures were predicted. This was done taking into account available structural data on extra- and intracellular parts of the receptors. Inspection of the extracellular segment mobility in the basal state revealed several modes of protein motion, although none of them allow TM domain dimerization. The calculated molecular dynamics of TM helices linked to intracellular domain led to a conclusion about autonomic behavior of the TM domain. Based on this data, the dimerization of TM domains was further simulated without extramembrane parts. The most probable models of TM dimeric structures were predicted and the free energy of helix-helix association in explicit lipid bilayers was evaluated. Two most energetically favorable models for IR and one for IGF-1R were delineated. Despite the lack of sequence homology, TM segments in both receptors pack in similar parallel dimers, thus suggesting a close activation mechanism. Acknowledgments: Russian Science Foundation (18-14-00375), Russian Foundation for Basic Research (18-54-15007).

## P-27-048
## A new approach to characterize apicoplast gyrases

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DNA gyrase is a bacterial enzyme with the unique ability to negatively supercoil DNA, which plays an important role during DNA replication process. Gyrase is a heterotetrameric enzyme with a $GyrA_2B_2$ subunit architecture. Besides bacteria, gyrase can also be found in the Apicomplexa phylum, including single-cell eukaryotic parasites such as *Plasmodium falciparum* (Pf, a causal agent of malaria) or *Toxoplasma gondii* (causal agent of toxoplasmosis). Due to its absence in humans and its complex reaction mechanism, gyrase is an attractive antibacterial drug target and may have potential for treating malaria or toxoplasmosis. In this study, we present a functional investigation of Apicomplexan gyrases (ApGyr). Ap gyrases are larger in size and differ in domain architecture from their well-studied bacterial counterparts, giving rise to divergent activities. Because of difficulties with the production of the full-length PfGyrA subunit we have developed an alternative approach. We have produced individual domains of both *E. coli* GyrA and PfGyrB subunits separately and reconstituted them as functional enzymes. Using this method, we were able to reveal properties of the holoenzyme, providing new information about its activity. Additionally, we tested a small molecule chosen on the basis of its ability bind PfGyrB in *in vivo* and *in vitro* assays. The compound altered DNA supercoiling efficiency, as well as DNA binding properties of the hybrid gyrase but not *E. coli* gyrase. These results are a first step towards unravelling the structure and mechanism of action of the ApGyr and their unique features may allow them to de developed as useful therapeutic targets.

## P-27-049
## Impact of cosolutes on an intrinsically disordered protein results in large enthalpy-entropy compensation effects

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Intrinsically disordered proteins (IDPs) are natively unstructured, functional proteins that are central to many signaling and regulation pathways. A common feature among IDPs is a disorder-to-


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order transition upon binding to their physiological interaction partner. Intrinsic disorder has been tied to multiple benefits, including accelerated association rates, or binding with low affinity, yet high specificity. Changes in the chemical environment, such as the presence of osmolytes or crowding agents, can have vast influences on IDPs, since their interactions usually involve a folding and a binding event, which are both heavily solvent dependent. Here, we demonstrate the impact of cosolutes on the interaction of the intrinsically disordered transcription factor c-Myb and its binding partner, the kinase-inducible interaction domain (KIX) of the CREB-binding protein. We measured temperature dependent kinetics, using temperature jump perturbation relaxation and binding affinities, using MicroScale Thermophoresis. While binding free energies are only marginally modulated by cosolutes, enthalpy and entropy of the interaction are very sensitive to the respective solvent conditions. For different cosolutes we observe substantial changes in enthalpy, both favorable and unfavorable, which are largely compensated by counter-acting entropic changes. This might be a potential mechanism by which c-Myb offsets changes in the chemical environment to maintain a roughly consistent binding affinity to KIX. We hypothesize this enthalpy-entropy compensation to be an additional benefit of IDPs to cope with changing cellular conditions and that it could have the physiological implication to sustain crucial cellular functions, such as transcription.

## P-27-050
## Structural characterization of the N-terminal part of apoptosis signal-regulating kinase 1 (ASK1)

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Apoptosis signal-regulating kinase 1 (ASK1) is an apical kinase of the mitogen-activated protein kinase cascade. Its activity is triggered by various stress stimuli such as reactive oxygen species (ROS), cytokines, endoplasmic reticulum (ER) stress or osmotic stress resulting in the activation of p38 and c-Jun N-terminal kinase metabolic pathways and leading to inflammation or cell death. Dysregulation of ASK1 is linked to several pathologies such as neurodegenerative and cardiovascular diseases and cancer, which makes this protein a potential target of therapeutic intervention. The activity of ASK1 is regulated through protein-protein interactions with 14-3-3 proteins and thioredoxin1 being among the most important negative regulators and tumor necrosis factor receptor-associated factors being an example of the positive regulator. Apart from that, ASK1 is also tightly regulated via oligomerization. Despite continual progress being made, the precise molecular mechanism of ASK1 regulation and the role of ASK1 oligomerization in this process still remain unclear to this day owing to the lack of structural data. In this study, we performed a biophysical and low-resolution structural analysis of ASK1 construct consisting of its central regulatory region (CRR) and catalytic domain (CD). Analytical ultracentrifugation analysis indicated a dimer formation in a concentration-dependent manner, which was subsequently confirmed by small angle X-ray scattering (SAXS). CORAL software was applied to build models of ASK1 monomers and dimers based on experimental scattering curves. Chemical crosslinking coupled to mass spectrometry was used as a complementary method to obtain distance restraints for further refinement of SAXS-based models. The data we obtained from various biophysical methods suggest that not only the isolated catalytic domain, but also ASK1-CRR-CD construct can form dimers in solution. This work was supported by the Czech Science Foundation (project 19-00121S).

## P-27-051
## Binding of double-positively charged substrates by *Serratia proteamaculans* oligopeptidase B studied by means of molecular dynamics

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Oligopeptidase B (OpB) is a two-domain, trypsin-like serine endopeptidase belonging to the prolyl-oligopeptidase (POP) family. OpBs have been shown to be pathogenic factors of leishmaniosis, trypanosomiasis, and other parasitic, and, presumably, bacterial diseases, and may be considered as candidate targets for anti-infective drugs development. Structural studies of OpBs and closely related POPs demonstrate the inherent flexibility of these molecules and importance of the molecular dynamics (MD) in investigation of catalytical activation of the enzymes. OpBs, including the enzyme from *S. proteamaculans* (PSP), which is model-object of this study, are able to hydrolyze substrates with a pair of sequential basic residues more efficiently than those containing single K or R residue, though the mechanism of secondary substrate specificity is still undiscovered. We aimed this work to the search of the structural factors determining secondary substrate specificity of PSP. For this purpose, we have created 3D models of wild type wtPSP in complexes with GRRG and GKRG peptide-substrates, carrying either R or K residue in the P2 position and R residue in the P1 position. Then we subjected these models to MD simulations and showed different behavior of these peptides in the substrate-binding pocket located in the interface between β-propeller and catalytical domains. We discovered that contacts of the P1 residue with the S1 substrate-binding center are mainly fixed, while contacts of the P2 residue may significantly vary depending on its nature and determine the orientation of the peptide in the substrate-binding pocket. The result of MD simulation provided explanation for our previous observation on the shift of the secondary substrate specificity of PSP-D649A mutant with preference to KR-type substrate compared to wtPSP with preference to RR-type substrate (Mikhailova, et al. Biochimie 2017). The Russian Science Foundation (#17-14-01256) supported this work.

## P-27-052
## NIQI approach – Novel Interesting Quadruplex Interaction Motif used for bioinformatics prediction of quadruplex binding proteins

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Quadruplexes are secondary DNA structures which can arise in guanine rich DNA sequences under certain conditions (positively charged ions, molecular crowding, protein binding and stabilization) *in vitro* and, also *in vivo*. They play important regulatory roles in many types of molecular processes including replication,


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transcription, translation, genome integrity maintenance, as well as many pathogenic processes like cancer or neurodegeneration. Recently, we have found that all to date known quadruplex binding proteins (QBPs) share common significant 20 amino acid long sequence motif (NIQI), which can be used for prediction of new potential QBPs. In this work we have analyzed complete set of human nucleic acid binding proteins for NIQI motif occurrence. Overall, we have predicted one hundred potential QBPs of median length 478 amino acid residues (min. 119 for Small nuclear ribonucleoprotein D1 polypeptide, max. 4911 for Lysine methyltransferase 2C) with highly significant NIQI motif ($q < 0.0001$) according to analysis in Find Individual Motif Occurrences (FIMO) tool. Moreover, we have done enrichment analyses and found that there is a high overrepresentation of some protein categories, mainly zinc-finger proteins, ribonucleoproteins and proteins containing homeodomains. Zinc-finger and homeodomain containing proteins bind DNA in sequence-specific manner, so the fact that some of them also contain NIQI is very interesting, whether from biological point of view or possible therapeutic applications. We believe that this work will help to discovery and subsequent characterization of new QBPs and speed-up research in this field remarkably. The discovery of the new QBPs and its characterization could lead to discovery of novel therapeutic targets. *The authors marked with an asterisk equally contributed to the work.

## P-27-053
## Identification of amyloidogenic regions in S1 ribosomal proteins from *Thermus thermophilus* and *Mycoplasma mobile*

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S1 is the largest protein component of bacterial ribosomes. *Thermus thermophilus* protein S1 includes five copies of the RNA-binding motif (so-called S1 motif) including about 70 amino acid residues separated by loops of 10 to 20 residues. Unlike this, *Mycoplasma mobile* protein S1 has a length of 111 residues and contains a single S1 motif. The aim of this research was to study the process of amyloidogenesis S1 proteins from *Thermus thermophilus* and *Mycoplasma mobile*. We have used bioinformatics programs (FoldAmyloid, ArchCandy, PASTA2.0, Waltz, and AGGRESCAN) and determined the percent amyloidogenicity of S1 proteins from the studied organisms. Amyloidogenic regions predicted by the bioinformatics tools were compared with the results of limited proteolysis followed by high-performance liquid chromatography and tandem mass spectrometry. Proteins were isolated and purified by dialysis under different buffer conditions. The sizes of protein particles and their associates were determined, using the method of measuring the dynamic light scattering, and correlograms of aggregate accumulation with increasing temperature were received. Electron microscopic images of preparations of S1 protein aggregates were obtained for each organism. The formation of protein aggregates was characterized using the spectra and kinetics of thioflavin T binding. Thus, we showed that S1 proteins from *Thermus thermophilus* and *Mycoplasma mobile* are able to form polymorphic aggregates. We have suggested that the S1 motif may not only be RNA-binding motif, but a domain involved in protein aggregation. This study was supported by the Russian Science Foundation (project no. 18-14-00321).

## P-27-054
## Optimization program of a novel inhibitor of the Cdc25B phosphatase activity: kinetic and structural studies of the inhibition mechanism and cytotoxic effects in melanoma cells

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Protein phosphorylation/dephosphorylation regulates crucial cellular processes, such as cell growth, differentiation, migration, survival, and apoptosis. In particular, cell division cycle 25 (Cdc25) enzymes are dual phosphatases, playing a pivotal role in the regulation of cell cycle progression. Because of its altered expression in different tumors, Cdc25 could be considered as a promising target for cancer therapy. In a previous work from my research group it has been demonstrated that the compound NSC28620 acts as a reversible inhibitor of Cdc25 ($K_i$ 5.3 μM) and affects the cell viability of some cancer cell lines at 200 μM. In order to identify more potent inhibitors of Cdc25, a lead optimization program on NSC28620 was undertaken and a group of thirty-one derivatives was considered. The properties exhibited by the novel molecules were investigated through kinetic measurements of the phosphatase activity sustained by the recombinant form of the catalytic domain of Cdc25B in the absence or in the presence of these inhibitors. Interestingly, some derivatives are endowed with a stronger inhibition power compared to the lead NSC28620 and the most active one (cpd 7j) had a $K_i$ value of 0.8 μM. The inhibition mechanism of the new compounds was evaluated through an inspection of the corresponding Lineweaver-Burk plots; this analysis suggested two main different types of reversible inhibition, *i.e.* non-competitive and un-competitive. Thanks to the presence of a unique tryptophan residue in Cdc25B, intrinsic fluorescence studies allowed an investigation on the protein region involved in the interaction with the various inhibitors. Finally, the cytotoxic effects of the most active compounds were evaluated in melanoma cell lines through MTT assay; at a low concentration, *i.e.* 2.5 μM, only one derivative (cpd 4a) significantly reduced the cell growth of melanoma cells in a time-dependent manner.

## P-27-055
## Structural basis for antigenic peptide precursor processing by ER aminopeptidase 1

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ER aminopeptidase 1 (ERAP1) in an intracellular enzyme that optimizes the peptide cargo of Major Histocompatibility Class I molecules (MHC-I) and regulates adaptive immune responses


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thus being an emerging target for immunotherapy applications. It has unusual substrate selectivity, preferring longer peptides to shorter ones and its trimming rates are influenced by sequence, although no clear preference motifs have yet to be identified, hindering efforts to predict the enzyme's complex effects on the cellular immunopeptidome. To help understand the mechanism of substrate selection by ERAP1 we set out to crystallize the enzyme in complex with substrate analogues. We used x-ray crystallography to solve two structures of ERAP1 at 1.62 A and 1.68 A with bound two substrate analogues, one 15mer and one 10mer, designed based on ERAP1-sensitive antigenic peptide precursors. The N-terminus of both peptides is found bound in the catalytic site resembling the transition-state intermediate formed during catalysis. Both peptides extend away from the catalytic site, along the internal cavity of the enzyme, making a series of atomic interactions that can influence selectivity. While both peptides extend along the base of domain II towards the domain II/domain IV junction, the 15mer diverges through the central region of the cavity and has its C-terminus stabilized in a pocket of domain IV by Tyr684, Lys685 and Arg807 in a manner reminiscent of carboxypeptidase recognition, while its middle portion is disordered. Analysis of the crystal structures suggests that the mechanism that underlies the unique specificity of ERAP1 revolves around sequence-dependent opportunistic binding in combination with specific C-terminal recognition for longer peptides. Our results provide a framework for understanding how ERAP1 influences the cellular immunopeptidome and adaptive immunity.

## P-27-056
## How folding and binding intertwine during protein complex formation provides an additional layer of functional regulation

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Intrinsically Disordered Proteins (IDPs) mediate highly diversified and crucial functions in living cells. While lacking a stable structure, a remarkable proportion of IDPs are capable to fold via interactions with (most commonly protein) partners. Assuming the classic binary classification of proteins, each partner in an interaction can be either ordered or disordered. Thus, there are three possible scenarios of the interplay between binding and folding: autonomous folding and independent binding (where all interacting partners are ordered), coupled folding and binding (where an IDP binds ordered partners) and mutual synergistic folding (involving exclusively IDPs). Recent advances in database development enabled us to identify a large amount of bound structures from all three classes, opening ways to assess the nature of these interactions through the sequence-structure-function paradigm. Since folding and binding share a similar biophysical background, these interactions can be described by the same approach, showing how the formation of structure from proteins with different structural states is mirrored at different levels (sequence, structure, function and regulation). High-level cellular processes not only utilize an interconnected network of all three basic interaction modes, but in extreme cases the same disordered region can be involved in different interaction modes. This creates competing interactions, comprising a switching mechanism that chooses between radically different outputs of the same regulatory subnetwork.

## P-27-057
## Structural and functional analysis of *Candida glabrata* Rpn4-like protein

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*Candida glabrata* causes 15% of all candidiasis cases. In difference of other pathogenic fungi *C. glabrata* is opportunistic pathogen and closely related to baker's yeast Saccharomyces cerevisiae. One of its unique features is the marked tolerance to oxidative stress and azole antifungals. In baker's yeast Rpn4 transcriptional factor provides tolerance to different kinds of stress including heat shock, DNA damage and oxidative stress. *RPN4* gene deletion leads to sensitivity to fluconazole and amphotericin antifungals. *C. glabrata* has the ortholog of ScRpn4, coding by *CAGL0K01727 g* gene. It's amino acid sequence close to ScRpn4 on 48% but the only similar domain is its DNA-binding region. We cloned this gene and characterized its activity in *S. cerevisiae* background. We have shown that CgRpn4 restores wild phenotype in rpn4-d genetic background in multiple stress conditions. Also we dissected its N-terminal region and found out that similar to ScRpn4 it contains transactivational domain. Unique short N-terminal sequence enriched in glutamine and aspartate residues also involved in its ability to activate proteasomal genes. In presence of oxidative agent 4-NQO CgRpn4 activates directly or indirectly genes involved in oxidative stress response including YAP1 and thioredoxin. In *C. glabrata* cells CgRpn4 expression is induced by heat, methane methylsulfonate and 4-NQO. Potential targets of CgRpn4 which include genes coding proteasome subunits and YAP1 are also induced in these conditions. We propose that in *C. glabrata* cells CgRpn4 is involved in stress response and possibly participates in its pathogenic properties. This work was supported by Russian foundation of basic research grant No. 18-34-00704.

## P-27-058
## Biochemical and biophysical properties of the human nucleoporin aggregates

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Amyloids are unbranched protein fibrils with a characteristic spatial structure and unusual features including high resistance to detergent or protease treatment. Numerous investigations of amyloids are in the top of interest due to increasing incidence of amyloid-associated disorders, for instance Alzheimer's disease, Parkinson's disease, type II diabetes etc. Previously we analyzed a set of human proteins, which physically interact with huntingtin protein (the expansion of repeats in the corresponding gene is linked with Huntington's disease development) according to BioGRID database, to the ability to form amyloid aggregates, and found one perspective candidate nucleoporin Nupl1 (Nup53). In this work we have shown that the protein forms amyloid-like aggregates in yeast and bacteria cells. For further investigation of amyloid properties of Nupl1 we obtained aggregates of the purified protein *in vitro* and investigated their properties. This analysis revealed that Nupl1 can form fibrils, which are resistant to proteases and detergents, and stained with amyloid specific dyes (Congo Red and Thioflavin T). Taking together these results allow us to suppose that Nupl1 is a new human


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amyloid. The research was supported by the Russian Science Foundation (17-74-10159) and research resource center "Molecular and Cell Technologies" of Saint-Petersburg State University.

## P-27-059
## Amyloidogenic interactions in regulation of autophagy

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Golgi-Associated plant Pathogenesis Related-protein 1 (GAPR-1) functions as a negative regulator of autophagy. The molecular mechanism of this regulation involves retention of Beclin 1, a major autophagy-related protein, at the Golgi. A Beclin 1-derived peptide that corresponds to the potential binding interface efficiently induces autophagy by competing with GAPR-1/Beclin 1 interaction. However, so far a direct interaction between GAPR-1 and Beclin 1 could not be observed. Therefore we hypothesized that GAPR-1/Beclin 1 interactions are based on oligomeric and/or amyloidogenic properties of both proteins. In this study, humanized yeast model system is used to study the amyloidogenic propensity of GAPR-1 and Beclin 1 and to investigate GAPR-1/Beclin 1 interactions. Protein segregation into Fluorescent Foci (FF) in the yeast cytosol has been shown to correlate with the propensity of a protein to form amyloid. Overexpression of GAPR-1 and Beclin 1 resulted in formation of FF in cytosol over time. Interestingly, in co-expression experiments the formation of Beclin 1 FF was inhibited and the number of GAPR-1 FF per cell were reduced, suggesting that the two proteins interact. These effects were efficiently reversed when mutant GAPR-1 or Beclin 1 lacking the suggested binding sites were used. Direct interaction between both proteins was confirmed by bimolecular fluorescence complementation analysis and by Beclin 1 re-localization to the GAPR-1 positive structures using plasma membrane-targeted GAPR-1. Finally, in a proof-of-principle experiment we show that Beclin 1 peptide can efficiently reverse the formation of FF in co-expression experiments, suggesting that GAPR-1/Beclin 1 interaction was interfered by the peptide. Together our results suggest that amyloidogenic interactions are involved in regulation of autophagy by regulating the interaction between GAPR-1 and Beclin 1.

## P-27-060
## Biophysical study of interaction between the scaffolding protein 14-3-3 and phosphorylated CaMKK2

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Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a member of the Ca2+/calmodulin-dependent kinase family involved in adiposity regulation, glucose homeostasis and cancer. CaMKK2 is an upstream activator of CaMKI, CaMKIV and the AMP-activated protein kinase (AMPK), and the AMPK:CaMKK2 complex has been shown to regulate energy balance by acting in the hypothalamus. The CaMKK2 is regulated through phosphorylation by various kinases including the cAMP-dependent protein kinase (PKA) and AMPK. It has been suggested that the phosphorylation of residues S100 and S511 by PKA creates two 14-3-3 binding motifs, however the role of 14-3-3 protein in the regulation of CaMKK2 is unclear. The goal of this study was the preparation of CaMKK2 stoichiometrically phosphorylated at S100 and S511 and the biophysical characterization of its complex 14-3-3 using analytical ultracentrifugation and small angle X-ray scattering (SAXS). Recombinant CaMKK2 was prepared as kinase dead mutant containing only two PKA phosphorylation sites Ser100 and Ser511 (CaMKK2 D330A, T145A, S495A). CaMKK2 was expressed in E. coli cells and purified using nickel chelating chromatography and size exclusion chromatography. Phosphorylation of CaMKK2 at S100 S511 by PKA was optimized to achieve stoichiometric phosphorylation at both sites and the result of phosphorylation reaction was monitored using phos-tag SDS-PAGE and mass spectrometry. Next, the interaction between phosphorylated CaMKK2 and 14-3-3 protein was investigated using sedimentation velocity analytical ultracentrifugation. These measurements revealed 1:2 molar stoichiometry between CaMKK2 and 14-3-3 and the binding affinity in low micromolar range. Data obtained from SAXS measurements were used for an ab initio reconstruction of the CaMKK2:14-3-3 complex. This work was supported by the Czech Science Foundation (project 19-00121S).

## P-27-061
## Investigation of DNA-binding properties of the UxuR and ExuR proteins, regulators of hexuronate metabolism in gammaproteobacteria

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Besides glycolysis that converts glucose into two molecules of pyruvate relieving free energy in the form of ATP and NADH, gammaproteobacteria can get energy from several alternative metabolic pathways induced in stress conditions. For example, *Escherichia coli* (E. coli) can use the hexuronates D-glucuronate and D-fructuronate as the sole carbon sources. These sugars are metabolized by the Ashwell pathway, which generates intermediates that are converted to pyruvate via the Entner–Doudoroff pathway. The hexuronate metabolism in *E. coli* is regulated by two related transcription factors from the GntR family, UxuR and ExuR, which have 46% identity. Using various genomic approaches the binding sites of ExuR and UxuR proteins on *E. coli* chromosomal DNA have been determined and a number of targets for the proteins, including autoregulation sites, have been identified. Bioinformatics analysis of the obtained results allowed to determine DNA consensuses which are specifically recognized by ExuR and UxuR. To measure the binding constants of selected oligonucleotides to the proteins the surface plasmon resonance has been chosen. The affinity of the UxuR protein for DNA fragments containing the region that is supposed to be specifically recognized by the UxuR protein is in the nanomolar range. The ExuR protein forms a less stable complex with a DNA fragment containing the putative region specifically recognized by the ExuR protein. A DNA fragment containing a nucleotide sequence that can be recognized by both proteins binds to proteins with different affinities. These results allow us to obtain stable DNA-protein complexes suitable for crystallization. This work was supported by Russian Scientific Foundation 18-14-00322.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-27-062
## Biochemical and structural characterization of an unusual cyanobacterial S-adenosyl-L-homocysteine hydrolase from *Synechocystis* sp. PCC 6803

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The mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 encodes in its genome *S*-adenosyl-L-homocysteine hydrolase (SAHase) of extremophilic archaeal origin. SAHases are essential enzymes involved in the regulation of cellular SAM-dependent methylation reactions, usually active as homotetramers or less commonly as homodimers. A SAHase subunit is comprised of two major domains, the cofactor ($NAD^+$) binding domain and substrate (SAH) binding domain, connected by a hinge element that is also a coordination site of an alkali metal cation, which influences the domain movement during the catalytic cycle. Typically, the highest activity and strongest ligand binding is observed in the presence of $K^+$ ions. The SAHase from *Synechocystis* (SynSAHase) is an exception in this respect. During size exclusion chromatography, applied as the last step of purification of recombinant SynSAHase, the protein was eluted in two distinct oligomeric states, corresponding to its dimeric and tetrameric forms. Small angle X-ray scattering clearly indicated that the oligomeric state of SynSAHase strongly depends on a protein concentration. In solution, the enzyme exists as an equilibrium of dimers and tetramers, shifted in the direction of tetramer formation at higher concentrations. Enzymatic and ITC studies demonstrated that in contrast to $K^+$-dependent SAHases, the activity and ligand binding of SynSAHase is not affected by the presence of any particular alkali ion. To explain this phenomenon, several crystal structures of SynSAHase were determined for the enzyme crystallized in the presence of various alkali cations. The structural data confirm that, while SynSAHase shares common structural features with other SAHases, there is no alkali metal coordinated by the cyanobacterial enzyme as a result of different spatial organization of the cation binding hinge region.

## P-27-063
## *In vitro* interaction of the postsynaptic GKAP and Shank-1 proteins

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Guanylate-kinase-associated-protein (GKAP) is a prominent member of scaffolding proteins of the postsynaptic density (PSD) in neural synapses. This largely intrinsically disordered protein can interact with several components of the PSD, including the PDZ domain of Shank proteins associated with neural disorders, such as mental retardation and autism. Although several interactions of GKAP have been described, many structural details remain unexplored. The PDZ domain of Shank can bind the C-terminus of GKAP, providing a link between PSD95 and Homer proteins. For this reason, the Shank-GKAP interaction can be a key regulator of PSD structure and function. To investigate this interaction in more detail, we designed and produced the C-terminus of GKAP, its globular GH1 domain followed by the PDZ binding segment, as well as the PDZ domain of Shank-1. Recombinant, 6xHis tagged proteins were expressed in BL21(DE3) cells, purified by immobilized metal affinity chromatography (IMAC), followed by ion exchange chromatography (IEC) and size exclusion chromatography (SEC). Purified protein samples were tested by PAGE and mass spectrometry prior to performing pilot NMR experiments. Interaction of the proteins was investigated by native PAGE, SEC and pull-down assays. Good quality NOESY and HSQC spectra suggested that both constructs have compact, well-folded globular structure. Preliminary results of pull-down assays and native PAGE suggested that our recombinant constructs are able to interact with each other *in vitro*. Detailed structural characterization of the proteins and their complex is under way.

## P-27-064
## Crystal structure of *Borrelia burgdorferi* outer surface protein BBK01 – a member of the paralogous gene family 12

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*Borrelia burgdorferi* as the causative agent of Lyme disease is transmitted to the mammalian host during the tick's blood meal. *Borrelia* is able to adjust the expression pattern of various outer surface proteins in order to adapt to the different encountered environments. *B. burgdorferi* outer surface proteins that are up-regulated once the tick starts the blood meal are believed to play some role in the pathogenesis of Lyme disease. We have determined the crystal structure of a previously uncharacterized *B. burgdorferi* outer surface protein BBK01 which belongs to the paralogous gene family 12 (PFam12). PFam12 contains in total 5 members which share 30 to 70% sequence identity and are up-regulated as the tick starts the blood meal, although the function for the PFam12 members is unknown. Crystal structure of BBK01 reveals a similar fold to the coiled coil domain of a structural maintenance of chromosomes (SMC) protein family members as found by DALI. Further research of the PFam12 member proteins is in progress to reveal the common function for all the PFam12 members. Structural and functional studies of *B. burgdorferi* up-regulated outer surface proteins provide an important contribution to the understanding of the pathogenesis of Lyme disease and potentially can be used to develop new strategies to fight against the disease. This work was supported by the ERDF grant Nr. 1.1.1.2/VIAA/1/16/144 "Structural and functional studies of Lyme disease agent Borrelia burgdorferi outer surface proteins to reveal the mechanisms of pathogenesis with the intention to create a new vaccine".

## P-27-065
## Residue-residue contacts in modeling protein structure

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Experimental determination of a molecular structure is difficult and not always feasible. Applying residue-residue contacts, predicted from protein sequences, greatly enhances modeling of the structure. Direct Contact Analysis (DCA) methods, using correlated mutations, stand out from other methods, however their efficiency is still insufficient. We enhanced their predictive power using machine learning and structural filters. Importantly, we


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designed a method to foresee modeling accuracy for each individual protein of interest, with an average error of 7 percentage points, replacing previously available mean accuracy over a numerous set of different proteins with experimentally solved structures. The studies show that for modelling unknown protein structure 30% of correctly predicted contacts can be sufficient for acceptable model quality. Nevertheless, methods using residue-residue contact maps need methods to differentiate between models of native and mirror orientation. Ramachandran plots of the structures are not suitable for big data sets and difficult for proteins rich in beta-sheets. Total energies of structures are often not helpful. We analyzed structural protein models obtained from contact maps of 1 305 SCOP domains from 7 structural classes and proposed an automated method for differentiating model orientations, independent of their secondary structures. The best algorithm used k-means clustering with three common energy terms: probability of amino acid assuming certain values of dihedral angles, Ramachandran preferences and Coulomb interactions. The accuracies were in the range between 0.68 and 0.76, with sensitivity and selectivity in the range between 0.68 and 0.87, depending on the structural class. Modeling methods based on contact maps can be applied to all fully-automated tools for protein structure reconstruction, especially those analyzing big sets of models.

## P-27-066
## Investigation of disease-causing germline mutations in postsynaptic density

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The postsynaptic density (PSD) is a complex and dynamic network of interacting proteins involved in synaptic transmission. Polymorphisms in some of the proteins of the PSD are associated with neuronal diseases such as Autism Spectrum Disorder, schizophrenia or Parkinson disease. The rapidly growing data on human genomic variations enables large scale studies of the distribution of variations in PSD proteins. Disease-causing germline mutations (DCMs) represent a special class with relatively weak phenotypes. In this study we investigated the effect of single nucleotide variations on protein structures from the PSD. Our approach reveals how DCMs might contribute to functional changes, in ordered and intrinsically disordered regions. Our results also suggest that proteins in PSD are much more exposed to single nucleotide variations compared to those of other proteins in the human proteome. Associating DCMs with protein structure and disease groups may provide a better understanding of the underlying mechanisms that govern disease emergence.

## P-27-067
## Amyloidogenic properties of human protein NOS1AP

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The gene *NOS1AP* encodes a cytosolic protein that binds to the signaling cascade component, neuronal nitric oxide synthase (nNOS). Moreover, the *NOS1AP* gene is linked with disorders from schizophrenia, post-traumatic stress disorder, and autism to cardiovascular disorders and breast cancer. NOS1AP/CAPON protein mediates signaling between complex of NMDA receptor, PSD95 and nNOS. This adapter protein is involved in neuronal nitric-oxide (NO) synthesis regulation via its association with nNOS/NOS1. It mediates the indirect interaction between NOS1 and RASD1, leading to an increase NOS1 ability to activate RASD1. Resulting from our bioinformatics analysis, NOS1AP protein was predicted as one of the components of the amyloid network surrounding APP (amyloid-beta precursor protein). Extracellular amyloid aggregates of NOS1AP in the system C-DAG (curli-dependent amyloid generator) were detected by red colony color on the medium supplied with the amyloid binding dye Congo Red and "apple-green" birefringence in polarized light. Fluorescent microscopy revealed the aggregation of EGFP-NOS1AP in yeast *Saccharomyces cerevisiae*. SDD-AGE proved out these aggregates were resistant to the detergent (SDS) treatment. Mammalian cell line HEK293 transiently expressed the same construct also represented the local aggregation of the investigated protein. Thus, NOS1AP protein shows amyloid properties, that is of great interest considering the fact it can interact with APP, so there is a chance the molecular mechanisms of neurodegenerative diseases can be more complex. The authors acknowledge the Resource Center "Centre for Molecular and Cell Technologies" of the Research Park of St Petersburg State University. The research was supported by the Russian Science Foundation (17-74-10159).

## P-27-068
## Protein–lipid interactions in glycophorin-like dimerization motifs of transmembrane helices

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Receptor tyrosine kinases (RTK) are vital players in cell signaling governing growth and proliferation. These integral membrane proteins work only in dimeric states, so the conformation of transmembrane dimer determines the signal transferred into cell. Here, we used modern molecular modeling techniques to study details of protein-protein and protein-lipid interactions in model systems containing monomers and dimers of several receptor tyrosine kinases with glycophorin-like dimerization motifs. Comparison of structural and dynamic aspects of ErbB family members and glycophorin A (GpA) revealed similarities in their properties, especially, for ErbB1, ErbB2 and ErbB4 receptors utilizing the same GpA-like motif for dimerization in their basal state. We demonstrated that they all have similar organization of TM domain's molecular surface in terms of both relief, hydrophobic properties and lipid binding sites resembling GpA pattern studied before. All these RTKs strongly interact with lipid acyl chains, forming stable binding sites both in monomeric and dimeric states, and the most prominent binding areas are located in monomers on the future GpA-like dimerization interfaces. Then, lipids distribution changes upon dimer formation. This is not the case for alternative packing geometries observed for the second state of ErbB1 and, especially, ErbB3. We found higher numbers of immobilized lipids near C-terminus in ErbB1 and ErbB2 active dimers, thus assuming that the existing structure of ErbB3 is also active. However, there is non-functional GpA-like motif in ErbB3 with some bound lipids present near the N-terminus, suspecting


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another structure for inactive receptor. However, despite considerable similarities, these RTKs have different hydrophobicity distributions along helices, that can be important in terms of preferable lipid environment. The work was funded by the Russian Academic Excellence Project '5-100' and Russian Foundation for Basic Research grant 18-54-15007.

## P-27-069
## Nisin/lipid II interaction in bacterial membrane: molecular dynamics study

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The worldwide rapid emergence of resistant bacteria put at threat the efficacy of antibiotics, thus the development of novel antibacterial agents is urgently needed. The cell wall precursor lipid II consisting the chemically conservative pyrophosphate group represents a promising pharmaceutical target. Antimicrobial peptides, that target lipid II, i.e. lantibiotic nisin, could be excellent prototypes for new generation antibiotics due to their low liability to develop resistance. Understanding of molecular mechanism of initial stages of membrane-bound lipid II recognition by water-soluble nisin is indispensable, in order to improve the peptide structure and properties into pharmaceutically applicable form. Here, we present a molecular dynamics simulation study of initial stages of the aforementioned recognition. In membrane environment, lipid II adopts very few conformations characterized by unique spatial arrangement of hydrogen bond acceptors in the pyrophosphate group at the bilayer surface. These acceptors are efficiently captured by NH groups of nisin, thus explaining its high selectivity to lipid II. Similarly, rings A and B of nisin, which are known to recognize lipid II, adopt the only stable conformation in the presence of dimethylpyrophosphate, which mimics the binding determinant of lipid II. Finally, we propose molecular model of nisin (rings A and B) / lipid II complex in bacterial membrane, which may be employed for design of novel antibiotic prototypes. Acknowledgements: "Molecular and Cellular Biology" RAS Programme, Russian Foundation for Basic Research (19-04-00350).

## P-27-070
## Structural and biochemical studies of an odorant binding protein from the malaria vector *Anopheles gambiae*

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*Anopheles gambiae* is the primary mosquito vector responsible for the transmission of malaria, *causing* more than 1 million *deaths* each year. Mosquitoes rely on *olfaction* to find mates, food, and sources of blood meals. Odorant binding proteins (OBPs) that mediate the initial steps in the transduction cascade of olfactory signals in insects have been suggested to play an essential role in the detection and transportation of semiochemicals to odorant receptors (ORs), and thus, they constitute promising targets for the design of novel repellent/attractant molecules. Therefore, a detailed knowledge of their 3D structures and functionalities may provide a valuable tool for the structure-based discovery of novel olfactory disruptors of insect host-seeking behavior to be used in more effective mosquito control strategies. Herein, we present the novel 3D crystal structure of AgamOBP, an OBP that displays the highest levels of expression in the female antenna. These levels also appear to be affected by the circadian cycle as they dramatically reduced in constant dark (DD) conditions compared to light-dark (LD) circles. Furthermore, co-crystallization and *fluorescence displacement experiments* revealed the ligand-binding site of AgamOBP as well as the binding modes and specificities of various natural volatile compounds with repellent properties. This information will contribute to the better understanding of the molecular basis of odorant perception. In addition, it will guide the generation of OBP-structure-based "olfactophore" models to be used in extensive virtual screening processes for the identification of novel candidate disruptors of host-seeking mosquito behavior. Such compounds, with enhanced binding affinity and specificity for a key OBP can be used at lower concentrations and be detected over longer distances, thus providing new effective repellents for the prevention of mosquito-borne diseases.

## P-27-071
## Structural insight into DAPK2 inhibition by 14-3-3 protein binding

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DAPK family of human calcium/calmodulin ($Ca^{2+}$/CaM) dependent serine/threonine kinases includes five proteins which regulate several biological functions in cell. They are most known for regulation of autophagy and apoptosis in caspase dependent or independent manner. The shortest member of the family, DAPK2, is 370 amino acid long protein with only three domains: catalytic domain (CD), autoregulatory domain (ARD), dimerization domain (DD) and unstructured N- and C- terminus. In basal conditions DAPK2 forms inactive homodimers. Prerequisites of this conformation is autophosphorylation on S318 in the ARD which happens to be primary $Ca^{2+}$/CaM binding site. In inactive conformation of DAPK2, S318 is buried deep in the basic loop of CD, preventing access of substrate. Canonical model of DAPK2 activation suggests that binding of $Ca^{2+}$/CaM pulls out the ARD from the CD, however in $Ca^{2+}$/CaM independent model of activation, phosphorylation on S299 is enough for DAPK2 to be catalytically active. Recently has been discovered that autoinhibitory conformation of DAPK2 is stabilized by interaction with 14-3-3 protein. Interestingly, 14-3-3 binding site, is predicted to be in the C-terminus, only 40 AA from $Ca^{2+}$/CaM binding site. Thus, primary focus of this study is to describe molecular mechanism of how DAPK2 is negatively regulated by 14-3-3 protein binding and how this interaction affects steps of DAPK2 activation by $Ca^{2+}$/CaM. Our preliminary data showed that 14-3-3 binds with a high affinity towards DAPK2 and that phosphorylated T369 is crucial for the interaction. Stoichiometry of 14-3-3/DAPK2 complex is 2:2 with $K_D$ in nanomolar range. Stability of the complex can be further stabilized using small molecules like fusicoccin. Thus, this study not only provides mechanistic insight into DAPK2 regulation but also presents DAPK2 as possible, easily and specifically modulable drug target during cancer treatment. This work was supported by Charles University project UNCE SCI/014.


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## P-27-072
## The interaction between Hsp104 and Hsp70 is important for thermotolerance and cells survival during the stress

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Hsp104 from *Saccharomyces cerevisiae* is a protein disaggregase. *Hsp104* gene plays a critical role in cell survival at high temperatures. Hsp104 consists of the N-domain, two AAA+-type nucleotide binding domains: NBD1 and NBD2 and a helical, mobile M-domain protruding from NBD1. Hsp104 forms hexamer with central channel through which the polypeptide during disaggregation are translocated. Hsp104 collaborates with the Hsp70 chaperons, to retrieve and reactivate stress-denatured proteins. The interaction with Hsp70 causes transmission of signal from the Hsp104 M-domain to NBD-1, thereby coupling ATP hydrolysis with polypeptide unfolding and translocation. The cooperation between Hsp70 and Hsp104 is important for the disaggregation process, but the mechanistic principle of cooperation are not well understood. The main objective of our research was to define the role of Hsp70 in the regulation of Hsp104 activity. Based on the comparison of amino acid sequences of the M domain from different Hsp100s, we identified phenylalanine located at the position 508 in Hsp104 as the key site of interaction with Hsp70. The substitution of phenylalanine to alanine in this site makes Hsp104 unable to interact with Hsp70 and in consequence to bind protein aggregates and perform disaggregation effectively. *In vivo* analysis shows that yeast carrying *hsp104 F508A* gene do not show resistance to temperature stress. Our results demonstrate that, the interaction between Hsp104 and Hsp70 contributes to the effective initiation of disaggregation process and plays an important role in cell survival during stress.

## P-27-073
## Hypochlorite-induced modifications of plasma fibrin-stabilizing factor identified by HPLC-MS/MS

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Plasma fibrin-stabilizing factor (pFXIII) being a member of the transglutaminase family exists as a heterotetrameric proenzyme FXIII-A2B2 consisting of two single-stranded catalytic A subunits (FXIII-A2), and two identical single-stranded inhibitory/carrier B subunits (FXIII-B2). The conversion of pFXIII into the enzymatic form FXIII-A2* is a multistage process. The important role of pFXIII in hemostasis is well known to lie in the covalent stabilization of fibrin clot and its protection from fibrinolytic degradation. Like many other blood plasma proteins, pFXIII is an oxidant-susceptible target. Currently, there is a limited number of studies on both identification of the oxidation sites in pFXIII brought about by its induced oxidation and by their influence on the structural and functional characteristics of pFXIII. A set of the oxidation sites in FXIII-A2 under hypochlorite-induced oxidation of pFXIII at different stages of its activation have been identified by mass spectrometry, and the extent as well as the chemical nature of these modifications have been explored. It was shown that amino acid residues susceptible to oxidative attack and the oxidation degree of these residues in FXIII-A2 derived from non-activated pFXIII, pFXIII activated by Ca2+ and fully activated pFXIII treated with thrombin and Ca2+ significantly differ. The experimental data have shown that the functional activity of FXIII-A2* is strongly depending upon the stage of pFXIII conversion at which the oxidation was carried out. That the modification degree of the enzyme is more pronounced than other intermediate protein forms can demonstrate both the greatest vulnerability of the catalytic subunit after the dissociation of the protein molecule upon its activation and the possible previously undescribed antioxidant function of the carrier subunit-B of pFXIII. The mass spectrometry measurements was supported by the Russian Science Foundation No 161400181.

## P-27-074
## Oxidation-induced modifications of the plasminogen and plasmin identified by HPLC-MS/MS

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Proteins are the primary targets for reactive oxygen species (ROS). Oxidative modifications of proteins may bring about violating their structure and functionality. Plasminogen is a precursor of plasmin, which plays a fundamental role in intravascular thrombolysis. At the same time, plasminogen is a completely unexplored plasma protein in relation to its post-translational oxidative modifications and, as a result, structural and functional disorders. For the first time, by using high-performance liquid chromatography combined with tandem mass spectrometry (HPLC MS/MS), the peculiarities of oxidation induced modifications of plasminogen and plasmin both have been analyzed. Likewise, the enzymatic activity of plasmin formed from plasminogen oxidized by different amount of hypochlorite (HOCL) were studied. The HPLC MS/MS data obtained indicated that mild amount of the oxidizing agent involved only KR-2, KR-4 and SP domains in oxidation while with higher amount of the oxidizer all the structural regions of plasminogen and plasmin were oxidized. The largest number of oxidation sites is given rise to by chemically damaged methionine and aromatic amino acids residues, among which tryptophans are the most accessible to the oxidizing agent. The plasmin oxidation resulted in forming the additional oxidation sites absent in oxidized plasminogen. Oxidative damage to the primary and secondary structure of plasminogen contributes to decreasing the functional activity of plasmin obtained from oxidized plasminogen that is confirmed by the results of electrophoresis. It is concluded that less folded structure of plasmin compared to compact structure of plasminogen


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become more vulnerable to ROS action. The antioxidant role of methionine residues in retaining the structural integrity of the crucial amino acid residues of plasminogen is discussed. The mass spectrometry measurements was supported by the Russian Science Foundation No. 161400181.

## P-27-075
## 3D structure of the mutant of carboxypeptidase T from *Thermoactinomyces vulgaris* with implanted S1′-subsite of pancreatic carboxypeptidase B in complex with the product analogue

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The mutant of bacterial carboxypeptidase T from Thermoactinomyces vulgaris (KPT5) with substitutions of amino acid residues in the S1'-site of the active center with residues corresponding to the S1'-site of pancreatic carboxypeptidase B, is crystallized with N-BOC-L-Leu, which occupies S1-site of the active center and is an analogue of the reaction product. The diffraction data set was collected from KPT5 crystal, the structure of KPT5 was solved at resolution of 1.40 A. The binding of N-BOC-L-Leu in the S1 site of KPT5 leads to conformational changes of five residues of the mobile loop located on the border of the S1 and S1′-sites, and the side groups of Tyr255 and Leu254 residues. In addition, conformational changes concern the active site residues Glu277 and Asp262. The observed changes differ from the conformational changes that takes place in wild-type CPT and N-BOC-L-Leu complex, which indicates the mutual influence of S1 and S1'-subsites in carboxypeptidase T. The work was supported by the Russian Science Foundation (Project 17-14-01256) in part of macromolecule production and Xray analysis and by the Central Research Institute for Mechanical Engineering, ROSKOSMOS in part of microgravity crystallization. *The authors marked with an asterisk equally contributed to the work.

## P-27-076
## Temperature-dependent structural changes in Cas3 protein in *Escherichia coli*

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The CRISPR-Cas system is a significant mechanism of bacteria and archaea that provide adaptive immunity against viruses and plasmids. It consists of DNA repeats separated by spacers of foreign origin (CRISPR locus), and *cas* genes responsible for various stages of defense. In *E. coli*, Cas3 protein is involved in a degradation of invader DNA as a last stage of defense. Recent studies showed that Cas3 activity is temperature-dependent and is lost at 37 °C, unless the protein is present in abundance. In this work, we wanted to investigate the mechanism of Cas3 activity loss by determining conformational changes of purified protein at different temperatures *in vitro*. We monitored structural changes by measuring a change of ellipticity using a circular dichroism method (CD) and by measuring intrinsic tryptophan fluorescence (ITF) using a fluorescence spectrometry method. Employing CD method, we observed a conformational change in helical region at 35 °C what was confirmed by ITF method. The obtained results are in an agreement with protein activity change *in vivo*. Furthermore, we introduced point mutations in three different α-helices in order to investigate which helical region is responsible for temperature-dependent activity of Cas3. Based on our *in vitro* and *in vivo* data, our results indicate a possible role for one α-helix that we discuss and propose a model of regulation. Overall, the results of this research will contribute to a better understanding of the regulation of Cas3 activity as well as to the progress of the CRISPR-Cas field.

## P-27-077
## Linker effects in single molecule protein folding from molecular simulations

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Single molecule force spectroscopy (smFS) experiments of protein mechanics have been extremely useful to study folding and unfolding behaviours and, more recently, even to resolve protein folding transition paths [1]. However, these experiments are inherently affected by the instrumental apparatus used to modulate the extension of the protein by the application of a pulling force. Recent analytical methods have been used to explain these effects both in the thermodynamics and kinetics. This is essential to understand the relevance of experimental results from these techniques. However, these theoretical methods are often validated against trajectory data from simulations on very simple one-dimensional models and then directly applied to the analysis and interpretation of smFS experimental results. Here I cover the gap between simple models and experiments using molecular simulations with an explicit chain model that recapitulates the most fundamental aspects of protein folding. The focus is on the simplest case of force lamp experiments, which allow monitoring reversible folding/unfolding transitions. I study a simple two-state protein where the effects of the linker turn to be dramatically important to understand the measured kinetics [3]. References: [1] The Power of Force: Insights into the Protein Folding Process Using Single-Molecule Force Spectroscopy. J Schöenfelder, D De Sancho & R Pérez-Jiménez, J. Mol. Biol. 428, 4245–4257 (2016). [2] Instrumental Effects in the Dynamics of an Ultrafast Folding Protein Under Mechanical Force, D. De Sancho*, J. Schönfelder, R. B. Best, R. Perez-Jimenez, V. Munoz. J. Phys. Chem. B, in press (2018).

## P-27-078
## Minichaperone-based fusion system for stabilization of hydrophobic proteins in stably soluble forms

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In this work, the new fusion system is described, able to maintain in soluble stable form initially insoluble proteins. For the proof of principle, two initially insoluble proteins were chosen, E6 from human papilloma virus (HPV) type 16, and N-terminal fragment of E2 from hepatitis C virus (HCV). Both these proteins are


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considered as candidates for corresponding therapeutic vaccines owing to their immunogenicity, and both cannot be used in this capacity because they are hydrophobic and practically insoluble in native buffers. In the new fusion system, both HPV E6 and HCV E2 are expressed as inclusion bodies, but are easily renatured from urea solution and retain their solubility in native conditions through standard biochemical procedures, such as concentration, storage in solution, freezing – thawing, and lyophilization – re-dissolving. As a leader for this fusion system, a new form of minichaperone – GroEL apical domain (GrAD, GroEL Apical Domain) – was designed. The sequence of GroEL from thermostable organism Thermus thermophilus was used as a template for GrAD, which included GroEL amino acid residues 190 – 333. The final form retained the thermostability of original GroEL, and this feature can be used as a first step of purification. In GrAD sequence, the codons encoding methionine residues were substituted for those encoding leucine residues. That permits easier purification of the target polypeptide after its chemical cleavage off the fusion by cyanogen bromide. Alternatively, in the linker between GrAD and the target polypeptide the recognition site of enterokinase for enzymatic cleavage is provided. Also, two more forms of GrAD were made, and their physico-chemical properties studied. These forms allow the variability of steric interactions between GrAD and the target polypeptide, which considerably widens the possibilities of using GrAD as a leader in fusion systems. *The authors marked with an asterisk equally contributed to the work.

## P-27-079
## Crystal structure of human AMACR provides insight into substrate recognition and catalytic mechanism

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Human α-methylacyl-CoA racemase (AMACR; P504S) plays a pivotal role in catalyzing a key chiral inversion step in the metabolism of branched-chain fatty acid, ibuprofen, and related drugs. Recently, AMACR was found to be overproduced in prostate cancer and has been used as a cancer biomarker and an attractive drug target. Moreover, human AMACR is associated with human diseases because of function deficiency caused by single base mutation such as the SNP mutants S52P and L107P. Here, we report the crystal structures of human AMACR in apo form and in complex with a substrate analog, isobutyryl-CoA (IBCoA). The structure of human AMACR presents an interlocked dimeric architecture. In addition, the structural information delineates the residues involved in catalysis and identifies a hydrophobic plateau for acyl or aromatic groups binding. For the binding effect of large side-chain substrates, the evidence implies that the hydrophilic side-chain would not contribute to binding. Finally, based on the results of MD simulation and *in vivo* thermal shift assay, we find that L107P and S52P mutants located very close to the binding pocket, and S52P are less stable than wild-type AMACR. These studies will shed new light on the drug development and understanding of AMACR function, and thus will be of great research and therapeutic value.

## P-27-080
## Changes in lysozyme's II-structure and aggregates formation as a result of its interactions with a gold surface

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Proteins have a natural tendency to form dimer or oligomer forms. In this work, we have defined conditions that indicate a change in the II-structure of the protein and induce the formation of unnatural aggregates. The adsorption of lysozyme (LYS) onto the gold surface under different conditions of pH, ionic strength and concentration was investigated using the Multi-Parameter Surface Plasmon Resonance (MP-SPR) and Quartz Crystal Microbalance (QCM-D). Experimental data shows that maximum adsorption efficiency was obtained at the pH=10, which corresponds to lysozyme's isoelectric point. Protein adsorption onto the gold surface was estimated according to Random Sequential Adsorption (RSA) model. This study shows the tendency of LYS to the formation of monolayer in *side-on* or *between* orientation depends on pH condition. Comparison of MP-SPR and QCM-D results allowed to determine the degree of hydration of protein's layers. The stability of lysozyme's structure in the adsorbed state was examined using Polarization-Modulation Infrared Reflection Absorption-Spectroscopy (PM-IRRAS) method. Analysis of the PM-IRRAS data shows that the interactions between lysozyme and the gold surface caused changes in its II-structure in comparison with Circular Dichroism measurements in solution. Additionally, the II-structure of lysozyme adsorbed at the different potential of gold was investigated. Strong misfolding of lysozyme in extreme pH conditions was observed. This enormous changes in lysozyme's structure are linked to the formation of aggregates with amyloid-like structure. It is to be expected that the change in protein's structure will have a big impact on changes in its biological properties and function. Acknowledgments: This work was partially supported by project NCN OPUS 2016/23/B/ST5/02788, InterDokMed POWR.03.02.00-00-I013/16, and NAWA PPN/BIL/2018/1/00103. *The authors marked with an asterisk equally contributed to the work.

## P-27-081
## Does the loop reconstruction method affect protein functionality?

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The majority of protein crystal structures submitted to Protein Data Bank (PDB) database is incomplete. The number and localization of missing residues is related to the structure quality which relies on the flexibility of particular region. Functionally important residues are usually very flexible since they need to be able to change their conformation. A good example of such residues are gating residues enabling the substrate to enter the buried active site or the product to be released to the environment. The most widely used technique to access the missing information in incomplete structures is homology modelling. Although the quality of obtained model is determined mostly by sequence similarities of used protein models and scoring functions assessing the


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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accuracy of each model were not designed to discriminate flexible solvent-exposed fragments of proteins. Here we would like to show a simple method to discard poor models based on their active site accessibility. As a case study we have used *Aspergillus niger* epoxide hydrolase structure (PDB ID: 1QO7). Epoxide hydrolases are members of wide-spread protein superfamily of alpha/beta hydrolases that consist of barrel-shaped core domain with flexible cap on its top. The *A. niger* structure is missing nine-amino-acid-long loop located at the protein surface in proximity of the entrance to the active site, just between the cap domain and the protein core. We decided to verify loop models using molecular dynamics and detailed analysis of water access to the active site using AQUA-DUCT software. The active site accessibility of each homology model is quite similar although the pathways used by water molecules depend highly on the model used for analysis. This approach allows to discard poor non-native protein structures and could be used to complement the homology models assessment.

## P-27-082
## Purification and structural characterization of EXOG a human mitochondrial inner membrane nuclease

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EXOG is a human nuclease that is present in the inner membrane of mitochondria. The enzyme belongs to a sugar non-specific family of nucleases and harbours both endonuclease and 5'-exonuclease activity. EXOG takes part in the DNA repair process and is thought to act as a scaffold for assembly of the DNA repair complex on mitochondrial inner membrane. Predicted N-terminal transmembrane region (TM, residues 16–38) folds into a helix and anchors the enzyme to the inner mitochondrial membrane. Here we show that the full length enzyme can be purified in the presence of different detergents. Moreover, the enzyme can be extracted from the membrane with the use of different detergents and polymers. We show that different extraction and purification methods affect the stability, structure and function of EXOG.

## P-27-083
## Structural view on the complex between the 14-3-3 protein and caspase-2

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Caspase-2 is an intracellular protease responsible for the proteolysis of cellular substrates directly involved in mediating apoptotic signaling cascades. Caspase-2 activation is inhibited by phosphorylation followed by binding to the scaffolding protein 14-3-3, which recognizes two phosphoserines located in the linker between the CARD domain and the large catalytic (p19) domain of the caspase-2 zymogen. This region with both 14-3-3 binding motifs also contains the nuclear localization sequence of caspase-2. Using biochemical and biophysical approaches, we performed a structural analysis of the 14-3-3zeta:caspase-2 complex to elucidate the structural details of this interaction and the role of 14-3-3 in the regulation of caspase-2 activation. The structure model proposed in this study suggests that phosphorylated caspase-2 and 14-3-3zeta form a compact and rigid complex in which the large (p19) and small (p12) catalytic domains of caspase-2 are positioned within the central channel of the 14-3-3 dimer and are stabilized through interactions with the C-terminal helices of both 14-3-3zeta protomers. In this conformation, the surface of the p12 domain, which is involved in caspase-2 activation by dimerization, is sterically occluded by the 14-3-3 dimer, thereby likely preventing caspase-2 activation. In addition, 14-3-3 protein binding to caspase-2 masks its nuclear localization sequence suggesting that 14-3-3 binding may regulate the subcellular localization of caspase-2.

## P-27-084
## Proteoforms of human S100A8 and S100A9 – a differential HDex-MS study

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S100A8 and S100A9 proteins exert a variety of significant intra- and extracellular roles in the human body, both in physiology and pathology. They constitute up to ~40% of the protein fraction of human neutrophils. Inflammatory processes induce expression of the proteins in every human cell. S100A8 and S100A9 are laboratory markers in diagnostics of rheumatoid arthritis, ulcerative colitis, Crohn's disease and established biomarkers of many types of human cancers including breast, prostate, pancreatic, liver or skin cancer. The functional diversity of human S100A8 and S100A9 relies on the formation of many different proteoforms. The proteins form homodimers, a very stable heterodimer (S100A8/S100A9, calprotectin) and other oligomeric forms. Proteomics studies identified phosphorylation and redox-based posttranslational modifications at methionine and cysteine side chains of the proteins. Besides, S100A8 and S100A9 are calcium and zinc-binding proteins, while the heterodimer calprotectin also binds manganese, nickel, and iron at a unique hexahistidine site. Our poster presents the application of hydrogen/deuterium exchange mass spectrometry (HDex-MS) to compare for the first time the structural dynamics of different S100A8 and S100A9 proteoforms. *The authors marked with an asterisk equally contributed to the work.

## P-27-085
## Interaction of proteins from *E. coli* DNA mismatch repair system with DNA fragment containing G-quadruplex

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DNA G-quadruplexes (G4) are curious biologically relevant non-canonical DNA structures. G4 DNA is believed to perform the regulatory roles in cellular processes and G4 binding functions of many proteins have been lately identified. However, in some cases the authentic assignment of G4-protein interactions is poorly understood. One example is the mismatch repair (MMR) system protein MutS which was shown to specifically interact with the tetramolecular G4. This recent evidence and the lack of data for other MMR proteins – MutL and MutH – puts a question of G4 being the signal for MMR initiation. Addressing this problem we constructed DNA system where intramolecular G4 $(GGGT)_4$-motif was incorporated in 76-bp duplex environment


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allowing the investigation of the G4 influence on the initial steps of *E. coli* MMR. Suggested DNA structure was verified via DNA footprinting and $^{1}$H-NMR. The interaction of MutS and G4 in the conditions providing different MutS conformations was characterized with apparent dissociation constants calculated from EMSA data. The nearly full independence from nucleotide cofactor present was shown implying the G4 binding mode to differ from the one with GT-mismatch. To elucidate ATPase activity in presence of G4 DNA, we employed malachite green assay for phosphate detection. For the first time we investigated the binding of MutL protein to G4 DNA. The significantly higher affinity to G4 as compared to other DNA molecules was discovered. We further subjected model G4 to cleavage by MutH protein. The DNA hydrolysis efficiency by MutH-MutL-MutS complex was demonstrated to be not dependent on G4 presence in DNA duplex. Therefore, despite the efficient interaction between G4 and both MutS and MutL with the affinity higher than to DNA with a mismatch, G4 DNA does not serve as a substrate for MMR initiation, and actual cellular function of G4-MutS and G4-MutL complex formation is yet to be found. This work was supported by RFBR grant No. 18-34-00768.

## P-27-086
## The interdependence between amyloid formation and virulence of Proteobacteria

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Proteobacteria represents largest phylum of the gram-negative bacteria including as various pathogens of humans, mammals and plants as symbiotic species some of which perform beneficial for multicellular host functions, like nitrogen fixation. Recent data obtained by several research groups suggest that the virulence of different pathogenic Proteobacteria species are associated with formation of highly ordered protein fibrils by proteins acting as the virulence factors. Such fibrils called amyloids, represent important structural component of biofilms playing crucial role in virulence of various *Proteobacteria* species, and they are related to bacterial toxin formation. In addition, we have previously found that M60 mucin metalloprotease of *Escherichia coli* that is involved in the pathogenesis of the enterotoxigenic strains of these bacteria, is amyloidogenic. Thus, repertoire of functions of amyloid-forming virulence factors of Proteobacteria is apparently wider than we expected so far. We analyzed abundance of amyloidogenic regions in the proteomes of more than 80 species of the order Rhizobiales belonging to the class Alphaproteobacteria using different bioinformatic algorithms. We found these regions tended to be overrepresented in the proteins associated with virulence of these bacteria and comprising so-called beta-barrel structure typical for outer membrane proteins. Further experimental verification including analysis of detergent resistance, fibril formation in the C-DAG system and ability to bind amyloid-specific dyes confirmed amyloid properties of such outer membrane proteins of agriculturally important nitrogen-fixing species *Rhizobium leguminosarum*. Overall, these data demonstrate that amyloid formation by virulence factors is important for capability of different bacterial species to colonize multicellular host. The study of amyloid proteins of *R. leguminosarum* was supported by the Russian Science Foundation, grant 17-16-01100.

## P-27-087
## Structural instability of FGF1 R50E mutant restricts its mitogenic potential

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FGF1 has been shown to interact with integrin alphavbeta3 through a specific binding site, involving Arg50 residue. The FGF1 mutant (R50E) with impaired integrin binding was found to be defective in proliferative response, although it was still able to bind to FGFR and heparin and induce activation of downstream signaling pathways. Here we demonstrate that the lack of mitogenic potential of R50E mutant is directly caused by its decreased thermodynamic stability and susceptibility to degradation *via* proteolysis. Introduction of three stabilizing mutations into R50E variant compensated the effect of destabilizing mutation and restored the proliferation potential of FGF1, while remaining defective in binding to integrin alphavbeta3. Our results suggest that the thermodynamic stability and resistance to degradation rather than interaction with integrin are required for mitogenic response of FGF1. Acknowledgments: The work was supported by the National Science Centre, Poland (Sonata Bis 2015/18/E/NZ3/00501). *The authors marked with an asterisk equally contributed to the work.

## P-27-088
## Selective Hsp70-dependent docking of Hsp104 to protein aggregates protects the cell from the toxicity of the disaggregase

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Hsp104 is a yeast chaperone that rescues misfolded proteins from aggregates associated with proteotoxic stress and aging. Hsp104-dependent protein disaggregation involves extraction of a polypeptide from an aggregate and its translocation through the central channel of the Hsp104 hexamer. This process relies on Hsp104 cooperation with the Hsp70 chaperone, which also plays an important role in the regulation of the disaggregase. Although the protein-unfolding activity of Hsp104 enables cells to survive stress, when uncontrolled, it becomes toxic. There is a trade-off: on one hand, this protein-remodeling machine must be powerful enough to tackle protein aggregates, on the other hand, it needs to be restricted to prevent promiscuous unfolding of disordered proteins with important cellular functions. Here, we investigated how collaboration with Hsp70 at the initial stages of protein disaggregation allows to maintain this balance. Using the hyperactive, toxic Hsp104 variant with disrupted Hsp70-binding site we demonstrate that the cooperation with Hsp70 shifts Hsp104 substrate specificity from non-aggregated, disordered substrates towards protein aggregates. Our results show that the Hsp70-mediated, selective recruitment for disaggregation makes the Hsp104 unfoldase less toxic and more productive.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-27-089

## Use of site-directed mutagenesis for structure-function studies of formate dehydrogenases from the bacterium *Staphylococcus aureus*

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$NAD^+$-dependent formate dehydrogenase (EC 1.2.1.2, FDH) has been found in different organisms: bacteria, yeasts, fungi and plants. FDH plays important role in vital activity of cells. The amount of FDH sharply increases under stress conditions. Under stress conditions pathogens form biofilms and at that time content of FDH in cell increases few times compared to plankton conditions. FDH is widely used in fine organic synthesis as a biocatalyst for NADH regeneration, but the drawback of known FDHs is low specific activity. Therefore study of FDHs is of high fundamental and practical interest. Systematic studies of FDHs from different sources are carried out in our laboratory. Now our studies are focused on FDH from the bacterium *Staphylococcus aureus* (SauFDH). Gene of this enzyme was cloned and expressed in *E. coli* cells. Previous experiments have shown that FDH from the bacterium *S. aureus* has outstanding thermal stability and high catalytic constant, while Michaelis constants for $NAD^+$ and formate are very high in comparison with described FDHs. At the same time, there is high value of catalytic constant. Crystals of SauFDH were obtained and 3D structures of apo- and holo-forms were determined with X-ray analysis. To identify promising amino changes in SauFDH for improvement of $K_M$ values we carried out comparative analysis of apo- and holo- forms of SauFDH structures, as well as structures of FDHs from bacterium *Pseudomonas* sp. 101, yeast *Ogataea parapolymorpha* DL-1, soya *Glycine max* and moss *Physcomitrella patens*. Computer structures of promising mutants were made and the best candidates were selected. New mutant SauFDHs were prepared, overexpressed in *E. coli*, purified and characterized. It was shown that one amino acid change leads to decrease of Michaelis constants for $NAD^+$ and formate in 4 and 2 times respectively. This work is supported by Russian Foundation for Basic Research, grants 17-04-01662 and 18-34-20098.

## P-27-090

## Diffusion of amyloid fibrils as observed in pulsed-field gradient NMR experiments

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Pulsed-field gradient NMR is an important tool to measure diffusion of proteins and protein assemblies and thus obtain insight into their structure and dynamics. For extended objects, such as amyloid fibrils, these experiments become difficult to interpret because in addition to translational diffusion they are also sensitive to rotational diffusion. We have constructed a mathematical theory describing the outcome of PFG NMR experiments on rod-like fibrils. These analytical results proved to be in excellent agreement with the predictions from our Monte-Carlo simulations. The effect of rotational diffusion is indeed significant. However, just like translational diffusion, rotational diffusion of a fibril is a slow process and registers as such in the PFG NMR experiments. Contrary to certain literature claims, this allows one to separate spectral signals from fibrils and other species that may be present in the sample (monomers, proteolytic fragments, etc.) based on their different diffusion properties. To test the validity of our theory, we have studied fibrils formed by protein Sup35NM derived from yeast translation termination factor Sup35p. The presence of disordered M domain in Sup35NM fibrils makes it possible to observe spectral signals from the said fibrils. Using this system, we have shown that the signals originating from the flexible tail of the peptide chains comprising the body of the fibril can be successfully separated from the similar signals representing monomers or proteolytic fragments of Sup35NM. This research was supported by RSF grant 15-14-20038. *The authors marked with an asterisk equally contributed to the work.

## P-27-091

## Proteomic screening revealed functional amyloid proteins in the seeds of garden pea *Pisum sativum* L.

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Amyloids represent highly ordered unbranched protein fibrils, which are stabilized by numerous intermolecular hydrogen bonds. This structure gives amyloids resistance to many physical and chemical factors, which is utilized by different organisms. Recently, functional amyloids were discovered in various organisms ranging from bacteria (biofilm formation, toxin storage and overcoming surface tension) to humans (hormone storage, antiviral response, tooth enamel and melanin polymerization). The only important group of organism where functional amyloids have not yet been found under native conditions *in vivo*, remains plants. Using the proteomic assay PSIA (Proteomic Screening and Identification of Amyloids), we have shown that the seeds of garden pea *Pisum sativum* L. are enriched with amyloids formed by the 7S globulin storage protein. The aggregates of this protein in seeds were resistant to the treatment with ionic detergent and they bound the amyloid specific dye Thioflavin T on pea seed cryosections and extracted protein bodies where this protein accumulates. The content of the aggregates increases along with the maturation of the seeds and drastically decreases at the seed germination. We have also shown that protein bodies containing amyloids of 7S globulin exhibit toxicity to yeast cells related with the lectin function of this protein. Taking together, our data show that amyloid formation by seed storage proteins might facilitate the preservation of proteins in mature dehydrated seeds and protect them from pathogenic fungi. This study was supported by the Russian Science Foundation (Grant No 17-16-01100).


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## P-27-092

## Crystal structure of a unique glucokinase from *Kluyveromyces lactis*

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Glucose is one of the main regulators of gene expression and metabolism in eukaryotic organisms. Glucose-dependent regulation requires the use of the phosphorylated glucose molecules provided by glucose-phosphorylating enzymes known as glucokinases or hexokinases. They catalyze the ATP-driven phosphorylation of glucose, yielding ADP and glucose-6-phosphate. Kluyveromyces lactis is an emerging tool in biotechnology. It has been employed as a host for recombinant protein expression e.g. to produce lactase or chymosin for industrial scale. Due to the growing importance of K. lactis in biotechnology, research on its biology and metabolism has a great value for both scientific and industrial reasons. KlGlk1 glucokinase was reported for the first time in 2007 and based on genome and functional analysis, it might act as an additional (to KlHxk1) glucose phosphorylation enzyme with another as yet unknown function. Here we present the crystal structure of KlGlk1 glucokinase from K. lactis at 2.6 A resolution and its comparative analysis to previously structurally characterized KlHxk1. Recombinant KlGlk1 was overexpressed in E. coli BL21 strain, purified and crystallized in the presence of potassium bromide and PEG 2000 MME. Diffraction data collection was performed at BESSY II 14.1 beamline at 100 K. The structure was solved by MR using KlHxk1 structure as a model. The asymmetric unit contains three KlGlk1 molecules and the unit cell belongs to C2221 space group with high solvent content (65%). The amino acid identity between KlGlk1 and KlHxk1 sequence is only 38%. Nevertheless, comparative analysis shows significant similarities and disclose almost identical fold of parts characterized by ordered secondary structure within both domains. Structural and functional characterization of KlGlk1 can help to understand the mechanisms responsible for glucose sensing and signaling therefore allowing for more intensive use of K. lactis for both scientific and industrial applications.

## P-27-093

## Structural basis for DNA recognition by FoxH1 pioneer transcription factor

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The FoxH1 protein, a forkhead box (Fox) family member, is a maternal transcription factor (TF) that plays a vital role in early embryonic development by the mediation of Nodal/TGF-β signaling. Mutations of FoxH1 are linked to human heart defects, holoprosencephaly, and cancer. FoxH1 has been recently proposed to act as a pioneer factor, preoccupying cis-regulatory elements to recruit other TFs, including TGF-β SMAD proteins, in order to accelerate the activation rates of target genes. These observations suggest that FoxH1 is able to identify its binding sites genome-wide in an autonomous and specific manner. To understand how FoxH1 mediates transcriptional activity, we solved four crystal structures of the DNA-binding domain (DBD) of fish and frog FoxH1 proteins, bound to various DNA duplexes containing a FoxH1 consensus binding sequence (TGTGGATT). The highest resolution structure was refined at 0.98 A. The FoxH1-DBD adopts the winged-helix fold with helix H3, the landmark of Fox proteins, contributing to the base-specific contacts in the major groove, while the wing 1 forms base-specific contacts in the minor groove, and both wings of FoxH1-DBD make additional contacts with the DNA backbone. We found that the N- and C-terminal extensions adjacent to the Fox canonical domain are structured and stabilize further the protein-DNA interactions. Our structural, biochemical, and bioinformatics analyses allow us to provide a model of high-affinity DNA binding for FoxH1 proteins in vertebrates. In addition, our structural analysis and biochemical assays provide a molecular basis for understanding disease-causing mutations in FoxH1-DBD.

## P-27-094

## Open-access Mustguseal platform for bioinformatic analysis in computational enzymology

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Comparative analysis of homologous proteins in a functionally diverse superfamily is a valuable tool at studying structure-function relationship, but represents a methodological challenge. We have developed an open-access platform available at https://biokinet.belozersky.msu.ru/mustguseal consisting of free on-line methods to study the structure-function relationship in proteins, to select the most promising hot-spots for implementation of novel functions, improvement of stability and evolvability of useful proteins/enzymes, and to design of their selective modulators. The key web-server Mustguseal can automatically collect and align thousands of homologous protein sequences and structures, and four sister web-methods are available for consequent analysis of the collected data: the Zebra web-server to identify variable amino acid residues responsible for functional diversity within a superfamily and to select hotspots for directed evolution or rational design experiments; the pocketZebra web-server to identify and rank binding sites in proteins by their functional significance and to select particular positions in the structure important for selective binding of substrates/inhibitors/effectors; the visualCMAT web-server to select and interpret correlated mutations/coevolving residues in protein structures; the Yosshi web-server to classify and study disulfide bonds in protein families as well as to select hot-spots for disulfide engineering. Integration of these bioinformatic web-tools provides an out-of-the-box easy-to-use solution, first of its kind, to systematically analyze all the available sequence and structural data related to a protein superfamily, thus promoting the value of bioinformatics for protein engineering and drug discovery. This work was funded by the Russian Foundation for Basic Research [18-29-13060] and carried out using the HPC computing resources at the Lomonosov Moscow State University supported by the project RFMEFI62117X0011.


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## P-27-095
## Bacterial production and NMR study of human endogenous neuromodulator Lynx2

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Human proteins from the Ly6/uPAR family share structural homology with snake α-neurotoxins and modulate functions of nicotinic acetylcholine receptors (nAChRs). Some of them are localized predominantly in the nervous system and were shown to regulate cognitive processes in the brain. Lynx2 is one of the poorly studied members of the Ly6/uPAR family. It is known that Lynx2 is membrane-tethered by GPI anchor protein, binds specifically to α4β2-nAChRs and is connected with anxiety-related behavior. To perform functional and structural studies of Lynx2, we developed for the first time the effective expression system in the form of *E. coli* cytoplasmic inclusion bodies, which allowed us to produce the water-soluble domain of human Lynx2 (ws-Lynx2) lacking GPI anchor. Careful screening of conditions for purification and renaturation permitted us to develop the optimal protocol and produce of milligram quantities of ws-Lynx2 and its $^{15}N^{13}C$ isotope-labeled analogue. NMR analysis confirmed correct folding of the recombinant protein with the predominant β-structure. The study was supported by the Russian Science Foundation (Project No. 19-74-20176).

## P-27-096
## Cryo-EM study of water-soluble extracellular domain of α7 nicotinic acetylcholine receptor

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Nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel, which is widely represented both in the central and peripheral nervous system, and in some of the non-neuronal tissues, including epithelium and immune cells. The pathophysiology of a number of diseases is associated with dysfunctions of this receptor, including neurodegenerative disorders like Alzheimer disease and schizophrenia. Here we present the results of structural cryo-EM study of the water-soluble analogue of the ligand-binding extracellular domain of a7 type nAChR. The domain was produced in *Pichia pastoris*, has an increased stability in solution, and demonstrates ligand-binding characteristics close to those of the native receptor. Analysis of the cryo-EM images of individual protein particles revealed the presence of a pentameric structure, confirming intact subunit assembly. To obtain better contrast and resolution, phase plate was used during data collection. As a result, the three dimensional structure of the domain with a diameter of ~ 9 nm and height ~ 7 nm was reconstructed. Obtained results open new perspectives for structural studies of the nAChR complexes with the ligands which escape crystallization. The work was supported by the Russian Science Foundation (project # 19-74-20163).

## P-27-097
## Mass spectrometry approach for GPCR ligand screening

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In the target-based approach of drug discovery G-protein coupled receptors (GPCRs) are key-players. About 40% of existing drugs are focused on this protein family comprised of about 800 members. There is an evident need for the development of GPCR ligand screening methods for speeding up the hit discovery process as well as hit-to-lead optimization. *In silico* methods shrink million libraries of potential drug candidates to thousands of potential hits. *In vitro* assays are used to verify the computational predictions and to select the best compounds. Mass-spectrometry approach to characterize the ligand-protein interaction is a developing and powerful tool. Although it is mostly a qualitative method, it allows ranking compounds by their affinity for a specific target reliably. In certain experimental conditions it also allows to estimate the dissociation constant Kd. It was shown that this method could be applied for a HT-compound screening of adenosine $A_{2A}$ receptor, cannabinoid receptors types 1 and 2 and several other receptors. In this work, we expand the method to other GPCR targets and propose a strategy to estimate the binding affinities for small-molecule compounds. This work was supported by the Russian President Grant for Governmental Support of Young Russian Scientists (project no. MK-5184.2018.4). *The authors marked with an asterisk equally contributed to the work.

## P-27-098
## Structural analysis and molecular dynamic processes in the human cystatin C at presence of human serum albumin (HSA) by means NMR spectroscopy

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The protein aggregation in many cases it leads to specific protein (or peptide) characteristic fiber-like shape. Such insoluble structural form named as amyloid featuring the loss of protein activity provided in many cases to disease state. The formation of such structures has been observed for many amyloidogenic proteins and peptides, including human cystatin C (*h*CC). In our studies, we explore structural alteration and molecular dynamic processes for *h*CC mutant in complex with human serum protein (HSA). HSA is a most abundant plasma transport protein, which is composed of three homologous domains. It is known, the HSA possible to bind and transport a wide variety of molecules including fatty acids, hormones, drugs and metal ions. The ability to bind such a vast and diverse group of molecules is reflected in its flexible three-dimensional structure. Ability of binding Alzheimer Aβ


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peptides is well documented [1]. The present studies report data for another amyloidogenic protein (*h*CC) and explore structural and dynamic alterations due to interaction with HSA. The NMR measurements were performed on $^{13}C$,$^{15}N$-double labeled *h*CC (V57G) protein with addition HSA free from fatty acids (Sigma-Aldrich). Mutant *h*CC (V57G) was selected for the studies because it is stable in monomeric form and reveals tendency to dimerize through domain swapping structure [2] which is difficult to analyze with NMR spectroscopy. The experimental data were acquired using Varian Inova 500 NMR spectrometer at temperature 298 K. The research was supported by grant 2017/27/B/ST4/00485 and partially supported by grant UMO-2016/23/B/ST5/02253 from National Science Centre (Poland).

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## P-27-099
## Crystallographic and cryo-EM studies of *E. coli* Lon protease

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Homooligomeric LonA proteases are key components of the protein quality control (PQC) system in the cells of bacteria and eukaryotes. They function in an energy-dependent manner and form a unique subclass of the $AAA^+$ proteins due to unusual N-terminal region, which has no analogues in other $AAA^+$ proteins of the PQC system. This region is composed of the N domain and the α-helical domain. The latter domain includes a coiled-coil fragment and is referred to as HI(CC). Whereas structures of various fragments of Lon composed either of individual domains or of their combination are available, no structure of any intact LonA protease, nor that of HI(CC) domain, has been published. We determined a new crystal structure of the fragment (235-584) of *Escherichia coli* LonA protease (*Ec*Lon) comprising the C-terminal part of the HI(CC) domain and the $AAA^+$ module. In this structure $AAA^+$ modules form an open ring helical hexamer, as was observed in the crystal structure of *Bacillus subtilis* LonA, as opposed to the closed ring hexamers of the proteolytic domain of *Ec*Lon and C-terminal fragment of *Meiothermus taiwanensis* LonA. Full-length *E. coli* LonA protease is also being investigated using cryo-EM, with both hexamers and dodecamers of the enzyme being visible. The symmetry of hexamers is partially broken, with only five molecules being well ordered while the sixth one is more flexible.

## P-27-100
## The QM/MM calculations and genetic evolution of biocatalysts optimization against organophosphorus toxins

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The optimization of organophosphorus toxins capturing by protein templates demands QM/MM calculations of chemical reactions and structural evolution of genetically encoded bioscavenger by rational design of appropriate mutations or/and combinatorial screening approaches. A two opposite critical starting points for template evolution are considered: (i) flexible immunoglobulin folds selected for catalysis and (ii) highly evolved enzymatic trap. The QM/MM calculations in the first case allowed to proceed with the virtual screening of effective mutants using supercomputer facilities. In the second case the ultrahigh-throughput screening of the representative library of mutated enzyme active sites allowed to redirect the reaction pathway. In both cases QM/MM calculations gave the mainstreams for genetic evolution of catalytic templates. Here we proposed QM/MM -based calculation of the reaction success rate estimation in case of immunoglobulin and enzymatic templates. We elaborated a semi-quantitative approach for prediction of the energetic parameters of calculated reactions which showed parameters very close to the experimentally obtained data. This ensured us in the very high predictive capacities for catalytic parameters of QM/MM estimations. The predicted mutants displayed a very good kinetic improvements mechanistically explained by refined X-Ray structures. Finally, the proposed QM/MM algorithms gave perspectives for tailored design of highly evolved genetically encoded organophosphorus scavengers. The work was supported by RFBR 17-54-30025.

## P-27-101
## Structural-dynamic NMR investigation of Trichobakin, a type I ribosome-inactivating protein from *Trichosanthes* sp. Bac Kan 8-98

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Ribosome-inactivating proteins (RIP) from plants are the toxins that inhibit protein synthesis in eukaryotic cells due to catalytic damage of the ribosomes. RIPs specifically cleave the N-glycosidic bond of the adenine nucleotide at the position 4605 of the conserved GAGA sequence of the sarcin-ricin loop of the 28S rRNA by the N-glycosidase activity, thus inhibiting protein synthesis and preventing the binding of the translation elongation factor 2. Trichobakin (TBK, 27 kDa) is the Type I RIP that was cloned from the plant *Trichosanthes* sp. Bac Kan 8-98, growing in the north of Vietnam. This enzyme is highly homologous to the Trichosanthin protein (TCS), but it has several times higher inhibitory activity of the protein synthesis in the rabbit reticulocyte lysate system. Therefore, it could be a promising biotechnological object for design of a recombinant toxin for effective


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inhibition of the cancer cells growth. For the future biotechnological use of TBK and its improvement, it is necessary to fully understand the mechanism of its functioning: from the penetration into the cells, recognition of the depurination site to the mechanism of catalytic N-glycosylation. In this study, the highly purified recombinant $^{15}N$,$^{13}C$-labeled TBK was obtained and investigated by the solution NMR spectroscopy. The $^{1}H$,$^{15}N$,$^{13}C$-chemical shifts assignments of the 93% backbone atoms were performed that allow to characterize the structural-dynamic properties of TBK in solution. NMR data analysis revealed that the TBK structure is composed of α-helices packing against a central core of antiparallel β-sheets clusters and high flexible loop 200-230, which is consistent with our preliminary data on accelerated Molecular Dynamics on the interval of 2.5 μs. This work will serve as a basis for further research on the interactions of the type I RIP with biomembranes and ribosomal proteins. NMR studies were supported by the Russian Science Foundation (project 19-74-30014).

## P-27-102
## The functional and structural study of PemIK_Sp toxin-antitoxin system from *Staphylococcus pseudintermedius*

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Toxin-antitoxin (TA) systems are widespread among bacteria. The systems are encoded in operons which may be located in mobile genetic elements and within the bacterial core genome. A toxin of type II TA system is a stable protein often with enzymatic activity while an antitoxin is an unstable and susceptible to degradation toxin's inhibitor. The study focuses on functional and structural analysis of proteins from PemIK_Sp, a TA system, chromosomally encoded in animal pathogen Staphylococcus pseudintermedius, in order to compare them to the homologous proteins from PemIK_Sa, the plasmid located TA system from Staphylococcus aureus, for which the role in virulence was postulated. pemIK_Sp operons from twenty S. pseudintermedius strains were amplified and sequenced. The pemK_Sp toxins and pemI_Sp antitoxins were cloned and recombinant proteins were produced in Escherichia coli. PemK_Sp toxins were tested for RNase activity and for the specificity of RNA cleavage, while PemI_Sp antitoxins for their ability to inhibit toxins' function. Possible cross-interactions between PemIK_Sp and PemIK_Sa system components were tested *in vitro* and *in vivo*. PemK_Sp toxin was crystallized. The PemIK_Sp system is fairly heterogeneous. Respectively, five and two length variants of PemI_Sp and PemK_Sp have been distinguished. Both toxins possess sequence specific RNase activity. PemK_Sp crystallizes in a form of a homodimer and adopts the PemK-like fold. All PemI_Sp antitoxins, save the shortest, have inhibitory function. Cross-interactions between PemIK_Sp and PemIK_Sa were observed, though they were less efficient than for cognate partners. The study points towards possible structural and functional consequences of plasmid-to-chromosome jump of pemIK TA system driving to the gradual loss of its function. The research was supported by the National Science Centre (NCN), Poland, grant no. UMO-2015/19/N/NZ1/00320 (to M.J.).

# Plant biotechnology

## P-28-001
## The new type of versatile antigen for the modern avian flu vaccine development

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M2e-domain of influenza virus A (IAV) membrane protein M2 is highly conservative in a large number of influenza A virus strains. It makes M2e a suitable target to devise a versatile IAV antigenic determinant. The present work addresses the synthesis of recombinant antigen containing an avian IAV M2e sequence and additional M2e consensus for H1N1, H2N2, H3N2 strains. We have designed the genetic construction with six repeats of M2e-sequence. The recombinant protein (24 kDa) was expressed in bacterial cells, then purified and named "TM2e". We have demonstrated that TM2e forms immunogenic complexes with spherical particles (SPs). As we have shown previously, SPs originate from the helical plant virus thermal remodeling and they can serve as an antigen-presenting platform as well as an adjuvant. For assembling the immunogenic complexes we used SPs from two helical plant viruses of different shape (Tobacco mosaic virus, rod-like virions; Alternanthera mosaic virus, filamentous virions). The TM2e-protein antigenic specificity was preserved within both types of complexes. The immunogenic complexes with the structurally modified plant viruses allow for developing the versatile veterinary marker vaccine since the antibody profile after the immunization by the proposed vaccine will include the antibodies against the adjuvant. Therefore, it will make the pathway of immunization evident: either through contact with a wild pathogen or by vaccination. The designed recombinant protein TM2e has a clear immunogenic potential to be harnessed for anti-flu vaccine. The presence of M2e consensus sequence for H1N1, H2N2, H3N2 strains within TM2e provides the opportunity to use the obtained antigen as a component of human vaccine as well. This vaccine will have the ability to protect not only from seasonal influenza infection, but also from high-contagious avian H5 IAV strain.

## P-28-002
## Biology of water chestnut *Trapa* L.

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Water chestnut (or water caltrop) *Trapa* L. is an aquatic plant, widely used as a food source and herb in Asia and Africa. In Europe it is at risk of extinction. It is important to study the biology of water chestnut to protect this species. The Southern Urals is one of the most northern and isolated habitats of this plant. We performed morphological and genetic analysis of *T. natans* in four lakes in the area, and studied the environmental conditions, including water chemistry. In spite of phenotypic variation, there was no genetic polymorphism between the plants from Southern Urals. It is to note that out of all DNA samples from different habitats in Russia, we observed high level of polymorphism only in those distinguished as *T. maximowiczii*. We prepared extracts from seed coats and kernels of water chestnut, gathered in lake Upkankul. Extracts from the seed coat, including peptide extract, inhibited the growth of pathogenic *E. coli* and *S. aureus*. We are investigating the molecular structure of these compounds. We obtained *in vitro* culture from the embryos of water chestnut. The seedlings were not susceptible to agrobacterium-mediated transformation. Explants from mature plants


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did not develop on the nutrient medium. Aquarium culture was successful, and it took 70–80 days for water chestnut to develop and produce seeds. Water chestnut benefits from mineralization and high level of organic compound, so eutrophication and stagnation are the main hazards to its habitats. Stable hydrological regime is a key to the survival of water chestnut and its successful aquaculture. The reported study was funded by Russian Science Foundation according to the research project No. 18-74-00056.

## P-28-003
## Sll0034 carboxypeptidase in involved in cell wall maturation in cyanobacterium *Synechocystis* sp. PCC6803

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Cyanobacterial cell is surrounded by a cell wall with peptidoglycan layer composed of chains of N-acetylglucosamine and N-acetylmuramic acid linked by β-1,4-glycosidic bonds and covalently linked oligopeptides, including D-alanine and D-glutamic acid. The peptidoglycan of cyanobacterial cell wall forms thicker layer and is more crosslinked than the one from other types of gram-negative bacteria. Little is known about enzymes involved in cyanobacterial cell wall metabolism. DD-carboxypeptidases are predicted to be involved in peptidoglycan elongation. Sll0777 and Sll0034 proteins are suspected to be engaged in peptidoglycan metabolism in cyanobacterium *Synechocystis* sp. PCC6803. The aim of this work was to construct deletion strain *sll0034*⁻ of *Synechocystis* sp. PCC6803, in order to verify the involvement of Sll0034 protein in cell wall physiology. Seamless cloning method was employed to prepare transformation vectors. Antibiotic resistance test proved that *sll0034*⁻ strain exhibits significantly increased sensitivity to carbenicillin, as compared to the wild type. Cell wall peptides from mutant and wild-type strains were isolated and analysed by HPLC. Scanning electron microscopy did not reveal any alterations in cell surface and photosynthetic activity of the deletion strain was not affected. Construction and characterization of the *sll0034*⁻ strain of *Synechocystis* sp. confirmed the involvement of Sll0034 carboxypeptidase in cyanobacterial cell wall maturation.

## P-28-004
## A new class of synthetic flavonoids as promising anti-*Candida* therapeutic agents

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The humanity is facing today with the rise and spread of opportunistic fungal infections which are growing up with the occurrence of diseases affecting immune system such as HIV/AIDS or cancers. The emergence of pathogenic multidrug resistant *Candida* strains demands new approaches in finding effective antifungal agents. Flavonoids could be a reliable solution due to their important antimicrobial activity and low cytotoxicity. We report here the potent *in vitro* antifungal activity of BrClflav – a novel tricyclic flavonoid with halogen substituents at the benzopyran core. The antifungal effects were tested using the minimum inhibitory concentration (MIC), minimum fungicidal concentrations (MFC) and time kill assays. A synergism test was performed using a checkerboard micro-dilution method. The antibiofilm activity, hyphal-form growth inhibition and sorbitol binding ability assays and scanning electron microscopy were also employed in order to evaluate the mode of action. Our results showed that different *Candida* strains were sensitive to BrClflav (MIC values ranging from 7.81 to 15.62 μg/mL). In liquid culture medium, our compound inhibited fungal growth up to 12 h at concentrations equivalent with 2 x MIC. BrClflav showed a fungicidal activity at a concentration of 31.25 μg/mL. A synergistic effect of BrClflav combined with fluconazole against *Candida albicans* was evidenced. Our flavonoid showed a good potential to influence *Candida* virulence factors, inhibiting the biofilm formation and yeast to hyphal transition. The mechanism of action is related to the impairment of the cell membrane and wall integrity. Based on the strong anticandidal activity, BrClflav has a promising potential for the design of new antifungal therapeutic agents.

## P-28-005
## Regulation of chloroplast development by alternative splicing

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Plastid-encoded RNA polymerase (PEP) plays a crucial role in chloroplast biogenesis by controlling expression of genes involved in photosynthesis. At least 12 PEP-associated proteins (PAPs) including FSD3/PAP4, regulate PEP activity and chloroplast development by modulating formation of the PEP complex. In this study, we identified FSD3S, a splicing variant of FSD3; the FSD3 and FSD3S transcripts encode proteins with identical N-termini, but different C-termini. Through characterization of FSD3 and FSD3S proteins, we found that the C-terminal region of FSD3S contains a transmembrane domain unlike FSD3. The transmembrane domain promoted FSD3S localization to chloroplast membrane. In contrast to FSD3S, FSD3 without the transmembrane domain localized to nucleoids in chloroplast. We also found that overexpression of FSD3S negatively regulates photosynthetic activity of chloroplasts. When expressions of genes involved in photosynthesis were analyzed in FSD3S-overexpressing plants, it was shown that FSD3S overexpression downregulates the expression of PEP-dependent photosynthetic genes. These suggest that FSD3S negatively regulates photosynthetic activity by reducing the expression of PEP-dependent photosynthetic genes. In addition, FSD3S failed to complement the defects of chloroplast development in the fsd3 mutant. Collectively, these results suggest that FSD3 and FSD3S with the distinct localization pattern have different functions in chloroplast development, and FSD3S negatively regulates expression of PEP-dependent chloroplast genes.

## P-28-006
## Genetic technologies as the base of better crop productivity in Russia

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The logic of the general development of the global Agricultural sector can be presented as a scenario of Biotechnological crops development. This study provides the results of the analysis of the current Russian R&D genetic strategies for Agriculture and the legislative, social, and educational barriers to its development. Over 931 standardized surveys of the Russian leading researchers, universities and the general public were performed during the study in May-July, 2018. The following main hypotheses were


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checked: (i) "legislation gaps" are "bottlenecks" for the development of genetic technologies in Russia; (ii) Russian scientific genetic R&D are financed insufficiently; (iii) most people are not ready to consume Foods containing GMOs; (iv) negative public perception of genetic technology products is supported by mass media, and *etc*. The results showed that only 5.3% of respondents believe that the financing of Russian genetic R&D is sufficient, and therefore, the Russian agricultural productivity is below world level (72.5% of respondents). At the same time, 52% of respondents noted that Russian agribusinesses are interested in using new technologies and 62% of respondents are ready to use GM Foods after a complete safety screening of the Russian Ministry of Health. Experts have recognized that plant genetic research is supported in Russia, but commercialization of the new Russian varieties based on genome reduction depends strictly on the appropriate governance and legal authorization. Legal uncertainty will create social and commercial barriers. We illustrate the ways of further development for plant breeding genomics – new Russian Subprogramme "The Development of Potato breeding and Seed production in the Russian Federation" of the Federal Scientific and Technical Program for the Development of Agriculture for the years 2017–2025. The work was partly supported by the grant RFBR 18-29-14067\18. *The authors marked with an asterisk equally contributed to the work.

## P-28-007
## Polyphenolic extract from buckwheat husks enhances ampicillin susceptibility of *Yersinia pseudotuberculosis* by adaptive changing of lysophosphatidylethanolamine content in bacterial envelope

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A growing number of antibiotic-resistant bacterial strains require a searching for new approaches to overcome bacterial resistance to known antibiotics. β-lactam antibiotics enter into Gram-negative bacteria *via* porins. However, mechanism of reducing permeability of porin channels remains the least understood among the possible reasons of antibiotic resistance. The accumulation of lysophosphatidylethanolamine (LPE) in cells of *Yersinia pseudotuberculosis* at elevated temperature, glucose availability and anaerobic conditions, which is typical for the parasitic phase of these Gram-negative bacteria, is accompanied by rearrangements in spatial structure of outer membrane protein OmpF that may impede the porin channel permeability for β-lactam antibiotics. In turn, plant polyphenols attract tremendous attention due to diverse biological activities. In particular, they can inhibit phospholipase A (PldA). The present study was aimed to test whether the changes in LPE content affect the resistance of bacteria to ampicillin. The addition of glucose to the culture medium was shown to increase simultaneously the level of LPE and minimal inhibition concentration (MIC) for ampicillin of *Y. pseudotuberculosis* cells. However, the co-administration of glucose and polyphenol extract from buckwheat husks (PEBH) restored the control MIC value, but PEBH had no effect on the low LPE cells growing in glucose-free medium. Therefore, the effect PEBH on susceptibility of *Y. pseudotuberculosis* to ampicillin is provided by the ability of PEBH to downregulate the LPE level in bacterial cells and thereby favor porin channel permeability. Thus, PEBH acts synergistically with ampicillin *in vivo* and can be used as antibiotic adjuvant in combination therapy to improve an ability of the conventional β-lactam antibiotics to cross the outer membranes of enteropathogenic Gram-negative bacteria. The work is supported by Russian Science Foundation (grant 15-15-00035-P). *The authors marked with an asterisk equally contributed to the work.

## P-28-008
## Effect of growth conditions on the growth rate, photosynthetic pigments content and pH value of new green microalga *Parachlorella kessleri*

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Green microalgae have flexible metabolism and able to grow under various conditions such as autotrophic, heterotrophic and mixotrophic conditions. These conditions may affect the photosynthetic pigments content and the growth rate of algae. The present work focuses on studying the effect of different growth conditions (photoautotrophic and photoheterotrophic, sulfur deprivation, presence of carbon sources and inhibitor, etc.) on the growth rate, pigments contents (chlorophylls (Chl) and carotenoids), and pH value of new green microalga *Parachlorella kessleri* RA-002 isolated from Armenia. The growth rate of *P. kessleri* was significantly higher during the algal growth under photoheterotrophic conditions in the presence of glucose as carbon source. Growth rates of *P. kessleri* decreased ~1.5-fold in sulfur-deprived conditions and by addition of the photosystem II inhibitor – diuron. Moreover, sulfur-deprived conditions affect the amount of algal photosynthetic pigments: the chlorophylls and total carotenoids content decreases ~1.4- and 1.8-fold, respectively. The highest amount of total carotenoids was found during *P. kessleri* cultivation in fructose containing medium. It was 1.4-fold higher than algae grown in the presence of glucose. In the presence of diuron the content of Chl *a* was not significantly changed, whereas Chl *b* and total carotenoids content decreased ~1.3–1.6-fold. Medium pH of algal culture grown under photoautotrophic conditions increased from 7.5 (initial pH) to ~8.0, which is associated with consumption of $CO_2$. Under photoheterotrophic conditions (glucose and fructose containing media) pH decreased up to ~7.0, which can be connected with the uptake of carbon sources and the formation of end-products of algal metabolism, such as $CO_2$. In the presence of diuron pH value did not undergo significant changes. Thus, the data obtained can be useful in biotechnology for regulating the growth of algae and the yield of biomass.


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## P-28-009
## Plant molecules and bacterial peptides synergetic action against quorum sensing

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Cell-to-cell communications in bacteria named "quorum sensing" (QS) is the ability to detect and to respond to population density by gene regulation. Many bacterial species use this mechanism to virulence factors induction and biofilm development that indicate QS as novel target for antimicrobial chemotherapy. Currently, numerous synthetic and natural compounds which led to quorum quenching (QQ) have been found, however their additive activity for QQ have not been evaluated. The goal of this study is to determine combined QQ activity of some plant-derived molecules and bacterial exoproducts. The compounds from *Scutellaria baicalensis* and *Eucalyptus obliqua* medicinal plants and supernatant from *Bacillus subtilis* 7048 probiotic strain were included in the study. The biosensor strain *Escherichia coli* JLD271 possessing luxI/luxR reporter plasmid *pAL103, and chemically synthesized C6-oxo-AHL autoinductor* were used for QS modeling. In this bioassay plant-derived molecules dianhydroglucitol; 5,6,7-trihydroxy-2-phenyl-chromen-4-one and 7,8-hydroxy-2 h-cromen-2-one (dihydroxycoumarin) decreased C6-oxo-AHL induced bioluminescence of sensor strain that was evaluated as QQ effect. The incubation of C6-oxo-AHL with *B. subtilis* 7048 supernatant decreased subsequent bioluminescence induction in sensor strain also. When plant-derived molecules and *B. subtilis* 7048 supernatant were used jointly, the expressed QQ activity was revealed for dihydroxycoumarin. We propose the mechanism of additive QQ effect through C6-oxo-AHL scavenging or degradation by bacterial exoproducts supplemented by dihydroxycoumarin-induced interruption of autoinductor perception. Taken together these results showed possibility of novel synbiotic containing bacterial strain and plant-derived molecules for cell-to-cell communication inhibition in pathogenic bacteria. The study was supported by Russian Science Foundation (project No. 0761-2019-0005).

## P-28-010
## Evaluation of *in vitro* antioxidant, antimicrobial and anticancer activities of the aerial part of *Cleome amblyocarpa* Barr. and Murb

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The present study was aimed to analyze the phytochemicals and to investigate the antioxidant, antimicrobial and anticancer activities of the aerial part of *Cleome amblyocarpa* Barr. and Murb, which is an important medicinal plant in Tunisia where it is widely used against colic and diabetes. Total phenolic content were estimated by the Folin–Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic acid equivalent (GAE) and total flavonoid content was estimated by aluminium chloride colorimetric method. Antioxidant activity was estimated by using different *in vitro* such as DPPH free radical scavenging activity, iron-chelating capability and ability to inhibit lipid peroxidation (TBARS). The antibacterial and antifungal activities of leaf and stem part of *Cleome amblyocarpa* were also investigated by the disc diffusion and microdilution methods. *In vitro* cytotoxicity of ethanolic extract of *C. amblyocarpa* was determined using crystal violet assay. *Cleome amblyocarpa* leaves possess high phenolic, flavonoid content and potential antioxidant activity, antimicrobial and anticancer activity in comparison to stem.

## P-28-011
## Morphological and functional changes in the small intestine and liver of laboratory animals after feeding with cavitation-treated feed

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The most promising and modern method of intensifying technological processes in food production is the use of liquid media activated by various means in combination with rational hydromechanical effects. Nowadays the activated liquid media with technologically significant functional properties get obtained by both electrochemical treatment and cavitative disintegration. A magnetostrictive transducer with 100 W power, 50 μm amplitude, and 27 kHz frequency was applied as the basic equipment for obtaining cavitation-treated products. We proposed to utilize zeolitized tuff (clinoptilolite) powder with the particle size of less than 1 mm for stronger effects of ultrasound on cellulosic waste. Broiler hens of Smena7 crosses were the object of our experiments to determine the effectiveness of the obtained product for animal feeding. Four groups (n = 30) of animals were under the same feeding and housing conditions during the preparatory period (14 days). Then animals of group I received starter mash; birds of group II – grower mash where 10% of the grain part were replaced by cavitation-treated wheat bran; for group III, 10% of the grain part were replaced by cavitation-treated wheat bran with the addition of zeolitized tuff powder (clinoptilolite) in amount of 1% with the particle size of less than 1 mm. The experiment lasted for 42 days. Liver and fragments of the small intestine were fixed in 10% buffered formalin and embedded in paraffin for the general histology analysis of organs. We revealed a significant increase of MEL in the intestinal mucosa of poultry when cavitation-treated products were added to their diet. At the same time, the most pronounced increase was in the experimental group with zeolitized tuff in comparison to the group that received cavitation-treated wheat bran. Studies were performed as part of research work for 2019–2020 at the Federal Research Center for Biological Systems and Agrotechnologies (No. 0761-2019-0005).

## *P-28-012*
## *Raphanus sativus* and *Amarantus sanguineus* transgenic plants transformed with *psl* gene

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Transgenic plants of *Raphanus sativus* and *Amarantus sanguineus* were obtained by floral dip method. At first, *A. tumefaciens* strain AGL-0, containing a genetic engineering construct, carrying the lectin gene *psl* under the control of the root-specific promoter, were cultured for 24 h on LB medium at a temperature of 28 °C. Next, the *A. tumefaciens* was centrifuged and resuspended in a solution containing 0.075 μM acetosyringone, 0.2% SilwetGold, 100 ng/l BAP and 30 g/l sucrose. Radish and amaranth inflorescences with buds and young flowers were immersed in the bacteria suspension for 1–2 min, then wrapped for 24 h. After seed collection the primary selection of


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transgenic radish and amaranth plants was carried out by keeping seeds obtained from transformed plants in hygromycin solution (150 mg/L) for a day. After that, the seeds were planted in containers with soil. Seeds were germinated in a climate chamber under optimal growth conditions. Plants with chlorosis were considered untransgenic. Green seedlings were subjected to qPCR to examine the expression gene psl, and then an immunochemical analysis to examine the presence of lectin on the root surface. Of the 500 radish and amaranth seeds treated with hygromycin, 27 radish plants and 55 amaranth transgenic plants were selected visually for further experiments. The RT-PCR analysis showed that efficiency of visual selection was 85% for radish and 20% for amaranth plants. Western blot analysis proved the presence PSL exclusively on root surface in about half of obtained transformed plants, where also the immunochemical analysis showed the localization PSL on surface of root hairs. In the future, the resulting transgenic plants will be used to create artificial symbiotic systems with rhizobia, recognizing the PSL lectin on root surface and capable of fixing atmospheric nitrogen in associative interactions without the formation of nodules. This work was supported by the grant of the RFBR 18-34-20004 mol_a_ved.

## P-28-013
## Stress regulating properties of two *Bacillus* sp. bacterial endophytes in plant culture *in vitro*

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*In vitro* environment imposes unfavorable conditions that lead to plant stress. Capacity of bacterial endophytes to regulate plant stress under *in vitro* conditions is largely unexplored. The aim of this study was to assess effect of two endophytic *Bacillus* sp. bacteria strains (Da4 and Oa4) on growth and oxidative stress response and gene expression in shoot culture *in vitro*. The two endophytic bacterial strains, isolated from apple buds, had contrasting effect on the apple cv. Gala shoot morphology after 3 weeks of co-cultivation. As compared to control, shoots inoculated with the strain Da4 had 2.2 and 1.8 fold larger biomass and adventitious shoot number, respectively. Meanwhile, strain Oa4 had inhibitory effect. Both endophytic *Bacillus* sp. strains reduced the shoot lipid injury level approx. 2.2 fold after 1 week of co-cultivation. The species-independent stress reducing and growth promoting effect of the strain Da4 was suggested by significant increase (~10%) in tobacco shoot biomass after 3 weeks of co-cultivation. Strain-specific regulation of expression of genes involved in jasmonic acid signaling pathway was observed. Strains Da4 and Oa4 upregulated *lox2* (~5 fold) and *aos* (~270 fold) genes, respectively. Meanwhile, expression levels of *coi1*, genes involved in salicylic acid *pr-1* and *wrky* as well as transcription factors *erf* regulating ethylene signaling pathway were similar. The results suggested that the two endophytic strains exploit an intricate interaction with plant tissues and had different plant stress regulating properties under *in vitro* conditions.

## P-28-014
## SUS4 and PHO1a are the main prolonged cold stress responsive genes in *Solanum tuberosum* tubers

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The postharvest quality of potato tubers strongly depends on carbohydrate metabolism. An important trend in potato breeding is the elimination of cold-induced sweetening (CIS). In the present study, 16 *Solanum tuberosum* cultivars contrasting in tuber total starch content and CIS resistance were analyzed for starch and sugar (sucrose, fructose, and glucose) content during prolonged cold storage (+3 °C; 8-month period September–April). HPLC analysis revealed that during cold storage, studied cultivars significantly differed in fructose and glucose accumulation dynamics (except cv. Saturna), while sucrose concentration in ten cultivars was unchanged, in four cultivars – decreased, and in two – showed a steep decrease in the middle storage point. Expression patterns of ten main carbohydrate metabolism genes, involved in sucrose and starch degradation, as well as the enzyme inhibitor genes, were evaluated. Eight of those genes (*PAIN-1*, *INH1*, *INH2*, *GBSS*, *GWD*, *BAM*, *AMY*, and *SBAI*) showed cultivar-specific expression pattern that did not correlate with starch and/or sugar content in tubers. The *SUS4* and *PHO1a* expression showed uniform growth during cold storage in all cultivars, except cv. Varyag. Although *PAIN-1* was reported as the crucial CIS-inducing gene, we observed that *PAIN-1* transcription was sharply downregulated in response to prolonged cold storage and, thus, does not involved in monosaccharide accumulation in potato tubers. We suppose that *SUS4* and *PHO1a* may serve as possible candidate genes for genome editing to obtain CIS resistant potato cultivars. The study was supported by the RFBR #18-29-07007 and Ministry of Science and Higher Education of the Russian Federation.

## P-28-015
## Pharmacological properties of *Salvia* species

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Nowadays, there is a great deal of interest in the discovery of drugs originated from natural products. Essential oils and extracts from Salvia species are a very promising source of bioactive compounds with a wide range of pharmacological properties, utilized as raw materials in the pharmaceutical industry. The genus *Salvia* belongs to Lamiaceae family and includes more than 900 species spread all over the world with bioactive compounds widely used in cosmetic industry. Since ancient years, Salvia species have been used in traditional medicine for the treatment of a wide range of diseases. Several studies have been carried out to verify the curative properties of Salvia species and find possible new pharmacological actions for these species. The majority of more than 120 bioactive compounds of *S. officinalis,* the most common species, were identified in its essential oil. The study of these components documented important properties such as antimicrobial, anticancer, antioxidant and anti-inflammatory. Particularly, essential oils of Salvia species have proved to have antimicrobial activity in a wide range of Gram-positive and Gram-negative bacteria. Also noteworthy is their inhibitory action in the growth of antibiotic-resistant bacterial strains. Pharmacological studies have also shown bioactive compounds of *S. officinalis* to have anti-inflammatory action, with some of these


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components showing greater activity than certain non-steroidal anti-inflammatory drugs. In addition, biological studies revealed extracts of Salvia species to have strong antioxidant activity through their free radical scavenging activity. Other studies have shown the promising action of Salvia essential oils against certain types of cancer cells. Further research is required to enhance the already existing results for Salvia species and to study the existence of new properties, especially in the case of endemic Salvia species and of species grown in different environmental conditions.

## P-28-016
## Novel approach for stabilization of *Bacillus anthracis* recombinant protective antigen – the main component of new generation vaccines against anthrax

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Modern vaccines against anthrax are highly needed because *Bacillus anthracis* is one of the most dangerous bioterrorist agents, its spore are highly resilient and can be easily aerosolized and disseminated. The important problem in new vaccines designing is the stabilization of anthrax protective antigen (PA). PA is a central toxin component playing a key role in the defense against *B. anthracis*. The majority of epitopes capable of generating antibodies with toxin-neutralizing activity are mapped on the all PA domains. Thus, full-size 83-kDa recombinant PA (rPA) is the most optimal for vaccine devising. The aluminum adjuvants are used in almost all candidate anthrax vaccines that are in clinical trials. However, there are data indicating that rPA adsorbed on aluminum hydroxide is unstable, can undergo proteolysis and loses its ability to induce neutralizing antibodies after storage. Here we propose novel approach of rPA stabilization based on adsorption of rPA on the surface of structurally modified plant viruses. Previously we have shown that thermal remodeling of rod-like tobacco mosaic virus leads to formation of RNA-free spherical particles (SPs). SPs are stable under physiological conditions, safe, biodegradable and have unique adsorption properties. In present work, we revealed that SPs could significantly lower proteolytic degradation of rPA in different temperature conditions (4 °C, 25 °C). Furthermore, we demonstrated the ability to lyophilize rPA-SPs compositions into dry formulation, while rPA retained its antigenic specificity. We suppose that adsorption of rPA on SPs surface play a crucial role in rPA stabilization. It should be noted that according to our data SPs can play not only the role of stabilizing platform but also an effective adjuvant and can be the basis for development of novel candidate vaccine. This work was supported by the Russian Science Foundation (Grant No. 18-14-00044).

## P-28-017
## Characteristics of cyanobacterial DnaJ homologue (sll1384) that binds to RuBisCO large subunit

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Carboxylase / oxygenase ribulose-1,5-bisphosphate (RuBisCO) is the key enzyme in global carbon cycle, responsible for attaching atmospheric $CO_2$ to pentose and therefore for initial step of biomass generation. Despite its dominant role in nature, the enzyme is a rather slow catalyst. A single enzyme molecule is able to carry out only several carboxylation reactions per second. Due to the fact that RuBisCO has been considered as an ineffective in catalyzing RuBP carboxylation, and that it catalyzes the unfavorable reaction of photorespiration, it is considered as a factor that is limiting the photosynthesis and, consequently, the obvious target of research for the increase in agricultural production efficiency by looking for ways to improve its kinetic parameters. The major obstacle to this venture is the complex RuBisCO biosynthesis process that requires a multiple chaperone proteins. The prokaryotic RuBisCO from *Synechocystis* sp. PCC6803, unlike other cyanobacteria, does not fold in *E. coli*, suggesting a lack of specialized folding factor or insufficient homology to existing factors in this bacterium. However, it has been showed recently, that plant RuBisCO assembly in *E. coli* cells with five chloroplast chaperones including BSD2 which has a zinc-finger domain what suggest that there might also be additional factor for cyanobacterial RuBisCO assembly in *E. coli* system and finding this out is the main goal of our studies. During some of our *in vitro* studies we showed that RuBisCO large subunit (RbcL) from *Synechocystis* sp. PCC6803 binds to one of seven DnaJ homologues (DnaJ sll1384) from derived cyanobacteria and we hypothesize that this particular protein is the missing factor in RuBisCO folding in *E. coli* cells. Here we present the characteristics of binding between DnaJ sll1384 and RbcL.

## P-28-018
## Development of the direct PCR method for tomato tissues

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The PCR method is mostly used to identify or amplify the specific gene in plant. To perform the PCR, usually genomic DNA is isolated and purified from homogenized plant tissue. This DNA extraction method is inefficient when handling a lot of samples because it requires cost and labor. Therefore, we established a method to identify plant genes by the direct PCR without homogenization of plant tissues and DNA extraction. To perform PCR directly in plant tissue without extracting DNA, three methods were used for tissue treatment: heat treatment, protease K treatment, and β-mercaptoethanol treatment method. The target genes were amplified by each method and the amplified DNA intensities were not significantly different between them. Therefore, the heat treatment method which is the easiest of the three methods, was used in this study. The ascorbate peroxidase 6 (APX 6) gene, a single copy of the tomato genome, was amplified directly by PCR using the heat treatment method without DNA extraction. The APX 6 gene was amplified in tomato leaves, mature green and breaker stage fruits. But the gene was not amplified in ripening fruit after orange stage. A different plant tissue treatment method should be develop to use the direct PCR method at the ripened tomato fruits. *The authors marked with an asterisk equally contributed to the work.


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## P-28-019
## Isolation of major phenolic compounds from the extract of *Prunella grandiflora* L. grown in Russia and its cytotoxic effect by the example of the model object of *Drosophila melanogaster*

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The effectiveness of medicine is accompanied by side effects of varying severity, including toxic when it comes to antitumor medication. The method we used is based on the use of cytostatic etoposide together with the extract of the medical plant *Prunella grandiflora L.* (*P. grandiflora*), which reduces the toxic effect in the model object *Drosophila melanogaster*. The data was obtained from *P. grandiflora* on the quantitative content of flavonoids and phenol carboxylic acids by HPLC (high performance liquid chromatography). In *P. grandiflora* rosmarinic acid is dominant and accounts for 70–89% of the amount of phenol carboxylic acids. In *P. grandiflora* up to 12.5% of the amount of phenol carboxylic acids of ferulic acid, up to 9.9% of syringic acid, up to 5.8% of *n*-coumaric acid, up to 2.8% of caffeic acid are noted. *P. grandiflora* contains up to 41.4% kaempferol in of the total amount of flavonoids, quercetin – up to 31%, rutin – up to 27.6%, luteolin – up to 13.9%, kaempferol-3-glycoside – up to 13.1%. Rosmarinic acid, kaempferol, quercetin and rutin, as the main compounds in the extract of *P. grandiflora*, have antitumor, antitoxic and antioxidant effects. To assess the overall toxic effect, the wild type strain of Oregon-R *Drosophila melanogaster* was used. For this, larvae were placed in a nutrient medium containing 1% extract, etoposide 800 μg / kg of the medium, or extract and etoposide together. Their lethality was estimated after the adult flies' departure. LD was calculated based on specimen deaths at different stages of development: in the control sample, the overall lethality was 48%, with the addition of etoposide 43.61%, the extract – 47%, and with the combined effect of the extract and etoposide – 28%. The cameral phase of the study showed the decrease in genotoxicity with the combined effect of etoposide and the *P. grandiflora* extract.

## P-28-020
## Size matters!? Influence of giant cells on ER body formation in *Arabidopsis thaliana*

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Since plants cannot run away from danger they have developed highly sophisticated defence strategies. A well-known defence system in plants is the mustard-oil bomb system. Here, glucosinolates are deglycosylated through myrosinases (β-glucosidases) and form so called isothiocyanates, chemicals toxic to herbivores. In the classical mustard-oil bomb system enzyme and substrate are stored in vacuoles of different cells and only mix upon break down of the cells. Recently it has been shown that *Arabidopsis thaliana* additionally harbours so-called ER (endoplasmic reticulum) bodies which are spindle shaped, 10 μm long organelles containing high amounts of β-glucosidases (PYK10/BGLU23; BGLU18). ER body formation can be induced extensively through wounding in leaves. Recently however, ER bodies have been found to be also constantly present in so called giant cells which are formed at the leaf margins, above the midrib and randomly throughout the leaf blade and are characterised by their large appearance as a result of endoreduplication. Furthermore, giant cells show a differing gene expression from their neighbouring cells. We found, that ER body formation does not depend on endoreduplication. Mutations that abolish endoreduplication and thus giant cell size but do not alter the general gene expression profile have minor effects to ER body formation. However, mutations in genes promoting giant cell identity lead to a massive reduction in gene expression of ER body related genes. Our studies give new insights into the complex regulation underlying the ER body formation.

## P-28-021
## Bicarbonate treatment improve photosynthesis efficiency in *Arabidopsis thaliana* npq 4-1 mutant with overexpression of genes coding βCA1 and βCA2 proteins

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The photosystem II (PSII) protein PsbS has an essential role in qE-type nonphotochemical quenching (NPQ), which protects plants from photodamage under excess light conditions. Transgenic tobacco plants with enhanced PsbS expression showed greater biomass production by 20%, and reduction in water loss per $CO_2$ assimilated by 25% compared to the wild-type, under laboratory and field conditions. Contrary, mutant plants that lack PsbS (*npq 4*) had impaired NPQ mechanism, and thus had a lower biomass production, seed yield, and used water less efficiently under laboratory and field conditions. β carbonic anhydrases (βCAs) catalyze reversible interconversion of $HCO_3^-$ to $CO_2$ and water, are also involved in $CO_2$ assimilation and bicarbonate ions uptake by roots in plants. Published researches showed that a single manipulation in β*CA*s genes does not influence significantly on plant productivity. While modification in a group of β*CA*s may improve physiological effect and phenotype. *Arabidopsis thaliana* β*CA1* and β*CA2* genes were overexpressed in *npq 4-1* mutant in single and double configuration using stable Agrobacterium-mediated plant transformation method. Transgenic homozygous plants with overexpression of β*CA1* and/or β*CA2* were generated. Based on Real Time RT-PCR analysis *npq 4-1* lines with the highest expression of β*CA1* and/or β*CA2* were chosen. Target genes expression level was higher in the range of 2-12 for β*CA1* gene and 90-600 for β*CA2* gene. Modifications of β*CA*s genes in *npq 4-1* are expected to reduce programmed cell death (PCD), improve biomass production and water use efficiency (WUE) under variable laboratory and field conditions. Preliminary results showed that *npq 4-1::*β*CAs* transgenic plants are more adapted to variable laboratory conditions in the light dependent manner.

## P-28-022
## Methods for the identification and quantitative analysis of biologically active substances from vitamin plants raw material

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The beneficial effect of solution extracted from fruits of *Rosae, Ribes nigrum* and *Sorbus aucuparia* on vasopermeability is associated with the content of flavonoids such as rutin, quercetin,


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myricetin, isorhamnetin, kaempferol, catechins and leukocyans. This group of biologically active substances is also responsible for the choleretic, diuretic, anti-inflammatory actions. Moreover, studies have also proved their antimicrobial action against bacterial and fungal biofilms. Consequently, it is advisable to develop a method of quantitative determination for these biologically active substances. In this study, we analyzed the content of flavonoids in vitamin mixtures No. 1 – *Fructus Rosae: Sorbus aucuparia* (50:50) and No. 2 – *Fructus Rosae*: *Ribes nigrum* (50:50) and their components. We established the importance of pH value for the complexation reaction of flavonoids with aluminum chloride. A 1.56–2.25 pH value during the complexation reaction contributes to an increase in the selectivity of the reaction on the ketone group of ring C and the hydroxyl group of ring A of flavonoids. We also determined the possibility of identifying the *Fructus Rosae* and vitamin mixture No. 1 by spectral characteristics: 323, 404, 526 nm and 311, 414, 540 nm, respectively. The kinetics of the complexation reaction for the vitamin mixtures, individual fruits of *Rosae, Ribes nigrum*, *Sorbus aucuparia* and infusion from the vitamin mixtures was also analyzed. The optimal time of complexation was 40–50 min. For the infusion, this process is longer, since it is competing with hydrolysis, and, therefore, requires more time to establish equilibrium. We have developed a method for spectrophotometric determination of total flavonoid content. The method was tested and validated for *Fructus Rosae*, *Ribes nigrum*, *Sorbus aucuparia*, vitamin mixtures and their infusion. The publication has been prepared with the support of the "RUDN University Program 5-100"

## P-28-023
## ICML – algorithm for gene classification from different species by expression patterns

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Over the past decade, the development of high-throughput sequencing technologies has resulted in the accumulation of a large number of genome sequences for a large amount of species. As a result, there is a great need for functional annotation of these new sequences. Functional annotation of genes in most cases is carried out by searching orthologs in the nearest species and transfer to them functional data of orthologs. The search for orthologs is based on sequence similarity; there a variety of tools for orthology assessment such as OrthoFinder, OMA, OrthoMCL, and many others. However these methods have limitations especially in large and complex genomes shaped by whole genome and segmental duplications. Also, despite widely accepted assumption that the orthologs share the same function, this is not always the case. In order to improve the accuracy of the transfer of functional annotation from model species to non-model ones, it is necessary to use additional information, such as the gene expression profiles. We developed ICML (Interspecific Classifier based on Machine Learning) – the approach that allows classification of genes according to their expression profiles between two species. The main problem that arises when comparing expression profiles in species with differing morphologies and/or developmental rates is the inability of direct matching of samples. This makes it impossible to use standard metrics (for example, Euclidean distance) for the assessment of the similarity of expression profiles. Thus we developed a new metrics based on machine learning methods. a proof of concept, we performed the comparison of the genes of two plant species belonging to distant taxonomic groups – *Arabidopsis thaliana* and *Fagopyrum esculentum*. We expect that the wider application of this algorithm will increase the accuracy of functional annotation in complex genomes. This research was funded by the Russian Science Foundation, grant number 17–14-01315.

## P-28-024
## C-terminal modification of TMV coat protein allows assembly of chimeric particles in plants suitable for "green" biotechnological applications

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Recombinant viruses based on cDNA copy of Tobacco mosaic virus (TMV) are useful for accumulation of chimeric particles carrying different immunogenic epitopes in plants. Earlier our group created vectors expressing conserved M2e and fp short antigens (23 and 14 aa) from Influenza A on the surface of TMV virions (Petukhova *et al*., 2013, 2014; Gasanova *et al*., 2016). Chemical conjugation of larger proteins to the particle could be efficient method to improve vaccination strategy. For this we constructed TMV genome containing additional reactive lysine at the C-terminus of coat protein (CP). Inserted sequence including lysine was optimized according to plant and viral codon usage. Agroinfiltration of *Nicotiana benthamiana* leaves led to delayed systemic spread of TMV-C-lys with symptoms different from the wild-type (wt) infection. We observed deformation (curling) of upper leaves (7–10 days post inoculation, d.p.i.) as well as necrotic lesions of stem and petioles of lower leaves (17–19 d.p.i.). Unlike TMV-wt and vector TMV-M2e-ser (Petukhova *et al*., 2014) plant growth and development were not disturbed. Modified particles were successfully purified from non-inoculated leaves (21 d.p.i.). Electron microscopy of preparations demonstrated that shape of TMV-C-lys virions varied from rigid to slightly curved rods (300–700 nm in size). The resulting particles can be a perspective platform for chemical conjugation (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/ N-Hydroxysuccinimide) with broad spectrum of Influenza (M1, NP) and other peptides and proteins. Stable complexes consisting of lengthy carrier and foreign protein(s) should be recognized by antigen-presenting cells (APC) such as dendritic cells and macrophages.

## P-28-025
## Effects of short-term drought on metabolism and nutritional properties of pea (*Pisum sativum*) seeds

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Drought is one of the major environmental stressors, affecting crop productivity. Plant response to dehydration is accompanied with metabolic changes, oxidative stress and enhanced generation of advanced glycation end products (AGEs), which are known to


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be pro-inflammatory in mammals, impacting on the pathogenesis of diabetes mellitus and ageing. The protein-rich seeds of legumes might be a target of such deleterious changes. However, the effects of drought-related protein damage on the quality of crops, as well as its influence on human health are still unstudied. Therefore, here we address the changes in seed quality, metabolome, protein AGE contents and nutritional properties of pea accompanying short-term drought, applied at the step of seed maturation. For this, pea plants were grown on vermiculite for 6 weeks before transfer to aqueous medium, to which 5 days later polyethylene glycol 8000 (PEG-8000) was supplemented. Two days later, the plants were transferred to PEG-free substrate and grown till the end of seed maturation. After evaluation of seed quality, seed metabolites were analyzed by GC- and LC-MS. Modulation of inflammation was evaluated with corresponding exhaustive enzymatic hydrolyzates in the model of human neuroblastoma cell line SH-SY5Y. For this, dynamics of proteins involved in inflammation-related signaling was assessed by Luminex xMAP multiparametric immunofluorescence technology. In parallel, profiles of individual AGEs were addressed in the same hydrolyzates by LC-MS/MS. Although no effect on AGE profiles was observed, clear alterations in primary metabolome and regulatory response were observed. This work is supported by the Russian Science Foundation (Grant No. 17-16-01042).

## P-28-026
## Characterization of the tomato P450-72 (SlP450-72) and analyze its function in tomato plant

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Plant P450s are necessary for the catalysis of many major reactions, typically in production of primary and secondary metabolites. The functions of these P450s involve the biochemical synthesis pathways include of terpenoids, fatty acids, lipids, as well biosynthesis of plant hormones. Also, they involve in many reactions that synthesis the metabolites which are related to defense mechanism. Plant P450s play such important roles in plants, and there are hundreds of genes in one plant. Tomato plants also contain 300 – 400 CYP genes in their genome, however many of them are unknown their function. The *SlP450-72* gene in CYP736A subfamily, which is not yet revealed its function in tomato, was isolated from tomato. To determine enzymatic character of the *SlP450-72*, it was heterologously expressed in *E. coli* and purified from them. The *SlP450-72* enzyme was analyzed its enzymatic activity and used for the substrate screening. In this study, we found out that the *SlP450-72* enzyme catalyze hydroxylation of resveratrol. To investigate its function in tomato plant, we developed the *SlP450-72* over-expressed transgenic tomato plants, and analyzed them.

## P-28-027
## Comparison of the CaCPR1 and CaCPR2 expression pattern in hot pepper

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The cytochrome P450 is the largest enzymatic protein family in plants and they plays important roles in development and defense system in plant. For P450 reaction, NADPH-cytochrome P450 reductase (CPR) required for electron transfer from NADPH to cytochrome P450. There are two CPR genes in the hot pepper genome which are *CaCPR1* and *CaCPR2*. The CaCPRs expression levels were quantitative by real-time PCR in various hot pepper tissues and under stress conditions such as JA treatment, SA treatment and drought condition. The *CaCPR1* expression level was gradually increased during fruit ripening. However, the *CaCPR2* gene was constitutively expressed in all tissues but the expression level was lower than the *CaCPR1*. Under the stress conditions, both of the *CaCPR1* and *CaCPR2* expression levels were increased, the *CaCPR1* expression was maintained high level compared to the *CaCPR2*. These results suggest that the CaCPR1 is major enzyme during plant development and under stress condition. In addition, under stress condition, both enzymes are increased because more enzymes are needed against stress. To investigate the enzymatic properties, two *CaCPRs* were isolated from hot pepper (*Capsicum annuum* L. *cv. Bukang*) and heterologously expressed in *Escherichia coli*. The enzymatic activities were assessed using protein and chemical substrates such as MTT and ferricyanide. *The authors marked with an asterisk equally contributed to the work.

## P-28-028
## D/H modification of plant peptides and microelements metabolome

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The aim was to investigate changes in the metabolism of peptides and essential microelements in plants at various D/H ratios in water. Before D/H control of chemical kinetics in water solutions under low deuterium concentrations were investigated and summarized in details for molecular systems and living cells. Peptide analysis was performed on a time-of-flight mass spectrometer, equipped with a UV laser. Zinc was analysed in plants by atomic absorption spectrometry with electro-thermal atomization and Zeeman effect and X-ray fluorescence energy dispersive. Water samples: deionized high ohmic water, (BD, D/H=140 ppm), Deuterium depleted water (DDW, D/H=12 ppm). The germination of moss in waters with different isotope ratios led to the change in the qualitative composition of proteins. A total of 300 proteins were determined. 58% of them were observed both in BD and DDW. 13% were observed only in DDW, and 29% were only in the water with a natural D/H isotope ratio. This change leads to differences in metabolism in the plant as a whole. For example, in growing plants (*Capsicum annuum L.*) in DDW, the zinc content increases by 1.5 times compared with BD. In addition, the technology of essential microelements enrichment in the leaves *Callisia fragrans L.* at different ratios of hydrogen isotopologues in water has been developed. In 12 days the zinc content in the leaves reached μg/g: 0.01–0.02 (BD without chelate); 0.43±0.05 (BD with chelate); 1.75±0.25 (DDW with chelate). As can be seen, the coefficient of zinc accumulation in leaves during incubation of shoots in DDW with zinc glycinate differs many times. Biogeochemical limitations of Zn accumulation in a medicinal plant can be overcome by a kinetic approach — modified water isotopic composition. Such a result will allow offering special metal-modified medicinal and food plants as supplements in zinc-deficient states in the future.


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## P-28-029
## A study on the antioxidant and antibacterial effect of plastochromanol-8

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Plastochromanol-8 (PC-8) is a natural component of plant tissues, discovered 50 years ago in the leaves of rubber trees. Together with tocopherols and tocotrienols, PC-8 belongs to a group of lipophilic antioxidants named tocochromanols (vitamin E). PC-8 can be found in leaves, seeds and other organs of higher plants. While tocopherols and tocotrienols have undergone extensive study, knowledge about the physiological functions of PC-8 is still limited in spite of its wide distribution throughout the plant kingdom. The aim of this study was to investigate the antioxidant and antibacterial properties of PC-8, with comparison to other tocochromanols. First, dozens of plant species underwent testing by high performance liquid chromatography, with a fluorescence detection, to examine the distribution of PC-8. In the majority of plant species studied, PC-8 was present. High contents of PC-8 were detected in: linseed, wheat germ and grapeseed oils as well as in *Crassula argentea* leaves. Then, the antioxidant activity of PC-8, and other tocochromanols, were measured in different liposomes based on the ability for lipid peroxidation inhibition. Two azoinitiators, the hydrophilic 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and the hydrophobic 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) were utilized to investigate the place of antioxidant action in the examined compounds. Finally, the antimicrobial activity of PC-8 underwent evaluation through the agar diffusion method on five bacteria species (*Bacillus cereus, B. subtilis, Escherichia coli, Staphylococcus aureus* and *S. faecalis*). Deeper understanding about sources and the physiological role of PC-8 is not only significant from a scientific point of view, but possible applications in medicine, pharmacy or cosmetology endorse its importance.

## P-28-030
## Anti-oxidant and wrinkle activities of constituents from Polygonaceae

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*Persicaria filiforme* is an annual plant in the family Polygonaceae which is distributed in whole of Korea. Since early times, this plant has been used as a folk medicine with beneficial effects for the treatment of various disease. However this plants has not been reported yet about bioactive cosmetic ingredients. In the course of screening for antioxidant ingredients by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl(DPPH) and ABTS radical scavenging assay a total methanolic extract of *Persicaria filiforme* was found to show potent antioxidant activity. To identify new active anti-wrinkle Ingredients, this study investigated the elastase inhibition assay of *Persicaria filiforme*. The results showed that the *Persicaria filiforme* methanolic extract has the 1,1-diphenyl-2-picrylhydrazyl(DPPH) and ABTS radical scavenging activity ($IC_{50}$ 24.3 and 9.5 μg/mL). In the elastase inhibition assay, the $IC_{50}$ of methanolic extract of *Persicaria filiforme* was 100 μg/mL. The *Persicaria filiforme* methanolic extract partitioned with n-hexane, $CH_2Cl_2$, EtOAc, n-BuOH, and aqueous fractions. The purification of EtOAc soluble layer by column chromatography separation and MPLC analysis to Compounds 1–4. It was identified as Quercetin, Gallic acid, Catechin, and Epicatechin. Structure was elucidated by a combination of 1D and 2D NMR, and MS spectrometry as well as comparison with reported literatures.

## P-28-031
## Determination of the signal sequence for thylakoid targeting in the OE23 transit peptide

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Chloroplasts have a complex structure, with three types of the suborganellar membrane, which are outer membrane, inner membrane and thylakoid membrane. Nuclear-encoded chloroplast protein requires the transit peptide signal for translocation to chloroplast. Particularly, thylakoid targeted proteins have bipartite transit peptide, one part is to target for chloroplast which is removed after stroma translocation and the other part is to target for thylakoid, also removed after translocation. To determine the signal sequence for thylakoid targeting, we used the transit peptide of 23 kDa oxygen-evolving protein (OE23). The OE23s are involved in photosynthesis and they have bipartite transit peptide to transfer thylakoid lumen. Four different transit peptides of the OE23 were isolated from tobacco (*Nicotiana tabacum* cv. Xanthi NC) and fused to *gfp* gene, then cloned into plant expression vector and transformed into tobacco. The GFP was localized into thylakoid lumen and the transit peptide was removed from GFP after localization. In order to determine which transit peptide sequence is mediated thylakoid targeting, we performed deletion experiment. The N-terminal 38 amino acids which are first cleaved from the GFP after translocation, is mediates translocation across the two membranes of chloroplast. To localize of protein into thylakoid majorly the Tat pathway signal which contain twin arginine, is needed. In this study, we confirm that the 20 AA from the cleavage site which is included twin arginine is mediated the translocation into thylakoid. Also only 10 AA from the cleavage site without twin arginine can mediated translocation into thylakoid. *The authors marked with an asterisk equally contributed to the work.

## P-28-032
## Investigation of flavonoids in *Mansoa alliacea* (Lam.) leaves

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Introduction *Mansoa alliacea* is a native Amazonian plant to South America, belonging to the family of Bignoniaceae. *Mansoa alliacea* is widely used by many of the indigenous peoples of the Amazon, with almost all parts of the plant being used. The screening for the phytochemicals present in the extracts of *Mansoa alliacea* (Lam.) revealed the presence of alkaloids, tannin, phenol, flavonoids, glycosides and sterol. The purpose of research was to determine the amount of flavonoids in *Mansoa alliacea* leaves. Methods About 1 g of medicinal plant material, is placed in a conical flask with a capacity of 250 mL, 50 mL of ethanol at 70% are added, and it was boiled in a reflux bath for 20 min. Then cool the extract to room temperature. The contents of the flask are thoroughly shaken and filtered through a dry paper filter into a dry flask with a capacity of 200 mL. Aliquot of 3 mL of extract in was transferred to 25 mL measure flask, 1 mL of $AlCl_3$ 2% solution and 2 drops of acetic acid 30% solution was


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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added, finally filled 70% ethanol up to the mark. The absorbance was measured after 60 min from the reaction beginning. Conclusion On the differential absorption spectrum, two maxima were detected at wavelengths of 391±2 and 438±1 nm, which made it possible to determine two groups of flavonoids, in terms of apigenin and quercetin. The content of flavonoids in terms of quercetin was 0,171±0,005%, apigenin content was equal 0,866±0,010. The publication has been prepared with the support of the "RUDN University Program 5-100"

## P-28-033
## Flavonoids and anthocyanins content diversity in rye forms

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Rye is a cereal traditional for Northern and Eastern Europe with very good resistance to abiotic stresses and ability to grow on poor soils. Because of relatively recent domestication and obligate cross-pollination rye still possesses a great diversity of gene variations leading to variations in content of secondary metabolites. In our study we evaluated anthocyanins and flavonoids content in grains of 24 rye forms and inbred lines (Peterhof collection of rye) by HPLC-ESI-MS. There were 4 types of grain colours: colourless, yellow, green, brown and violet. Only in green and violet seeds anthocyanins were detected: mostly, cyanidin and peonidin as aglycones and rutinoside and glucoside as sugar residues. Variations of anthocyanins content are considerable even in cases of closely related rye forms and forms with the same phenotype. Variability of detected flavonoids and other secondary metabolites correlates with the grain colour: the least in colourless seeds and yellow-seeded forms, the greatest in violet seeds. The most interesting differences in flavonoids content were revealed for 5 lines (vi1, vi2, vi3, vi4 and vi6) which carry recessive mutations, leading to anthocyaninless phenotype. The differences between lines in content of flavonoids, including colorless precursors of anthocyanins, allowed us to suggest candidate genes for vi mutations. Our results confirmed wide variability of flavonoids and anthocyanins content in rye. These substances are involved in plant pigmentation, UV filtration and also are a valuable elements of food with anti-inflammatory, anti-cancer activities. Wide variability existing in rye is a good source for breeding of specialized rye cultivars as well as a genetic reserve of the valuable traits to be used in related cereals, including wheat. This study was supported by RFBR (grant No 19-016-00205) and state budget (project "Genetics and breeding of rye on the base of natural hereditary diversity").

## P-28-034
## Genetic diversity of CAD genes in *Linum usitatissimum* L.

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Flax (*Linum usitatissimum* L.) is an important crop that is used for production of seed and fiber. The quality of flax fiber is determined by many factors, and lignin content is an essential characteristic, which is important for the use of fiber in textile and composite productions. Cinnamyl alcohol dehydrogenase (CAD) is involved in the control of lignin formation, therefore the study of *CAD* genes is necessary for understanding of lignin biosynthesis and breeding of improved cultivars. Polymorphism of *CAD* genes was estimated in 288 flax cultivars and lines with lignin content varied from 2% to 7.5% (Institute for Flax, Torzhok, Russia). DNA was extracted from pools of at least 40 seedlings for each cultivar and line using CTAB protocol. Primers were designed for amplification of full sequence of *CAD* genes and their probable promoter region using overlapping DNA fragments with 450–500 bp in length. DNA libraries for high-throughput sequencing were obtained by two successive polymerase chain reactions according to the Illumina recommendations for 16S metagenomic sequencing library preparation with modifications. Evrogen (Russia) reagents were used in preparation of DNA libraries, whose quality and concentration were evaluated by Qubit 2.0 fluorometer (Life Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies). High-throughput sequencing was performed on MiSeq Illumina with 300 + 300 read length. The pipeline was developed for bioinformatics analysis of the sequencing data. The average coverage of *CAD* genes for an individual sample was over 100x. Polymorphism of the genes within studied flax cultivars and lines was assessed. Obtained results contribute to evaluation of genetic diversity of flax and determination of association between *CAD* allelic variants and lignin content in flax fibers. This study was funded by the Presidium of the Russian Academy of Sciences, Program No. 41 «Biodiversity of natural systems and biological resources of Russia».

# Natural networks and systems

## P-29-001
## Identification of unique molecular signature of browning in human primary adipocytes from deep and subcutaneous neck fat

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There are two types of thermogenic adipocytes, classical brown and beige (BAT) which are UCP1-positive dissipating energy as heat. BAT markers have been well studied in rodents but detailed molecular studies are still lacking in humans where BAT is interspersed at several sites and may serve as a target of anti-obesity therapies. Our study aims to identify the unique signature of browning in human primary adipocytes from the different anatomical location by analyzing global gene expression patterns. Preadipocytes were obtained from subcutaneous (SC) and deep neck (DN) and differentiated to white and brown adipocytes. We analyzed differential gene expressions by total RNA sequencing, molecular pathways by KEGG Mapper, genetic constraint by ExAC and verified several genes of interest associated with adipocytes browning. We identified 37 genes which are closely clustered to UCP1. Out of those 13 genes have been already described to play a role in thermogenesis (CIDEA, CKMT1A/B), while the roles of the others are still unclear (ANO5, FAM151a). Several pathways were represented, such as retinoic acid biosynthesis which was upregulated (CPT1, CYP261B), while extracellular matrix organization pathways were among the downregulated ones (COL, ITGF). Mitochondrial creatine kinases, CKMT1a/b, are reported to play role in UCP1-independent thermogenesis; UCP1 and CKMT1a were expressed higher in DN, as compared to SC adipocytes and this was verified by RT-qPCR. Several


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transporters were expressed higher in DN, such as transporter of amino acids (SLC7A10), glutamate (SLC25A18) and pyruvate (SLC16A7). Our data proves that progenitors from DN fat can be differentiated to browning adipocytes at a greater extent than SC ones. We have started to investigate revealed molecular elements not linked yet to browning by deleting, inhibiting or overexpressing them This work is supported by the GINOP-2.3.2-15-2016-00006 project co-financed by the European Union

## P-29-002
## S2B (Specific-Specific Betweenness): a novel method to prioritize cross-disease associated genes and its appliance in motor neuron degeneration as case of study

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Cell functions are accomplished by the interaction of numerous components on biological networks. In the same way, patho-phenotypes are the result of molecular alterations that cannot be deciphered through reductionist approaches. Indeed, the discovery of new associations between genes and diseases is crucial for the understanding of pathological mechanisms and for the establishment of new therapeutic targets. It is commonly assumed that proteins that physically interact are more likely involved in similar functions. Likewise, proteins that interact with disease-genes are also suspected to be involved in the same disease. Thus, we conjecture that diseases with similar phenotypes are triggered by alterations in proteins localized in a close neighborhood. We present a novel network-based method called S2B (Specific-Specific Betweenness) for disease-associated genes prioritization able to identify proteins linking two disease phenotypes. S2B was applied to Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy diseases, both characterized by motor neuron degeneration. By using artificial disease modules, we saw that proteins with higher S2B scores are more likely part of the disease modules overlap. Interestingly, S2B method was robust to interactomic artificial noise and disease-associated gene list size incompleteness. S2B candidates are enriched in functions associated to motor neuron degeneration and pinpoint interesting pathways that might bring novel cross-disease mechanistic hypotheses. Additionally, several S2B candidates were already associated to other mental or neuro-muscular disorders. These results were recently published in a peer-review journal and S2B function, disease-gene associations and Protein-Protein Interaction (PPI) data is publicly available. Work supported by BioSys-PhD programme by FCT(PT) (PD/BD/128109/2016) and EU Joint Programme supported through FR/ANR, GE/BMBF, PT/FCT and SP/ISCIII (UID/MULTI/04046/2013 JPND-CD/0002/2013).

## P-29-003
## Natural yeast-like fungi strains producing potential antibacterial agents

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Lack of strict control of antimicrobial therapeutic and veterinary agents' usage has resulted in spread of antibiotic resistance among pathogenic bacteria forcing the humankind to expand spectra of new antibacterial drugs. Natural compounds with the efficacy verified as a result of natural selection till now constitute a source of material for further optimization in laboratory conditions. Our study is aimed at finding and investigation of low-molecular weight antimicrobial compounds, which are produced by the strains of the unique collection of yeast and yeast-like fungi. This collection, containing more than 2500 strains, was formed by B.F. Yarovoy and V.P. Stepanova during several expeditions to extreme regions of Russia: the Kuril Islands, the Kamchatka Peninsula and Sakhalin Island. The diversity of species from extreme natural areas is under-investigated, and our research may contribute to the discovery of previously unknown antimicrobial agents. We perform the analysis of yeast and yeast-like fungi culture liquids applying high-throughput bacterial system that expresses specific reporter fluorescent proteins in the presence of substances impairing translation or inducing SOS response in the analyzed mixture. In our experiment, we use fresh culture of bacterial strain with the test system and analyze two fluorescence signals of reporter proteins (ex/em wavelengths: 553/574 nm and 588/633 nm) normalized to the bacterial culture growth in the presence of the culture liquids samples to evaluate the presence of potential antimicrobials. We have analyzed 600 samples from the collection and identified samples inhibiting bacterial cell growth, as well as enhancing the expression of SOS response reporter proteins. No samples inhibiting translation were found pointing to the fact that this mechanism of combating bacteria is not characteristic for yeast and yeast-like fungi. This work is supported by the RFBR grant 17-00-00368.

## P-29-004
## Tick-borne pathogens in sympatric areas of *D. reticulatus* and *I. ricinus* ticks in Latvia

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Ticks and transmitted pathogens are important in human and animal health, and they are examples of the ecological complexity of parasitic associations in a landscape. These natural networks are highly structured, and the recent studies indicate that ticks and hosts interact along the shared environmental gradient, while pathogens are linked to groups of phylogenetically close reservoirs. In this study, we analysed the presence of two epidemically-important pathogens, Tick-borne encephalitis virus (TBEV) and Borrelia, in sympatric areas of *I. ricinus* and *D. reticulatus* ticks which were recently appeared in several regions of Baltic States. In total, 1960 ticks were collected, geolocated and analysed. RNA and DNA were isolated, and the samples were screened for the presence of TBEV and Borrelia using molecular RT-PCR and PCR methods. The results showed that 21.9% of *I. ricinus* and 2.6% of *D. reticulatus* ticks were Borrelia-positive; this difference was statistically significant ($P < 0.0001$). By contrast, TBEV was detected in similar proportions of *I. ricinus* and *D. reticulatus* ticks: 0.9% and 1.1%, respectively ($P > 0.05$). The availability of suitable vertebrate hosts and environment facilitate the establishment of foci of ticks and pathogens. *D. reticulatus* tick, which is spreading in Latvia from the South, is a generalist tick with a wide host range similarly to *I. ricinus*. It seems that TBEV could pervade between sympatric tick species through feeding on the same animals indicating existence of strong interaction and transmission networks. This is also dangerous factor for other coinfection outburst. On the other hand, the distinct difference in Borrelia prevalence indicate the complexity of such interaction. Another possibility is that zoonotic potential and /or a general life-cycle of *D. reticulatus* ticks is less suitable for Borrelia and has to be addressed in further studies.


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Acknowledgements. This work was supported by the ERDF grant Nr.1.1.1.1/16/A/044.

## P-29-005
## Cellulolytic and antagonistic activity of bacterial strains isolated from the rumen of *Rangifer tarandus*

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The feature of the microorganisms of rumen microbiocenosis is the ability to form a number of digestive enzymes, including cellulases, which allows ruminants effectively use the energy of feeds rich in fiber. The digestion of plant feed in reindeer (*Rangifer tarandus*) occurs, as in other ruminants, due to cellulolytic enzymes produced by rumen symbiotic microorganisms. Reindeer are one of the special animals that live in conditions of poor diet, a significant part of which are lichens and fiber. Cellulolytic bacterial strains were isolated from samples of fresh reindeer's rumen content from Yamalo-Nenets and Nenets Autonomous Districts on the medium containing different sources of cellulose. A total of 63 associations decomposed cellulose in aerobic and anaerobic conditions was isolate. Then their ability to degrade cellulose was evaluated by the method of Churlis. The isolates No. 14, 15, 21, and 26 turned out to be the most active, decomposing 44–62% of the cellulose. The level of antagonistic activity of most active isolates to the *Escherichia coli*, as well as to pathogenic fungi of the genus *Fusarium oxysporum* and *Fusarium sporotrichioides* was determined by the method of wells. In relation to *E. coli*, antagonism was almost not observed. In relation to *F. oxysporum*, high antagonism in isolates No. 7, 15 and 26 was shown. High antagonistic activity to *F. sporotrichioides* was detected in isolates No. 7, 14, 15, and 24. The obtained data allowed to make a conclusion about the ability of bacterial strains isolated from the rumen to synthesize cellulolytic enzymes carrying out cellulose biodegradation, which probably allows them to gain a competitive advantage in the rumen of the reindeer. The ability to show antagonistic properties against bacteria and fungi allows us to consider isolates as possible probiotics. The research was carried out with the support of the grant of the Russian Science Foundation (RSCF) No. 17-76-20026.

## P-29-006
## Novel insights into candidate tumor suppressor protein CTCF from multi-omics data mining

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The advent of high-throughput profiling technologies, such as genomics, transcriptomics and proteomics, has resulted into accumulation of vast amount of publicly available multiple omics data in the biomedical domain. Integrative bioinformatics approaches have proven useful for investigation of complex diseases by enabling the combined statistical evaluation of rapidly growing multi-omics datasets. This study focuses on the elucidation of the CCCTC-binding factor (CTCF) protein's putative role in cancer by use of the multi-omics data mining approach. Despite the growing body of research on diverse aspects of CTCF, which is recognized as a master weaver of the genome, the findings on its possible implication in cancer remain largely inconclusive. Current evidence only loosely links CTCF to cancer as a candidate tumor suppressor gene, mainly due to the lack of experimental results and missing meta-analysis studies. The emergence of experimental multi-omics data offers the unprecedented opportunity to fill the gap in this understudied field. In this study, multiple high-dimensional experimental datasets were retrieved from the selected omics databases and mined to address this issue. The data analysis pipeline, based on multiple bioinformatics software, implemented diverse machine learning and visualization algorithms. The employed integrative bioinformatics approach leveraged CTCF-related experimental observations on multiple omics levels by reconstructing the putative CTCF protein-protein interaction map and linked this multifunctional protein with distinct molecular processes. This network-based computational analysis results in a broader functional annotation of the CTCF protein and facilitates elucidation of its putative role cancer. The presented work complements experimental research on candidate tumor suppressor protein CTCF by yielding novel insights into its possible implication in cancer and highlights usability of the integrative multi-omics approach.

## P-29-007
## Community structure and metabolic networks inferred from environmental DNA sequences

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Microbial communities are key members in the functioning of all ecosystems. However, the vast majority of these organisms cannot be recovered as axenic cultures under standard laboratory conditions, and their roles in natural systems remain unknown. Next-generation sequencing technologies have revolutionized microbiome research. These techniques have allowed a deep understanding of microbial ecology and function of environmental microbial communities. Here, using new sequencing methods of DNA analyses we report microbial diversity in seven coastal Arctic glaciers from the Svalbard archipelago. Some of these glaciers terminate in a calving front, greatly affecting the physical and chemical characteristics of the sea to which they discharge. Glaciers are populated by a large number of microorganisms including bacteria, archaea, microeukaryotes and viruses. Several factors such as solar radiation, nutrient availability and water content greatly determine the diversity and abundance of these microbial populations, the type of metabolism and the biogeochemical cycles. Global warming is a worldwide environmental problem, having a great impact on the Arctic region, due to the change of air temperature and precipitation. As a consequence, the glacial ice melts and englacial materials are being transported into the ocean. This research allowed the identification the microbial diversity and the reconstruction of the metabolic networks and biogeochemical cycles in polar glacier habitats. Our results demonstrate an interchange between glacier and marine microbial populations as well as the presence of some indicator species as possible sentinels for bacterial transport between glaciers and their downstream seawaters. The consequence of this process could be the alteration of the water composition of the fiords producing serious consequences throughout the marine ecosystem and in the cycling of globally important elements.(PI14/00705)


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## P-29-008
## A platform using engineered bacteriophage bioconjugates for detection and identification of surface-exposed receptors on living mammalian cells

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The specific interaction between eukaryotic cell receptors and their ligands is widely analyzed in both fundamental studies and drug development research. Here we designed the platform in which we claim to combine phage display and flow cytometry techniques for visualization and selection of eukaryotic cells according on their receptors' specificity. We optimized protocol for *in vivo* enzymatic biotinylation of bacteriophage M13K07 carrying exposed peptide ligand and subsequent staining of transgenic antigen-specific B cells with obtained conjugate. Flow cytometry analysis revealed increased efficiency of antigen-specific B cells visualization in comparison with utilization of chemically synthesized peptides. We suggest that engineered bacteriophage bioconjugates may be used for identification of new peptide ligands for receptors, exposed on the cell surface of alive mammalian cells in their native environment. This study was supported by Russian Science Foundation grant #17-74-30019.

## P-29-009
## The insight into the interactions between biosurfactants and plasma membrane in living cells

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Biosurfactants are biological surface-active compounds produced as secondary metabolites by diverse microorganisms. They are a class of amphipathic molecules including glycolipids, phospholipids and lipopeptides. It has been reported that biosurfactants possess various biological activities which make them excellent candidates for use in medicine. Several lipopeptides exhibit antitumor properties causing inhibition of cells proliferation, morphological changes and induction of apoptosis in cancer cells. The activity also includes plasma membrane permeabilization and reorganization. However the exact mechanism of their interaction with lipid bilayers is still unknown. Biological membranes are heterogeneous, asymmetrical structures composed of lipids and proteins remaining in constant motion. Various interactions between plasma membrane components form sterol-rich nanodomains which can drive the activity and location of crucial membrane proteins such as receptors, channels and effectors. The influence of several biosurfactants on plasma membrane dynamics was investigated by spot variation Fluorescence Correlation Spectroscopy (svFCS). This innovative, confocal microscopy-based technique enables the detection of single molecules and can be used to study the lateral organization of the plasma membrane. Our experiments confirm that several biosurfactants destabilize membrane structures changing the diffusion properties of various plasma membrane components. Also, cytotoxicity tests show that cells expressing proteins which impact plasma membrane organization are more sensitive to biosurfactants treatment. This increased sensitivity can be a result of the additional effect of membrane domains destabilization. Our observations support the hypothesis that biosurfactants interact with cell membranes affecting their composition and lateral organization which is crucial for cell functionality and viability.

# RNA in pathogenesis and therapy

## P-30-001
## RNA sequencing – different approaches to identification of molecular targets of investigated protein

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RNA sequencing (RNAseq) is a powerful method that in the field of transcriptome analysis allows precise measurement of levels of transcripts and their isoforms. The aim of our study was to identify RNA species regulated by a protein called MCPIP2. MCPIP2 belongs to the family of proteins which participate in the regulation of inflammation through the degradation of inflammatory transcripts and modification of cell signaling pathways. Two approaches for identification of MCPIP2-interacting RNA molecules were employed. In the first attempt the transcriptome for RNAseq analysis was obtained from the cells modified by the use of Sleeping Beauty transposon system. This system allows for overexpression of the gene of interest (GOI) at the "physiological" level thus avoiding cellular stress accompanying other overexpression systems. Additionally, the expression of the GOI can be doxycycline-induced. Using this system we have compared the level of transcripts, known MCPIP2 targets and negative controls, in doxycycline-induced and control knock-out cells. Our data indicate that the system itself influences the changes in the level of transcripts thus excludes its use for transcriptome analysis. Due to this obstacles second approach, based on RNA immunoprecipitation (RIP), was applied. RIP allows to eliminate the background RNA molecules and enriches the sample in the transcripts directly interacting with investigated protein. Similar as in the first approach we have compared the level of known MCPIP2 targets and negative controls in immunoprecipitated samples. Obtained results indicate that RIP is a perfect method for investigating the RNA molecules interacting with particular protein. Here we compare this two approaches, and show that the way of samples preparation can result in obtaining the false results.

## P-30-002
## A microRNA panel for diagnostics and identification of pathomechanisms in Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related dementia. Among its major challenges is identifying molecular signatures characteristic for the early AD stage in patients with Mild Cognitive Impairment (MCI-AD), which could serve for deciphering the AD pathomechanism and also as non-invasive, easy-to-access biomarkers. Using qRT-PCR we compared the microRNA (miRNA) profiles in blood plasma of 15 MCI-AD patients, whose diagnoses were confirmed by cerebrospinal fluid (CSF) biomarkers, with 20 AD patients and 15 non-demented, age-matched individuals (CTR). 6 miRNAs (3 not yet reported in AD context and 3 reported in AD blood) were selected as the most promising biomarker candidates differentiating early AD from controls with the highest fold changes (from 1.32 to 14.72), consistent significance, specificities from 0.78 to 1 and sensitivities from 0.75 to 1. Further, using bioinformatic tools (TargetScan, MirTarBbase and KEGG), we identified putative mRNA targets of the selected differential


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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miRNAs in AD blood, including key proteins involved in AD pathophysiology. We confirmed by qRT-PCR and immunoblotting that hsa-miR-483-5p, one of the mostly upregulated miRNAs in AD blood, regulates ERK1 at both mRNA and protein level and binds Tau mRNA in luciferase assay. Moreover, we found that hsa-miR-483-5p mediated lowering of ERK1 resulted in reduced phosphorylation of Tau protein in neuronal cells. These result support the hypothesis that miRNAs could report the particular molecular mechanisms underlying AD pathology.

## P-30-003
## Towards exploiting RNA binding to govern the self-assembly of TDP-43

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The aggregation of TAR DNA-binding protein 43 (TDP-43) as toxic cytoplasmic deposits is one of the hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Like many proteins associated to neurodegeneration, TDP-43 has a native nucleic acid binding function. Mounting evidence indicates that interactions with RNA act as one the main modulators of protein phase transitions in the cell—including aberrant aggregation. Yet, interactions with RNA do not appear to influence unidirectionally aggregation transitions. In the TDP-43 case, binding to its cognate DNA and RNA can prevent the formation of oligomers and larger aggregates, and enhance solubility. In contrast, RNA can also induce this protein to attain a highly toxic misfolded conformation. To shed light into how the native RNA binding ability of TDP-43 modulates its pathological self-assembly, we analyzed the binding affinity of different RNAs derived from a previously known high-affinity UG-rich consensus motif, and we tested their effect on the aggregation kinetics of a TDP-43 aggregation model. The RNA binding affinity of the TDP-43 model is strongly dependent on sequence composition and specificity, and the increase on RNA length by repetition of smaller units tends to reduce affinity. Remarkably, we identify a trend between increasing binding affinity and reduced protein aggregation. But this effect on aggregation cannot be explained based on binding affinity solely, as we also notice the influence of sequence specificity; and, interestingly, the increase of RNA length by unit repetition potentiates the effect on aggregation of the original RNA units. Notably, a 3-fold repetition of the high affinity RNA motif completely abrogates aggregation. Our work lays the ground for dissecting the interplay between functional RNA binding and pathological aggregation. This effort may eventually open a new avenue in the search for therapies for a subset of neurological disorders. *The authors marked with an asterisk equally contributed to the work.

## P-30-004
## miRNA-regulated restriction of transgene expression to the heart is limited by interindividual variability in cardiac miRNA-122 level

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Successful gene therapy requires efficient, tissue targeted expression of the chosen therapeutic gene. In case of the heart, high transduction efficacy and stable expression *in vivo* can be achieved through utilisation of adeno-associated vectors serotype 9 (AAV9) as a delivery system. However, intravenous administration of this vector provides transfer of the transgene also to the liver and skeletal muscles. A promising approach to restrict expression to selected tissue is the incorporation of miRNA target sites in the vector sequence. It was demonstrated that efficient targeting of transgene expression from AAV9 to the heart can be provided by application of the vectors concomitantly regulated by miRNA-122 and miRNA-206, which are abundant in liver and skeletal muscles, respectively. In our studies, we prepared AAV9 vectors for heme oxygenase-1 (HO-1) overexpression with miRNA-122 and miRNA-206 target sequences localised in 3'UTR of the construct, to enable heart-specific transgene expression. We successfully validated functionality of these vectors *in vitro* in murine cardiomyocyte (HL-1), hepatocyte (AML12) and myoblast (C2C12) cell lines. In the next step, we evaluated the distribution of transgene expression *in vivo*, after administration of AAV9 encoding HO-1 to HO-1 KO mice of C57Bl/6xFVB strain. Surprisingly, although AAV genomes were detected in the heart, HO-1 expression was not observed. We found that even though miRNA-122 was described as liver-specific, it can also be present in the heart of various common mice strains, for example C57Bl/6J, FVB or DBA. To further establish how these results reflect the situation in humans, we checked miRNA-122 level in heart biopsies collected from various cardiomyopathy patients undergoing heart transplantation surgery. It turned out that the majority of the samples showed significant miRNA-122 expression, what prevents universal therapeutic utilisation of such regulation strategy. Supported by 2014/14/E/NZ1/00139 (NCN)

## P-30-005
## RNAi knockdown of UBR ubiquitin ligases results in ROS upregulation in cancer cells *in vitro*

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Reactive oxygen species (ROS) play an essential role in tumor progression and metastasis. Upregulated ROS facilitates cell proliferation, genomic instability, DNA mutations and the release of tumorigenic signals. Increased ROS production is a feature of cancer cells and can serve as a specific trigger for the activation of anticancer therapeutics. ROS-responsive moieties can be attached to small molecules or to siRNA and act as an "on" switch in the presence of increased ROS. UBR ubiquitin ligases


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of the N-end rule pathway are actively involved in the regulation of various cellular processes including proliferation, migration and apoptosis, which are hallmarks of cancer cells. Previously, we evaluated the four UBR ubiquitin ligases of the N-end rule as new targets for cancer treatment. UBRs can be effectively downregulated by siRNA embedded in lipid nanoparticles *in vitro* and *in vivo* and this negatively affects cell migration and proliferation as well as increases cell sensitivity to pro-apoptotic signals. Accurate ROS measurements showed that knockdown of UBRs leads to increased ROS production in hepatocarcinoma cells *in vitro*, while ROS levels in normal AML-12 hepatocytes were less affected. In this project we evaluate a novel approach for selective activation of ROS-sensitive cancer prodrugs by simultaneous downregulation of Ubrs of the N-end rule using siRNA. The work was supported by the NGP Skoltech-MIT program (validation of Ubr knockdown) and a grant from the Russian Science Foundation No. 19-44-04111 (ROS studies). *The authors marked with an asterisk equally contributed to the work.

## Molecular biology of aging

### P-31-001
### Activity of human AP-endonuclease APE1 on damaged G-quadruplex structures

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Apurinic/apyrimidinic sites (AP-sites) are one of the most widespread DNA lesions. More than 95% of AP-sites arising in mammalian DNA are processed by AP-endonuclease 1 (APE1). Although canonical B-form is thought to be prevalent in human genome, DNA is able to adopt many noncanonical forms. Such DNA sites are often localized in genome regions having important biological significance. Some regions of such nature have an increased susceptibility to oxidative damage. In particular those are G-rich sequences observed in single-stranded telomeric repeats (TTAGGG in humans). The AP-sites occurring in such regions due to their being prone to create single-strand breaks in DNA are a threat to genomic stability. Left unrepaired such lesions can lead directly to shortening of telomeric regions which is known to result in premature aging and development of various diseases including neurodegeneration. At present the information about the ways and consequences of DNA repair in the telomeric region is insufficient. Therefore the main goal of this work was to elucidate mechanism of AP-sites processing by APE1 in TTAGGG tandem repeats. The model DNA-substrates forming G-quadruplex (G4) structures with single F-site (AP-site analogue) in core or loop regions were constructed. Their structures were proved by CD-spectroscopy. Analysis of product accumulation using gel-electrophoresis has shown that APE1 excises both types of G4. The process of DNA binding and catalysis was also monitored in the real-time by the stopped-flow method with detection of the FRET-signal. Taking together, obtained data revealed the ability of APE1 to excise AP-sites from different positions of G-quadruplex structures analogous to those in telomeric repeats. This work was supported by grants from the Russian Science Foundation (No 18-14-00135) and Russian Foundation for Basic Research (No 19-04-00012).

### P-31-002
### Muc17 functional maturation and expression in intestine is age-dependent

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Transmembrane mucin Muc17 is a dynamic glycoprotein expressed in apical membranes of intestinal epithelial cells (IECs). Muc17 extends up to 1 μm into the intestinal lumen, which makes Muc17 an ideal docking site for luminal gut bacteria. However, the Muc17 function is still unknown. Our aim is to determine the role of Muc17 in the small and large intestines. Muc17 and a panel of innate immunity components were analyzed in ileum and colon of mice 0, 3, 9 14 and 24 days after birth (P0, P3, P9, P14 and P24). Muc17 tissue localization was determined using immunofluorescence (IF), whereas gene expression of Muc17 and innate immunity components was assessed by RT-qPCR. Surprisingly, IF revealed that Muc17 resided intracellularly in ileum from P0 to P14 mice and was mobilized to IECs apical surface between P14 and P24, coinciding with weaning. Time-resolved gene expression analysis revealed a significant upregulation of Muc17 upon weaning that correlated with upregulation of cytokines such as Il-10 and Il-18 as well as antimicrobial proteins Reg3-β, Reg3-γ and Zg16. By contrast, Muc17 in the distal colon was intracellular from P0 to P3 and reached apical membrane of IECs by P9, corresponding to the period of early microbial colonization. Mobilization of Muc17 to apical membranes in colon was reflected in a significant upregulation of Muc17 gene expression, which coincided with upregulation of Toll-like receptors (TLRs) and the adaptors Myd88 and Trif. Our results suggest that the functional maturity of Muc17, hallmarked by apical expression, is age-dependent. In the ileum, Muc17 expression is trigged upon weaning and an adult-type microbiota through a TLR-independent signalling. By contrast, Muc17 expression in distal colon is TLR-dependent and triggered by the initial colonization of distal colon by gut microbiota. We suggest that Muc17 maturation depends on microbiota and plays a yet undefined role in microbial-host interactions.

### P-31-003
### A novel role for POLO kinase in the establishment of the non-random inheritance pattern of the spindle pole bodies in *Saccharomyces cerevisiae*

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In many cases cell divisions are asymmetric, and cellular components are differentially inherited by the resulting cells. The uneven distribution of these molecules constitutes a mechanism for the generation of cellular diversity that is essential during development, cell differentiation and aging. An interesting phenomenon associated to asymmetric cell division is the differential segregation of the spindle-associated microtubule-organizing centers (MTOCs). The budding yeast *Saccharomyces cerevisiae* represents an ideal model to study this process, since it displays a non-random inheritance pattern of the spindle pole bodies (SPB, the centrosome equivalent). Specifically, during budding yeast mitosis the old SPB inherited from the previous cell cycle is segregated to the bud, while the new one is retained in the mother cell. Remarkably, asymmetric inheritance of the centrosomes has been also described in higher eukaryotes, where the age of the


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centrosome can specify cell fate and the disruption of this inheritance pattern can have detrimental consequences, related to an accelerated aging and the development of cancer or neurodegenerative disorders. The members of the POLO family of protein kinases seem to be important players in the regulation of the differential distribution of MTOCs, although their precise roles in this process are still largely unknown. Using budding yeast as a model, we shed light on the mechanisms by which POLO-like kinases control MTOC inheritance in asymmetric cell divisions. Our results demonstrate that Cdc5, the POLO homolog in *S. cerevisiae,* ensures the unequal segregation of SPBs during budding yeast mitosis by acting as a molecular timer that facilitates a timely and sequential recruitment of key determinants of this process on the SPBs.

## P-31-004
## Stem cell like features in polyploid triple negative breast cancer cells favour overcoming the doxorubicin-induced cell senescence

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MDA-MB-231 (mt TP53) cancer cells treated with doxorubicin underwent reversible cell senescence, as they were still capable of recovering the clonogenic growth. At day 4 after treatment most of the cells expressed typical hallmarks of cell senescence, such as: arrest in G2/M phase, increased level of cell cycle inhibitor, p21, enlarged size, flattened morphology, SA-β-gal increased activity, lipofuscin accumulation, and increased lipid content. Moreover, they secreted proinflammatory cytokines, thus displaying features of senescence-associated secretory phenotype (SASP). At day 9 after treatment DNA image cytometry revealed a protracted arrest in S phase, premature mitosis, and mitotic slippage into polyploidy <8n and <16n. Most of the cells were both Ki67- and BrdU- positive, which proves that they were endo-cycling. Moreover, senescent/polyploid cells were characterised by persistent DNA damage, as revealed by accumulation of γH2AX and 53BP1 foci, and activated DNA damage response-DDR, manifested by increased level of ATR, ATM, NHEJ and HR regulators indicating DNA repair. Interestingly, we observed sorting of γH2AH-positive DNA. The cells also had higher expression of nuclear cyclin B1 and Mos kinase, which prevented degradation of cyclin B1. Both proteins are likely driving the meiotic component of endopolyploidy, which un-couples cells from apoptosis (lack of PARP cleavage). Interestingly, polyploid senescence cells were positive for stem cells markers, namely NANOG, OCT4 and SOX2 (demonstrated by IF, western blot and PCR). <19 days after treatment the giant polyploid cells depolyploidised and supposedly released mitotically active descendents. What is more important, daughter cells were characterised by lower DDR signaling than the initial cells. We conclude that senescence halts cell divisions, but not DNA replication, which together with induced stemness enables the cells to produce vital progeny. This research was funded by grant UMO-2015/17/B/NZ3/03531.

## P-31-005
## Novel 17ß-HSD10 inhibitors as small molecules for neurodegenerative disorders or cancer

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Mitochondria are the unique organelles, central place of cell energetic metabolism, with important role in maintaining homeostasis, regulation of cell death, but also in many neurodegenerative disorders. 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10), a mitochondrial protein playing role in various physiological pathways, mainly in steroid metabolism, was found to be overexpressed in Alzheimer's disease (AD) and several types of cancer. In AD 17β-HSD10 bind β-amyloid resulting in increased oxidative stress, cell toxicity, and neuronal impairment. Modulation of this enzyme could be a possibility for both cancer and neurodegenerative disorders treatment. For testing of novel 1-(benzo[d]thiazol-2-yl)-3-phenylurea-based inhibitors, the recombinant enzyme was produced in *E. coli* and purified using chromatographic methods. Enzymatic activity assay was performed spectrophotometrically in a microplate reader at 37 °C, using acetoacetyl-CoA as a substrate. Kinetic parameters of enzyme were determined, and inhibitors were screened for their inhibitory ability. Over 15 compounds with $IC_{50} \leq 10$ μM were found and kinetic study of 3 selected compounds (K900, K901, K903) determined the uncompetitive mechanism of inhibition. The most promising HSD-10 inhibitors are further studied using *in vitro* and *in vivo* methods with implication to neurodegenerative disorders and/or cancer. The study was supported by Specific Research Project of Faculty of Science, University of Hradec Kralove (No. 2113-2019).

## P-31-006
## Effects of interfering transmembrane peptides on neuraminidase-1 activity

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Elastin is an essential component of numerous human tissues and plays a critical role in elasticity of skin, lungs and arteries. During vascular aging, the elastin network is degraded generating elastin-derived peptides (EDP). The ERC (Elastin Receptor Complex) is a membrane heterotrimeric receptor composed, amongst others, of a membrane-bound neuraminidase, NEU-1. Binding of EDP to the ERC induces the activation of signaling pathways associated with biological effects notably the development of diseases such as atherosclerosis, cancer and diabetes. Previous studies of our laboratory have shown that NEU-1 catalytic activity is linked to its ability to homodimerize. Thus, NEU-1 constitutes a key pharmacological target to fight against deleterious effects of EDP. The aim of this work is to develop by biological/biochemical experiments and molecular dynamic (MD) simulations a


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transmembrane interfering peptide (pI) able to inhibit specifically NEU-1 dimerization. Peptides are delivered into cells using two strategies, TAT peptides, which are cell-penetrating peptides, or lithium dodecyl sulfate micelles. No cellular toxicity was observed in both approaches. Confocal microscopy underlines a colocalization between pI and NEU-1 at the plasma membrane and coimmunoprecipitation experiments show an interaction between pI and NEU-1. Furthermore, sialidase activity assays point out the ability of pI to inhibit NEU-1 homodimerization (47.4±5%; 51±11%) and its associated sialidase activity (21.4±9%; 47±12%). Preliminary MD simulation studies emphasize that both pI and the transmembrane domain of NEU-1 are stable and helix integrity is conserved in lipid bilayer environments. Moreover, the formation of a spontaneous dimer between NEU-1 and pI was identified. Further MD analyses underline the biological relevance of our membrane model. These results reveal the ability of pI to bind to NEU-1, inhibit its dimerization and sialidase activity.

## P-31-007
## Unique role of Cdc25B in inducing senescence in normal human fibroblasts

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Cdc25B is a dual specificity phosphatase that activates CDK complexes and regulates progression through the cell division cycle. Cdc25B is responsible for activation of Cdk1/cyclin B during G2/M transition. Cdc25B activates Cdk1 by dephosphorylating the inhibitory residues Thr14 and Tyr15. Elevated *CDC25B* level is frequently observed in many cancer types. It is considered that Cdc25B enhances the growth and survival of cancer cells through activating the Cdk1/cyclin B complex. Interestingly, Cdc25B is frequently detected in premalignant tumors, suggesting a role of CDC25B in early-stage cancer formation. Since senescent cells are frequently detected in premalignant tumors and senescence is known to have an important role in cancer formation, the role of Cdc25B in senescence is analyzed. We found elevated Cdc25B level by ectopically expressing *CDC25B* caused growth inhibition, increased senescence associated-β-galactosidase activity, reduced Cdk1, and elevated p53 levels in human normal fibroblasts. The senescence phenotypes induced by *CDC25B* expression did not arrest cell cycle progression at G2/M phase and was not fully mediated through Cdk1. We found p53 is required for the Cdc25B-induced senescence as depleting p53 reduced the senescence phenotypes in both normal fibroblasts and cancer cells. The p53 protein is a key mediator of DNA damage response pathway that is activated by various DNA damages. The effect of Cdc25B on p53 was next analyzed. We found Cdc25B did not induce DNA damage response, suggesting that activation of p53 was not mediated through DNA damage induction by Cdc25B. We found Cdc25B increased the stability of p53 to induce senescence. The results identified a unique role of Cdc25B in mediating senescence through stabilization of p53 protein. Since the elevation of Cdc25B level and senescent cells are frequently detected in pre-malignant tumors, our finding might define a critical role of Cdc25B in early-stage tumorigenesis.

## P-31-008
## *In vitro* gut microbiota fermentation of *Pleurotus ostreatus*: impact on bone metabolism parameters and on the molecular basis of osteoporosis

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Recent data have highlighted the role of gut microbiota on bone health and dietary manipulation of gut microbial ecosystem might offer a plausible target in the fight against bone degenerative diseases. We investigated the effects of the *in vitro* fermentation products of an edible mushroom (*Pleurotus ostreatus*) on human osteoblasts and further elucidated differences on potential effects based on gut microbiota inoculum origin (healthy vs. subjects with osteopenia). Lyophilized P. ostreatus IK1123 mushroom (2% w/v, POWS) and its rich in β-glucan extract (1% w/v, POWSE) were fermented in six-plicate in an *in vitro* static batch-culture model, using faecal inoculum from healthy (n=3) and osteopenic postmenopausal women (n=3). Positive (inulin) and negative (no substrate) controls were also included. Supernatants were collected after 24 h of fermentation, lyophilized and reconstituted with culture medium for MG-63 human cells. The effect of processed fermentation supernatants in the viability of MG-63 osteoblast-like cells was evaluated by the MTT assay, whereas the evaluation of bone metabolism parameters, namely OPG (Osteoprotegerin) and RANKL (Receptor activator of Nuclear factor kappa-B ligand) was performed by ELISA. In the case of OPG, inulin treatment induced similar levels compared to control. POWSE treatment resulted in lower RANKL levels compared to control in healthy subjects ($P = 0.034$), whereas a similar trend was indicative in the case of osteopenia ($P = 0.076$). Inulin and POWSE demonstrated similar positive effects regarding OPG-to-RANKL ratio in comparison to control. POWS induced a drastic decrease in both bone metabolism markers and their ratio compared to control and inulin, especially in the case of osteopenic subjects. In conclusion, the potential beneficial activity of P. ostreatus bioactive extract on bone physiology has been currently uncovered, offering insight into the molecular basis of osteoporosis and new alternatives in pharmacotherapy choices.

## P-31-009
## Regulatory T cells induces telomerase-associated aging of effector lymphocytes

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The suppression of effector T, B, and NK lymphocytes by regulatory T cells (Tregs) is an important mechanism for the maintenance of immune tolerance. The proliferative activity of suppressed effector cells remains unexplored. We studied the mechanism of contact-independent Treg actions on target lymphocytes through the induction of telomerase inhibition leading to cellular aging. We isolated human or mouse Tregs from peripheral blood and co-cultivated them with autologous T, B or


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NK lymphocytes in porous membrane system. We found that both human and mouse Tregs could induce alternative splicing of mRNA of telomerase catalytic subunit hTERT and inhibition of telomerase activity in all types of target lymphocytes. Prolong proliferation of target cells resulted in telomere attrition, cell cycle arrest in G0/G1 phase and developing of aged phenotype. The investigation of gene expression in aged responder lymphocytes revealed changes in the expression levels of genes involved in cell cycle regulation. Such cells were unable to divide and expressed senescence-associated beta-galactosidase. Further cultivation of aged lymphocytes resulted in the developing of apoptosis. The results demonstrated that the long-proliferative phenotype of human and mouse lymphocytes can be reversed by continuous contact-independent exposure to Tregs. The present work suggests that regulation of the immune response can modulate the onset of Treg-driven telomerase-dependent aging of effector lymphocytes.

## P-31-010
## Soluble klotho regulates the function of salivary glands through activating KLF4 pathways

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The dysfunction of salivary glands in the elderly commonly induces dry mouth, infections, and dental caries caused by a lack of saliva. This study was undertaken to determine genetic and functional changes of salivary glands using klotho (−/−) aging mouse model. Here, we found the attenuation of KLF4 expression in klotho(−/−) mouse tissues. Soluble klotho (sKL) overexpression induced KLF4 transcription and KLF4-related proteins including mTOR, AMPK, and SOD1/2. siRNA Klotho silencing significantly down-regulated KLF4 expression. Also, we monitored the function of salivary glands and sKL and/or KLF4-responses and demonstrated that sKL increases the expression of salivary gland functional markers (α-amylase, ZO-1, and Aqua5) in the primary cultured salivary gland cells from klotho (+/+) and/or (−/−) mice. In 3D culture system, the cell spheres aggregates were observed in sKL or KLF4 expressed cells and exhibited higher expression levels of salivary gland function-related proteins compared to non-transfected cells. These results suggest that activation of the sKL mediated KLF4 signaling pathways contribute to potentiating the function of salivary glands.

## P-31-011
## Potential role of elastin carbamylation in the alteration of vascular wall properties: application to aortic aneurysm development

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Background: The arterial wall is subjected to important structural modifications and to matrix remodeling during aging and cardiovascular diseases. Molecular aging of matrix proteins, which is due to non-enzymatic post-translational modifications, such as glycation or carbamylation, has been identified as a potential mechanism involved in these structural alterations. Carbamylation is characterized by the binding of cyanate, which mainly derives from the dissociation of urea, to protein amino groups. The aim of this study was to determine how elastin carbamylation may participate in the alteration of vascular wall properties and in the development of aortic aneurysm. Materials and methods: The morphology of *in vitro* carbamylated bovine elastin and its sensitivity to pancreatic elastase degradation has been evaluated. In addition, a murine model fed with cyanate in drinking water was used for evaluating *in vivo* the impact of carbamylation on aortic elastic fiber stiffness by atomic force microscopy (AFM) and measure of aortic pulse wave velocity (aPWV). Besides, the role of protein carbamylation in the development of aortic aneurysm was assessed using a murine model of cyanate-diet $ApoE^{-/-}$ mice receiving angiotensin II by subcutaneous diffusion. Results: *In vitro* carbamylated elastin exhibited the same morphology as control elastin but was more sensitive to pancreatic elastase degradation. *In vivo* studies showed that vascular wall contains carbamylated elastin. Feeding mice with cyanate led to an increased carbamylation rate of aortic matrix proteins which was associated with an increased stiffness of elastic fibers assessed by AFM at 3 weeks and an increased aPWV at 7 weeks. Besides, the percentages of mice with aneurysm and of occurrence of ruptured aneurysms were decreased with the cyanate diet, whereas aneurysm area was not modified. Conclusion: These results suggest that carbamylation of elastin may participate in structural and mechanical alterations of vascular wall.


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## P-31-012
## The effect of PTPN11 gene knockdown and vemurafenib treatment in thyroid tumor model with BRAF V600E mutation

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The V600E mutation of BRAF gene is found in various types of malignant tumors, especially in melanoma and thyroid tumors, and is associated with a poor response to traditional chemotherapy as well as with a poor clinical prognosis for patients. The occurrence of V600E mutation leads to a multiple enhancement of BRAF kinase activity and, as a result, to the constitutive activation of the components of the MAPK/ERK signaling cascade. The drug resistance of this type of cancer may be associated with the activity of the protein tyrosine phosphatase SHP-2 encoded by *PTPN11* gene. Phosphatase SHP-2 is a key mediator of the PD-1 and BTLA signaling pathways, and a regulator of cell survival and proliferation. The mechanism of the involvement of SHP-2 in the activation of the MAPK/ERK signaling cascade is not fully understood. Nevertheless it was shown for intestine cancer cell models and mouse model of pulmonary adenocarcinoma that reactivation of the cascade mediated by EGFR could be prevented by inhibition of SHP-2. This study was conducted in order to assess the effect of a selective BRAF-V600E inhibitor vemurafenib (PLX4032) and the *PTPN11* gene knockdown with siRNA to the viability of complex blockade of signaling growth factors in a thyroid tumors. Using the thyroid follicular epithelial cell line transduced with a lentivirus containing BRAF V600E mutation, we showed that the suppression of BRAF V600E by vemurafenib leads to increase of *PTPN11* gene expression in 2, 3 and 5 times in 2, 3 and 4 days after the treatment, respectively. Also, qRT-PCR analysis demonstrated that si-mediated knockdown of *PTPN11* reduces the expression of some senescence-related genes. Thus, it can be assumed that inhibition of *PTPN11* expression could reduce BRAF-V600E-induced cell senescence. This work was supported by the Russian Foundation for Basic Research (Grant No. 18-315-00 171) and by the Program of fundamental research for state academies for 2013-2020, research topic 01201363823.

## P-31-013
## *In vitro* studies regarding the protective effects of procaine and GH3 against lipid peroxidation

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Procaine, as a compound with local anesthetic action, binds to membrane constituents and along its metabolites, affects several biochemical and cellular processes, including mitochondrial function and structure. Gerovital H3 (GH3) is a procaine-based anti-ageing drug. Considering that oxidative stress is linked to aging and several metabolic diseases, the anti-aging properties of GH3 may be attributed, at least in part, to an antioxidant effect (AE). As such, the objective of our study was the assessment of GH3 and procaine AE on isolated rat mitochondria and lipoprotein concentrates from human serum, using a previously optimized Amplex Red (AR)-based method for determining lipid peroxidation (LPO). Crude liver mitochondrial fraction obtained from a Wistar rat was incubated with 0.05 M succinate and treated with different procaine and GH3 volumes, corresponding to final concentrations of 0.5, 1.0, 2.0, 5.0, and 10 mM procaine hydrochloride. Serum lipoprotein concentrates were isolated from four healthy adult volunteers following centrifugation (Ultra-10k Centrifugal Filter Units 10,000 NMWL). LPO was assessed using an AR working solution of 75 and, respectively, 300 μM working solution for mitochondrial and serum concentrates samples. Results were expressed as LPO inhibition in percentage (%), calculated from the recorded relative fluorescence units. Procaine and GH3 showed a dose-dependent inhibition of free radical generation and LPO in both rat liver mitochondria and serum lipoproteins. However, procaine was showed a higher AE in the mitochondria, while GH3 for serum lipoproteins. These effects could be explained considering the different lipid microenvironments and antioxidant defense systems. Our results indicate that GH3 exerts a protective role against cellular and systemic oxidative stress. The observed antioxidant action may be part of its anti-aging effect, pointing to a possible use of this drug in metabolic maladies frequently found in elderly patients.

## P-31-014
## Variation of satellite 3 (1q12) content during replicative senescence of cultivated human skin fibroblasts

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Introduction: Human pericentromeric heterochromatin harbors many tandem repeats, including satellite 3. 1q12 is a hot spot for rearrangements. Using *in situ* hybridization, it was shown that during senescence of cultivated human skin fibroblasts (HSF) an augmentation of the area of sites of chromosome 1 hybridized with the probe for satellite 3 (1q12) (SatIII) occurs in the interphase nuclei. One of the causes of this phenomenon can be expansion of the SatIII in the genome. Methods: Five HSFs were derived from 5 donors of different age. The cells were cultivated until they completely stopped to divide (replicative senescence, RS). During cultivation, we examined the content of SatIII and telomere repeat (TR) at consecutive passages using nonradioactive quantitative hybridization (NQH) of the DNA with the probe for satellite 3 sub-fraction. Results: SatIII content in DNA at first passages varied from 10 up to 25 pg/ng DNA, while at final passages, it varied from 20 up to 41 pg/ng. During RS, an increase in SatIII content by a factor of 1.5 to 2.1 was observed in the DNA of all HSFs. The highest ratio of SatIII increase with cell divisions was found in those HSFs, which had an initially higher SatIII content. Cells with a larger Hayflick limit (66 and 61 passages) initially contained the minimum SatIII amounts, and their SatIII increase rate during senescence was the slowest. The TR content expectedly decreased by a factor of 2.5 to 5.1 during cultivation in all the HSFs. For each HSF, a negative correlation was found between the contents of TR and SatIII. Conclusions: (i) During RS, elevation of the SatIII content occurs in the genome. (ii) The SatIII content is negatively correlated with the abundance of the TR. (iii) A SatIII content to TR abundance ratio in the DNA can be a good potential marker of replicative senescence to forecast the rate of achieving the Hayflick limit by a certain cell culture. The work is supported by RSF grant #18-15-00437.


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## P-31-015
## *In vitro* investigation of toxicity and regeneration activities of collagen

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Background: Collagen is the most widely abundant proteins in the human body. The use of collagen-based biomaterials in the field of regenerative medicine applications has been intensively growing over the recent years. Collagen has a major advantage in being biodegradable, biocompatible, highly versatile and easily available. Methods: Collagen from natural sources compared to commercial collagen was investigated using *in vitro* methods regarding cytotoxicity and regenerative activity. Cytotoxicity and proliferation testing was performed using the LDH and MTS assays, on ATCC-CRL-9855 cell lines, in standard conditions, at different cell concentrations (10,000–50,000 cells/well), at different times of exposure (24 h, 48 h) and different collagen concentrations (15–150 μg/mL). xCELLigence cell index (CI) impedance measurements were performed according to the instructions of the supplier. Results: Two collagen types were investigated and did not express significant cytotoxic effects (LDH and MTS assays) over the concentrations ranging 15–150 μg/mL. Our results showed comparative effects of novel tested collagen and commercial type. We obtained a cellular proliferation/regeneration induced by collagen compounds, in a dose-dependent manner by xCELLigence assay. Conclusions: Our data suggests that the xCELLigence analysis offers dynamic live cell monitoring and combines high data acquisition rates with ease of handling. The combination of *in vitro* assays could provide an effective screening system for the discovery of potential innovative biomaterials based on collagen for regenerative medicine applications. Acknowledgment: Partially supported by the grant COP A 1.2.3., ID: P_40_197/2016, Ctr. 52/2016 and by Ministry of Research and Innovation in Romania, under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018.

## P-31-016
## Conformational variability and intramembrane recognition possibly associated with abnormal cleavage of APP transmembrane domain

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Bitopic proteins having only one helical transmembrane (TM) domain are a class of biologically significant membrane proteins, including the type I receptors and amyloid precursor protein (APP), which are involved in regulating the homeostasis of human organism and recognized as substrate by γ-secretase. Amyloid Aβ-peptides forming plaques in brain during Alzheimer disease (AD) are the products of sequential intramembrane cleavage of APP. A lot of mutations associated with AD familial forms were found in the APP transmembrane (TM) domain and juxtamembrane (JM) regions. We designed highly productive systems of bacterial and cell-free expression and easy purification procedure for 13C/15N-isotope labeled APP JM-TM fragments of different length (corresponding to the sequential cleavage steps of APP) and with several familial AD mutations, as well as the TM fragments of γ-secretase. The fragments were solubilized in membrane-mimicking complexes (detergent micelles and lipid bicelles), which allows to acquire proper high-resolution NMR spectra despite low sample stability and to characterize their structural-dynamic properties. Molecular Dynamics relaxation of obtained NMR structures of the fragments in hydrated explicit lipid bilayers provided a detailed atomistic picture of the intra- and intermolecular interactions within membrane. The mutant APP JM-TM fragments are shown to be promising objects for elaboration the molecular aspects of the abnormal recognition and sequential proteolysis by γ-secretase, revealing a straightforward mechanism of the pathogenesis associated with some familial AD mutation as well as with aging. Bioengineering work, NMR and SAXS studies were supported by the Russian Foundation for Basic Research (projects 17-04-02045, 17-00-00489 and 18-54-74001) and the RAS program "Molecular and cellular biology". MD simulations and computational data analysis were sponsored by the Russian Science Foundation (project 18-14-00375).

## P-31-017
## Antioxidant properties and geroprotective activity of fucoxanthin on human lung fibroblasts

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The marine carotenoid fucoxanthin is widely distributed in algae such as brown seaweeds, macroalgae and diatoms. There are numerous studies demonstrating anti-inflammatory, -cancer, -obesity, and antioxidant bioactivities of the fucoxanthin. It has shown that the fucoxanthin can increase the lifespan of D. melanogaster and C. elegans. In this work, we analyzed antioxidant and geroprotective properties of the fucoxanthin on diploid human lung fibroblasts (LECH-4) of the 12th ("young") and 18th ("old") passages. Evaluation of the fucoxanthin cytotoxicity was performed using a Resazurin Assay Kit (Abcam). For further experiments, the following its concentrations were used: 100 nM, 1 μM, and 5 μM. The cells in 40% confluence were subjected to the fucoxanthin treatment for 48 h, followed by 100 μM H2O2 treatment for 1 h. The intracellular ROS level was measured by a 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) method. For cell senescence detection, we used a Senescence β-Galactosidase Staining Kit (Cell Signaling). The results indicated that the fucoxanthin could reduce ROS contents. Their concentration of 5 μM had the most pronounced effect. Fucoxanthin reduced beta-galactosidase staining of senescent fibroblasts at 1 μM and 5 μM concentrations compared to control cells. Fucoxanthin treatment at 100 nM concentration showed no effects. Sizes of senescent cells are typically larger than younger ones. Also, senescent cells contain more lysosomes and mitochondria that leads to increasing of granularity. Our results demonstrated decreasing the mean cell size and granularity according to forward scatter (FSC) and side scatter (SSC) parameters. Altogether, we observed "rejuvenation" of the


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treated fibroblasts and showed dose-dependent ROS scavenge capacity of the fucoxanthin. Thus, we suggest that the fucoxanthin may be used as a potential geroprotector for prevention of premature aging. This work was funded by the Russian Science Foundation grant no. 17-74-30030.

## P-31-018
## Life history and mitochondrial traits in aging research: Preliminary studies on *Paramacrobiotus* sp. (*richtersi* group)

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Tardigrades are considered as model organisms for many astrobiological and physiological studies. The main reason for their application is their capability to enter a large variety of dormant stages including anhydrobiosis, defined as the ability to survive almost complete drying without sustaining damages. In response to dehydration, tardigrades form a so-called "tun" and in this stage can tolerate complete lack of liquid water, and survive to the next period of favourable environmental conditions, suitable for the active life. Importantly, 20 years ago two models were proposed to explain the effect of anhydrobiosis on ageing, although up to now their predictions have been rarely tested. In a nutshell, the two models predict the presence (the "Picture of Dorian Gray") or not (the "Sleeping Beauty") observable ageing symptoms for the animals undergoing anhydrobiosis. The validation of the proposed ageing scenarios requires first of all the detailed description of the life history of studied species. In the present study we used *Paramacrobiotus* sp. (*richtersi* group) as a model species for the "Sleeping Beauty" and the "Picture of Dorian Grey" model validation. We studied life history traits of this species with special care on lifespan and fecundity. In the next step, we will analyse how anhydrobiosis affects these parameters, as well as mitochondria functioning, is known to contribute to ageing and to successful anhydrobiosis. The obtained data will allow for better understanding of the role of mitochondria dysfunction in ageing of *Paramacrobiotus* sp. and the impact of anhydrobiosis on species ageing. This, in turn, is crucial for testing "Sleeping Beauty" and "Picture of Dorian Gray" models as well as to understand the process of ageing and the involvement of mitochondria in this process. The work was supported by the research grant of the National Science Centre, Poland, NCN 2016/21/B/NZ4/00131.

## P-31-019
## Postmitotic ATM-deficient neurons develop senescence-like phenotype with disturbances in autophagy

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The definition of cellular senescence has been traditionally built around proliferation arrest of dividing cells. Yet, some recent data suggest that non-dividing, terminally differentiated cells like neurons may enter a similar state. As we reported in previous studies, neurons in primary culture exhibit features of senescence, so do neurons in some areas of ageing brain, according to sparse data published on this topic. Ataxia-telangiectasia (AT) is a recessive human disease caused by mutations in ATM gene, characterised by radiosensitivity and neurodegeneration. Signs of senescence have been shown in AT-derived fibroblasts. No previous study, however, dealt with senescence in AT neurons, even though brain is the organ where many symptoms of the disease are pronounced. With that in mind, the aim of the study was to determine the phenotype of AT neurons in terms of senescence and to look at the role of ATM in this process. The study was conducted using human neurons derived from induced pluripotent stem cells. Primary human fibroblasts obtained from patients with and without ATM protein were reprogrammed to induced pluripotent stem cells, that were in turn differentiated into neurons. Several markers of senescence were assessed in postmitotic neurons after the differentiation, revealing increased level of senescence-associated β-galactosidase, IL-6 and IL-8 release and GATA4 transcription factor in neurons lacking ATM. In addition to that, our experiments pointed to disturbances in autophagy and mitophagy with accumulation of LC3B II, p62 and Parkin. ATM-deficient neurons appeared to be highly susceptible to oxidative stress, both from external and internal sources. Further experiments revealed that oxidative stress may be the underlying factor behind the observed phenotype of senescence in neurons. Overall the study gives an account of ATM-dependent senescence in postmitotic cells and, possibly, extends our understanding of some symptoms in AT disease.

## P-31-020
## Protein kinase CK2 regulates expression of histone H3 Lys 9 trimethylase SUV39 h1 in a p53-dependent manner during senescence-associated heterochromatic foci formation

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Senescence-associated heterochromatic foci (SAHFs), which are visible as distinct DNA foci upon 4′,6-Diamidino-2-phenylindole (DAPI) staining of senescent cells, result from condensation of chromatin into characteristically punctate heterochromatic domains. SAHFs repress the expression of gene encoding cell cycle progression-associated protein such as cyclin D1 and contain markers of transcriptionally silent heterochromatin including trimethylated histone H3 Lys 9 (H3K9me3) and heterochromatin proteins 1 γ (HP1γ). We have previously shown that CK2 downregulation induces senescence-associated β-galactosidase (SA-β-gal) activity and activation of the p53 and $p21^{Cip1/WAF1}$ axis, suggesting that cellular senescence is mediated via the p53-$p21^{Cip1/WAF1}$ pathway in CK2-downregulated cells. We here present evidence that CK2 downregulation induces H3K9me3, SAHFs formation, and reduction of cyclin D1 expression in HCT116 and MCF-7 cells. CK2 downregulation-mediated H3K9me3 is associated with induction of H3K9 trimethylase SUV39 h1. Pharmacological inhibition of SUV39 h1 significantly attenuated induction of SA-β-gal activity, H3K9me3, and SAHFs formation in CK2-downregulated cells. In addition, the PI3K-AKT-mTOR-reactive oxygen species-p53 pathway is necessary for CK2 downregulation-mediated H3K9me3. Furthermore, CK2 downregulation promotes SUV39 h1 stability by inhibiting proteasomal degradation of SUV39 h1 in a p53-dependent manner. Taken together, these results demonstrate that CK2 downregulation leads to H3K9me3 and SAHFs formation by increasing SUV39 h1 in a p53-dependent manner.


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## P-31-021
## The influence of early and late senescent vascular smooth muscle cells on the T cells functions

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Cellular senescence is a highly dynamic and complex process, during which the properties of senescent cells continuously evolve and diversify from early to late senescent state. One of the most important features of senescent cells is their ability to secrete high amounts of soluble factors, known as senescence-associated secretory phenotype (SASP). It was demonstrated that those early and late senescent cells, and particularly their SASP, may actively modulate neighboring microenvironment. Senescence of vascular smooth muscle cells (VSMCs), the most abundant cell type in the arterial wall, was shown to contribute to the development of atherosclerotic plaque mainly due to diminished proliferation potential that limits efficient plaque repair as well as secretion of SASP factors. Those factors may favor proinflammatory state of atherosclerotic plaque by affecting immune cells. Thus, the aims of our studies were (i) to characterize morphology and ultrastructure of early and late senescent VSMCs as well as to compare the changes in expression of senescence markers; (ii) to investigate the influence of senescent cells conditioned medium (CM) and VSMC-T cells co-culture on T cells activation and proliferation. To this end T cells were isolated from buffy coats of blood samples and activated with agonistic antibodies to CD3, CD28 and CD2 in the presence of conditioned medium or in co-culture with VSMCs. Using flow cytometry we measured the expression of markers of activation (CD25, CD69 and CD38) and characterized proliferation of $CD4^+$ and $CD8^+$ T cells. Our data indicates that late senescent VSMCs per se as well as factors secreted by these cells increase the activation level of T cells and influence on their proliferation. This work is supported by grant National Science Centre UMO 2014/15/B/NZ3/01150.

## P-31-022
## Role of giant polyploid cells in reversibility of cancer cell senescence

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Clinically relevant, moderate doses of anti-cancer drugs can induce proliferation arrest instead of apoptosis, a process frequently accompanied by increased cell size and granularity, which overall can be considered as cell senescence. The presence of giant, senescent cancer cells in tumors correlates with significantly worse patient prognosis, indicating that cancer cell senescence could be reversible. This study is aimed at establishing the role of senescent cells polyploidy in their escape from proliferation arrest. Three different cancer cell lines (breast MCF-7, colon HCT116, and lung A549 cells) were treated with several clinically used chemotherapeutics (doxorubicin, 5-fluorouracil, irinotecan, methotrexate, oxaliplatin, and paclitaxel). Upon irinotecan, methotrexate, paclitaxel, and doxorubicin treatment cells display senescence markers in all tested cell lines. However, 'senescence escape' is observed only when senescent cells also undergo polyploidization/depolyploidization, which was visualized by confocal and electron microscopy. Accordingly, in giant, polyploid cells we observe many perinuclear blebs filled with DNA and/or proteins, either involved in senescence signaling pathway, p53/21, or in cell cycle, Ki67. With the use of transmission electron microscopy and 3View system we reveal the blebs formation, their more detailed structure and their disconnecting from the nucleus. Overall, we show that tested cancer cells respond similarly to different chemotherapeutics in terms of senescence. Moreover, we documented that polyploidization is indispensable for cancer cells to escape senescence via removal of excess DNA and protein.

# Plant–environment interaction

## P-32-001
## Transcriptomic and proteomic response to temperature stress in cauliflower *Brassica oleracea* var. botrytis

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Plants as sessile organisms with no ability to move are constantly exposed to the detrimental environmental conditions such as high or low temperature. During evolution, plants have developed a number of adaptive strategies to adverse conditions both at the morphological and physiological as well as on biochemical and molecular level. Organellar RNA-binding proteins (RBPs) have many regulatory functions and are involved in stress response in plants. Using qPCR we checked the level of different RBPs and chose two of them which are the most variable after stress treatment. Confocal microscopy analysis showed a speckles like pattern throughout protoplast suggesting mitochondrial localization. To further extend our study we performed transcriptomics and proteomics analysis. Results from these experiments proved the crucial role of RNA-binding proteins in temperature stress response.

## P-32-002
## Effect of warm-acclimation rate on fatty acid composition and thermotropic behavior of major membrane lipids from marine algae *Saccharina japonica* and *Ulva lactuca*

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The world ocean is the main reservoir of global warming heat. The influence of the rate of ocean temperature rise on marine hydrobionts is of special interest. Ectothermic organisms adjust


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fatty acid composition of membrane lipids to maintain their optimal liquid-crystalline state at environmental temperature changes. The present work was aimed to study the ability of marine algae *Saccharina japonica* and *Ulva lactuca* to adapt the membrane lipid matrix at different rates of warm-acclimation. Algae collected in the Sea of Japan in winter at 4 °C were acclimated to 20 °C during 1 or 8 days. Fatty acid (FA) composition and the crystalline-liquid crystalline phase transitions of photosynthetic lipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) from both algae acclimated as well as betaine lipid 1,2-diacylglyceryl-3-O-4′-(N,N,N-trimethyl)-homoserine (DGTS) from U. lactuca were studied by GC, LC-MS and DSC, respectively. It was shown the decrease in unsaturation index (UI) and unsaturated/saturated FA ratio at warm-acclimation that corresponds to seasonal changes. At slower acclimation, the effect was less pronounced in MGDG and DGDG, and even was reversed in these glycolipids from S. japonica. The ratio *n*-3/*n*-6 polyunsaturated FA (PUFA) almost did not change, although this ratio significantly reduces from winter to summer. In general, the number of molecular species with PUFAs decreased. DSC thermograms of these lipids, as a rule, changed in the same direction as during warm-acclimatization. The temperatures of the main peaks (Tmax) of glycolipids was shifted to lower values unlike changes in UI. Phase transition of DGTS shifted more adequately, towards higher temperatures, and Tmax reached the summer value even at 1-day acclimation. U. lactuca reacted more adequately than S. japonica. The work is supported by Ministry of Science and Higher Education of Russia (the state assignment 6.5736.2017/6.7). *The authors marked with an asterisk equally contributed to the work.

## P-32-003
## Role of a novel *Medicago truncatula* ABC transporter in nodulation and lateral root development

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Nodulation, as well as a whole root architecture, is controlled by a mixture of phytohormones in legumes. One of them, cytokinin, is essential for the inner root cell division at the onset of nodule formation. What is more, it is proven that cytokinin is strongly influencing nodule and lateral root development in response to environmental stimuli. Drought and nitrogen starvation play here a significant role, affecting nodule and lateral root formation in opposite manner. While drought suppresses nodulation and supports lateral roots emergence, nitrogen shortage increases number of nodules and decreases lateral roots density. The exact mechanism of cytokinin action in such regulation has not been clarified yet. Due to widespread presence of ATP driven membrane transporters in plants we decided to investigate their role in nodule and lateral root formation. With a use of model legume *Medicago truncatula* joined with molecular approaches and phenotypic studies, we have identified an ABC transporter present in root vascular bundles, its apical meristem and in developing nodules. Its expression is influenced by cytokinins and symbiotic bacteria, as well as nitrogen starvation and drought stress. Mutant plants lacking this transporter have higher lateral root density under nitrogen limitation and form more nodules than corresponding wild type. We propose that this transporter takes part in the negative regulation of nodulation and lateral root formation. Our results have implications both in legume crop research and determination of the fundamental molecular processes involved in drought response and nodulation. National Science Centre supports this work: UMO-2015/19/B/NZ9/03548.

## P-32-004
## Aphid-triggered oxidative damages of nucleic acids and lipids in maize (*Zea mays* L.) seedlings

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The purpose of the study was to evaluate the impact of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) females infestation on the level of selected biochemical markers of oxidative damages of DNA, RNA and lipids in maize (*Zea mays* L.) host plants. Seedlings (14-day-old) of two tested maize varieties (Złota Karłowa and Waza – susceptible and highly resistant, respectively) were infested with 30 or 60 apterous females of *R. padi* for 3–96 h. Uninfested seedlings of each maize cultivar were used as control ones. The content of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in genomic DNA, 8-hydroxyguanosine (8-OHG) in total RNA and malondialdehyde (MDA) in insect-stressed and control plants were monitored. The aphids' infestation did not evoke significant alternations in the content of 8-OHdG in genomic DNA in seedlings of Złota Karłowa and Waza cultivars. On the other hand, bird cherry-oat aphid infestation caused an increment in the amount of 8-OHG in total RNA in seedling leaves of both studied maize cultivars, in comparison with the controls. Aphid-susceptible Złota Karłowa seedlings characterized with about 2-fold higher increases in 8-OHG content in total RNA than more resistant Waza plants. Furthermore, insects' infestation resulted in augmented accumulation of malondialdehyde (MDA) in maize seedlings. The maximal elevation in MDA content occurred at 24-h aphids' infestation, and reached about 2-fold higher increases in Złota Karłowa plants, compared to Waza seedlings. It has been evidenced oxidative damages of total RNA and lipids as a consequence of aphid-induced oxidative responses of the host plant tissues. The research was financially supported by the National Science Centre (NSC; Poland) under the grant no. 2016/21/B/NZ9/00612.

## P-32-005
## Assessment of the genotoxicity of water and soil in the places of storage of reserves of old pesticides by *Allium* test

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Pesticides and persistent organic pollutants (POPs) are a serious environmental problem for almost all countries of the world. The toxic and mutagenic activity of samples of natural water and water extracts of soils collected near the storage sites of old and prohibited pesticides located in the villages of Kyzylkairat, Amangeldy, Beskainar, Belbulak and near farmland, where pesticides were used in the past (Taukaraturyk village, Talgar area, Almaty region) was investigated. *Allium cepa L.* was used as a biological test system. Purified water served as a control. The mitotic activity and the frequency of disturbances at different stages of mitosis in onion root meristem were evaluated. Chemical analysis showed that in all soil samples the total content of 23 POPs-Pesticides exceeds the maximum permissible concentration (MPC). The most polluted soil was from Kyzylkairat and


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Beskainar (≥MPC 60-120 times). In Taukaraturyk, where there are no former storage facilities – MPC ≥17 times. It was established that the level of mitotic activity of *Allium cepa* cells in all samples was slightly reduced compared with the control. In soil samples, the frequency of micronuclei in interphase cells (0.79–3.25%) is higher than in water samples (0.47–0.99%) (control – 0.32%). However, the analysis of disorders at different stages of mitosis, on the contrary, revealed a high mutagenicity of water samples. Their average level (9.64–24.5%) was 2–5 times higher than the control value (5.03%), which indicates the presence in the water of substances of mitoso-modifying and genotoxic action. The results of cytogenetic studies correspond to the data of chemical analysis. The highest frequency of violations recorded in the evaluation of samples of water and soil from villages Kyzylkairat and Beskainar. The work was carried out as part of the Scientific-Technical Program No. BR05236379. *The authors marked with an asterisk equally contributed to the work.

## P-32-006
## Comparative analysis of cell viability of *Triticum vulgare* after exposure to copper nanoparticles

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Among the wide range of nanoparticles (NP), copper-based NP, which are among the five most used by industrial enterprises all over the world, attract considerable interest. Metal ions belonging to the group comprising Cu, Cd, Hg, Ni and Zn, cause toxicity in living systems. Some of the NP of metals are known to be able to release toxic ions from their colloidal matrix. The object of study – the seeds of *Triticum vulgare* Vill. Cu° powders (54 ± 2.06 nm) are provided by Plasmotherm LLC (Russia). The NPs were intensively pipetted, processed in an ultrasonic bath for 30 min. Disinfection field in 0.01% $KMnO_4$ for 10 min and the seeds were washed with distilled water, soaked in gauze and left for 3 h at t = 37 °C. The swollen seeds were transferred onto wet filter paper and germinated in a climatic chamber (Agilent, USA) under 12-h illumination, t = 22 ± 1 °C and humidity 80 ± 5% within 48 h. Sprouted seeds (20 pieces) were transferred to wet filter paper in separate plastic cups and 5 mL of metal NP suspensions were added in concentrations of 0.0255 to 1 M and left for 48 h. Cell viability (JS) of cells was measured by the change in enzymatic activity of reductases (Cell Cell Kit-8 (CKK-8), WST-8 patent No. 2.251.850, Canada). The results of the WST test showed that the CuЧ NPs caused an unstable and a sharp decrease in the yield of formazan immediately after the start of the exposure (1 h). The pronounced effect began to manifest itself after 24 h of exposure and reached 89% compared with intact plants. We assume that such suppression of the enzymatic activity of reductases is an early response to the metal and a reliable ($P < 0.05$) cytotoxic effect of NP in wheat roots was recorded after 48 h of incubation with metals in doses of 0.05 and 0.1 M. Studies have been performed on research work for 2019-2020 at the Federal Research Center for Biological Systems and Agrotechnologies (No. 0761-2019-0003, No. 0761-2019-0004).

## P-32-007
## Research on the implementation of biological effects $MoO_3$ nanoparticles

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The rapid development of nanotechnology and the production of new nanomaterials should imply priority research on the toxicity of the latter. The data on molybdenum-containing nanoparticles (NP) are especially contradictory and insufficient. Plants, vermiculture and microorganisms, as highly informative experimental models, are indispensable living organisms for the study of the specific action of NP. The studies used spherical MoO3 NPs ("Plasmotherm", Russia) with a size of 92 ± 0.3 nM. The object of the study was the seeds of wheat Triticum vulgare Vill. Seeds were germinated in soil with 50, 100, 250, 500 and 1000 mg/kg with a day length of 16 h (26 °C), 8 h (19 °C), and an air humidity of 50%. After 21 days, based on the data on the length of leaves and roots of seedlings, the effect of inhibition was calculated. The study of the effect of MoO3 NP on plant growth of Triticum aestivum L. showed the presence of a pronounced toxic effect in all variants of the experiment. A mild toxic effect was noted for MoO3 concentrations of NPs 50–250 mg/kg, medium, for concentrations of 500 mg/kg, and strong or unacceptable, for concentrations of 1000 mg/kg. An increase in the concentration of $MoO_3$ NPs over 250 mg/kg causes suppression of the growth of the plant root system, and the magnitude of the phytotoxic effect increases by 40% at a concentration of 500 mg/kg of NPs of $MoO_3$ and 72.2% at 1000 mg/kg. In the presence of inhibition and stimulation of plant growth, an inverse linear relationship is observed between the concentration of pollutant in the soil and the number of roots ($r = -0{,}78$, при $P \leq 0.05$), the minimum value of the number of roots is noted for a nanoparticle concentration of 1000 mg/kg. Studies have been performed on research work for 2019-2020 at the Federal Research Center for Biological Systems and Agrotechnologies (No. 0761-2019-0003, No. 0761-2019-0011).

## P-32-008
## Plant molecules influence on luminescent *Escherichia coli* K12 TG1 with constitutive expressed *luxCDABE* genes

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The relationship between the microorganism and the microorganism can be mediated by small molecules, that influence on different cell subsystems. But overall inhibition effect on metabolism may be detected by whole luminescent bacteria. In this conditions using recombinant *E. coli*, carrying *lux*-gene cassette are convenient, fast and accurate method. The aim of this study is determine plant molecules influence on recombinant luminescent strain *E. coli*. We used molecules selected from *Scutellaria baicalensis (SB)* and *Eucalyptus viminalis (EV)*, which tested on recombinant luminescent strain *E. coli* K12 TG1 with *luxCDABE*-genes of natural marine bacteria *Photobacterium leiongnathi* 54D10. Serial two-fold dilutions of molecules from 2.5 to 0.001 mg/mL were prepared in 96-well plate ("Microlite 2+", Thermo). It was revealed that from the entire spectrum of the tested compounds, 6


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small molecules of plant origin did not significantly affect the bacterial biosensor. 7,8-dihydroxy-2 h-cromen-2-one (*SB*) showed a high degree of toxicity and its action was characterized by an EC50 value of 0.156 mg/mL. At the other hand shikimic acid (*EV*) was demonstrate more low toxicity with an EC50 value of 1.2 mg/mL. The final ranking of compounds according to the EC50 values allows us to build a series of increasing toxicity dianhydroglucitol <flavone <5,6,7-trihydroxy-2-phenyl-chromen-4-one <oleic acid amide <2(3H)-furanone <3,4-dihydroxyoxolan-2-one <shikimic acid <7,8-dihydroxy-2 h-cromen-2-one. Using plants contain this molecules can affect to normal bacterial microflora by metabolism inhibition. The project was supported by the Russian Science Foundation (No. 16-16-10048).

## P-32-009
## Study of the ability of strains isolated from the reindeer's rumen to biodestruction of mycotoxins

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Reindeer (Rangifer tarandus) – is an unique animal that can exist in a poor diet. Lichens occupy from 15 to 70% of the total amount of feed depending on the season. Lichens are producers of a number of harmful metabolites like usnic acid and mycotoxins. Mycotoxins are low-molecular secondary metabolites produced by microscopic mold fungi. When it ingested in humans and animals with food, they can have a damaging effect – inhibit protein synthesis, disrupt vascular permeability, suppress immunity, etc. The search for microorganisms-destructors of mycotoxins is an important task. We isolated strains of bacteria from the healthy reindeer's rumen and studied their ability to decompose mycotoxins. The ability of the most active cellulose-decomposing isolates (No. 7, 14, 15, 21, 26) to transform mycotoxins was analyzed. The process of incubation was carried out in tubes with isolated 1-day strains of bacteria and mycotoxins in the following quantities: aflatoxins – 12 μg/L, ochratoxin A – 15 μg/L, T-2 toxin – 60 μg/L, zearalenone – 100 μg/L, deoxynivalenol – 1000 μg/L. The duration of incubation was 3 days at 38 °C in a thermostat with regular stirring. Using the method of ELISA was determined the decrease in the amount of mycotoxin in solution. The results showed that all of the isolates studied had the ability to biodegrade at least two mycotoxins used in the study. Isolate No. 26 was of particular interest, which was capable of biotransformation of all studied mycotoxins (the level of biodegradation ranged from 13.4 to 87.5% with $P \leq 0.05$). All studied isolates possessed properties for the degradation of aflatoxin and T-2 toxin. At the same time, the most pronounced biotransformation ability was observed in relation to aflatoxins (from 22.8 to 75.1% with $P \leq 0.05$). The research was carried out with the support of the grant of the Russian Science Foundation (RSCF) No. 17-76-20026.

## P-32-010
## INH2, PAIN-1 and SUS4 co-expression data reveals that only sucrose synthase can serve as a sign of cold-resistance in wild potato species

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Potato cold resistance is associated with increased content of reducing sugars. Wild tuber-bearing relatives of cultivated *S. tuberosum* (*Solanum* section Petota) represent a rich source of genetic variants. We characterized structural variability of vacuolar invertase inhibitor INH2 in eight wild potato species, which are commonly used as breeding material, and found that the detected polymorphisms did not affect the tertiary structure of INH2 homologs. The co-expression of *INH2* and saccharolytic enzymes *PAIN-1* and *SUS4* genes was assessed in leaves before and after cold treatment in four wild species differing in cold resistance. Cold exposure induced *INH2* transcription in *S. demissum* and *S. chacoense* but not in *S. vernei* and *S. stoloniferum*, suggesting species-specific role of INH2 in cold stress response of potato. In cold-stored leaves, cold-resistant S. vernei demonstrated sharply increased *PAIN-1* and *SUS4* expression and unchanged *INH2* levels, indicating increased sucrose hydrolysis, whereas cold-sensitive *S. stoloniferum* had constant *INH2*, *PAIN-1*, and *SUS4* expression and should have the same content of glucose and fructose after cold exposure. *S. demissum* and *S. chacoense* with medium cold resistance showed a different mode of *INH2*, *PAIN-1*, and *SUS4* co-expression. It can be hypothesized that in *S. demissum*, cold tolerance is maintained by the *SUS4* upregulation in parallel with *INH2* downregulation and unchanged *PAIN-1* levels, whereas *S. chacoense* behaves as a cold-sensitive species because significant *SUS4* and *PAIN-1* downregulation together with *INH2* upregulation should decrease the content of reducing sugars. Our data suggest that the potato cold response may depend on the balance between activities of vacInv inhibitor INH2 and saccharolytic enzymes PAIN-1 and SUS4. The study was supported by the RFBR (18-29-07007 and 18-016-00108) and Ministry of Science and Higher Education of the Russian Federation.

## P-32-011
## The combination of *Quercus cortex* extract and probiotic changes rumen microbiome of cattle

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The ban on feed antibiotics for rearing farm animals makes it necessary to seek alternatives actively, including plant extracts and probiotics. In this regard, research on joint use of these substances for the internal environment of ruminants are interesting. The effect of extracts of *Quercus cortex* (QC), where we previously found antiquorum substances, and probiotics (*Bifidobacterium adolescentis; BA*) was assessed. Ruminal fluid (control) was obtained from animals' rumen fistula (diet: 80% – sudan grass and alfalfa hay, 20% – grain feed); combination of RF and BA (1 test); the combination of RF, QC and BA (2 test). The combination of RF and BA increases the number of *Bacteroidetes* phylum (by 32.0% of control; $P \leq 0.05$), and decreases *Proteobacteria* – by 19.0% ($P \leq 0.05$); in addition, the number of representatives of *Bacilli* classes (by 14.9%; $P \leq 0.05$), *Gammaproteobacteria* (by 7.9%; $P \leq 0.05$) decreases, and the *Clostridia* (by 13.2%; $P \leq 0.05$) increases. The combination of RF, QC and BA reduces the number of *Clostridia* (by 5.7%; $P \leq 0.05$), *Bacteroidia* (by 12.2%; $P \leq 0.05$), members of the genus *Escherichia* (by 2.0%) and the number of representatives of *Gammaproteobacteria* taxon (by 54.1%; $P \leq 0.05$). The combination of RF and BA is characterized by a significant increase in the number of *Ascomycota* mushrooms – by 22.9% ($P \leq 0.05$) and *Neocallimastigomycota* – 16.5% ($P \leq 0.05$). The combination of RF, QC and BA contributed to an increase in the number of representatives of microscopic fungi of *Chytridiomycota* by


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32.6% ($P \leq 0.05$). Thus, the combination of QC and BA may be interesting to decrease pathogenic bacteria in cattle. Research was held under project No. 0761-2019-0005.

## P-32-012
## Effect of exogenous application of methyl jasmonate on wheat tolerance to cadmium

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Methyl jasmonate (MJ) is methyl esterol jasmonic acid is generally regarded as plant growth regulator that mediates various aspects of gene and metabolic regulation. MJ play important roles for regulating the defense mechanisms to biotic stresses. In recent years, MJ has been focus of much attention because its ability to increase the plant tolerance to abiotic stresses. Therefore, this study aims to investigate whether or not exogenous MJ also exerts protective effects on wheat seedlings under cadmium (Cd) stress. 7-days-old winter wheat seedlings (cv. Moskovskaya 39) subjected to cadmium sulphate (100 μM) treatment for 1 week. MJ application was made 24 h prior cadmium impact. Results of cadmium accumulation showed, that wheat seedlings treated with MJ have decreased uptake and accumulation of Cd in shoots and roots. Exposure to Cd resulted in decrease in shoot length and fresh/dry weight of wheat throughout growth period. Application of MJ has almost no effect on plant's length whereas leads to increase in fresh and dry weight and first leaf area. Exogenous application of MJ leads to increase in *MYB33* gene expression, encoding transcription factor prior to cadmium effect. While under Cd stress MJ untreated seedling has higher *MYB33* transcript level. It might be due to activization of signaling pathway that activated defense reactions even before stress. MJ treated seedlings have enhance mRNA content of *GS1* and *PCS1* genes, encoding glutathione synthetase and phytochelatin synthases respectively. These enzymes regulate the synthesis of glutathione and phytochelatins that play a key role in cadmium detoxification. MJ can reduce the negative effect of cadmium on plants and improve its tolerance through the increase in *GS1* and *PCS1* gene expression and further synthesis of glutathione and phytochelatins. The study was carried out under a state order (project No. 0221-2017-0051).

## P-32-013
## Influence of FNR-TROL interaction on directing of photosynthetic electrons

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Ferredoxin NADP+ oxidoreductase (FNR) catalyses reversible reaction of electron transfer from ferredoxin (Fd) to NADP+ as the last step of linear electron flow during photosynthesis. FNR can be bound to the protein complexes on thylakoid membranes with thylakoid rhodanese-like protein (TROL) and translocon on the inner envelope chloroplast membrane (Tic62), but also as a soluble protein in stroma. It is envisaged that Fd efficiently passes electrons to alternative pathways when FNR is not sequestered to the TROL. FNR protein is shown to be involved in oxidative stress responses. We are using the common C3 model plant organism *Arabidopsis thaliana* L. Col-0 that has two leaf FNR isoforms and *Zea mays*, a C4 plant that contains three isoforms. We have chosen maize FNR1 since it has been shown that ZmFNR1 strongly binds to the TROL. Maize FNR1 gene was obtained from Arizona Genomic Institute and FLAG and HA tags were PCR-added to the gene to enable posterior isolation and analyses of protein complexes via co-immuno precipitation assays. Using Gateway cloning technology, we have made Arabidopsis transgenic plants containing *Z. mays* FNR1 bearing FLAG and HA tags. By crossing those plants with the Arabidopsis plants that accumulate TROL with amino acid or domain changes, we have created specific experimental platforms that will be used to explore TROL structures that are involved in the dynamic FNR binding under different light conditions. Photosynthesis in C4 plants has higher efficacy and higher yield than in C3 plants and this research on FNR1 shuttle between C3 and C4 plants should provide better insight in to the differences between these two photosynthetic pathways. Finally, we hope to create the C3 plant that has more effective responses to oxidative stress.

## P-32-014
## Cold plasma treatment stimulates germination due to rapid changes induced in the content of radish seed phytohormones

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The aim of this study was to evaluate effects of air cold plasma (CP) on germination dynamics of radish (*Raphanus sativus*) seeds and to estimate how changes in germination correlate with the content of phytohormones in dry seeds. Freshly harvested as well as stored for 3 and 5 months radish seeds were treated for 1, 3, 5, 7 or 10 min with a scalable dielectric barrier discharge CP device. Germination tests *in vitro* were performed and methanol extracts of dry seed homogenates were prepared for phytohormone analysis by HPLC. The dynamics of changes in the content of seed phytohormones after CP treatment was also estimated. For freshly harvested radish seeds CP treatment increased germination percentage and germination rate in all experimental groups, and these changes most strongly correlated with the decrease in the content of abscisic acid (ABA) and increase in gibberellin (GA) although changes in amounts of auxins, cytokinins, and salicylic acid were also determined. CP-induced decrease in seed ABA content was detected 1 h after treatment and it was further decreasing during the following 4 days. The decrease in ABA/GA ratio was determined during after-ripening of radish seeds (3 and 5 months after harvest) along with dormancy loss that was followed by the decreased ability of CP treatment to stimulate germination. We report that the extent of the observed effects on germination is dependent on seed dormancy status characterized by seed hormonal balance (the most important is the ratio between ABA/GA). The obtained results show that stimulation of germination by CP treatment on the molecular level can be explained by the induced changes in the ratio of the main phytohormones involved in the control of seed dormancy and germination, i.e., CP treatment works in a similar way as other seed dormancy breaking treatments. The obtained results indicate also the potential of CP treatment for use in plant biotechnology. *The authors marked with an asterisk equally contributed to the work.


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## P-32-015

## Changes in seed phytohormones induced by treatment of common sunflower seeds with cold plasma are associated with changes in germination and seedling growth

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The changes induced by the pre-sowing treatment of common sunflower (*Helianthus annuus*) seeds with cold plasma (CP) in kinetics of seed germination, phytohormone content, seedling growth and photosynthetic efficiency in seedling leaves were studied. The effects of CP treatment (duration 5, 7, 9 and 11 min) were determined by seed germination *in vitro* and in the substrate, morphometric analysis of seedlings was performed 5 and 30 days after sowing, seed phytohormone content was determined using HPLC analysis, the photosynthetic performance index (PI) was determined by chlorophyll fluorimeter Handy Pea+. The only noticeable effect of CP treatment on sunflower seed germination was slight (13%) inhibition of germination in the substrate (but not *in vitro*), however treatment had obvious effect on phytohormone content in dry seeds: the content of abscisic acid (ABA) decreased by 36–40%, while the amount of gibberellins (GA) increased after 7 min (43%) but decreased (50%) after 11 min of CP treatment. CP 11 min treatment increased the amount of stress hormone salicylic acid (SA) by 23%. Such changes were associated with different effects on seedling growth 30 days after sowing: seedlings from CP 7 min treated group were 16% heavier and 20% longer in comparison to the control, but number of leaves and leaf weight decreased in seedlings from CP 11 min treated group (by 10% and 15%, respectively). CP effects on sunflower seedling growth were consistent with changes in photosynthetic activity – seed treatment with CP for 7 min activated photosynthesis on sunflower seedlings (PI increased by 19%) but 11 min treatment had strong negative effect: PI was 57% smaller in comparison to the control. In conclusion, CP treatment induced changes in amounts of seed phytohormones ABA, GA and SA can be responsible not only for the effects on germination but also may be related to changes in seedling growth and photosynthetic efficiency on the later stages of plant development. *The authors marked with an asterisk equally contributed to the work.

## P-32-016

## Study of plant systemic signaling proteins in response to nitrogen, phosphate, and potassium deficiency using quantitative proteomics approach

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Primary macronutrients nitrogen (N), phosphate (Pi), and potassium (K) are major elements to build-up fundamental biomolecules, function in energy transfer and the regulation of enzyme activity in plants. In response to the limited macronutrient availability in soil, plants display a high degree of adaptive responses, depending on both local and systemic regulation networks to coordinate nutrient homeostasis within the whole plant. There are several long-distance traveling signals in plants such as RNA which can systemically migrate through phloem; however, those long-distance proteins under nutrient shortage are still elusive. Among 3000–4000 identified proteins in the phloem sap of heterografted cucumber/watermelon, about 700 proteins could be translocated in the phloem sap under normal condition, while 471, 714, and 640 cucumber proteins were found to be systemically translocated in the phloem sap under N, Pi, and K deficiency, respectively. When plants encountered N, Pi, K-deficiency, there were 93, 113, and 326 mobile proteins with significant change in abundance, respectively. Using BLAST, the closest matched *Arabidopsis thaliana* proteins were extracted by the sequence of these quantified cucumber mobile proteins and further analyzed by PANTHERN (Protein ANalysis THrough Evolutionary Relationships) classification system and agriGO for function grouping and identification of enriched Gene Ontology terms. Those long-distance translocated cucumber proteins with more than 1.3- or less than 0.76-fold change in abundance are involved in carbohydrate metabolic process, molecular biosynthetic process, ubiquitin/protein degradation, and cytokinesis. Interestingly, some potential RNA-binding proteins were also identified. These mobile proteins with change in quantities in response to macronutrient deficiency should provide better understanding of the mobile signaling mechanisms and homeostasis for nutrient starvation.

## P-32-017

## Reversible light-dependent carotenoid transfer between water-soluble proteins suggests a new way to modulate the OCP-based photoprotection in cyanobacteria

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Carotenoids are essential components of all photosynthetic organisms and many non-photosynthetic organisms such as fungi and animals. These molecules have pronounced photoprotective and antioxidant properties and participate in visual sensation. Cyanobacteria utilize carotenoids to dissipate excessive light energy absorbed by their light harvesting complexes, phycobilisomes, through the action of the water-soluble photoactive Orange Carotenoid Protein (OCP), which is composed of the N- and C-terminal domains coordinating the carotenoid. OCP is downregulated by the so-called Fluorescence Recovery Protein (FRP); however, many cyanobacteria possess no genes for FRP. Maturation of OCP carotenoprotein has to involve the extraction of carotenoids from the membrane, but the mechanism of this process is largely unknown. Recently, it was shown that the naturally expressed C-terminal domain homolog of OCP (called CTDH) can extract carotenoid molecules from the cell membrane and transfer them to OCP and/or the N-terminal domain homologs of OCP (called HCPs). Here we show that the carotenoid transfer between CTDH and OCP-related proteins can go in the opposite direction and revealed the corresponding metastable heterodimeric intermediates. Moreover, we discovered a light-dependent carotenoid transfer process when OCP photoactivation led to the domain separation and transient carotenoid migration into



FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675

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CTDH, associated by protein dimerization, whereas in the dark the reversal of this process was observed. Such an observation suggests that CTDHs may participate not only in OCP and HCP maturation but also in modulation of the OCP activity through the light-dependent carotenoid arrest. Our hypothesis was supported by the CTDH-dependent recovery of phycobilisome fluorescence after the OCP action. These results suggest a new possible mechanism of the OCP activity modulation in cyanobacteria where FRP is absent. Partially supported by RFBR (18-04-00691).

## P-32-018
## Analysis of the resistance of *A. thaliana* transgenic plants expressing the RNA aptamers to the HopU1 effector protein, to the effects of the phytopathogen *P. syringae* pv. tomato DC3000

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We have selected two RNA aptamers (H1 and H2) to the third type transport system (TTS) effector protein HopU1 of the *P. syringae* pv. tomato (Pss) bacteria using the SELEX method. The effector protein HopU1 is an ADP-ribosyltransferase interacting with RNA-binding proteins thereby suppressing plant immunity. Next the plasmid vectors for the constitutive expression of the RNA aptamers in *A. thaliana* plants were created. The A. thaliana plants were transformed using agrobacteria delivery via floral dip. We have obtained more than 5 independent lines of plants carrying one genomic insert for each vector. To determine the effect of the aptamer expression on the development of a protective response in plants, the leaves of the selected transgenic plants were infected with the bacteria *P. syringae* pv. tomato (Pss) with a subsequent assessment of the growth rates of the pathogen populations (cfu/cm2 of leaf surface at various time intervals after inoculation). We found significant differences in the rates of infection between transgenic and wild-type plants. The number of pathogen colonies in wild-type plants on the day 3 of inoculation exceeded the number of colonies in the transgenic plants by almost 150 times for H1 and 138 times for the plants of the H2 line. The study has shown that compared to the wild-type plants A. thaliana transgenic plants expressing the H1 and H2 aptamers are highly resistant to the bacterial phytopathogen P. syringae pv. tomato (Pss). This work was supported by a grant from the Russian Foundation for Basic Research No. 16-04-01002 and Russian government assignment No. 0112-2019-0002.

## P-32-019
## MPK4 as a molecular switch that controls growth optimization in changing environmental conditions

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Plants are sessile. Their survival depends on the ability to quickly perceive the signal, react to it and adapt to suboptimal environmental conditions. Mitogen-activated protein kinase (MAPK) signaling pathways form an advanced system which play a key role in adaptive processes in plants. MAPK modules are involved in stress and hormonal responses, regulation of cell division, growth, differentiation and programmed cell death. Although MAPKs regulate global changes in gene expression in the medium and long term, they also affect cytoplasmic infrastructure and operate in the short term. Interactions between MAPKs and other signaling molecules, such as reactive oxygen species and hormones are important for maintaining the plant cell homeostasis in response to various stresses. The data previously presented clearly show the importance of MAPK in abiotic stress signaling in plants. Study of the *MPK4* gene promoter sequence, are important for understanding the role of MPK4 in optimizing plant growth under changing environmental conditions. Various *cis* regulatory elements (CREs) were identified in the *MPK4* promoter sequence. Most of them were connected with response to various environmental stimuli, such as: high and low temperature, drought, $CO_2$ concentration and light. In addition, several important CREs related to hormone response have been identified. The occurrence of hormones related CREs: TCA, TGA and GARE in the regulatory region of MPK4 may indicate cooperation between MPK4 and hormones such as auxin, GAs and SA in the regulation of many important processes for optimal plant growth. The experiments made it possible to determine the impact of environmental conditions and plant hormones on the role of MPK4 protein as a molecular switch that controls growth in variable environmental conditions. Moreover, the obtained results broaden the knowledge on how the transduction of hormonal signals through MPK4 kinase modulates the plant's response to various stress factors.

## P-32-020
## Differentiation of defensive metabolite biosynthesis in Brassicaceae plants

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Aliphatic (AGs) and indolic (IGs) glucosinolates are specialized metabolites involved in the environmental interaction of Brassicaceae plants. They determine plant constitutive defences, but their accumulation and metabolism can be also affected by abiotic and biotic factors. For instance, the loss of cellular integrity of the plant tissue as a result of wounding by insect or pathogen attack activates glucosinolate hydrolysis leading to the formation of unstable intermediates, which can rearrange into several types of bioactive products. Interestingly, published results suggest that some species belonging to the Brassicaceae family can be devoid of at least one of these two glucosinolate types, for instance, IGs were not found in leaves of *Capsella rubella*. This seems surprising considering the important functions of these compounds in the interactions with the environment. In this study we focused on glucosinolate biosynthetic capacity of selected species closely related with *Capsella rubella* including *Arabidopsis thaliana*, *Capsella grandiflora*, *Capsella bursa-pastoris*, *Camelina sativa* and *Neslia paniculata*. To this end we analysed metabolite extracts from different organs and at different stages of development using UPLC coupled with double quadrupole mass spectrometer and investigated expression profiles of genes linked with glucosinolate biosynthesis. It is well known that *A. thaliana* accumulates IGs and short-chain AGs at each developmental stage and in every organ. In contrast, we detected long-chain AGs in young seedlings, roots and siliques of other species. Moreover, we observed only low amounts of IGs and only in inflorescences and siliques of these species. Correspondingly, our investigations revealed that expression of the key IG biosynthetic genes, including *CYP83B1* and *MYB34* in *C. rubella* and its relatives is not detectable or significantly lower than in *A. thaliana*.


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## P-32-021
## Comparative proteomic analysis of two potato varieties under biotic and complex stresses

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The study of plant resistance mechanisms to biotic and abiotic stresses is one of a problem in plant breeding. In order to determine changes in biological pathways induced by individual and complex stresses, we performed proteomic profiling of two potato varieties differ in resistance to heat stress (28 °C) and infection with potato virus Y (PVY) – cultivars Gala (resistant genotype) and Chicago (susceptible genotype). Label-free quantification and isobaric tags for relative and absolute quantitation (iTRAQ) approaches were used for quantitative proteomic analysis. The proteome of susceptible genotype (cv. Chicago) showed a weak response to both an individual (viral infection or heat stress) and complex stresses. In contrast, nuclear proteins such as calreticulin, small nuclear ribonucleoproteins (snRNPs) and small nuclear ribonucleoprotein E were up-regulated in resistant genotype (cv. Gala). snRNPs are known to be involved in a spliceosome assembly and can be functionally linked to another nuclear protein – fibrillarin. In addition, the abundance of a number of ribosomal proteins, acidic class II 1.3-beta-glucanases, multicystatin and antioxidant enzymes have also been increased. We also detected that proteins of the primary metabolism, such as glyceraldehyde 3-phosphate dehydrogenase A, plastocyanin and peptidyl-prolyl cis-trans isomerase were down-regulated in resistant genotype. The further studies are needed to determine the contribution of the most promising protein targets to establish of resistant to complex stresses. This work was supported by Research Grant No. 14.W03.31.0003 from the Government of the Russian Federation.

## P-32-022
## The evaluation of some antioxidant and epigenetic markers of flooding tolerances in wheat (*Triticum aestivum* L.) genotypes

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Flooding is one of the remarkable abiotic stresses for many crops, in which the gas exchange between soil and air decreases and root hypoxia stress triggers the formation of reactive oxygen species (ROS) and induces oxidative stress in plants; this influences on photosynthesis, growth, development, significant reduction of productivity. To elucidate the mechanisms involved in different flooding sensitivity and tolerance the flooding-induced changes in enzymatic and non-enzymatic antioxidant systems, electrolyte leakage and global DNA methylation in the leaf were evaluated in some Latvian commercial winter wheat (*Triticum aestivum* L.) varieties. Etiolated wheat seedlings were germinated on moist filter paper at 26 °C for 24 h before being transferred to black soil. Growth of seedlings has occurred in a climate chamber under controlled conditions (light/dark cycle, day/night, temperature, relative humidity and light intensity). The 1-week seedling was separated in two groups: one group has continued growth in this condition, but others group was subjected to flooding. After 1-week flooding the first leaves were cut; contents of phenolic compounds determined by DPPH (using diphenyl-p-picrylhydrazyl radical), and FRAP (ferric reducing antioxidant power) methods, ABTS used to observe the reaction kinetics of specific enzymes, free radical scavenging capacity, malondialdehyde level (MDA), carotenoids content, activity of catalase (CAT) and superoxide dismutase (SOD) was measured, the electrolyte leakage was determined and the global DNA methylation level was investigated measured by pyrosequencing, used LUminometric Methylation Assay (LUMA). It was shown, that the tolerance to the flooding may be improved by increased antioxidant capacity and decreased of membrane lipid peroxidation and the global DNA methylation level in leaf reflects a tolerance to the flooding stress of the wheat.

## P-32-023
## Nucleolar fibrillarin as a negative regulator of resistance to biotic and abiotic stresses mediated by salicylic acid

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Fibrillarin is one of the major nucleolar proteins. In addition to its traditional function in methylation and processing of prerRNA, fibrillarin participates in virus-host interactions. Previously we have shown that under a heat-stress condition (28 °C), potato virus Y (PVY) accumulates to markedly increased levels compared with normal temperature (22 °C) and significantly aggravates these symptoms in infected potato plants. Knockdown of fibrillarin achieved by TRV-mediated VIGS notably attenuates these symptoms and suppresses the virus infection. In contrast, overproduction of fibrillarin in the potato transgenic lines significantly accelerates all of these processes, suggesting involvement of fibrillarin in the responses of potato plants to heat stress combined or uncombined with PVY infection. Here we studied the possible molecular basis of fibrillarin effect. Overexpression of fibrillarin dramatically inhibits the expression level of PR-genes (PR1/GluIII (PR2)). An increase of the expression level of PR genes (3–5 times higher than the level in control) is observed only in response to a viral infection at normal temperature. Treatment with salicylic acid (SA) partially compensates for the effect of fibrillarin overexpression, which indicates a functional connection between the SA mediated pathway and fibrillarin functions in the response to PVY infection. Expression level of HSP genes (heat shock protein) decreases with fibrillarin overexpression, especially at high temperature, and the SA treatment completely compensates this inhibition. We assume that fibrillarin is a negative regulator of SA-induced resistance to viral infection (PVY), heat stress, and, obviously, combined stress caused by PVY and high temperature. This work was supported by Research Grant No. 14.W03.31.0003 from the Government of the Russian Federation.

## P-32-024
## The CaCYP707A70 gene expression analysis under drought stress in hot pepper

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Control of stomatal aperture is important for drought stress resistance. Under drought stress condition, plants promote synthesis of the abscisic acid (ABA) hormone that triggers stomatal


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closure and inhibits stomatal opening in guard cells on the leaf surface. The endogenous ABA level is modulated by the balance of its biosynthesis and catabolism. The major ABA catabolic pathway is oxidative degradation to 8′-hydroxy ABA and it is catalyzed by the cytochrome P450 CYP707A family. To investigate the roles of the CYP707A family under drought condition, CaCYP707A70 which is one of CYP707A family gene from Hot pepper (*Capsicum annuum* L.) was analyzed during drought stress treatment. The expression level of gene was increased until 3 day after watering and decreased from that day. In addition, the expression level was increased after 1 day of subsequent rehydration, however continuously decreased thereafter. The results were assumed that the CaCYP707A70 gene expression is down regulated in drought stressed conditions and up regulated after re watering to modulate ABA levels in Hot pepper. To identify the catalytic function of the CYP707A70 gene, it was cloned into *E. coli* expression vector and heterologously produced in *E. coli* system. This enzyme was catalyzed ABA to 8′-hydroxy ABA, which is inactive form of ABA, and it was converted to phaseic acid and dihydrophaseic acid. To investigate function of the CYP707A70 gene in plant, this gene was cloned into plant expression vector and transformed into tobacco (*Nicotiana tabacum* cv. Xanthi NC). The over-expressed transgenic tobacco plants showed more sensitive on drought stress compare to non-transgenic tobacco. *The authors marked with an asterisk equally contributed to the work.

## P-32-025
## The role of cytokinin transporter in nodulation process

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Cytokinins (CKs) constitute a plant-specific hormones, involved in numerous growth and developmental programs as well as responses to environmental cues. In legumes, which have ability to form symbiotic interactions with nitrogen-fixing bacteria, role of cytokinins in the beneficial associations cannot be overestimated. Activation of cytokinin signaling pathway in the root cortex and as a consequence their *de novo* biosynthesis, triggers inception of the new root-derived symbiotic organ, called nodule. Interestingly, epidermal cytokinins are being proposed as signaling molecules joining outer and inner root tissue responses. However, elucidation of the molecular mechanism of their translocation between tissues/cells in legumes is still elusive. Here we address a question about putative role of selected, root expressed ABC (ATP-binding cassette) transporter in modulation of nitrogen-fixing symbiosis, in model legume plant *Medicago truncatula*. Transcript of this transporter accumulates upon symbiotic bacteria, isolated Nod factor, as well as cytokinin treatment. *Rhizobium*-dependent increment in its mRNA is restricted to the infection zone. Additionally tissue-specific expression pattern of this transporter is strictly regulated in symbiotic context and observed in epidermis and root cortex. Analyzed protein is located in the plasma membrane and translocates bioactive cytokinins in an ATP-dependent manner. Disruption of this transporter results in nodulation impairment and affects cytokinin signaling. In light of our studies, role of ABC transporters in N-fixing symbiosis through cytokinin translocation can be postulated. National Science Centre supports this work: UMO-2015/19/B/NZ9/03548.

## P-32-026
## Hormonomic approach to the understanding of winter wheat flowering induction

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Hormonomics is the profiling of a large group of phytohormones and their metabolites. Phytohormones are a vital element of the regulation of various aspects of plant development. A complex network of more than one plant hormone is involved in controlling plant responses to environmental stimuli. Flowering is the main physiological process enabling plant propagation. An annual plant, wheat, requires the exposure to cold and long days to enable the transition to flowering – vernalization. Though some areas are covered, considerable gaps in our knowledge and understanding still exist in the control of hormonal networks and crosstalk between different hormones during the transition to flowering. Exogenously applied plant regulators can only partially substitute vernalization in time and dose-dependent manner. The fungal secondary metabolite, zearalenone (ZEN), posses high activity through shortening a period of flowering during the vernalization. The aim of the study was to test if the effect of ZEN leading to accelerated flowering in the winter wheat is followed by specific hormonal balance during vernalization. The experiments were carried out *in vitro*. The isolated winter wheat embryos were grown on MS medium with ZEN and MS medium without ZEN (control). Both were subjected to vernalization and afterward transferred to the vegetation tunnel. After 120 days of vegetation, the degree of apical meristem development was determined and targeted phytohormone profiling in apexes was conducted (UHPLC-MS/MS). Among the control plants, only 10% achieved a generative developmental phase. In contrast, plants vernalized on in the presence of ZEN have reached the generative phase in 80% already after 6 days of cooling, and after 15 days all plants were generative. ZEN activity as a promoter of generative winter plant induction consists of the specific determination of mutual proportions of gibberellins, cytokinins, auxins, abscisic acid, jasmonates, and ethylene.

## P-32-027
## Role of miR159 in tomato plants undergoing drought stress

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Climate changes and environmental stresses have significant implications on global crop production being water scarcity one of the most threat for agriculture as it limits crop yields. Because of this, alternatives are urgently needed for improving plant adaptation to drought stress. A biotechnological strategy might be provided by microRNAs (miRNAs) due to their regulatory role in gene expression. Drought-responsive miRNAs have been reported in many plant species and some of them are involved in complex regulatory networks via transcription factors implicated in water stress signaling. The role of *miR159* was explored in tomato plants undergoing drought stress by analyzing the expression of *Sly-miR159* and its targets *SlMYB* transcription factor genes in tomato plants of *Solanum lycopersicum* Mill. cv. Ailsa Craig grown in deprived water conditions or attacked by the Colorado potato beetle, a devastating coleopteran pest of Solanaceae plants. Results showed the potential involvement of this miRNA in adaptation to water deficit conditions in tomato plants, which could be mediated by differential stress-specific MYB transcription factors. The targeting of *SlMYB33* transcription factor


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transcript by *Sly-miR159* correlated with accumulation of the osmoprotective compounds proline and putrescine, highlighting the potential role of this miRNA in adaptation to water deficit conditions.

## P-32-028
## Is silicon involved in plant stress reactions?

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Silicon is one of the most abundant element on the Earth. Despite this, its concentration in living organisms, among them in plants, is extremely low. The probable reason is its low chemical activity in the environmental conditions of biosphere on the Earth. However, many authors published some interesting articles about presence and putative role of silicon in plants, and the interest in this subject is constantly growing. There are some data about possible silicon contribution to plant stress reactions. We have conducted experiments on soya growing in the field, where plants need to cope the environmental stresses. Some of the plants were sprayed with solution of sodium metasilicate in water (1775 g/L). The aim of the experiment was to track the changes in stress related markers (abscisic acid and free proline) and changes in proteome of plants living in the field conditions. Samples of leaves were collected after 1 h, 3 and 30 days after the treatment. We have found substantial changes in examined parameters: ABA and proline concentrations increased in plants treated with the silicon, and we have found some upregulated proteins too. We have discussed the observed effect and their links with the stress.

## P-32-029
## Functional analysis of movement proteins encoded by Hibiscus green spot virus

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Viral cell-to-cell transport in plants requires specialized virus-encoded movement proteins (MPs), which provide delivery of viral genomes to plasmodesmata and their further translocation to neighboring cells. Genomes of many plant viruses carry 'triple gene block' (TGB), a conserved gene module coding for three MPs termed TGB1, TGB2 and TGB3. Recently, we have demonstrated that the genome of Hibiscus green spot virus (HGSV, the genus Higrevirus) contains a novel transport module, a 'binary movement block' (BMB), consisting of two MP genes designated as BMB1 and BMB2. The HGSV BMB1 and BMB2 proteins are necessary and sufficient for cell-to-cell transport of a reporter virus construct. Evolutionary, BMB is distantly related to TGB. Similarly to TGB1, BMB1 has a helicase domain; however it is closer in sequence to viral replicative helicases than to TGB1 proteins. BMB2, like both TGB2 and TGB3, is a highly hydrophobic protein, which has a marginal sequence similarity to TGB2 and is functionally similar to TGB3, combining therefore features of the two TGB proteins. Transient co-expression of BMB1 and BMB2 fused to fluorescent proteins has revealed that the BMB2 protein can direct BMB1 to peripheral membrane bodies, which are derivative of the endoplasmic reticulum (ER) membranes located in close vicinity of plasmodesmata, to the plasmodesmata interior, and to neighboring cells. BMB2-directed transport to plasmodesmata-associated sites depends on functional integrity of the ER/actin network and can occur via lateral translocation along the ER membranes. Collectively, these data demonstrate that plant virus cell-to-cell movement is closely associated with ER membranes and is accompanied by modifications of ER structures. This work was supported by Joint Research Project funded by French National Center for Scientific Research (grant PRC 252520 CNRS/RFBR 2017) and Russian Foundation for Basic Research (grant 18-54-15003).

## P-32-030
## Genetic diversity of *Linum usitatissimum* L. pathogens

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Flax (*Linum usitatissimum* L.) is used for fiber and seed production, however, pathogens decrease the crop yield. The aim of our work was to assess the genetic diversity of fungal pathogens of flax using deep sequencing. One hundred strains were obtained from Institute for Flax (Torzhok, Russia), including 24 strains of *Fusarium oxysporum*, 3 strains of *Fusarium avenaceum*, 5 strains of *Fusarium culmorum*, 8 strains of *Fusarium moniliforme*, 4 strains of *Fusarium gibbosum*, 2 strains of *Fusarium semitectum*, 3 strains of *Fusarium sporotrichiella*, 5 strains of *Fusarium solani*, 9 strains of *Melampsora lini*, 20 strains of *Colletotrichum lini*, 8 strains of *Septoria linicola*, and 9 strains of *Aureobasidium pullulans*. Samples were homogenized with MagNA Lyser (Roche) and DNA was purified using CTAB protocol. Genes encoding translation elongation factor 1-alpha (tef1), RNA polymerase II subunits (RPB1 and RPB2), and beta-tubulin (tub2) and internal transcribed spacer (ITS) region of the rRNA gene were chosen for evaluation of genetic diversity of fungal pathogens of flax based on the literature data. The method proposed by Illumina for 16S metagenomic sequencing library preparation was the basis for our study of the polymorphism of flax pathogens. Primers for the chosen genes with overhang adapters were used at the first stage of DNA library preparation followed by the second stage of amplification using Nextera XT index primers. DNA libraries for 100 strains were obtained and sequenced on MiSeq Illumina with 300 + 300 nucleotide read length. About 4 thousand reads were generated for each strain. The results of our study allow assessment of the genetic diversity of flax pathogens and determination of DNA sequences that are the most promising for use as genetic markers for identification of fungus that cause *L. usitatissimum* diseases. This work was financially supported by the Russian President Grant MK-5828.2018.4.

## P-32-031
## Comprehensive impact of typical to the Baltic Sea salinity levels to photosynthesis, photosynthetic pigments, the activity of catalase and other metabolic components in the *Elodea canadensis* (Michx.)

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Salinity is a major abiotic stress in plants worldwide. The effect of different levels of salinity on some physio-biochemical parameters of invasive plant Canadian waterweed (*Elodea canadensis*


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Michx) investigated. Our focus was on the effect of NaCl (applied at 0, 10, 25, 50 or 100 mM) on leaf pigments, total soluble proteins, chlorophyll fluorescence (Fv/Fm), proline concentration, rate of superoxide ($O2^{\cdot-}$) production, superoxide dismutase, and catalase activity. More notable changes were observed with the concentration of carotenoids in chloroplasts, it significantly increased as early as in the first week of the experiment and by the third week, concentrations of carotenoids on the average increased by almost 68% (25 mM NaCl) and by 75% (50 mM NaCl) compared with control. By the end of the third week in the samples with concentration of 25 mM NaCl there was a decrease of Chl *a* by 62% and Chl *b* by 43%, with the concentration of 50 mM NaCl on the third week there was the reduction of Chl *a* by 72%, Chl *b* by 60%. Thus, high NaCl concentrations caused a great reduction in photosynthesis activity. Significant inhibition of photosynthesis by high salinity (100 mM NaCl) seems to be associated with the photosystem II (PSII) complex. Salinity stress decreases the PSII activity and inhibits the quantum yield of PSII electron transport. Leaf protein content also decreased as salinity increased, which suggests either a possible disruption in protein synthesis mechanism or more likely, an increase in proteolytic activity. The accumulation of proline played a key role in the plant's stress-induced adjustment to NaCl. The rate of formation of superoxide varies within ~1.5–1.6 μmol/h (Control, 10 mM, 25 mM NaCl), and ~3.35 μmol/h (100 mM NaCl). Changes of other biochemical parameters were also significant, physiological responses measured and reflected. This study partly funded by the Daugavpils University Programme: Grants for the Research, Project Nr. 14-95/16

## P-32-032
## Diversity of proline/hydroxyproline-rich glycopeptides from dandelion (*Taraxacum officinale*) flowers with high specific antimicrobial activity against pigment-generating plant pathogenic fungi

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Discovery of the cellular and molecular aspects of plant innate immunity toward biotic environmental stress factors is focused on fundamental and potential applications. Recently, the most relevant analysis of the composition of plant antimicrobial molecules was a comparison between wild and cultivated plants to identify the specific determinants that provide this resistance. These molecular determinants usually belong to a wide diversity of plant defense polypeptides whose functionalities are involved in antimicrobial and signal compounds, enzymes, enzyme inhibitors, and ribosome-inactivators. In previous work, a peptidomic analysis of dandelion (*Taraxacum officinale* Wigg.) flowers found five highly homologous hydroxyproline/proline-rich glycopeptides. These close-specifically antifungal molecules were isolated and purified from the plant tissue. Further work showed that this action is stipulated by the hyphal inhibition of "colored" or "pigment-generating" fungi (*Aspergillus, Bipolaris, Alternaria*), which are found in large quantities in the pigment melanin. These peptides were found to effectively bind (more than 55–65%) with the primary polymers generated by the fungal cell wall architecture (chitin, high and low-molecular weight chitosan, and beta-1,3-glucan). These procedures were performed *in vitro* by the "butch-method" after which reversed-phase high-performance liquid chromatography was used to quantify the unbound peptides. These results represent a significant investment in studies of the molecular aspects of the antimicrobial action of this novel class of plant glycopeptides. This work is supported by the Russian Science Foundation (grant No. 18-74-10073).

## P-32-033
## Roundup® resistance trait traced in genomes of maize inbred lines

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Glyphosate is an active compound of a systemic, nonselective and most widely used herbicide in the world – Roundup. It causes broader range of physiological alterations than previously assumed and some plants gain higher level of resistance without the need to use genetic engineering methods. The holistic understanding of Roundup mechanism of action is of great importance since it has been shown that glyphosate affects the growth of plants not only by inhibiting EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) but also through altering several crucial physiological processes (e.g., photosynthesis, carbon metabolism, mineral nutrition, oxidative events). To study the genetic variations between genomes of plants that are naturally tolerant of and sensitive to Roundup, we used two *Zea mays* L. lines traditionally bred in Poland. To overcome the complexity of the maize genome two sequencing technologies (Illumina and SMRT PacBio) were employed. Corrected PacBio reads were used to identify the genome structure variations (SV), and single nucleotide polymorphism and insertions-deletions (indels) were revealed using Illumina short reads. We identified 11 thousand structural variants, 4 million SNPs and approximately 800 thousand indels differentiating the two genomes. Detailed analyses allowed us to identify 20 variations within the EPSPS gene, but all of them were predicted to have moderate or unknown effects on gene expression. Other genes of the shikimate pathway encoding bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase and chorismate synthase were altered by variants predicted to have a high impact on gene expression. Additionally, high-impact variants located within the genes involved in the active transport of glyphosate through the cell membrane encoding phosphate transporters as well as multidrug and toxic compound extrusion have been identified. The work is supported by a grant no. UMO-2012/06/A/NZ9/00125 from National Science Centre, Poland. *The authors marked with an asterisk equally contributed to the work.

## P-32-034
## Small RNA-Seq highlights importance of microRNAs in soybean chilling stress

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Soy is an annual legume grown for its edible bean. Exceptional nutritional value of soybean, with high protein (40%) and oil (20%) contents, make it one of the staple crops. Political situation in EU greatly hampers the cultivation of GM crops, meanwhile extensive meat production enforces the need for substantial import of soy meal mainly from Argentina. Additionally, in Polish climate soybean is exposed to chilling stress, which impairs yield quality. Thus, in order to provide a sustainable source of soybean we must turn to other solutions, namely development of


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a chilling resistant cultivars. We aimed to decipher the role of miRNAs and their target genes in plant chilling stress response, by determining the changes in their expression levels. Four soybean cultivars were employed for the comprehensive investigation of molecular basis of abiotic stress responses. Swedish cultivar Fiskeby V alongside with Polish Augusta, were representing stress tolerant cultivars, whereas Japanese variety Toyomusume was considered to be susceptible to chilling. Lastly, wild soybean *Glycine soja* was used as a reference. Small RNAs were isolated from explants of soybean cultivated in stress and control conditions. Harvested samples consisted of roots and leaflets from seedlings and trifoliates of plants at vegetative growth stage (V1). Assessment of the differential expression of miRNAs was performed using high throughput sequencing of 72 small RNA libraries (accounting for three biological replicates). Additionally, degradome sequencing was conducted in order to analyze the RNA degradation patterns and confirm the activity of miRNAs. The work is supported by a grant no. UMO-2014/15/B/NZ9/02312 from National Science Centre, Poland and the Ministry of Science and Higher Education of the Republic of Poland by the KNOW program. *The authors marked with an asterisk equally contributed to the work.

## Synthetic networks and systems

### P-33-001
### The expression of Epstein-Barr virus capsid proteins in yeast cells

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Epstein-Barr virus (EBV) is a gammaherpesvirus that infects greater than 90% of the general population and can cause severe diseases, like Burkitt's and Hodgkin's lymphomas, nasopharyngeal carcinoma and multiple sclerosis. The oncogenic mechanisms EBV virus uses inside human cells are still not fully understood and a vaccine against EBV is not yet created. Recombinant EBV capsids are a good source of information for studying this virus. It was previously shown that six proteins are required for EBV capsid assembly in insect cells. The aim of this research is to analyse the expression of EBV proteins and achieve the formation of EBV particles in yeast *Saccharomyces cerevisiae* cells. EBV capsids can be used in medical diagnostics, cancer therapy or for the creation of the vaccine against EBV. Yeast strain was created with six necessary genes for recombinant EBV capsid formation integrated into the genome. The expression of the EBV proteins was determined by SDS-PAGE gel electrophoresis and the localization of the proteins was observed by fluorescent microscopy. All six capsid-forming EBV proteins were successfully synthesized for the first time in yeast expression system. These promising results can help to get a deeper understanding about mechanisms responsible for the formation of EBV particles in yeast cells and its further appliance for the treatment and prevention of life-threatening diseases associated with EBV infection.

### P-33-002
### Network inference for oil accumulation system control in oleaginous yeast *Lipomyces starkeyi*

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The final goal of synthetic biology or systems biology is artificial control of cell system, such as bioproduction ability in microorganisms and so on. To realize those artificial control, revelation of the regulatory mechanism which is occurred in the cell is important. The oleaginous yeast *L. starkeyi* is reported as an ascomycetous yeast with efficient lipid biosynthesis system, and those products are expected to use for industrial field as functional food and biodiesel ingredients. Even though the whole genome of *L. starkeyi* is uncovered at 2016 and its lipid biosynthesis pathway have been estimated, the gene regulatory mechanism of lipid biosynthesis in *L. starkeyi* is remained unclear. To clarify the gene regulatory mechanism which works in oil accumulation system in *L. starkeyi*, we applied our developed network inference method, based on Structural Equation Modelling (SEM), to systematic measured profiles. First, we compiled 40 time-series data measured in several types of *L. starkeyi* strains with high oil accumulation abilities. To clarification of gene regulatory mechanism as a network model, genes which are arranged as variables in network model should be selected for construction of an initial model in SEM calculation process. In this study, we improved our gene selection method based on cross correlation to summarize the time preceding information from gene expressions measured in 8 time points. Cross correlation is usually utilized as a measure of similarity between two waves in signal processing by a time-lag application, and we defined the values of lags ranged from $-4$ to $+2$. By this improved method, we selected 14 genes as regulatory factors for oil accumulation in *L. starkeyi*, including unknown function genes, to infer the regulatory relationships among them. In the inferred model, only 6 genes among the selected 14 genes were considered to affect the volume of oil accumulation, and this system is controlled in closed and specific system. *The authors marked with an asterisk equally contributed to the work.

### P-33-003
### Automatic processing and quantification of histological images

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The rapid development of fluorescent, electronic, atomic-force microscopy, tomography and real-time bioimaging greatly increased available tools in life science investigations. While qualitative visualization of samples are often sufficient, for many tasks the quantitative analysis of images could provide the deeper insight into the issue. Here we present the simple algorithm for characterization of histological images. The approach is based on automatic determination of the direction of the fibrous structures in the tissue and calculation of their vector's sum. Briefly, the object of analysis is determined by color vector. Then the image is binarized and meshed. For every element distribution of the mean intercept length is restored and orientation of the objects can be calculated by using approximation of the mean intercept length. The efficiency of proposed algorithm was analyzed by using the collagen fibers recovery in rats after skin injury. The skin injuries were treated either with standard antiseptic of with


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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protease ficin which was reported previously as efficient tool to remove bacterial biofilms. After full recovery of the wound the skin samples were taken and microscopic images were analyzed. In the control group the tissue recovery was 48 ± 8%, while in ficin-treated samples the recovery was 78 ± 7% suggesting promising wound-healing activity of ficin. The research was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities, project No. 1.12878.2018/12.1.

## Multicomponent complexes

### P-34-001
### Investigations of Dps/DNA crystallization conditions and the structure of biocrystals according to SAXS and cryo-electron microscopy data

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The ability to maintain biological integrity and adapt to changes in the environment during periods of stress is essential for the survival of any organism. Bacteria have evolved a multitude of ways to survive under stressful conditions including the expression of specific stress mediation proteins. One of such proteins vital in stress survival is the DNA-binding protein from starved cells (Dps), which is conserved in more than 300 bacterial species. Dps is highly expressed in stationary phase, it is also involved in the cellular response to oxidative, UV, thermal, and pH shocks. Dps binds to both supercoiled and linear DNA to form a dense biocrystal structure. Although the crystal structure of the Dps dodecamer has been solved, no atomic-scale structure of Dps–DNA assemblies currently exists, and little is known about complex formation and biocrystallization processes. Not resolved in the E. coli Dps crystal structure were the disordered N-terminal regions of each Dps monomer, which extend outward from the dodecamer and contain several lysines proposed to contribute to DNA binding. Removal of large sections of the N-terminal region reduces DNA condensation by Dps, however the exact molecular interactions that cause DNA binding remain unclear. Our investigations represent new insight into Dps/DNA crystallization processes from SAXS and cryo-electron microscopy data. Using SAXS data we obtained the full-length structure of Dps protein and analyzed the processes of Dps/DNA complex formation depending on buffer conditions. During our investigations it was found that Dps formed with DNA several types of crystals. Utilizing cryo-electron microscopy, we report here structural models of these Dps/DNA biocrystals. Obtained results can help us to advance understanding of the mechanisms of protection of the bacterial genome from stress factors. This work was supported by the Russian Science Foundation (project No. 18-74-10071).

### P-34-002
### Surface display on food grade bacteria: characterization of domains for anchoring to the surface of *Lactococcus lactis*

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Display of recombinant proteins on the bacterial surface is an emerging research area with wide range of biotechnological applications. Because of its recognized safety, lactic acid bacterium (LAB) *Lactococcus lactis* represents an attractive host for surface display and a promising vector for *in situ* delivery of bioactive proteins. To achieve surface display, recombinant proteins are usually anchored to the cell wall of LAB through anchoring domains. Different types of surface anchoring domains have been described for LAB, with LPXTG-type domains and lysin motif (LysM) domains being among the most frequently applied in *Lactococcus lactis*. Regardless of the available options, alternative surface display approaches are being sought. The goal of the present study was to characterize new anchoring domains for surface display on *Lactococcus lactis*, and to evaluate their applicability as an alternative to established anchoring domains, especially to the LysM repeats-containing non-covalent anchor of AcmA. We prepared genetic constructs consisting of secretion signal, reporter protein (B domain or DARPin) and surface anchoring domain of lactococcal or phage origin. 13 non-covalent and 2 covalent anchoring domains were tested with flow cytometry to evaluate surface display of fusion proteins via their ability to bind the Fc region of staphylococcal protein A (B domain-containing fusion proteins) or the Fc region of human IgGs (DARPin-containing fusion proteins). One of the anchoring domains, AM12, demonstrated comparable extent of surface display to that achieved with AcmA. In further studies, AM12 enabled display of CXCL8-binding evasin-3 on *Lactococcus lactis*, as well as heterologous display, to a level similar to that achieved with AcmA. To conclude, we have demonstrated effective use of different protein anchors in *Lactococcus lactis*. AM12, in particular, represents a promising alternative to established approaches for surface display on *Lactococcus lactis*.

### P-34-003
### The impact of $F_OF_1$-ATPase on $H_2$ producing hydrogenase activity in *Escherichia coli* during mixed carbon sources fermentation

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*Escherichia coli* has four membrane-bound [Ni-Fe]-hydrogenases (Hyd), which reversibly oxidize $H_2$ to $H^+$. In this work, the role of the $F_OF_1$-ATPase in $H_2$ production was first investigated during mixed carbon sources (glucose, glycerol, formate) fermentation by *E. coli* at different pHs (7.5, 6.5, 5.5) using wild type and mutant strains with defects in various Hyd and/or formate dehydrogenase (FDH) enzymes. $F_OF_1$-ATPase specific inhibitor *N,N′-dicyclohexylcarbodiimide* (DCCD) had an inhibitory effect on the duration, but not on the rate of $H_2$ production in wild type at pH 7.5. The external pH of growth medium of *selC* (lacking formate dehydrogenases) single and *hyaB hybC selC* (lacking Hyd-1 and Hyd-2 large subunits and formate dehydrogenases) triple mutants was acidified more by ~ 0.3 units compared to wild type. No effect of DCCD on $H_2$ production at pH 6.5 was determined. But DCCD had a negative influence on Hyd enzymes activity at pH 5.5. The external pH was acidified by 0.7–0.8 units in *selC* mutant growth medium compared with wild type. The


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overwhelming effect of the DCCD on the specific growth rate was detected at pH 7.5 and 6.5, and the effect of DCCD at pH 5.5 was almost excluded, except for *selC* mutant (reduction by 25%). It was shown that specific growth rate was increased by ~ 65% and ~ 50% in wild type and hypF (lacking all Hyd enzymes) mutant with DCCD at pH 6.5. Taken together, it is suggested that FOF1-ATPase has metabolic cross talk with FDH and Hyd enzymes and has a great contribution in $H_2$ production at pH 7.5 and 5.5. It was shown that the main role of FDH in the neutralization of both external and intracellular formate during fermentation. Acidification of Hyd deficient mutant's external medium and the change of FOF1-ATPase activity indicate an interaction between FOF1-ATPase, Hyd and FDH enzymes in terms of $H_2$ metabolism or $H^+$ transport in the membrane, which function to regulate intracellular pH and thus proton motive force. *The authors marked with an asterisk equally contributed to the work.

## P-34-004
## The effect of the mixture acetate and glycerol on *E. coli* growth and $H_2$ production during fermentation

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It is well known that *Escherichia coli* is able to ferment sugars and/or glycerol for producing molecular hydrogen ($H_2$). $H_2$ produces via four multiple and reversible [Ni-Fe] Hyd enzymes. This study describes growth and the total $H_2$ production in batch cultures during utilization of the mixture of acetate (5 g/L) and glycerol (10 g/L) at various pHs (7.5, 6.5, 5.5) in *E. coli* wild type and different mutant strains with defects in Hyd 1 or Hyd-2. It was shown that specific growth rate (μ) of *E. coli* wild type was 0.289 $h^{-1}$, which was similar for mutant strains at pH 7.5 and 6.5. Only in *hybC* (lacking large subunit of Hyd-2) mutant μ was increased ~ 1.3 fold compared to wild type at pH 7.5. It has been determined that in batch tests at pH 7.5 and 6.5 wild type strain evolved $H_2$ during long time ~ 168 h. No $H_2$ production was observed in all strains at pH 5.5. Interestingly, *hyaB* (lacking large subunit of Hyd-1) and *hybC* mutants have exhibited the same results at pH 7.5 and 6.5, especially $H_2$ generation was ~ 150 h. This is a prolonged period compared to acetate alone fermentation. In *hyaB* or *hybC* mutants, $H_2$ production was detected earlier than in wild type at pH 7.5. But at pH 6.5 only in *hybC* mutant earlier $H_2$ production was detected suggesting an important role of Hyd-2 under these conditions. Taken together, it can be concluded that cell growth and $H_2$ generation depends on external pH and carbon sources. Particularly, Hyd-1 and Hyd-2 work towards $H_2$ oxidation which is in contrast to glycerol only fermentation suggesting that acetate affects Hyd enzymes working direction. Moreover, $H_2$ was produced earlier and μ was higher mainly in *hybC* mutant assuming that at pH 7.5 $H_2$ is inhibitory for cells. *The authors marked with an asterisk equally contributed to the work.

## P-34-005
## Mapping the Su(Hw) domain that is required for promoter repression in the *Drosophila* ovary

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The Su(Hw) is a DNA-binding protein in *Drosophila*, which interacts with CP190 and Mod(mdg4)-67.2 proteins in the well-known insulator complex. The Su(Hw) is involved in development as a transcriptional repressor of neuronal genes in oocytes. To characterize Su(Hw) domains involved in repression, we selected neural genes whose promoters are bound by Su(Hw) (Rph, cg32017, Hs3st-A, Syn2, mAcR-60C). The deletion of aa 88–202 region at the N-terminus of Su(Hw) ($Su(Hw)^{\Delta 114}$) prevents CP190 recruitment to the Su(Hw) complex. We compared the recruitment of Su(Hw) and CP190 to the model promoters in $Su(Hw)^+$ and $Su(Hw)^{\Delta 114}$ transgenic lines in the absence or presence of the *mod(mdg4)*$^{u1}$ mutation inactivating the Mod(mdg4)-67.2. No significant effect of the 114 bp deletion on Su(Hw) recruitment was observed even in the absence of Mod(mdg4)-67.2. Thus, Mod(mdg4)-67.2 and CP190 are not required for the Su(Hw) binding. The RT-qPCR analysis in the ovaries and heads showed that the $Su(Hw)^{\Delta 114}$ did not lead to derepression of model genes. Therefore, the Mod(mdg4)-67.2 and CP190 are not involved in repression. The Su(Hw) C-terminal domain contains the enhancer-blocking (aa 716–892) region. We generated different Su(Hw) mutants and compared their effect on the expression of model genes. The $Su(Hw)^{\Delta 283}$ line expressed the protein lacking aa 760–778 that are critical for enhancer-blocking activity. The $Su(Hw)^{e7}$ and $Su(Hw)^{J}$ transgenes produced truncated proteins lacking 223 and 150 aa, respectively. Both deletions affect the Mod(mdg4)-67.2 interacting domain. In $Su(Hw)^{J}$, $Su(Hw)^{e7}$, or $Su(Hw)^{\Delta 283}$ females, the expression level in the ovaries proved to be increased up to 5-fold, while that in the heads remained unchanged. These results show that the enhancer-blocking domain mediates the Su(Hw) dependent repression. This work was supported by Russian Science Foundation grant No. 18-14-00295.

## P-34-006
## Role of dcu transporters in proton ATPase dependent proton flux during glucose fermentation at pH 7.5

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The C4-dicarboxylate or dcu family of transporters are exchanging succinate, malate, fumarate etc. Depending on external conditions dcu system can transport succinate with H+ symport. H+ transport across the cell membranes can be done via proton transporters, proton ATPase and various ion or other exchange systems. Current study revealed total and DCCD inhibited (specific inhibitor of proton ATPase) H+ flux in *E. coli* BW25113 wild type and dcuD, dcuABC and dcuABCD mutants. When glucose was supplemented total H+ flux in wild type was 4.02 mM/min. DCCD inhibited H+ flux ~ 30%. The same involvement of proton ATPase was shown for dcuACBD mutant. In dcuD mutant total H+ flux was decreased by 1.6 fold suggesting that DcuD has significant role in total H+ flux. But interestingly no difference was detected in DCCD inhibited H+ flux.


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Only in dcuACB mutant total H+ flux was ~ 1.6 fold higher than in wild type. In this mutant DCCD inhibited H+ flux by 56%. When glycerol was supplemented the total H+ flux was similar in wild type and all mutants but only difference was determined in DCCD inhibited fluxes. Especially, in wild type cells it was inhibited by 90%, in dcuACBD mutant only by 34% and no inhibition was shown for dcuD and dcuACB mutants. Even in dcuACB mutant addition of DCCD increased H+ flux by 2.2 fold compared to the assays without DCCD. In the assays with succinate addition only in wild type cells H+ flux was inhibited by DCCD. Interestingly in dcuACB mutant total flux was ~ 2.1 fold higher than in wild type and in DCCD inhibited assays H+ influx was determined. Moreover, in dcuD mutant total flux was significantly lower than in wild type by 2.7 fold and DCCD inhibited flux stimulated the H+ efflux. Taken together it is suggested that Dcu family transporters are directly related to proton ATPase dependent H+ flux and interact together to regulate total H+ flux. Moreover, Dcu have also compensatory uptake or efflux function to regulate the H+ concentration in the cell.

## P-34-007
## Amino acid plasma membrane transporter SLC6A14 interacts with cytosolic heat shock protein HSP90beta

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SLC6A14 is a plasma membrane amino acid transporter transporting all neutral and basic amino acids in a Na/Cl dependent way. It is overexpressed in many types of cancer, supporting growth and proliferation of transformed cells. The first step in transporter trafficking to the cell surface, i.e. the exit from endoplasmic reticulum (ER) was demonstrated to depend on specific interaction with COPII component – SEC24C. Several cytosolic heat shock proteins were detected by mass spectroscopy in SLC6A14 proteome, including HSP90beta. A direct interaction of the overexpressed transporter with HSP90beta was confirmed in immunoprecipitation, immunofluorescence, surface biotinylation and in proximity ligation assay. Treatment with HSP90beta inhibitor – radicicol resulted in a dramatic decrease of SLC6A14 presence in the plasma membrane, an effect of the total transporter diminution. These results indicate that a plasma membrane protein folding can be controlled not only by ER chaperones, but also those localized in the cytosol. The ATPase activity of purified recombinant HSP90beta was assayed in an *in vitro* system by fluorescence intensity measurements. Several fragments of the transporter N- and C-termini localized in cytoplasm were selected and HSP activity was measured in the presence of the corresponding peptides. The results indicate involvement of SLC6A14 C-terminal fragment proximal to the last transmembrane domain in HSP activity, pointing to the same fragment of the transporter binding HSP90beta and SEC24C in the relay of a correct folding and further trafficking to the plasma membrane. This study was financed by a grant 2015/19/B/NZ3/00049 from the National Science Centre in Poland.

## P-34-008
## Synthesis and characterization of ovocystatin–albumin bioconjugates

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Ovocystatin, a protease inhibitor isolated from chicken egg white, is considered as a drug for the supportive treatment of diseases characterized by excessive cathepsin activity e.g. cancer and rheumatoid arthritis. Albumin is used in the pharmaceutical preparations as a carrier for active substance and has be shown to accumulate at the inflammatory or cancerous sites, and that is why it can be a tool for so-called targeted therapy. The aim of the study was to develop conjugates of ovocystatin and bovine serum albumin using different cross-linking agents. SMCC and SM(PEG)4 crosslinkers (Thermo Scientific) were used for conjugation. The efficiency of the conjugation was better for linker with PEG spacer arm as evaluated by electrophoresis, Western-blot and particle size analysis. The SM(PEG)4 conjugate showed higher inhibitory activity against papain than the SMCC conjugate. Both conjugates contained some dimeric form and trace amounts of free albumin. Isoelectric focusing was used for precise determination of isoelectric point of the SM(PEG)4 conjugate. It was shown that pI of the conjugate (4.4) was different from that of the albumin (4.7). This finding was the base for proceeding with chromatofocusing on PBE94 column in order to separate the conjugate from free albumin. Elution with the pH gradient of 4.0–7.0 allowed a partial separation of the two proteins. Chromatofocusing using narrow pH gradient could result with successful separation of the conjugate from the redundant albumin. In conclusion, heterobifunctional amine-to-sulfhydryl crosslinkers that contain NHS-ester and maleimide reactive group at opposite ends seem to be suitable for synthesis of stable ovocystatin conjugates. *The authors marked with an asterisk equally contributed to the work.

## P-34-009
## The role of several subunits of *Escherichia coli* hydrogenase 4 in hydrogen production during fermentation of various glucose concentrations at pH 7.5

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*Escherichia coli* is one of the well-studied microbes producing molecular hydrogen ($H_2$) via 4 membrane-bound [NiFe]-hydrogenases (Hyd), and the optimization requires good understanding of both structure and working mechanisms of enzymes. Experiments were done to clarify the role of individual subunits of Hyd-4 and glucose concentrations effect on $H_2$ production. When cells were grown in the presence of 0.2% glucose at pH 7.5 $H_2$ production rates ($V_{H2}$) in wild type cells were 5.44 and 2.7 mV/min/mg dry weight, respectively, in the assays with 0.2% and 0.8% glucose added. In *hyfD* and *hyfF*, but not in *hyfB* mutants $H_2$ generation in the assays added with 0.2% glucose was enhanced ~ 1.7 fold, compared to wild type. Interestingly when 0.8% glucose was added only in *hyfB* $V_{H2}$ was 2.6-fold higher compared to wild type. $H_2$ evolution was obviously enhanced by 2.6-fold compared to wild type in *hyfG* single mutant coding large subunit of Hyd-4 when 0.2% glucose were added, but no differences were observed when 0.8% glucose were added. This means that during fermentation of low concentration of glucose HyfG is working towards $H_2$ uptake. In other *hyf* mutants $H_2$ production was the same as in wild type. Based on these data it can be concluded that the expression and function of HyfB, HyfD and HyfF subunits strongly depends on glucose concentration. It is possible that under the above conditions these subunits are responsible for excretion of protons from the cell. The results point out that component of these multiple systems can create different functional complexes depending on conditions. *The authors marked with an asterisk equally contributed to the work.


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## P-34-010
## Crystal structure of the complex ribosomal silencing factor S and ribosomal protein L14 from *Staphylococcus aureus*

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*Staphylococcus aureus* (*Sa*) is a pathogenic bacterium and the causative agent of different diseases: meningitis, pneumonia, sepsis and etc. Combating with *Sa* is complicated by the high viability of the bacteria and its potent resistance to different types of drugs. More than a half of them have a peptidyl-transferase center on a 70S ribosome as the target. The probable solution of this problem is searching the new targets for drugs not on the 70S ribosome, but in the 70S formation process. We focused on one of these steps: influencing a ribosomal silencing factor S (RsfS) to 50S ribosome subunit. This factor binds with L14 protein on the large subunit and takes out the 50S from a recycling process. This process is important to save energy and ensure survival in a starvation period. For a detailed analysis of the interactions between RsfS and the 50S ribosomal subunit in *Sa*, the crystal structure of the complex of recombinant *Sa*RsfS and *Sa*L14 was obtained at 2.27 A resolution. Two heterodimers with the wide interface into each complex were found in the asymmetric unit: C-tail two α-helixes (α1, α2), loop 8, loop 10 from SaL14 and four of five β-strands of β-sheet (β2, β3, β4, β5), two α-helixes (α3, α4), loop 2 and loop 4 from *Sa*RsfS. The heterodimer is maintained by 14 H-bonds and hydrophobic interactions. Based on the number of hydrogen bonds on one amino acid we assume the stability of heterodimer is mostly provided by Arg97, Arg107 on *Sa*L14 and by Glu70, Asp81 on *Sa*RsfS. This correlates with published information about structures and functions of RsfS from *M. tuberculosis* and *E. coli*. Violation of these contacts by drugs is one of the ways to reduce the viability of *Sa*. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

## P-34-011
## Liposomes as tool for structural studies of membrane proteins: synchrotron small-angle X-ray scattering approach

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Membrane proteins play an essential role in such biological processes like ion transport, signal transduction, and electron transfer to name a few. Membrane proteins are notoriously difficult to study, but the ability to do so could aid in the development of therapeutic drugs and novel technologies. Micelles, amphiphilic polymers, bicelles, nanodiscs or liposomes can facilitate membrane protein studies. In the present study the liposomes prepared from the lipids obtained from two different sources were investigated. Small-angle X-ray scattering (SAXS) technique has been employed to structural investigation of two types of liposomes. The first type is liposomes that were prepared from synthetic lipids. These lipids are regularly used for liposome studies. The second one is liposomes formed from the lipids, extracted from the Influenza A virus. Liposomes, containing natural lipids are a more approximate model of the cell membrane. A novel method for structural characterization of liposomes and micelles by small angle X-ray scattering was applied in this study. The method is based on quasi-atomistic approach. Thus, two types of lipids were structurally studied, and a comparison characterization of synthetic and natural liposomes was provided. In the result, it was found that natural liposomes with 24 h incubation are prone to the formation of multilayer structures in contrast to synthetic ones. The work has been supported in part by RFBR grants 18-54-00019 and 17-00-00487.

## P-34-012
## Lactic acid bacteria as a platform for intestinal delivery of therapeutic proteins derived from albumin-binding domain scaffold

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Lactic acid bacteria (LAB) represent an interesting alternative for the treatment of inflammatory bowel disease and infections with Shiga toxin-producing bacteria. LAB advantages, i.e. safety and probiotic activity, can be further strengthened by genetic modification. The aim of our work was to establish LAB as a delivery platform for therapeutic protein release in the gastrointestinal tract. Delivery platform includes combination of production, secretion and surface display, in *Lactococcus lactis,* of artificial binding proteins, based on a scaffold of albumin-binding domain (ABD). Previously, we have selected binders against Shiga toxin 1 B subunit (S1B binders) and confirmed their functional display on *L. lactis*. In the present study, we focused on the delivery of binders of p19 subunit of human IL-23 cytokine (ILP binders) and human IL-23 receptor (REX binders). In the first part of our work we prepared lactococcal expression plasmids with ILP030, ILP317 and ILP323 variants in fusion with Usp45 secretion signal, FLAG tag sequence and peptidoglycan-binding C terminus of AcmA. All ILP fusion proteins were effectively displayed on the surface of lactococcal cells; however, ILP317 showed the best binding of human IL-23. In the second part of our study, we prepared genetic constructs for three REX binders (REX009, REX115 and REX125) (i) for surface display on *L. lactis* cells by fusing them with Usp45 secretion signal and C terminus of AcmA, and (ii) for secretion into the growth medium via Usp45 secretion signal. We have confirmed expression of all REX variants on the surface of lactococcal cells and showed binding to the IL-23 receptor by flow cytometry. We observed significant binding of secreted REX009 to soluble recombinant IL-23 receptor. To summarize, we have successfully developed LAB with surface displayed ABD-derived binders of IL-23 and IL-23 receptor. This represents a possibility for new, synergistic therapeutic approach for intestinal inflammation.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-34-013
## Development of an expression system for bispecific antibodies

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Production of full-length bispecific antibodies is more challenging than the expression of conventional antibodies due to the double number of heavy and light chain genes that need to be expressed. Typically, this problem is solved using four expression plasmids for transfection. The goal of this work was to create two expression plasmids for the efficient production of full-length bispecific IgG1 antibodies. The sequences encoding the variable domains of the immunoglobulin heavy and light chains were linked to the constant domains of human antibodies. Also, Kozak sequence, leader peptide sequence and restriction endonucleases recognition sites for further cloning were added. Knob-into-hole mutations were introduced into the CH3 domains of heavy chains for correct assembly of the bispecific antibody. We also introduced mutations that reduce binding to Protein A into one of the heavy chains of the bispecific antibody, in order to improve the purity of the protein by using gradient elution in affinity chromatography. Between the light and heavy chain genes we inserted an IRES element – regulatory region of eukaryotic mRNA and their viruses, which provides cap-independent, or internal translation initiation for efficient heavy chain translation. The resulting bicistronic sequence was placed under the control of the CMV promoter. We obtained two expression plasmids, each containing the heavy and light chain genes for one part of the bispecific antibody. The bispecific antibody was transiently expressed in CHO cells and then purified from culture supernatant by affinity and size-exclusion chromatography. Expression yield and biochemical parameters of the bispecific antibody were analyzed. This work was supported by a subsidy of the Ministry of Science and Higher Education of the Russian Federation (RFMEFI60417X0189). *The authors marked with an asterisk equally contributed to the work.

## P-34-014
## Disulfide polycationic amphiphiles for stimuli-responsive siRNA delivery

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Gene silencing provided by RNA interference mechanism is one of the most promising approaches for the treatment of diseases caused by overexpressed genes. Small interfering RNA (siRNA) delivered to the target cell may complementary binding target mRNA preventing undesirable protein synthesis. Since siRNA is unstable in biological medium, special delivery systems, for example cationic liposomes, are needed for siRNA delivery to the cells. In this work, we synthesised two novel polycationic amphiphiles and studied siRNA delivery efficiency mediated by cationic liposomes based on them and zwitter-ionic helper lipid DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine). Amphiphiles are provided with disulfide groups making them sensitive to degradation by intracellular reducing agents such as glutathione for a better siRNA release into the cytoplasm. Dynamic light scattering studies were carried out to determine hydrodynamic diameters as well as zeta-potentials of cationic liposomes and their complexes with siRNA. Transfection efficiency was investigated by a delivery of siRNA into transgenic BHK IR780 cells expressing green fluorescent protein. It was demonstrated that both disulfide cationic liposomes provided high green fluorescent protein synthesis inhibition up to 10% as compared with untreated cells. Moreover, results were exceeded those demonstrated by commercial transfectant Lipofectamine 2000. In conclusion, cationic liposomes based on novel disulfide polycationic amphiphiles are promising carriers for delivery of siRNA into eukaryotic cells. This research was supported by Russian Science Foundation (grant 18-73-00270). E.V. Shmendel is a recipient of Presidential fellowship 1199.2018.4. *The authors marked with an asterisk equally contributed to the work.

## P-34-015
## PorU – the ultimate player in protein secretion in type IX bacteria secretion system

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Deciphering mechanisms underlaying protein secretion is of special interest because of its crucial role in bacterial pathogenesis. Here, we present data about type IX secretion system (T9SS) which is present in prominent periodontitis pathogens *Porphyromonas gingivalis* and *Tannerella forsythia*. T9SS is found in Gram negative bacteria belonging to phylum Bacteroidetes. In those bacteria the inner membrane transfer is done by the Sec translocon. Further steps of protein secretion (periplasm crossing and outer membrane release) involve around 17 proteins building the T9SS translocon. Presented work focus on the ultimate player engaged in secretion process, the PorU protein. The 130 kDa PorU protein is localized in outer membrane facing the extracellular milieu. The PorU is a sortase- an enzyme that cleaves the C-terminal secretion signal (the C-terminal domain, CTD) and attaches the A-lipopolysaccharide moiety. The deletion of the PorU protein blocks T9SS cargos secretion. This is manifested by the loss of the black pigmentation of *P. gingivalis*, lack of proteolytic gingipains' activity, and the accumulation of unprocessed client proteins in periplasm. Moreover, the deletion of PorU increase the expression of other T9SS components. The PorU mutant studies revealed the presence of few conserved essential amino acid residues, and the presence of uncleaved CTD domain in this protein. Finally, the switch of *P. gingivalis* PorU for *T. forsythia* ortholog shows that, though the protein is expressed it cannot restore the function. This observation shows that however, the Bacteroidetes protein secretion system is highly conserved, it can possess major differences between species which need to be studied more deeply. This study was supported by the NCN funding: 2014/15/D/NZ6/02546

## P-34-016
## The relationship between proton ATPase and Dcu transport system during glucose fermentation at pH 6.5

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During anaerobic growth *E. coli* can use $C_4$-dicarboxylate (succinate, fumarate, malate etc.) as energy and electron sources. In this study total and DCCD inhibited ATPase activity was investigated in *E. coli* BW25113 wild type and *dcuACB*, *dcuACBD* and *dcuD* mutants encoding different $C_4$-dicarboxylate carriers. These


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carriers transport succinate ions with $H^+$ via symport. It is well known that $H^+$ can be transported mainly through the proton $F_OF_1$-ATPase or different carriers/transporters. ATPase activity of membrane vesicles was determined by measuring the amount of inorganic phosphate ($P_i$) produced in the reaction of membrane vesicles with ATP in the assay mixture. Total ATPase activity in wild type was 172.98 nM $P_i$/min/μg protein. In *dcu* mutants the ATPase activity was ~ 1.5–1.9 times lower compared to wild type. The highest proton ATPase activity was detected in *E. coli* wild type. In the other cases proton $F_oF_1$-ATPase activity was lowered by ~ 86%, 83% and 62%, respectively in *dcuD, dcuACBD* and *dcuACB* mutants. In *dcuD* mutant $K^+$ ions stimulated the total ATPase activity ~ 2 fold compared to wild type. Moreover, when succinate was added the total ATPase activity was lowered ~ 1.3 fold in *dcuACB* and ~ 1.4 fold higher in *dcuD*. Interestingly, $K^+$ ions stimulated total ATPase activity ~ 1.3 fold in *dcuD* and inhibited ~ 1.2 fold in *dcuACB*. In addition, DCCD inhibited ATPase activity was increased ~ 1.2 fold in *dcuD* and decreased in succinate assays ~ 1.8 fold compared to wild type. The presence of succinate in the mixture decreased the DCCD inhibited ATPase activity ~ 2.8, 1.3, and 3.4 fold respectively in *dcuD, dcuACBD* and *dcuACB* in presence of $K^+$ ions. It might be concluded that *dcu* transport system is connected directly with ATPase. DcuD has the main role for proton transport across the membrane. *The authors marked with an asterisk equally contributed to the work.

## P-34-017
## The role of *Escherichia coli* hydrogenase 3 subunits in hydrogen production during fermentation of high glucose concentration at different pHs

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*E. coli* Hyd-3 is known as the main producer of molecular hydrogen ($H_2$) during glucose fermentation. However, it was shown in this study that not all subunits are responsible for $H_2$ formation to occur and can change their function depending on glucose concentration and pH values. Hyd-3 works to produce $H_2$ during 8 g/L (high) glucose fermentation at pH 5.5. Moreover, it seems that glucose high concentration accelerates $H_2$ formation during the time and more subunits function towards $H_2$ production at pH 5.5, compared to pH 7.5. *E. coli* BW25113 wild type and all single mutants of Hyd-3 were used in this experiment. Measurements were done with bacteria grown in 8 g/L glucose at pH 7.5 and 5.5 in anaerobic conditions, then 8 g/L glucose was added in the assays. Wild type showed $H_2$ production rate of 2.39 mV/min/mg dry weight and 2.58 mV/min/mg dry weight, at pH 7.5 and 5.5, respectively. *hycB* mutation adduced ~ 2.4 fold the $H_2$ production rate and *hycG* mutation, as *hycI,* had no significant role at pH 7.5, compared to wild type, whereas, no $H_2$ production was detected at pH 5.5. In *hycE* mutant $H_2$ production was lowered ~ 3.5 fold, compared to wild type, at pH 7.5, but not at 5.5, where production was absent. HycH deletion did not have an essential role in $H_2$ production. *hycA* single mutation did not show any differences, compared to wild type. *hycC*, *hycD* and *hycF* single mutants showed lack of $H_2$ production in both conditions. Taken together it can be concluded that the functions of HycG and HycI are more vital during fermentation of high glucose concentration, and HycH does not work to produce $H_2$ in different pHs. *The authors marked with an asterisk equally contributed to the work.

## P-34-019
## Immunoproteasome inhibition increase infection of U937 cells with lentiviruses

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Ubiquitin-proteasome system (UPS) degrades most intracellular proteins. The UPS principal element is a proteasome, within which the proteolysis is performed by three paired catalytic subunits. Besides standard proteasomes (StPs) many cells contain intermediate (IntPs) and some cells – immunoproteasomes (IPs). These proteasomes differ from StPs by complete (IPs) or incomplete (IntPs) substitution of standard catalytic subunits with the "immune" subunits. IPs and IntPs display differing comparing with StPs proteolytic activity, generate altered set of peptides and play important role in antigen presentation and stress response. Viral infection can modulate proteasome activity and expression of proteasome subunits. Moreover, it was demonstrated that basal proteasome activity in the cell determines efficacy of lentiviral infection, however, the role of different proteasome forms was insufficiently addressed. Human histiocytic lymphoma U937 cells were used. Expression level of catalytic proteasome subunits was determined and a high amount of immune subunit beta5i transcripts was revealed, confirming elevated levels of IntPs and IPs in these cells. Cells were treated with non-toxic concentrations of MG132 – pan proteasome inhibitor and ONX-0914 – inhibitor of beta5i. Six hours post treatment the cells were infected with HIV-1 VSV G pseudoviruses. If using 50 nM of ONX-914 only a marginal effect on infectivity was revealed, while chymotrypsin-like proteasome activity was suppressed by 69% and beta5i subunit-specific activity – by 80%. When 100 nM of ONX-0914 was used, the lentiviral infection was increased by 50%, at the same time, chymotrypsin-like proteasome activity was decreased by 75% and beta5i activity – by 80%. LDL receptors level was unchanged in the presence of 100 nM of the ONX-914. Obtained results allow suggesting increased risk of lentiviral infection after IP-specific inhibitors use. The study was supported by Russian Science Foundation grant No. 18-74-10095.

# Cell signaling in tumor biology

## P-35-001
## Cell proliferation-related expression of alpha-taxilin, a candidate tumor marker, in the murine gastrointestinal tract

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α-Taxilin has been identified as a binding partner of the syntaxin family which is involved in intracellular vesicle traffic. Accumulating evidence has shown that α-taxilin protein is overexpressed in various tumor cells such as glioma, hepatocellular carcinoma, and renal cell carcinoma, leading to the possibility that α-taxilin is a candidate tumor marker. Moreover, since a recent report has shown that the fusion protein of α-taxilin and FGFR2 may be involved in tumorigenesis of biliary duct cancer, α-taxilin is gaining prominence in the cancer research field. Then, we examined expression of α-taxilin by western blot and immunochemical analyses in the murine gastrointestinal tract where cell renewal vigorously occurs. α-Taxilin was expressed in the majority of the


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gastrointestinal tract and prominently present in epithelial cells positive for Ki-67, a marker of actively proliferating cells. In the corpus and antrum of the stomach, α-taxilin was expressed in cells localized in the isthmus and at the lower part of the gland, respectively, but not in surface mucous, parietal or mucous neck-zymogenic cells. In the small intestine, α-taxilin was abundantly expressed in a transient-amplifying cells but also significantly expressed in a minority of cells just above the Paneth cells and crypt base columnar cells, both of which were positive for musashi-1, a stem cell marker. During development of the small intestine, α-taxilin was expressed in Ki-67-positive epithelial cells. Blocking cell proliferation by inhibition of the Notch cascade using a γ-secretase inhibitor, led to a decrease in α-taxilin and Ki-67 positive cells in the stomach. These results suggest that expression of α-taxilin is regulated in parallel with cell proliferation in the gastrointestinal tract and imply that α-taxilin is involved in tumor cell proliferation.

## P-35-002

## Endoplasmic reticulum stress: major player in size-dependent inhibition of P-glycoprotein by silver nanoparticles in multidrug-resistant breast cancer cells

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Development of multidrug resistance (MDR) is a major obstacle of successful chemotherapy, therefore, innovative approaches to defeat MDR are imperative. Although the remarkable anti-cancer propensity of silver nanoparticles (AgNP) has been verified and their potential application in MDR cancer has been proposed, the nanoparticle size-dependent cellular events directing P-glycoprotein (Pgp) expression and activity in MDR cancer have never been addressed. Therefore, in the present study we examined nanoparticle size-dependent cellular features in multidrug resistant breast cancer cells. In this study we report that 75 nm AgNPs inhibited significantly Pgp efflux activity in drug-resistant breast cancer cells and potentiated the apoptotic effect of doxorubicin, which features were not observed upon 5 nm AgNP treatment. Although both sized AgNPs induced significant production of reactive oxygen species and mitochondrial damage, 5 nm AgNPs were more potent than 75 nm AgNPs in this respect, therefore, these features cannot be accounted for the reduced transport activity of ATP-driven pumps observed after 75 nm AgNP treatments. Instead we found that 75 nm AgNPs depleted endoplasmic reticulum (ER) calcium stores, caused notable ER stress and decreased plasma membrane positioning of Pgp. Our study suggests that AgNPs are potent inhibitors of Pgp function and are promising agents for sensitizing multidrug resistant breast cancers to anticancer drugs. This potency is determined by their size, since 75 nm AgNPs are more efficient than smaller counterparts. This is a highly relevant finding as it renders AgNPs attractive candidates in rational design of therapeutically useful agents for tumor targeting. In the present study we provide evidence that exploitation of ER stress can be a propitious target in defeating multidrug resistance in cancers.

## P-35-003

## Investigate the differences between the different angiogenic and antiangiogenic markers before and after treatment in several types of cancer

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Cancer is a type of disease in which cells proliferate in an uncontrolled way sometimes resulting in death, and which is seen with increasing frequency today. Cancer spreads by penetrating the blood vessels and lymphatic system, and studies have shown that this invasion is facilitated by angiogenesis. In order to examine this mechanism in a more detailed way, we aimed that examine the difference between levels of the angiogenic and antiangiogenic markers VEGF, MMP, endostatin and thrombospondin-1 in patients with various operable cancers. Methods: The study will consist of preoperative and postoperative groups from patients with various cancer. Blood was collected from each patient the day before surgery and on the 30th after surgery. NF-κB, VEGF, MMP, Endostatin and Thrombospondin-1 were evaluated in serum samples isolated from peripheral blood and banked at −80 °C. Patient plasma samples were analyzed by an enzyme-linked immunosorbent assay (ELISA) with commercial kits for NF-κB, VEGF, MMP, Endostatin and Thrombospondin-1. Result: There was no significant difference in age, gender and weight of these groups ($P > 0.05$). When the control group was compared with the pre-op group, we observed that the levels of TSP, ES, NF-κB in the pre-op group were high in each of the various types of cancer, while VEGF was low in all but the breast. MMP was found to be high in all cancer types. When pre-op and post-op were compared, there was an increase in TSP, ES, NF-κB, VEGF and MMP levels in the colon and thyroid, while there was no statistically increase in the others ($P < 0.05$). Conclusion: In this study which we have done in various types of cancer, we think that all of the markers in the preoperative are high, there will be a height in the markers in the post-operative period, and depending on the duration of treatment will increase ES and TSP-1. We think that this study should be tried in more case groups and longer treatment groups.

## P-35-005

## Hepatocellular carcinoma pluripotent stem-like cells induced by long-term treatment with sorafenib

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Sorafenib resistance remains a major problem for the effective treatment of hepatocellular carcinoma (HCC) as sorafenib is the main standard clinical treatment against the advanced form of this disease. Drug resistance has been frequently associated with the emergence of cancer stem cells. In this study, we have generated two cell lines from the hepatocellular carcinoma cells HepG2 and Huh7 which are resistant to the chemotherapeutic drug sorafenib. HepG2 and Huh7 cells were cultured continuously for 12 months with a step-wise increase of Sorafenib concentrations (starting at 0.75 μM and increasing the concentration by 0.15 μM at each passage up to a final concentration of 8 μM). Surviving cells were selected and designated as HepG2SF1 and Huh7SF1 cells. HepG2 and Huh7 parental cells


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were cultured in parallel without Sorafenib and served as controls. Both HepG2SF1 and Huh7SF1 cells were resistant to sorafenib-induced cell death compared with their parental cells as inferred from MTT cell viability and cell counting assays. Interestingly, the survival pathway PI3K/Akt/mTOR was upregulated in HepG2SF1 and Huh7SF1 cells, in good agreement with previous reports demonstrating activation of this signaling pathway in chemotherapy-resistant cells. To investigate whether sorafenib resistance was associated with the development of stem cells, we determined the expression of the stem cell markers CD133, ALDH1A1, α-Fetoprotein, ABCB1A, Nanog and Oct4. Western blot, cell cytometry and qPCR analyses showed that all the markers analyzed increased in the sorafenib-resistant HepG2SF1 and Huh7SF1 cells. Moreover, those cells had a greater tumorigenic potential, colony forming ability and the capacity to differentiate into neurons or astrocytes when cultured in the appropriate defined medium. These results indicate that long-term treatment with sorafenib can induce pluripotent stem-like features of HCC cells.

## P-35-006
## The antimetastatic effect of DNase I is associated with a decrease in the number of SINEs and LINEs in the blood of mice with different metastatic tumors

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Taking into account recently obtained data indicating the participation of circulating extracellular DNA (exDNA) in tumorigenesis, DNases have been again considered as potential antitumor and antimetastatic agents. Previously, using several tumor murine models we have shown the antimetastatic activity of bovine pancreatic DNase I, which correlates with an increase of DNase activity and a decrease of exDNA concentration in the blood serum. To find the possible targets of DNase I, we compared the exDNA profile of mice with LLC before and after the treatment with DNase I and healthy mice, using next-generation sequencing (NGS). We found that upon DNase I treatment, the inhibition of metastasis correlated with a number of strong alterations in the patterns of exDNA. The major difference was the excess abundance of 224 types of tandem repeat in the blood of mice with LLC, significantly reduced after DNase I treatment. The most important result obtained is that DNase I caused the decrease of fragments of mobile genetic elements (SINEs and LINEs, both known as markers of carcinogenesis) in the mice with LLC, to the level of healthy mice. To prove the connection of SINEs and LINEs with metastasis spreading, we analyzed their abundance in the blood serum exDNA of mice with different metastatic tumors – LLC, melanoma B16 and resistant lymphosarcoma $RLS_{40}$. We found that DNase I treating caused dose-dependent decrease of over-presented SINE and LINE elements to the levels of healthy animals in the case of B16 and LLC, and only slightly affected their levels in the case of $RLS_{40}$. Thus, based on the obtained data we can assume that circulating SINEs and LINEs could have a crucial role in tumorigenesis, and are the molecular targets of DNase I in the bloodstream in the implementation of antimetastatic effect. This research has been supported by the Russian State funded budget project (VI.62.1.3, 0309-2016-0005). *The authors marked with an asterisk equally contributed to the work.

## P-35-007
## The effectiveness of multi-targeted treatment in head and neck squamous cell carcinoma cell lines

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide with relatively frequent recurrence rate following conventional treatment or single molecular targeted therapy and multi-targeted chemotherapy may improve the effects of HNSCC treatment. The aim of this study was to evaluate the effects of the concomitant inhibition of EGFR or PI3K signaling with the inhibition of Wnt canonical signaling or the inhibition of the activity of histone demethylases KDM4 or KDM6 in CAL27 (tongue) and FaDu (hypopharynx) cell lines. The inhibitors of EGFR, which are already used in the treatment of HNSCC, show limited effectiveness in monotherapy and PI3K signaling is another oncogenic pathway frequently disrupted in HNSCC. The action of the inhibitors of these pathways can be enhanced by the concomitant treatment of cells with the inhibitors of Wnt signaling or histone demethylases. The combination of EGFR or PI3K inhibitors with compounds targeting Wnt pathway or KDM4/6 in most cases enhanced the cytostatic/cytotoxic effects analyzed by resazurin assay when compared to single inhibitor treatment. Multi-targeted treatments led to stronger down-regulation of the expression of *BIRC5* and *Cyclin D1* which are related to the regulation of apoptosis and cell cycle. Flow cytometric analyzes of cell cycle distribution (propidium iodide staining) and the induction of apoptosis (phosphatidylserine externalization or caspase 3/7 activation) showed, that the co-treatment of cells with PI3K and Wnt or KDM4/6 inhibitors led to more potent accumulation of cells in G2/M phase. Similar effects were observed in apoptotic rate analyzes. Our results support the assumption that multi-targeted treatment is able to improve the results of therapy of HNSCC. In this regard, the combinations of EGFR or PI3K signaling inhibitors with Wnt signaling or KDM4/KDM6 inhibitors seem very promising. This work was supported by grant from PUMS No. 502-14-03302403-10649.

## P-35-008
## Targeting breast cancer stem cells

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Triple-negative breast cancer (TNBC) tend to be more aggressive than other types of breast cancer. It is thought that the Cancer stem cell (CSC) population found in these tumors is quite resistant to conventional chemotherapy such as Paclitaxel and doxorubicin. CSC are mesenchymal cells which grow slowly and there are no drugs that can be used to kill them. We found that CSC targeting drugs like Midostaurin and Elesclomol are potential candidates for treating CSC cells. Using previously published gene list, we classified breast cancer cell lines into CSC-like and Non-CSC-like. Then we classified the same cells into molecular groups, Luminal, Basal A and B using another published gene list. Both classifications showed that Basal B cell-lines behave like CSC when Luminal and Basal A show non-CSC-like behavior. We identified 15 genes which were expressed differentially between CSC and Non-CSC-like-cell lines. These identified genes were then validated in silico in 10 other datasets. These genes showed worse prognosis in Paclitaxel treated patients. Using drug


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sensitivity data, we predicted drugs to target CSC-like (Elesclomol and Midostaurin) and non-CSC-like cell lines (Panobinostat and Lapatinib). Selected genes were then validated *in vitro*. Additionally, we screened selected cell lines with identified drugs. Midostaurin and Elesclomol showed increased growth inhibition activity against CSC-like cells while Panobinostat and Lapatinib showed increased cytotoxicity against non-CSC-like cells as predicted. Moreover, cell lines showed increased sensitivity to Midostaurin and resistance to Lapatinib upon 3D cell culture. And when epithelial genes were knocked down for, cells showed increased resistance to Midostaurin and sensitivity to Lapatinib as expected. So, in conclusion, CSC cells can be targeted by using Midostaurin and Elesclomol. And a combinational therapy with conventional drugs might lead to a better prognosis of breast cancer patients.

## P-35-009
## Adaptor protein Ruk/CIN85 induces transcriptomic reprogramming required for breast cancer cells invasion and metastasis

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Adaptor protein Ruk/CIN85 is highly upregulated in human tumors and its expression levels correlate with tumor stage and poor prognosis. The aim of present study was to investigate possible molecular mechanisms providing cancer cell reprogramming into invasive and metastatic state. Murine breast adenocarcinoma 4T1 cells with stable overexpression and downregulation of Ruk/CIN85 were used as a model. Invasiveness *in vitro* was studied by Boyden chamber assay and metastatic potential *in vivo* – using experimental metastasis model. Gene expression levels were estimated by RT-qPCR. MMP-2 and MMP-9 activities were analyzed by gelatin zymography. It was demonstrated that Ruk/CIN85 overexpression in 4T1 cells resulted in elevated invasiveness and metastatic potential, while Ruk/CIN85 knockdown led to opposite effects. Expression of more than 35 invasiveness- and metastasis-related genes was analyzed in Ruk/CIN85 up-/down-regulated 4T1 cells. It was found that overexpression of Ruk/CIN85 is associated with increased expression of EMT markers, such as Vimentin, Zeb1/2, Twist, Snail, reprogramming and CSCs markers KLF4, Nanog, CD44, and decreased expression of adhesion molecules (TJP, ICAM1, Integrin β1, Cofilin) and ECM degrading enzymes (MMP-2, MMP-9, Cathepsin D). In contrast, Ruk/CIN85-downregulated 4T1 cells showed increased expression of E-cadherin, adhesion molecules, especially ICAM1, ECM degrading enzymes MMP-2, MMP-9, Cathepsin D, while expression of EMT-driving transcription factors Zeb1/2, Snail, Twist was significantly decreased. The changes in MMP-2 and MMP-9 expression levels in Ruk/CIN85 Up/Down cells correlated with their enzymatic activity. The data obtained indicate that Ruk/CIN85-overexpressing 4T1 cells possess increased malignant properties probably mediated by hybrid mesenchymal-amoeboid phenotype. At the same time, knockdown of Ruk/CIN85 induces epithelial-like phenotype of 4T1 cells thereby suppressing their invasiveness and metastasis.

## P-35-010
## Investigation of the effects of GW8510 pharmacological inhibitor on *in vitro* pancreatic cancer model

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In this study, all genome gene expression microarray data obtained from tissue samples taken from 176 pancreatic cancer patients in TCGA (Cancer Genome Atlas) database and clinical findings of patients were evaluated. As a result of this evaluation, we showed that Ribonucleotide Reductase Regulatory subunit M2 (RRM2) gene which encodes the RRM2 protein is highly expressed in patients with pancreatic cancer and it is statistically significant that this high expression correlates with low survival rate and poor prognosis in patients ($P$ = 1.62e-4).Within the scope of this study, cell viability, apoptosis and cell cycle were investigated in the pancreatic cancer *in vitro* model. However, the combination of gemcitabine chemotherapeutic agent and the GW8510 pharmacological inhibitor has been tested and analysed with the CompuSyn combination analysis program. Apoptosis and cell cycle assays were performed using flow cytometry. Analysis of the wound healing model was carried out with the program Image-J. The effect of GW8510 on the RRM2 protein levels was determined using the ELISA method according to the manufacturer's instructions. According to the results, it was observed that the cell viability was inhibited around 47% in PANC-1 cells treated with GW8510. When the combination analysis was performed, it was determined that the combination of GW8510 and Gemcitabine showed synergistic interaction. Results of apoptosis analysis of the respective combination dose showed 35% apoptosis. On the other hand cell cycle experiments showed that the combination dose affected cells remained in the Sub-G1 phase at a higher rate than GW8510- treated cells. In addition, the inhibitory effect of GW8510 agent on RRM2 protein levels in PANC-1 cells was shown for the first time with this study. It has been found that the possible efficacy of the GW8510 agent in the treatment of pancreatic cancer disease by the inhibition of the RRM2 gene is not negligible.

## P-35-011
## Characterisation of the integrin αv adhesome in human melanoma cells RPMI-7951

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Integrins are transmembrane receptors, often overexpressed in melanoma, that can modulate response to antitumour therapy. They transmit signals from extracellular matrix (ECM) to cytoskeleton through a multimolecular complex of signalling and adaptor proteins called integrin adhesion complex (IAC). The total sum of proteins of the IAC (adhesome) represents a pool of insufficiently investigated potential targets for tumour therapy. Our previous results in human melanoma cells RPMI-7951 have shown that targeted knockdown of integrin subunit αv via siRNA increased sensitivity to antitumour drugs: cisplatin, paclitaxel or vincristine, and drastically decreased *in vitro* migration and invasion. The goal of this study was: (i) to define adhesome of RPMI-7951 cells that have assembled their own ECM, (ii) to determine key integrins these cells use to form IACs and (iii) to


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define IAC components dependent on integrin αv in order to pinpoint proteins involved in the observed phenotype of increased sensitivity to drugs and decreased motility. IACs were isolated from RPMI-7951 cells transfected with control or integrin αv-specific siRNA plated on uncoated Petri dishes, upon crosslinking with DTBP and analysed by mass spectrometry. We identified 344 proteins, with a minimum number of spectra 4, in at least one of the three replicas. 31.9% of proteins are focal adhesion- and 25.5% ECM- associated proteins. Data suggest that RPMI-7951 preferentially form IACs via integrin αvβ5. Eighty-eight proteins, whose expression was changed at least 2 times in RPMI-7951 cells after integrin αv knockdown, were defined as integrin αv adhesome. These proteins might have a role in the observed phenotype of increased sensitivity to antitumour drugs and reduced motility. Further studies of IAC proteins could lead to the identification of potential target molecules for combined use with antitumour drugs for the metastatic melanoma treatment.

## P-35-012
## Subunit of PBAF remodeling complex, PHF10 regulates expression of cell cycle regulatory proteins

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SWI/SNF are chromatin-remodeling complexes that regulate gene expression by changing the position of the nucleosomes. Human tumors often harbor mutations of the SWI/SNF complexes subunits, leading to tumor progression and resistance to chemotherapy. PHF10 is a subunit of the PBAF (mammalian SWI/SNF) complex that is important for the processes of cell differentiation, cell proliferation and programmed cell death. However, its functions especially during carcinogenesis are poorly understood. The aim of our research was to study the role of PHF10 in cell cycle regulation and to examine the effect of cell cycle regulators on the expression of PHF10. We examined PHF10 expression in primary and transformed cell lines. In both cell types PHF10 is expressed in all phases of the cell cycle, and its transcription is increased in transformed cell lines compared to primary fibroblasts and epithelial cells. Furthermore, PHF10 is regulated by cell cycle regulatory proteins. We observed that the overexpression of p27 in cells leads to an increase of PHF10. The knockout of β-catenin and c-Myc caused decrease of PHF10 expression in cancer cells lines. Thereby, the expression of PHF10 is regulated by β-catenin, c-Myc and p27. Genetic inhibition of PHF10 leads to changes in levels of cell cycle regulatory proteins such as the increase of p21, p27 and the decrease of cyclin D1, and accumulation of cells in G1 phase of cell cycle. Full knockout of PHF10 in HCT116 lead to prolongation of G1 phase, and slowing of cell proliferation. Knockout cells showed aberrant increase in *egr1* and *c-fos* genes expression in conditions of high density of PHF10-KO cells. Moreover, mutant cells showed resistance to bortezomib, compared with wild-type cells. Overall, PHF10 is associated with cell cycle proteins and oncogenes, involved in oncogenic transformation, making it a potential target for anti-cancer therapy or a marker of carcinogenesis. This study was supported by RFBR grant #17-54-33031.

## P-35-013
## Transcription factor Snail leads to resistance of breast cancer cells to hypoxia

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A significant part of cell responses to long-term hypoxia is associated with the stimulation of the processes of epithelial-mesenchymal transition (EMT). Transcription factors (TFs) of the Snail family are the most studied as stimulators of EMT. Additionally, they promote the development of tumor progression, migration and invasion of tumor cells. Thereby, the aim of our research was to study of the adaptive mechanisms of human breast cancer (BC) cells to hypoxia and the analysis of the role of the TF Snail in this process. We used human BC cells MCF-7, MDA-MB-231 and HBL-100 which were cultured in standard DMEM containing 10% fetal calf serum at 37 °C and 5% $CO_2$. For hypoxia modeling, the cells were cultured in a $CO_2$-incubator maintaining $O_2$ concentration at 1%. The genetic inhibition of the Snail leads to increased sensitivity of BC cells to hypoxia, and block of cell cycle in the S phase. Under the influence of chronic hypoxia in BC cells (MCF7, HBL-100) E-cadherin redistributes in the cytoplasm, and β-catenin and Snail are activated and translocated to the cell nuclear. BC cells were more sensitive to hypoxia in high density, where Snail expression is decreased. We used a chemical inhibitor of p53-Snail interaction GN25, to examine if blocking of this interaction could lead to increased sensitivity in hypoxia. We have shown that this compound has a greater cytotoxic effect in normoxia than hypoxia. The separation of Snail and p53 causes apoptosis but also activation of Snail and decrease of E-cadherin expression. In conclusion, targeted inhibition of Snail-associated signaling pathways can be considered as a possible approach to the growth regulation of tumors with hypoxic regions. This work was supported by RFBR grant No. 18-015-00422 A.

## P-35-014
## The role of *de novo* expressed sirtuin 3 in triple negative human breast cancer cells upon oxidative stress

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Hyperoxic treatment (inducer of reactive oxygen species) was shown to support some tumorigenic properties, but finally suppresses growth of certain mammary carcinoma cells. While estrogen receptor α cancers are more receptive to hormonal therapy, triple negative breast cancers (TNBC) are characterized by an aggressive behaviour and the lack of targeted therapeutic strategies. Sirtuin 3 (Sirt3), a major mitochondrial NAD+-dependent deacetylase, has bifunctional role in cancer tumorigenesis, acting as both oncogene and tumor suppressor, depending on the tissue and cancer-type specific metabolic programs. Due to strikingly reduced Sirt3 level in many breast cancer cells, we hypothesized it would have tumor suppressive effect in TNBC cells. Therefore, we stably transfected MDA-MB-231 cells with Flag-tagged Sirt-3 or empty plasmid, as confirmed by real time PCR, western blot analysis and confocal microscopy. We found the optimal duration of hyperoxic treatment which induces DNA damage in the cells but macroscopically does not influence the vitality of the


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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cells. We are using western blot to monitor the expression of proteins involved in mitochondrial biogenesis, glycolysis, metabolic regulation and antioxidant defence. To assess the differences in the growth rate and metabolic activity upon expression of Sirt3 and normoxic/hyperoxic treatment, we are using MTT test and colony-forming cell assay. Furthermore, we are checking the mitochondrial function through cellular respiration and FACS analysis, by monitoring the mitochondrial mass, mitochondrial potential and ROS production. The surprising finding that Sirt-3 markedly promoted growth of MDA-MB-231 cells in normoxic conditions, whereas hyperoxia markedly inhibited the growth of the Sirt3-overexpressing cells compared to control cells gave us a rationale for more detailed studies on Sirt3 and hyperoxia as an adjuvant tumor therapy in TNBC cells. *The authors marked with an asterisk equally contributed to the work.

## P-35-015
## Hypoxia in breast cancer cells and targeting HIF-1alpha signaling by novel derivatives of quinoxaline-2-carbonitrile 1,4-dioxide

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Solid cancers commonly contain wide hypoxic areas. A major cause of tumor hypoxia is the formation of aberrant blood vessels in neoplastic tissue, particularly in rapidly progressing cancers. Hypoxia is associated with aggressive tumor phenotypes, drug resistance, and poor clinical prognosis. Based on these observations we aimed to create novel class of compounds with high activity under hypoxic conditions. We have developed a series of bioreductive hypoxic cytotoxins (prodrugs) that are activated in hypoxic tissue and therefore selectively kill hypoxic tumor cells. These compounds are low-activity agents in normoxia that require enzymatic activation under hypoxia. One- or two-electron reductases generate radical that is either partially reoxidized to the original prodrug in normoxic cells or is converted into its cytotoxic species in hypoxic cells. Therefore, the use of hypoxic reductases is a very promising approach for the enzymatic activation of the compounds in hypoxia. So, 3-phenylquinoxaline-2-carbonitrile 1,4-dioxides with amino residues in quinoxaline ring were synthesized and were assayed for cytotoxicity against of human breast cancer cell lines (MCF-7 and MDA-MB-231) under hypoxic (1% $O_2$) and normoxic (21% $O_2$) conditions. The most potent compounds LCTA-2645 and LCTA-2647 have $IC_{50}$ values lower than 0.2 mM in hypoxia and hypoxic cytotoxicity ratio (HCR) values 32 and 37, respectively. HIF-1$\alpha$ activity assessed by reporter analysis was effectively inhibited by compound LCTA-2647. Moreover, LCTA-2647 treatment caused partial suppression of AP-1 transcription complex activity in hypoxia. These results suggest that quinoxaline 1,4-dioxides seem to be promising bioreductive agents for selective targeting of HIF-1/AP-1 signaling pathways in hypoxia for improving cancer treatment efficacy. The experiments were supported by RFBR grants 18-53-34005 (chemistry) and 18-015-00422 (biology).

## P-35-016
## Nanobased fluorescent sensor system for zearalenone detection

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Fluorescent sensor system based on nanostructured molecularly imprinted polymer (MIP) membrane was developed and used for determination of zearalenone and its metabolite – zearalenol. Zearalenone is an estrogenic mycotoxin, which people can be exposed to through the diet. It has been reported that zearalenone and its metabolites were associated with development of hormone-dependent tumors and breast cancer. To evaluate the risk of breast cancer related to zearalenone exposure, levels of mycotoxins can be performed by direct detection in urine. The MIP membranes capable for recognition of zearalenone were synthesized using a fragment-based approach with cyclododecyl 2,4-dihydroxybenzoate as a dummy template and ethylene glycol methacrylate phosphate as functional monomer. Triethyleneglycoldimethacrylate was used as a cross-linker. The sensor responses are generated by the UV-irradiation, which initiates natural fluorescence of the adsorbed zearalenone, while intensity of fluorescence is proportional to the analyte concentration in the sample. Sensor responses of the fluorescent system were investigated as a function of both type and concentration of the functional monomer in the initial monomer mixture used for the membranes' synthesis, as well as the sample composition. The influence of pH, ionic strength, and buffer concentration on sensor responses were investigated. The fluorescent sensor system based on the optimized MIP membranes provided a possibility of zearalenone detection within the range 1–25 μg/mL demonstrating detection limit (3б) of 1 μg/mL. The MIP membranes provide selective recognition of zearalenone as compared to their close structural analogues. Storage stability of the proposed sensor systems is 12 months. The brand new sensor systems are easy-to-use and provide fast, efficient and inexpensive analysis to evaluate the risk of breast cancer related to zearalenone exposure.

## P-35-017
## Synthetic lethality of hyperthermia and inhibitors of DNA-PKcs and ATM

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Thermal sensitization of cells to DNA-damaging agents has been known for a long time and is a case of synthetic lethality. Temperature-dependent radio(chemo)sensitivity is thought to arise because DNA repair systems are inhibited in hyperthermia (heat shock). However, the fact that hyperthermia itself induces DNA lesions has been omitted in the majority of relevant studies. Recently, we have demonstrated that hyperthermia could induce both single-stranded (in the S phase) and double-stranded (in G1 and G2) DNA breaks. Here, we demonstrate that hyperthermia induces replication stress and associated DNA-PK- and ATM-dependent DNA damage response. When such a response is prevented, double-stranded DNA breaks arise in S phase, and the cells subsequently die. We have investigated the mechanisms underlying hyperthermia-induced sensitization of S-phase cells to PIKK inhibitors. Moreover, we show that heat shock can inhibit some of the proteins involved in fork protecting (BRCA1,


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BRCA2, XRCC1). Our current data support the idea that during heat shock replication forks are not well protected and may further be processed by structure specific nucleases such as Mus81 and SLX4. The work was supported by Russian Science Foundation (17-74-20030).

## P-35-018
## Intra-tumor hypoxia as a modulator of endothelial cell activity: *in vitro* model of pathological angiogenesis

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Besides tumor cells, the tumor microenvironment harbors a variety of non-cancer cells such as endothelial cells (ECs). ECs make the tumor angiogenesis, thus influencing tumor progression, metastasis and the recruitment of immune cells. Hypoxia is a critical parameter modulating the tumor microenvironment, it is thus crucial to understand the activity of ECs in low $pO_2$. Aim of the experiments was to study the effect of pathologic microenvironment (low $pO_2$ and cancer-derived factors) on the activity of ECs. Murine adult, brain derived endothelial cells (MBr MEC FVB) were cultured in hypoxia (1% $pO_2$) and normoxia (~ 19% $pO_2$) or with conditioned medium from kidney cancer cells (RenCa). The content of factors secreted by cancer cells was checked using membrane-based antibody array. Tube formation assay was performed in Matrigel to test *in vitro* the angiogenic ability. ECs were tested for their secretion of VEGF (by Real-Time PCR and ELISA), expression of surface markers (by flow cytometry) and cytoplasmic proteins (by Western blot). After exposure of ECs to hypoxia, the expression of PD-L1 was found to be enhanced and cells produced more VEGF. Low $pO_2$ and factors secreted by cancer cells influenced the ability to form vessels *in-vitro*, causing faster formation of irregularly shaped vessels. ECs cultured in low $pO_2$ or in conditioned medium from cancer cells expressed less Claudin protein, suggesting a permeabilization of the vessels. Hypoxia induces changes of ECs activity. Low $pO_2$ upregulated PD-L1 what may influence the infiltration of the tumor by immune cells. A decreased level of Claudin may indicate a disturbance of tight cell-cell connections and therefore potentially participate to the pathologic character of tumor angiogenesis and help metastatic dissemination. Together with inducing the proangiogenic response in tumor cells hypoxia shapes the pathologic features of the formed vessels (Funded by Opus CRU/WIM/1238/2017 and Kosciuszko 579/2016/DA grants).

## P-35-019
## Mechanisms of resistance in prostate cancer: AMPK as a therapeutic target

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Prostate cancer (PCa) is the second cause of cancer death in men. It can progress and become resistant to conventional therapies such as androgen deprivation therapy (ADT). Among the causes that can contribute to the failure of treatments highlight the epithelial-neuroendocrine transition (NED), a phenotypic change in which prostate cancer cells differentiate into neuroendocrine cells, and the presence of cancer stem cells (CSC), a subpopulation of tumoral cells with the capacity for self-renewal and differentiation. In this study, we aim to explore the relationship between both processes by inducing CSC in a long term neuroendocrine cancer model. We show that long-term androgen deprivation induced CSC phenotype. The LNCaP-NE cells maintained in these conditions of hormonal deprivation exhibited a decrease in sensitivity to docetaxel as well as an increase in NED, drug resistance and pluripotent stem cells markers. In addition, we studied the role of the metabolic sensor AMP-activated kinase (AMPK) in the CSC acquired phenotype. Analysis of AMPK revealed that LNCaP-NE cells had higher levels of total AMPK compared with their parental cells. Interestingly, the modulation of AMPK regulated the expression of neuroendocrine markers in CSC-like cells. These results suggest that the positive regulation of AMPK induces the transition of LNCaP cells to neuroendocrine cells while the negative regulation of this kinase reverses it. Therefore, AMPK could be a key therapeutic target in resistance mechanisms of PCa and its regulation could be a novel strategy to overcome drug resistance.

## P-35-020
## Determination of drug sensitivity subgroups in acute myeloblastic leukemia by the detection of gene-based cancer biomarkers and the prediction of prognosis and treatment

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Local bone marrow renin angiotensin system (BM RAS) specific to the hematopoietic BM microenvironment plays important roles in the development of neoplastic malignant blood cells. It functions in primitive embryonic hematopoiesis confirm the hypothesis that it may play a role in neoplastic hematopoiesis and it has been demonstrated that RAS related genes may be potentially biomarkers for acute myeloid leukemia (AML). Each of the patient's tumors has its unique gene expression profile. So it is seen that pathological and morphological classification of patients is insufficient to distinguish heterogeneity of a cancer. Therefore, for many years, the researchers aimed to identify homogeneous cancer subgroups of patients and to elucidate the molecular mechanism of the disease. In this work potentially biomarker genes determined for AML through the association of identified RAS gene based homogeneous subgroups and most effective drugs for each subgroup. The cancer genome project (CGP) database from Welcome trust Sanger and the 6-Model IC50 calculation method have been used. Two chemotherapy agents were found to be the most effective drugs for each homogenous subgroup. Three important potentially biomarker genes were identified to predict the patient prognosis and response to treatment. Better prognostic prediction has been obtained when these genes combined together. It has been demonstrated that two of these genes can contribute to cell sensitivity by mediating autophagy and the third one may contribute to cell resistance associated with resistance mechanisms by TGF-beta. In our study, an exemplary and original approach to how a large database can be used for the detection of a suitable chemotherapeutic agent and biomarker gene for acute myeloid leukemia has been described. Before the treatment, this gene panel can make the prediction of patient prognosis and it can directs clinicians to the effective treatments. *The authors marked with an asterisk equally contributed to the work.


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## P-35-021
## Novel mechanism of action of natural plant defensin PvD1 involves a modulatory effect of CD63 and CD9 expression level in breast cancer cells derived exosomes

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Communication between cancer cells regulates a cascade of events essential for disease development and progression. This communication process can be mediated by several signalling pathways. Exosomes are nano-sized vesicles produced by tumor cells capable of transferring proteins and genetic material to recipient cells and thus acting as cells' messengers. Formation of exosomes is regulated either by exosomal sorting complex required for transport (ESCRT) or mediated by the lipid composition of the endosomal membrane. In both pathways proteins from the tetraspanins family have been reported to play an important role. Consequently, many of these, such as CD9, CD63, CD81 or CD105, are highly enriched in exosomes and can serve as exosomal markers. Tetraspanins are also important molecular scaffolds playing a role in signalling, adhesion or cell invasion processes. The goal of this work is to study the effect of the natural plant defensin $Pv\mathrm{D}_1$ on the production of exosomes by breast cancer cells. Our data focus on the modulatory effect of $Pv\mathrm{D}_1$ on the expression level of CD63 and CD9 tetraspanins in exosomes isolated from MCF-7 cells. Additionally, we examined the interaction of $Pv\mathrm{D}_1$ with biological membranes, employing a combination of biophysical and imaging techniques including dynamic light scattering (DLS), surface plasmon resonance (SPR) and atomic force microscopy (AFM). This work can potentially lead to a better understanding of the exosome-mediated anticancer action of $Pv\mathrm{D}_1$ and envision an innovative strategy for treating cancer, either as a therapy alone or as a co-adjuvant in conventional treatments.

## P-35-022
## Identification of microenvironmental regulation and therapeutic targeting of oncogenic eEF2K in pancreatic cancer

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Eukaryotic elongation factor 2 kinase (eEF2K) is overexpressed in cancer cells and its overexpression is correlated with poor prognosis in several types of cancer. It has been known that pancreatic cancer (PaCa) has a unique tumor microenvironment (TME) which contributes all stages of tumorigenesis. In this study, the effects of interaction between PaCa cells and macrophages on eEF2K levels and aggressive tumor behaviours were investigated. PANC1 cells cocultured with macrophages, and then the changes in eEF2K expression, cell migration and invasion were analyzed using western blot, migration and invasion assays, respectively. The effects of the interaction between these two cell types on monocyte chemoattractant protein-1 (MCP-1) levels, one of the most important chemokines in TME, were analyzed using ELISA. eEF2K was stably overexpressed in PANC1 cells, and MCP-1 levels were measured using western blot to detect the possible interaction between eEF2K and MCP-1. Then, eEF2K was silenced using siRNA both *in vitro* and *in vivo*, and the changes in MCP-1 levels, cell migration and invasion, and the number of tumor-infiltrated pro-tumorigenic macrophages were analyzed. As a result, the interaction between these two cell types caused an increase in eEF2K and MCP-1 levels and thus accelerated aggressive tumor behaviours. In addition, a bidirectional interaction was found between MCP-1 and eEF2K. MCP-1 also caused differentiation of monocytes to pro-tumorigenic macrophages. *In vitro* silencing of EF2K decreased MCP-1 expression, cell invasion and migration. *In vivo* inhibition of EF2K also decreased MCP-1 expression levels, tumor volume and the number of tumor-infiltrated pro-tumorigenic macrophages. Consequently, targeting both tumor cells and macrophages through EF2K inhibition might be a promising strategy for PaCa treatment.

## P-35-023
## Sensitizing glioma cells to fatty acid oxidation inhibitor by inhibition of SLC22A5 transporter activity

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Gliomas are the most common primary malignant brain tumor in adults, but current treatment for glioblastoma multiforme (GBM) is insufficient. It was shown that primary-cultured human GBM cells depend on fatty acid oxidation (FAO) for proliferation, and that FAO inhibition *in vivo* by etomoxir prolongs survival in the mouse model. Etomoxir, an inhibitor of FAO rate-limiting enzyme – carnitine palmitoyltransferase 1 (CPT1), was tested in clinical trials, which were terminated due to high toxicity. Carnitine is a CPT1 substrate, and SLC22A5 is the only high-affinity carnitine transporter in the plasma membrane expressed in the central nervous system. HEK293 cells overexpressing SLC22A5 show increased transport of several chemotherapeutics, what may inhibit carnitine transport into the cell. This study is aimed at establishing the role of SLC22A5 in glioma cells and at assessing its potential for sensitizing them to lower, less toxic doses of etomoxir. Analysis of data from REMBRANDT showed higher SLC22A5 mRNA expression in glioma patient-derived tissues when compared to normal tissues, an observation confirmed by a Western Blot protein analysis of astrocytes and several glioma cell lines. Out of several tested drugs, transported by SLC22A5, vinorelbine and vincristine are most efficient in carnitine transport inhibition in U87-MG, LN229 and T89G glioma cells. The viability (MTT test) is reduced and cytotoxicity (CellTox Green assay) and apoptosis (Annexin V assay) increased when the foregoing are used in combination with etomoxir. Proper carnitine delivery by SLC22A5 transporter is important for glioblastoma metabolism and SLC22A5-transported chemotherapeutics induce cell death both by their regular mechanism of action and by inhibition of carnitine delivery to glioma cells, thus sensitizing them to lower, less toxic doses of etomoxir. This project is financed by grant 2016/23/N/NZ3/02430 from National Science Centre in Poland.


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## P-35-024
## Identification of a novel ERK-dependent protein interaction, important for productive binding of hypoxia inducible factor-1a to chromatin

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Hypoxia inducible factor-1 (HIF-1) is a central transcriptional regulator of genes associated with adaptive responses to cellular oxygen levels. Oxygen controls HIF-1 by destabilizing its HIF-1a subunit via hydroxylation, subsequent ubiquitination and proteasomal degradation. However, HIF-1a activation also heavily depends on oxygen-independent mechanisms involving its direct phosphorylation and/or interaction with other proteins. We have previously shown that HIF-1a transcriptional activity is stimulated by ERK-mediated phosphorylation of Ser641/Ser643. This modification 'masks' an adjacent nuclear export signal and results in the efficient nuclear accumulation of HIF-1a. By pull-down assays, mass spectrometry and immunoprecipitation experiments, we now show that this modification promotes the interaction of HIF-1a with nucleophosmin (NPM1), a hypoxia-inducible multifunctional protein that is also implicated in euchromatin binding and genomic stability. Furthermore, pull-down experiments demonstrate direct binding of a phosphomimetic HIF-1a form to NPM1. HIF-1a immunoprecipitation indicates that NPM1 is required for (i) HIF-1a association with acetylated histones, (ii) activation of HIF-1-target genes, (iii) metabolic adaptation of cancer cells to hypoxia and (iv) inhibition of apoptosis. Furthermore, sequential chromatin immunoprecipitation (ChIP/re-ChIP) shows that NPM1 associates with a HIF-1-inducible promoter which can be simultaneously occupied by HIF-1α only under conditions that promote its phosphorylation by ERKs and interaction with NPM1. Transcriptome analysis using Quant-RNA-seq after HIF-1a or NPM1 silencing under hypoxia reveals a significant number of genes, the hypoxic expression of which depends on both proteins. Our data suggest that phosphorylation-dependent binding of HIF-1a to NPM1 is essential for productive association of HIF-1a with chromatin components leading to HIF-1 activation and adaptation of cancer cells to oxygen deprivation.

## P-35-025
## The importance of PFKFB4 overexpression for the progression of malignant melanoma

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While in most solid tumors low oxygen (hypoxic) conditions occur only at a certain stage of cancer growth, when the tumor mass is insufficiently vascularized, in skin, mild hypoxia is a natural state. In consequence, malignant melanoma, tumor derived from melanocytes residing in the dermal-epidermal junction, develops in constantly hypoxic environment. Since hypoxia via hypoxia inducible factor 1 (HIF-1) induces glycolysis, which is essential for malignant melanoma growth/survival and which enhancement is associated with worse clinical outcome, using semi-quantitative RT-PCR we have checked the influence of hypoxia on the expression of HIF-1 target genes involved in glucose breakdown in melanoma cells. Examined cells showed high basal glycolytic profile and the only gene with low expression in normoxia and clear induction in hypoxia was *PFKFB4,* one coding the cancer specific isoenzyme of phosphofructokinase II. Observation that *PFKFB4* gene can be crucial for the enhancement of glycolytic pathway in melanoma under hypoxia prompted us to investigate its basal expression in the panel of melanoma cell lines from different stages of carcinogenesis. The levels of expression varied markedly between analyzed cell lines and we found melanoma cell lines with high expression of PFKFB4 even under normoxia. Next, melanoma cells were exposed to first- in-class PFKFB4 specific inhibitor under both normoxia and hypoxia, what resulted in melanoma growth inhibition. For further analysis of PFKFB4 importance in melanoma biology, we analyzed the mRNA expression of PFKFB4 in 214 melanoma patients using publicly available data set GSE65904. The Kaplan-Meier survival analysis for patients with high and low expression level of PFKFB4 revealed that high PFKFB4 expression contributes to shorter overall survival and distant metastasis free survival. Our study suggests that currently available anti-melanoma therapeutic strategies may significantly benefit from agents targeting PFKFB4 activity.

## P-35-026
## Role of c-Met receptor and MCPIP1 in tumor resistance to RTKs

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The main objective of our study was to determine the role of c-Met and anti-inflammatory protein MCPIP1 in acquisition of resistance to RTKs inhibitors in ccRCC. Clear cell renal cell carcinoma (ccRCC) treatment with small molecules that inhibit multiple receptor tyrosine kinases (RTKs), showed in approximately 38% of patients significant tumor control. However, despite the efficacy of treatment, ccRCC often develops resistance to targeted drugs and the majority of patients who receive such treatment exhibit progressive disease after 1 year. Several hypotheses have been proposed regarding the mechanisms underlying resistance to RTKs inhibitors, but the precise pathways have not been fully elucidated. Our study shows that short term (24 h) and long term (3 weeks) stimulation with sunitinib or sorafenib induce expression of phosphorylated c-Met receptor, STAT3 and Src. Furthermore, stimulation with sunitinib resulted in the acquisition of cancer stem cells features with strong up-regulation of e-cadherin. In addition, after sunitinib and sorafenib treatment we observed increased lung metastasis and decrease in protein level of MCPIP1. Interestingly, after MCPIP1 overexpression, we observed c-Met downregulation compared to control cells, which is regulated by RNase activity of MCPIP1. Moreover, our results show that in patient samples, together with MCPIP1 downregulation, level of phosphorylated and total c-Met receptor increase. We showed that acquisition of therapy resistance in ccRCC may be affected by MCPIP1 decrease, together with phosphorylation of c-Met receptor and it can be partially reversed by overexpressing the MCPIP1 protein, which may act as a potent tumor suppressor. Proposed research may help in understanding the mechanisms responsible for tumor resistance to targeted therapy. This study was supported by research grants from the NSC 2013/09/D/NZ/00249, 2017/25/N/NZ5/03014, 2017/26/E/NZ5/00691 and grant from Jagiellonian University BMN 16/2017.


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## P-35-027

## Ferroptosis, a non-apoptotic cellular death is silenced in melanoma cancer cells (Me45) after ionizing radiation

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Aim of the studies were to investigated a different types of cellular death in melanoma cancer cells (Me45), after ionizing radiation (IR) with focusing on the Fe-dependent ferroptosis pathway. This death resulted from oxidative stress and lipid peroxidation products accumulation in treated cells. Some of the cancer cells, e.g. melanoma cancers presented high resistance to ferroptosis induction. Materials and methods: Me45 cancer cells were treated with 4 Gy of IR, with addition of specific ferroptosis inductor, RSL-3 (1000 ng/mL). At a different time points (1, 6 12 and 24 h) after IR exposition variable levels of oxidative stress were observed, measured with reactive oxygen (ROS) and nitrogen species (NOS). Using cytometric Annexin-V assay both, apoptosis and necrosis were investigated, together with cell cycle. For cellular proliferation and viability 24 h MTT assay and fluorescence microscopy imaging were performed. Results and conclusions: As a consequence of irradiation a cellular proliferation and viability were decreased, followed by 24 h MTT assay. However, the cellular death were not confirmed by cytometric Annexin-V apoptosis assay, where either apoptosis nor necrosis were observed. Cell cycle analysis excluded also presence of sub-G1 subpopulation for death cells, so the conclusion was only cell cycle inhibition, here in G0/G1 phase. The confirmation of these results came also from fluorescence images, where mostly mononuclear cells were observed, with rare mitosis. All that results brought us to the conclusion, that irradiated Me45 cells, also after RSL-3 addition do not responded with any of the apoptosis-like death, also ferroptosis were not induced. Me 45 cells obtained ability to silence ferroptosis. The work was supported by grant No. 02/010/BK_18/0102 from Silesian University of Technology in Gliwice, Poland.

## P-35-028

## MIM1, the Mcl-1 inhibitor induces cells death in human COLO829 melanoma cells trough intrinsic apoptosis pathway

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Malignant melanoma is characterised by higher expression of Mcl-1 protein, than normal melanocytes or begin nevi, which indicates that dysregulation of apoptosis via overexpression of Mcl-1 protein is responsible for malignant melanoma development and progression. Moreover, current data provides supporting evidence for a critical role of Mcl-1 protein in low chemosensitivity of melanoma. Therefore, the antiapoptotic protein Mcl-1 is a potential treatment target for malignant melanoma also in advanced stage. MIM1 is a specific Mcl-1 protein inhibitor able to induce Mcl-1-dependent cancer cells death. The aim of this study was to check whether the inhibition of Mcl-1 protein by the use of a selective Mcl-1 inhibitor is sufficient to induce apoptosis in melanoma cells. Analysis of apoptosis was performed by fluorescence image cytometer NucleoCounter NC-3000. The obtained results demonstrated that MIM1 induces apoptosis in COLO829 melanoma cells. Early apoptotic cells (Annexin V-positive) were detected after 16 h exposure of studied cells to MIM1, whereas DNA fragmentation – the final step of apoptosis in COLO829 cells was observed after prolongation of incubation time up to 72 h. Summarizing, this data suggests that targeting Mcl-1 protein by selective Mcl-1 inhibitors is a promising target in malignant melanoma treatment and provides convincing evidence that MIM1, which inhibits Mcl-1 protein is sufficient to induce apoptosis in melanoma cells. This research was funded by Medical University of Silesia in Katowice (KNW-1-O85/N/8/O)

## P-35-029

## Curcumin induces apoptosis and necroptosis of prostate cancer PC-3 cells through ROS-mediated pathway under lactic acidosis

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Lactic acid buildup and resultant acidic extracellular pH in tumor microenvironment has an important role in conferring tumor progression, metastasis and resistance to therapy. Curcumin, a phenolic compound extracted from Curcuma longa, is known to have chemotherapeutic and chemopreventive effects in diverse cancers, including prostate cancer, but the involved mechanism still remains elusive, and requires further investigation. Herein, we studied the underlying mechanism(s) of action of curcumin on prostate cancer PC-3 cells in the lactic acid-containing medium. At concentration showing no toxicity on normal prostate epithelial RWPE-1 and HPrEC cells, curcumin induced a preferential cytotoxicity in PC-3 cells. Curcumin concurrently increased the number of cells in the sub-G0/G1 peak of the cell cycle and the annexin V-PE-positive fraction, accompanied by increased reactive oxygen species (ROS) levels, mitochondrial membrane depolarization, and decreased cellular ATP content. Meanwhile, curcumin increased the amount of cleaved forms of caspase- 3 and its downstream substrate PARP with an increased Bax/Bcl-2 ratio as apoptosis mediators, as well as phospho (p)-RIPK3 and p-MLKL proteins as necroptosis mediators. Pretreatment with apoptosis inhibitor Q-VD-Oph or necroptosis inhibitor necrostatin-1 recovered cell viability inhibited by curcumin. Moreover, Curcumin treatment increased the levels of various damage-sensing molecules such as p-histone H2A.X, p-ATM-Ser1981, p-ATRSer428, p-CHK1Ser345, and p-CHK2Thr68. The series of phenomena caused by curcumin effectively reversed after pretreatment with radical scavenger *N*-acetylcysteine. Collectively, this study revealed the molecular mechanism by which curcumin under lactic acidosis concurrently led to apoptosis and necroptosis through DNA damage and mitochondrial impairments in PC-3 cells, indicating that ROS served as upstream molecules of curcumin-driven cytotoxicity.

## P-35-030

## Characterization of vestigial-like 1, a novel prognostic biomarker and therapeutic target of gastric cancer

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Gastric cancer (GC) is highly heterogeneous, which impedes the development and implementation of targeted therapies for precision medicine. Vestigial-like 1 (VGLL1) is a coactivator of TEA domain family member 4 (TEAD4) of the HIPPO pathway.


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However, the molecular function of VGLL1 in GC remains unclear. Here, we evaluated its potential as a therapeutic target for GC by investigating its clinical relevance and molecular characteristics. We conducted microarray analysis of GC tissues to evaluate the clinical significance of *VGLL1* expression. We revealed that its expression correlated with PIK3CA expression and that it is a prognostic biomarker for survival in GC. VGLL1 was found to regulate the proliferation and migration of GC cells *in vitro* and metastasis *in vivo* in a mouse model. *VGLL1* expression was also regulated by the PI3K–AKT–β-catenin pathway. Moreover, transforming growth factor-β was shown to activate VGLL1 via phosphorylation by the RSK2/ERK pathway. Phosphorylated VGLL1 induced the expression of *MMP9* by enhancing the formation of a TEAD4–VGLL1 complex at the *MMP9* promoter. In conclusion, VGLL1, a novel prognostic biomarker and therapeutic target for GC, is regulated by both PI3K/AKT/β-catenin and RSK2/ERK signaling to promote MMP9-mediated proliferation and metastasis in GC.

## P-35-031
## Licochalcone A inhibits melanoma cell growth and migration via arresting cell cycle and suppressing Akt phosphorylation

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Cancer metastasis is the hallmark of malignant tumors and is characterized by cells that lose their cell–cell contact, cross the basement membrane, invade the stroma, spread across blood vessels, and form new neoplastic tissue at sites other than that of the original tumor. Therefore, cell adhesion, migration, and proteolysis of extra-cellular matrix (ECM) proteins are regarded as important steps in the development of metastasis. Licochalcone A (LA), a natural chalcone derived from the roots and rhizomes of *Glycyrrhiza* spp., exhibits multiple biological activities such as antibacterial, antioxidant, anti-inflammatory, antimalarial, antiviral, and antitumor effects. Although the antimetastatic effect of LA has been reported, the detailed mechanism remains unclear. Therefore, we further delineated its antimetastatic properties *in vitro* and *in vivo*. In the study, we investigated the effects and possible mechanisms of action of LA on migration by the metastatic melanoma cell line B16-F10. The data revealed LA (10–30 μM) markedly inhibited B16-F10 growth as detected by the MTT assay. LA revealed a concentration-dependent inhibition of migration of B16-F10. Moreover, LA arrested the B16-F10 cell cycle at the G0/G1 phase. LA also inhibited the phosphorylation of Akt in B16-F10 cells. In addition, histopathological analysis showed that B16-F10 melanoma cells had a high metastatic potency to the host in the lungs, which was markedly reversed by LA. LA significantly reduced a massive tumor cell proliferation around the bronchioles of the lung and inhibited fibrosis and increased alveolar space, suggesting that LA possess a potent antimetastatic effect. We demonstrated that LA caused cell-cycle arrest by suppressing the Akt signaling in B16-F10 melanoma cells. LA also significantly inhibited the migration of B16-F10 *in vitro* and *in vivo*. These findings indicate that LA may provide a therapeutic potential for treating cancer metastasis.

## P-35-032
## Extracellular cystatin F downregulates cytotoxicity of cytotoxic T lymphocytes

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Cystatin F is an endogenous protein inhibitor that regulates the activity of cysteine cathepsins. In cytotoxic lymphocytes cathepsins C and H activate granzymes, while cathepsin L is involved in perforin activation. Since perforin and granzymes are major effector molecules used by cytotoxic lymphocytes to kill transformed or virally infected cells, defects in their activation can affect the killing potential of cytotoxic lymphocytes. Therefore, endogenously expressed intracellular cystatin F as well as extracellular cystatin F secreted by by-stander cells and internalised into cytotoxic cells could attenuate cytotoxicity of cytotoxic cells. We have demonstrated that extracellular full-length cystatin F after internalisation decreased activities of cathepsins C and H in a cytotoxic T cell line, TALL-104, resulting in decreased activities of granzymes A and B. This led to reduced ability of TALL-104 cells to kill their target cells. Next, we tested the effect of N-terminally truncated cystatin F, which in contrast to full-length cystatin F can directly inhibit cathepsins' activity and does not need prior activation, and found that the effect of N-terminally truncated form is even more pronounced. On the other hand, neither of cystatin F forms affected perforin or cathepsin C processing. Importantly, we demonstrated that even cystatin F present in target cells can reduce the killing potential of cytotoxic cells. To conclude, cystatin F is an important regulator of proteolytic activity of cysteine cathepsins in cytotoxic T cells, regulating their ability to eliminate transformed or virally infected cells and thus affecting the immune response in cancer and infectious diseases.

## P-35-033
## Morphological change of human cancer cells induced by body fluid of *Mastigias papua*

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Since induced pluripotent stem (iPS) cells which have the ability of differentiation to any kinds of cells had discovered in 2006, the researches of cell differentiation have been ongoing actively. Besides, more recently, the techniques of the cell conversion to target cells not via iPS cell have been researching all over the world. For example, the overexpression of the transcription factors (Ascl1, Brn2 and Myt1l) in the human somatic cell is widely known to induce the differentiation to neurons efficiently. The cell differentiations are also the biological phenomena not only in human but in the other animals, especially, are considered to be repeated frequently in metamorphosis animals. *Mastigias papua*, which is belong to cnidarian, one of the perfect metamorphosis animals, changes the morphology in the growing stage of the life cycle (polyp, strobila, ephyra and medusa). Therefore, *Mastigias papua* is possible to have potential factors caused cell differentiation. In this research, first of all, *Mastigias papua* body fluid was added into HeLa cells, human malignant epithelial cells. As a result, the drastic morphological change from typical HeLa cells to slender cells which have neurite was occurred. Next,


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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whether the human morphological changed cells induced by the body fluid of jellyfish were neurons or not was verified. Beta III Tubulin protein, which is known as a protein specific expressed in neuron, was detected in the morphological changed cells induced by the body fluid of jellyfish. Therefore, the potential factors which can induce morphological change from HeLa cells to neurons were suggested to exist in the body fluid of *Mastigias papua*. In addition to the above results, the discussion of the growth stage both cellular morphology and protein expression in the neuron-like cells using specific antibodies will be reported.

## P-35-034
## Tilorone promotes the emergence of cytotoxic active lymphocytes

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There are several mechanisms to stimulate the immune response. Earlier, we have shown that the incubation of PBMCs (peripheral blood mononuclear cells) of healthy donors with Tag7 and IL2 induce appearance of cytotoxic lymphocyte subpopulations that can kill HLA (−) cells. Our recent studies have discovered that the low-molecular weight synthetic compound Tilorone (2, 7-Bis [2-(diethylamino) ethoxy]-9-fluorenone dihydrochloride) shows the same effect on PBMCs. The purpose of this work was to describe the mechanisms of the occurrence of cytotoxic lymphocyte populations under the action of Tilorone. We have demonstrated that Tilorone cause the appearance of lymphocytes subpopulations which are capable to recognize and kill HLA (−) tumor cells. The cytotoxic activity on day 4 is caused by NK and CD4 lymphocytes and on day 6 by CD4 and CD8 cytotoxic lymphocytes. Activated T cells are able to induce programmed cell death through FasL-Fas interaction, while NKs kill via secretion of granzymes. It was also shown that tumor cells are recognized due to the expression of stress molecules – MicA (ligand for NKG2D on the surface of NK and CD8) and Hsp70 (ligand for Tag7 on the surface of CD4) and this interaction is critical to trigger programmed cell death. We have shown that monocytes are involved in Tilorone signaling. The removal of monocytes by magnetic separation from PBMCs at the early stages of activation completely stopped the appearance of cytotoxic lymphocyte subpopulations. It was established that the effect of Tilorone on PBMCs leads to increase subpopulation of dendritic cells which are required to transmit activation signal to effector cells. Thus, we have shown that Tilorone causes the activation of cytotoxic lymphocyte subpopulations, using monocytes as an intermediary in signal transmission. This work was supported by Russian Science Foundation grant N 15–14–00031-P. *The authors marked with an asterisk equally contributed to the work.

## P-35-035
## Sesamol inhibits melanoma cell growth and migration via suppressing the phosphorylation of Akt and p38 MAPK

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Malignant melanoma is the cutaneous malignancy with highest mortality rate showing increased incidence in recent years, and 30% of patients with malignant melanoma reportedly have multi-organ metastases. The median overall survival in patients with metastases was of 5.3 months. Sesamol has a wide range of biological activities, including anti-mutagenic, antioxidant, anti-inflammatory, antiatherogenic, and antiplatelet effects. However, the effect of sesamol in melanoma remains unclear. Therefore, we further investigated the detailed mechanism underling antimelanoma activity of sesamol. In the present study, B16-F10 mouse melanoma cells were treated with sesamol (50–300 μM) for the indicated times (0–48 h). The data showed that sesamol (100–300 μM) markedly reduced cell viability of B16-F10 mouse melanoma cells, as measured by the MTT assay, indicating that sesamol significantly inhibited melanoma cell growth. Sesamol (300 μM) also induced melanoma cell apoptosis and caspase-3 activation, as detected by flow cytometry and western blotting, respectively. These findings revealed that sesamol could inhibit melanoma cell growth, in part, through the induction of cell apoptosis. In addition, sesamol could attenuate melanoma cell migration, as detected by the wound healing assay. Moreover, sesamol could reduce the phosphorylation of Akt and p38 MAPK. In conclusion, we demonstrated that sesamol significantly induced melanoma cell apoptosis and inhibited melanoma cell migration, at least in part, through suppressing Akt and p38 MAPK signaling pathways. These findings also suggest that sesamol may provide therapeutic potentials for treating patients with melanoma.

## P-35-036
## A novel class of endogenous lipid ligands for the orphan GPR55 receptor inducing apoptosis of cancer cells via PLC and IP3R activation

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The orphan G protein-coupled receptor 55 (GPR55) is activated by lysophosphatidylinositol (LPI) and some endocannabinoids. Activated by LPI, GPR55 stimulates cancer cell proliferation; its high expression level is often associated with bad prognosis for cancer patients. Anandamide, in turn, suppresses cancer cell growth signaling via GPR55. We show for the first time that endogenous lipids acyl dopamines (NADA), which also activate cannabinoid and vanilloid receptors, are novel GPR55 ligands and induce apoptosis of cancer cell lines expressing GPR55. This was demonstrated using inhibitor assay and siRNA knockdown. Signal transduction steps from GPR55 were shown using key enzyme inhibitors, direct $Ca^{2+}$ measurement using fluorescent dyes, and a CREB luciferase reporter assay. The ability of NADA to induce apoptosis via GPR55 was validated using a panel of human cancer cell lines with known GPR55 expression (SW620, MDA-MB-231, PANC-1, DU 145, PC-3). LPI was used as a control. In all cell lines tested, NADA induced apoptosis with $EC_{50}$ in the range 15–49 μM, while LPI was not toxic. GPR55 inactivation using selective inhibitors, as well as siRNA knockdown led to a drop of NADA cytotoxicity by 60–80% depending on the cell line; the remainder of the toxicity was blocked by the CB1 and TRPV1 receptor inhibitors. Downstream of the GPR55, NADA induced PLC and IP3R activation, $Ca^{2+}$ liberation from the intracellular stores, and activation of CaMKII/IV and CREB with concomitant neuronal NO synthase expression and oxidative stress induction. The observed $EC_{50}$ values for NADA toxicity via GPR55 were in range with those reported for proliferation stimulation with LPI (1–10 μM), and the signal transduction pathway, at least until the $Ca^{2+}$ liberation, was in agreement with the reported in the literature for GPR55. Thus, NADA are a new class of GPR55 ligands with an inverse activity compared to LPI. The work was in part supported by the RFBR grants 19-04-00302 and 17-00-00105. *The authors marked with an asterisk equally contributed to the work.


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## P-35-037
## Selective heme oxygenase-1 gene silencing overcomes proteasome inhibitor chemoresistance in multiple myeloma cells

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Despite recent advances in proteasome inhibitor and immunomodulatory drug-based therapies, multiple myeloma (MM) remains with a poor prognosis, mainly because of chemoresistance. Our previous studies showed that Heme Oxygenase-1(HO-1), a cytoprotective microsomal enzyme catalyzing the degradation of heme, is involved in chemoresistance mechanisms. The aim of the present study was to evaluate the role of HO-1 in MM cells (U266) to Bortezomib (BTZ) resistance. All experiments were performed on a MM cell line (U266) resistant to BTZ following specific HO-1 gene silencing. Cell viability, oxidative stress and autophagy were assessed by cytofluorometric analysis and endoplasmic reticulum (ER) associated proteins (i.e. BIP, IRE1a and PERK) were evaluated by real time PCR and western blot analysis. We showed that BTZ induced a significant increase in HO-1 mRNA after 3 h of treatment and peaked after 6 h. Furthermore, such expression correlated with the induction of ER stress related proteins. Selective HO-1 gene silencing using specific shRNA, resulted in a significant decrease of BIP, IRE1a and PERK mRNA expression, thus suggesting that BTZ-induced ER stress is mediated via HO-1 activation. In addition, this set of experiments also showed that HO-1 silencing results in a significant reduction of autophagy as measured by p62, Lc3BII and beclin expression and autophagosome formation. Interestingly, U266 resistant to BTZ (U266R) express higher levels of HO-1 compared to naïve cells (U266S) and exhibited a significant increase of HO-1 nuclear translocation. HO-1 gene silencing restored BTZ sensitivity in U266R. Such results were further confirmed in a clinical setting using primary MM cells obtained from BTZ refractory patients. Taken all together, our results suggest that HO-1 plays a major role in MM resistance to BTZ and may represent a possible target to overcome such issue improving the outcome of MM patients.

## P-35-038
## Cysteine cathepsins B and X: increased expression in cancer stem cells

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Tumors consist of phenotypically and functionally heterogenous population of cells that differ in the degree of differentiation, invasiveness and ability for metastasis formation. Along with differentiated cancer cells tumors contain also a subpopulation of cancer stem cells (CSCs). CSCs represent a small subset of cells with a tumorigenic potential that can give rise to a disease relapse. They are intrinsically resistant to the convenient antitumor therapies and are one of the main causes of the failure of cancer treatment. Therefore, there is an urge need for identification of new drugs and therapeutic approaches that will be, in addition to differentiated cells, effective also towards CSCs. Promising approach to enhance CSCs directed therapy could be targeting proteolytic enzymes associated with tumor progression, such as cathepsins B and X. In cancer the expression and activity of these redundant lysosomal cysteine carboxypeptidases is increased and they have an important role in degradation of extracellular matrix, tumor invasion, migration, metastasis and angiogenesis. Moreover, we have shown that their increased expression is associated with less differentiated cell phenotype. To test our hypothesis, we evaluated expression of cathepsins B and X in cells with CSC phenotype (CD44+/CD24-), isolated from breast cancer cell lines, based on their ability to form tumorspheres *in vitro*. We demonstrated that expression and activity of cathepsins B and X were significantly increased in the CSCs, following tumorsphere formation compared to their expression in single adherent cells as demonstrated by western blot, ELISA and enzyme kinetics assays. Therefore, inhibition of cathepsins B and X could serve as promising approach for CSC directed treatment in cancer that may improve the effectiveness of current antitumor therapy.

## P-35-039
## Targeting RSK4 prevents both chemoresistance and metastasis in lung and bladder cancer

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Lung and bladder cancers are aggressive and fast spreading form of cancer with unmet clinical needs. We have identified RSK4, a member of the p90 ribosomal S6 kinases (RSKs) family, as a promoter of drug resistance and metastasis in lung and bladder cancer cells, and silencing this kinase sensitises to therapy and hinders metastasis *in vitro* and *in vivo*. We have determined the crystal structures of RSK4 in both its inactive and active states, revealing differences from those seen previously for other AGC kinases. We have shown that the βB-sheet, a unique structural feature of RSKs and MSKs, plays a key role in RSK4 regulation and activation. Finally, drug screening revealed several floxacin antibiotics as potent RSK4 activation inhibitors, and trovafloxacin reproduces all effects of RSK4 silencing. Through crystallography and Markov transient analysis, we have proposed a mechanism for the action of this compound. Hence, we suggest that RSK4 inhibition represents a novel therapeutic strategy for treating lung and bladder cancers. *The authors marked with an asterisk equally contributed to the work.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-35-040
## Transfection of easily oxidizable GC-rich DNA fragments into MCF7 cells

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The cell-free DNA (cfDNA) of patients suffering from various diseases is characterized with accrued GC-rich fragments, such as ribosomal repeats (rDNA), which contain many easily oxidizable Gn (n > 2) motifs. The oxidized DNA penetrates breast cancer cells (MCF7 cell line) and changes their physiology. We hypothesized that easily oxidizable GC-rich fragments can penetrate into the cancer cells and be expressed. We have proved this through experiments with cell culturing in the presence of pEGFP and pEGFP-rDNA plasmids added to the medium. The plasmids carried the EGFP gene as a marker. We have estimated the level of pEGFP-rDNA and pEGFP in the cell using qPCR. The pEGFP-rDNA accumulates in the cells in larger quantities than pEGFP. The pEGFP-rDNA could be detected in the cell even after 72 h of incubation. The rDNA fragments increased the oxidation rate, the speed of plasmid transfection and the level of EGFP expression. We have estimated the content of 8-oxodG in the cell using flow cytometry. The pEGFP-rDNA caused an increase in the number of cells with a high level of 8-oxodG (a 2–3-fold increase, $P < 0.05$). The level of 8-oxodG was maximum within the first few hours and decreased in a day after the plasmid had been added to the medium. The pEGFP did not elevate the level of 8-oxodG ($P > 0.05$). We observed (using fluorescence microscopy), that 8-oxodG was localized in the nucleus and near the nucleoli of about a half of the cells only after adding pEGFP-rDNA. So, the easily oxidizable GC-rich cfDNA can easily penetrate cells and be expressed. These findings are important for understanding the factors of cancer cell survival during the therapy. The cfDNA can carry mutant genes of the tumor genome that trigger carcinogenesis in healthy cells to lead to metastases. The work was supported by funding within the frameworks of Project No. 0517-2018-0003 under the "Biomedical technologies: innovative developments" program of the Russian Academy of Science Presidium.

## P-35-041
## The role of DNA sensors TLR9 and AIM2 in signal transduction when cell-free DNA acts on MCF7 cells

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DNA freely circulating in blood stream has been termed cell-free DNA (cfDNA). DNA-sensors TLR9 and AIM2 recognize cfDNA fragments, which penetrate the cells. TLR9 favors the cancer cell survival via TLR9-MyD88-NF-kB signaling pathway activation, whereas AIM2 induces apoptosis in the cell. The content of transcribed region of ribosomal DNA (rDNA) in cfDNA isolated from breast cancer patients (N = 68) was several times higher than in cfDNA isolated from healthy controls (N = 76, $P < 10^{-8}$). Plasmids pBR322-rDNA and pBR322 were used. The pBR322-rDNA plasmid (50 ng/mL) induced a several times increase in the amount of TLR9 RNA and protein as early as in 30 min. In 2 h, the amount of TLR9 RNA and protein decreased, but remained above the reference level within the next 48 h. The pBR322 vector also induced TLR9 expression, but in a much smaller degree. The peak amounts of TLR9 RNA and protein were observed in 2 h, while the effect completely disappeared in 24 h. When the effect disappeared, considerable inhibition of AIM2 expression occurred at the levels of both RNA and the protein. When using pBR322 vector, the amount of AIM2 RNA and protein decreased, but insignificantly ($P > 0.05$). In this case the protein was virtually not detected in the cytoplasm, but transmigrated inside the nucleus in almost every cell of the cell pool. In the nucleus, AIM2 is located as compact structures in some certain regions. The nucleoli are distinctly contrasting, i.e. AIM2 never penetrates the structures. In the presence of pBR322 vector, AIM2 migrates to the nuclei of approximately a half of the cells. The other cells expresses almost no AIM2. Thus, stimulation of TLR9 expression in MCF7 is followed by an arrest of expression of the AIM2 receptor, so, GC-rich cfDNA enhances survivability of MCF7 cancer cells. This work is supported by the Project No. 0517-2018-0003 under the "Biomedical technologies: innovative developments" program of the Russian Academy of Science Presidium.

## P-35-042
## Combination of ribonuclease binase and Akt1/2 kinase inhibitor blocks two alternative survival pathways in Kasumi-1 cells

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Ribonucleases (RNases) are considered as agents for adjuvant therapy of tumors which effects on malignant cells and on the products of their activity, including circulating RNA. RNase from *Bacillus pumilus* (binase) has a selective toxic effect on various malignant cells. The basis of the molecular mechanism of binase is the induction of apoptosis in tumor cells, and the sensitivity of cells to binase is determined by the presence of certain oncogenes. In myelogenous leukemia Kasumi-1 cells binase block the KIT-PI3K signaling pathway, however, some cells are resistant to the toxic action of binase. We hypothesized that when binase blocked the KIT signaling pathway, cell survival may be due to the alternative proliferative pathway mediated by Akt kinase, since it is known that Akt kinase is constitutively activated in Kasumi-1 cells and this activation is not associated with PI3K. In this work, the toxic effect of the Akt1/2 kinase inhibitor and its combination with binase on Kasumi-1 cells was studied. Akt1/2 inhibitor reduces the survival of Kasumi-1 cells in a dose-dependent manner. With the simultaneous action of binase and an Akt inhibitor on Kasumi-1 cells, their effects summarized. So, at an inhibitor concentration of 5 μM, cell viability is reduced by 18%, and binase at a concentration of 4 μM results in a 50% decrease in cell survival. Together, binase and the Akt1/2 inhibitor reduce cell survival by 70%. The increase in the percentage of apoptotic cells in population due to the action of binase and the Akt1/2 inhibitor is also summarized. In the case of simultaneous use concentrations of cytotoxicants, sufficient for the total elimination of cells, several times lower than in the case of individual use. This shows the possibility of the combined use of binase with other chemotherapeutic agents to enhance the antitumor effects. Supported by RFBR #17-00-00061.


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## P-35-043
## WMJ-J-2, a novel hydroxamate-based histone deacetylase inhibitor, induced colorectal cancer cell death via LKB1-p53-miR320a-survivin/NRP1 signaling

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Recent development in drug discovery has highlighted the broad biological and pharmacological properties of a key pharmacophore, hydroxamate. Growing evidence showed that hydroxamate-based histone deacetylase inhibitors (HDACis) have emerged as a powerful new class of therapeutic agents acting through the regulation of the acetylation states of cellular targets. In this study, we explored the anti-tumor mechanisms of a novel aliphatic hydroxamate-based HDACi, WMJ-J-2, in colorectal cancer (CRC) cells. WMJ-J-2 reduced cell viability in different CRC cell lines. WMJ-J-2 caused survivin or NRP-1 reduction in CRC cells. Survivin siRNA or NRP-1 siRNA significantly induced cell apoptosis in CRC cells. Results from microarray analysis showed that WMJ-J-2 up-regulates 66 microRNAs (miRNAs) in CRC cells. Among these 66 miRNAs, eight miRNAs including miR-320a are down-regulated with tumor progression in CRC patients according to the data of The Cancer Genome Atlas (*TCGA*) project. WMJ-J-2 induced p53 phosphorylation and acetylation. WMJ-J-2 induced miR-320a expression was reduced in p53-null CRC cells. Transfection of cells with anti-miR320a reduced WMJ-J-2's effects in reducing survivin and NRP-1 levels. WMJ-J-2 also caused LKB1 phosphorylation, while LKB1 siRNA diminished WMJ-J-2-induced p53 phosphorylation. Moreover, WMJ-J-2-caused p53 acetylation was abolished in the presence of anacardic acid (a HAT inhibitor). Both anacardic acid and LKB1 siRNA are capable of reducing WMJ-J-2's inhibitory effects on survivin and NRP-1 levels. Furthermore, WMJ-J-2 suppressed the growth of subcutaneous CRC xenografts *in vivo*. Together these results suggests that WMJ-J-2-induced CRC cell death may involve LKB1-p53-miR320a-survivin or NRP1 signaling cascade. HDACs inhibition may also contribute to WMJ-J-2's actions in CRC cells. The present study also suggests that WMJ-J-2 may be a potential lead compound and warrant the clinical development in the treatment of CRCs.

## P-35-044
## A novel 2-aminobenzimidazole derivative, ABM, exhibits anti-angiogenesis effects via targeting VEGFR-2 signaling

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Background and purpose: Benzimidazole derivatives exhibit broad-spectrum pharmacological properties including anti-microbial, anti-diabetic and anti-tumor activity. However, whether benzimidazole derivatives are effective in suppressing angiogenesis and its underlying mechanisms remain incompletely understood. In this study, we aim to characterize the anti-angiogenic mechanisms of a novel 2-aminobenzimidazole derivative, ABM, in an effort to develop novel angiogenesis inhibitor. Experimental approach: MTT, BrdU, migration and invasion assays and immunoblotting were used to examine ABM's effects on vascular endothelial growth factor (VEGF)-A-induced endothelial cell proliferation, migration, invasion, as well as signaling molecules activation. ABM's *ex vivo* and *in vivo* anti-angiogenic effects were determined using tube formation assay, aorta ring sprouting assay, Matrigel plug assay and a metastasis mouse model. Key results: ABM inhibited VEGF-A-induced cell proliferation, migration, invasion and endothelial tube formation of human umbilical vascular endothelial cells (HUVECs). ABM also reduced VEGF-A-induced microvessel sprouting *ex vivo* and suppressed VEGF-A- or tumor cells-induced neovascularization *in vivo*. AMB was also shown to attenuate B16F10 melanoma lung metastasis. In addition, ABM suppressed the VEGF-A-induced phosphorylation of VEGFR-2 and its downstream signaling molecules in HUVECs. Results from computer modeling further demonstrated that ABM may binds to VEGFR-2 with high affinity. Conclusions and implications: ABM may suppress endothelial remodeling and inhibit angiogenesis via targeting VEGF-A-VEGFR-2 signaling. These results also support the role of ABM as a potential lead compound and warrant the clinical development in the treatment of cancer and angiogenesis-related diseases.

## P-35-045
## Differential cytotoxic effects of esculetin compared with vinblastine and paclitaxel on tumour prostate PC-3 cells

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The coumarin esculetin shows antioxidant action on some cell types, both by scavenging ROS and by decreasing ROS production. We have previously demonstrated the induction of apoptosis by esculetin on NB4 human leukaemia cells. Metastatic prostate cancer is one of the most therapy-resistant human neoplasms. PC-3 cells do not express androgen receptor and is one of the most commonly used prostate cancer cell lines established from bone-derived metastases. Commonly used chemotherapy drugs for metastatic prostate cancer include paclitaxel and vinblastine, among other drugs. In this work, we show different cytotoxic effects of the antioxidant esculetin with respect to vinblastine and paclitaxel on tumor prostate cells PC-3. Esculetin decreases metabolic activity of PC-3 cells in a time and concentration dependent way whereas the metabolic activity of vinblastine- or paclitaxel-treated PC-3 cells is not time-dependent. However, PC-3 cells treated with a high 250 μM esculetin for 48 or 72 h show apoptosis levels similar to those produced by 50 μM vinblastine at these incubation times or by 200 μM paclitaxel at 19 h. Vinblastine and paclitaxel produced cell cycle arrest in G2/M phase after incubation for 19 h. In contrast, esculetin does not seem significantly to affect cell cycle. Thus, a differential mechanism of esculetin on PC-3 prostate cells could be inferred. These results and further studies on such a mechanism could be relevant to design new therapies against resistant prostate cancer.
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## P-35-046
## Microarray-predicted molecular effect of WT1 isoforms in MCF-7 breast cancer cell line

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Breast cancer is a prominent disease in women worldwide. The highest distribution of breast cancer subtype is luminal A (ER+, PR+/−, HER2−). Recent study reported that the luminal subtype showed significantly higher expression of the transcription factor, Wilms' tumor 1 (WT1), than the others. The role of WT1 and its isoform in breast carcinogenesis still need to be discovered. In this study, we investigated the molecular effect of stable WT1 overexpression by its various isoform [isoform: A(−/−), B (+/−), C(−/+) and D(+/+)] in MCF-7 cell line which represent luminal A subtype. Gene expressions were analyzed by microarray using Affymetrix u133 plus 2.0 chips. Gene level data were acquired using Chipster and further analyzed by Ingenuity Pathway Analysis (IPA) tool. Results showed that only 3.4% of the differentially expressed genes was induced by all four WT1 isoforms overexpression, while 21.6%, 6.9%, 7.6% and 25.3% were unique for isoform A, B, C and D, respectively. A great number of WT1 induced genes were enriched in disease categories related to cellular movements, migratory capabilities, angiogenesis and cell death of tumor cells. The isoforms had differentiated effects based on the expressional changes, and while WT1 overexpression induced the production of EGFR ligands, it also affected remodeling of the extracellular matrix and altered the TGF-b route. WT1 isoform A and D seemed to have more aggressive genotype when compared with isoform C and D. The isoform A and D significantly upregulated of PDGFRA, AREG, CTGF/CCN2 while isoform C and D had an opposite effect. Induction of MAPK signaling may explain the differential aggressiveness of the various isoforms. Keywords: WT1 isoform, molecular pathway, MCF-7, aggressiveness, microarray.

## P-35-047
## Evaluation of a panel of non-pathogenic enterovirus strains as potential oncolytic agents for the therapy of malignant lymphomas

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Despite the achievement in the treatment of lymphatic system tumors, there remains the problem of frequent relapses and the resistance to chemotherapy. 42 short-term tissue cultures of lymphoid tumors were obtained from biopsies that included 20 follicular lymphomas (15 of the first and second cytological types, 5 – of the third cytological type), 4 mantle cell lymphomas, 7 marginal zone lymphomas, 2 Burkitt lymphomas, 3 chronic lymphocytic leukemia, 2 diffuse large B-cell lymphoma and 3-high malignancy B-cell lymphoma and 1 hairy-cell leukemia sample. Every culture was plated on 96-well plates, and then tested for sensitivity and replication ability for 6 viruses: Coxsackievirus B5 (Live Enterovirus Vaccine 14 strain, LEV14), Coxsackievirus B6 (live enterovirus vaccine 15, LEV15), Coxsackievirus A7 (Live Enterovirus Vaccine 8 strain, LEV8), Echovirus 12 (Live Enterovirus Vaccine 7 strain, LEV7), Type 1 Poliovirus, Sabin strain (PV1S), and Vesicular Stomatitis Virus, Indiana strain (VSV-I). The short-term tissue cultures of lymphoid tumors demonstrated differential sensitivity and replication ability for these viruses, which varied in the wide range. Among the studied viral strains LEV14, PV1S and LEV7 demonstrated the stringiest tropism for lymphoid tumor cells. These non-pathogenic enterovirus strains could represent a perspective agents for the therapy of lymphomas. The VSV-I has shown the activity only in few follicular lymphomas among those sensitive for all of the other tested viruses. Acknowledgments: This study was supported by grant from the Center of Strategic Planning, The Ministry of Healthcare of the Russian Federation, project code 1.1096. *The authors marked with an asterisk equally contributed to the work.

## P-35-048
## Thioredoxin/thioredoxin reductase system and redox-dependent regulation of adaptive antioxidant response under formation of cancer cell resistance to cisplatin

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Thioredoxin/thioredoxin reductase (Trx/TrxR) system is multifunctional and participates in regulation of the most important vital processes of the cell such as proliferation, gene transcription, DNA reparation, apoptosis and cellular signaling through the transient oxidation/reduction of key Cys-residues in regulatory proteins. Here we studied the character of Trx/TrxR expression, which involve in the control of the cellular redox state, under the development of resistance of human ovarian carcinoma SKOV-3 cells to cisplatin (CDDP), which has the prooxidant effect. The growth of *TRXDR1* and *TRXRD2* genes expression was found in resistant SKOV3/CDDP cells. Elevation of Trx and TrxR activity was also noticed in resistant cells. In according to the increase of Trx/TrxR functional activity, it was detected a decrease in ROS cellular level due to enhanced expression of key antioxidant enzymes (*SOD2*, *CAT*, *GPX1, HO-1*) and depression of NADPH-oxidase (*NOX5*) gene. In addition, an increase in GSH/GSSG ratio as well as redox-dependent activity of transcription factor Nrf2 were observed in resistant SKOV3/CDDP cells. The obtained results are discussed in the direction of the possible leader role of Trx/TrxR system in the redox-dependent development of the adaptive antioxidant response as an important process in the mechanism of formation of cancer cell resistance to cisplatin. The publication was prepared with the support of the "RUDN University Program 5-100".


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## P-35-049

## Cancer cell specific anticancer effect of extracts of *Coptis chinensis* in EGFR-TKI resistant lung cancer cell *in vitro* and *in vivo*

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The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) gefitinib is an effective therapeutic drug against non-small cell lung cancers (NSCLCs) harboring EGFR mutations. However, both adaptive and acquired resistance significantly limits the efficacy of EGFR-TKI and thus the current chemotherapeutic strategies for NSCLCs. There is therefore a need to overcome this resistance. In this study, we investigated the anticancer potential of natural extracts of *Coptis chinensis* (ECC) against the gefitinib-resistant (GR) lung cancer cells PC9GR, A549GR, and HCC827GR. ECC inhibited cell viability, migration, and invasion, and induced apoptosis effectively *in vitro* GR cells with limited minimal cytotoxic effect on BEAS-2B cells, and tumor regression *in vivo* zebra fish models. These effects were associated with the suppression of EGFR-AKT signaling and anti-apoptotic proteins Mcl-1 and Bcl-2, which were overexpressed in GR cells. Moreover, combination treatment with ECC and gefitinib enhanced the sensitivity of GR cells to gefitinib. These results indicate the potential of ECC in the treatment of NSCLC, particularly in combination with EGFR-TKI therapy in EGFR-TKI resistance.

## P-35-050

## Discovery of LDD-1075 as a potent FLT3 inhibitor

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Fms-like tyrosine kinase 3 (FLT3) is a valuable pharmacological target in the treatment of acute myeloid leukemia (AML). LDD-1075 and LDD-1076 are indirubin derivatives, and LDD-1075 is the ester form of LDD-1076. LDD-1076 exhibited a potent *in vitro* FLT3 kinase activity inhibition with an $IC_{50}$ of 7.89 nM, while LDD-1075 showed a relatively weak activity against FLT3 ($IC_{50}$ 3.19 μM). In contrast with the results of the FLT3 kinase activity inhibition assay, the LDD-1076 did not affect the growth of the MV4-11 cell line, which harbors the constitutively activated form of the FLT3 mutation. Interestingly, LDD-1075 exhibited a strong cytotoxic effect against the MV4-11 cells. When LDD-1075 was incubated with the MV4-11 cell lysate, the formation of LDD-1076 was observed. The LDD-1075 treatment inhibited the FLT3 phosphorylation along with the phosphorylation of the STAT5 protein, which is a downstream signal transducer of FLT3. The LDD-1075 treatment induced the apoptosis induction and cell cycle arrest at the G1 phase. This study shows that the LDD-1076 formed by the bioconversion of LDD-1075 is a potent FLT3 inhibitor with an anti-leukemic activity.

## P-35-051

## Regulation of expression of genes from the Grainyhead-like family (GRHL) – new molecular insights into cancer development

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Genes from the Grainyhead-like family are found in all animal species and fungi that were studied so far. In mammals there are three genes that belong to this family: Grainyhead-like 1 (GRHL1), Grainyhead-like 2 (GRHL2) and Grainyhead-like 3 (GRHL3). The expression of these genes is tissue- and developmentally-specific, and occurs primarily in epithelia. Development of many types of cancer is often accompanied by changes in the levels of expression of the genes from the GRHL family. GRHL genes are directly involved in the process of carcinogenesis. In order to predict transcription factors regulating the expression of GRHL genes, we conducted bioinformatic analyses of the promoter regions for each gene: GRHL1, GRHL2, and GRHL3. Subsequently, using appropriate experimental methods (chromatin immunoprecipitation, quantitative real time PCR, etc.) we verified whether the predicted transcription factors indeed regulate the expression of GRHL genes. In our project we discovered additional transcription factors regulating the expression of GRHL genes. Consequently, our findings may allow to identify novel drug targets in signaling pathways whose activation or inhibition may lead to changes in the levels of expression of GRHL genes. This work is supported by the National Science Centre grant 2016/21/B/NZ1/00279. *The authors marked with an asterisk equally contributed to the work.

## P-35-052

## The novel tetracarboranylchlorin derivative for binary anticancer treatment: rapid tumor elimination via superoxide anion production

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Tetrapyrrolic macrocycles are suitable for a variety of chemical modifications aimed at new agents for binary antitumor treatment. In particular, the conjugation of the carborane cage to the 5,10,15,20-tetrakis(pentafluorophenyl)-17,18-dihydro porphyrin macrocycle, a modification designed for tumor sensitization in photodynamic (PDT) and boron neutron capture (BNCT) therapies. Further exploring the potential of modified tetrapyrrolic compounds as photo/radiosensitizers we synthesized a chlorin derivative carrying four *closo*-carborane cages (total 44 $^{10}B$ atoms) and 16 fluorine atoms at the periphery of the macrocycle (fluorinated tetracarboranylchlorin, compound 1). Compound 1 was readily soluble in water and showed a negligible dark cytotoxicity. Fluorine and boron substituents did not alter the photoactivation of 1 *in vitro*. Intracellular accumulation of the bulky compound 1 reached maximum by 36 h. A monochromatic light illumination of human and rodent cell lines loaded with low


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micromolar concentrations of 1 triggered rapid (within a few minutes) photonecrosis as determined by the entry of propidium iodide into the cells. This effect was mechanistically associated with generation of superoxide anion radicals as registered with an intracellular fluorescent probe. *In vivo* experiments demonstrated that 1 (administered i.p. or i.v. up to 5 mg/kg) caused no significant toxicity. Illumination of subcutaneous B16 melanoma transplants (syngeneic C57BL/6 mice) or C6 rat glioma xenografts (immunocompromised mice) after i.p. injection with 1 (5 mg/kg) led to a decrease of tumor foci, tumor growth retardation and cure of a cohort of animals. Furthermore, experimental BNCT resulted in a growth retardation of C6 derived tumors compared to mock-irradiated animals. Photo/radionecrosis emerges as an important mechanism of tumor cell elimination if other death pathways are not functional. The publication was prepared with the support of the RUDN University Program "5-100".

## P-35-053
## Glutamine can serve as a sensitizing factor for chemotherapy of solid tumors

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Metabolic transformation of cancer cells includes increased dependency on glutamine metabolism termed "glutamine addiction". Glutamine, the most abundant amino acid in the circulation, is substrate for the synthesis and import of other amino acids, proteosynthesis, synthesis of nucleotides, and anaplerotic reactions of the Krebs cycle. Glutamine has also an antioxidative role through its involvement in the synthesis and regeneration of glutathione. The aim of this project was to study the effect of asparaginase-mediated glutamine depletion on oxidative stress and apoptosis of cell lines derived from solid tumors and on control fibroblasts. Asparaginase is an approved drug for the long-term therapy of acute lymphoblastic leukemia, which decreases the blood levels of asparagine and glutamine. Surprisingly, simulation of short-term, but not long-term, asparaginase treatment sensitizes tumor cells to apoptosis induced by hydrogen peroxide but not to chemotherapeutic effect of doxorubicin, oxaliplatinum, and paclitaxel. For this effect, a simultaneous depletion of glutamine and asparagine is required. Acute depletion is accompanied by a decrease of antioxidant capacity, lower activity of mechanistic target of rapamycin complex 1 and changes in levels of some intracellular metabolites. On the other hand, all the described phenomena are absent in fibroblasts. In conclusion, while asparaginase is clinically used for the long-term chemotherapy of leukemia, this study suggests a potential role of its acute exposure as a chemosensitizing factor in the therapy of solid tumors.

## P-35-054
## VS-5584 enhances the anti-proliferative effect of ponatinib on chronic myeloid leukemia cells

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Ponatinib is a third generation tyrosine kinase inhibitor that targets BCR-ABL1 fusion protein for selective treatment of chronic myeloid leukemia (CML). PI3K/Akt/mTOR pathway known to be active in CML and Leukemia stem cells (LSC). VS-5584 is a selective PI3K-mTOR dual-inhibitor. We aimed to investigate the mechanisms of anti-proliferative effects of ponatinib and VS-5584 combination on CML cells and LSCs at molecular level. Cytotoxic effects of VS-5584, ponatinib and their combinations on K562 and LSC cell lines were measured with WST8 assay. Cell cycle regulations were investigated with BD Cycletest Kit by flow cytometry. The quantitations of transcriptional activities of E2F/DP1, Myc/Max, C/EBP with dual-luciferase reporter assay after transfection of appropriate vectors were analyzed. PI- (viable) and CD133+ (stem) cells stained with CFSE to assess quiescent cell fraction. Expressions of genes related to cell cycle, NFkB, MAPK, PI3K/Akt/mTOR, and JAK/STAT signaling pathways evaluated by qRT-PCR. Ponatinib and VS-5584 combination was synergistic and inhibited proliferation via $G_0/G_1$ arrest in K562 and LSC. CFSE staining results revealed that quiescent LSCs in $G_0$ phase reduced LSC after VS-5584 treatment. Activations of E2F/DP1, Myc/Max and suppression of C/EBP with combination treatment contributed to the regulation of gene expression profiles. In K562 and/or LSC cells treated with the combination of ponatinib and VS-5584, expressions of CCNB1, Myc, AKT1, MTOR, EIF4E, CRKL, RPS6KA1, SOCS3, NFKBIA, FOS, GRB2, JUN, and NRAS were down-regulated more prominently than those treated with ponatinib. VS-5584 enhanced the ponatinib related upregulation of CEBPA in both cell lines. Also, the highest upregulation of CDKN2C in LSC observed with the combination treatment. The combination of ponatinib and VS-5584 can be a promising treatment strategy that can inhibit the uncontrolled proliferation of CML blasts and LSCs.

## P-35-055
## Exosomes derived from prostate cancer cells after treatment with ruthenium and copper metallodendrimers

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Prostate cancer (PCa) is the fifth leading cause of cancer death in men globally. PCa is rarely symptomatic early in its course, thus it is extremely critical to detect and diagnose it in its earliest stages. Prostate-Specific Antigen (PSA) is the most widely used serum biomarker of PCa, but this marker falls short in its diagnostic ability. Therefore, it is necessary to find new biomarkers for PCa risk, early detection, and prognosis. In recent years, a novel diagnostic biomedical tool with high potential has been identified in extracellular nanovesicles or exosomes; they are released by cells and contain detailed molecular information on the cell of origin including tumor hallmarks. Moreover, the use of metallodendrimers as delivery systems of drugs are promising agents for cancer therapy. For this purpose, PC3 cells were treated for 72 h with metallodendrimers at the doses of IC50 for each of them; two of them with ruthenium in their dendritic periphery (27Ru, 8Ru) and the other compound with copper


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(2Cu). Finally, the medium was centrifuged and filtrated in order to isolate the exosomes and the size of medium exosomes was characterized by transmission electron microscope. We could detect the presence of numerous vesicles with a size of 30–50 nm; when these vesicles were immunolabelled with CD-9 or CD-63 antibodies (present in the exosomes membranes), we observed that in the medium treated with 27 Ru the number of vesicles was higher in comparison with the other groups. The results show an inhibitory effect of both 8Ru and 2Cu metallodendrimers on exosomes release from PC3 cells and therefore support the application of these delivery systems in advanced prostate cancer treatment.

## P-35-056
## Regulation of S100A10 expression by Grainyhead-like 2 transcription factor

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Dysregulated expression of genes encoding the Grainyhead-like 2 (GRHL2) transcription factor and S100 protein family members is a common cause of malignant transformation. Depending on the cancer type, the disease can be caused by either up- or down-regulation of these proteins. GRHL2, due to its various regulatory functions, may act as a tumor suppressor or oncogene while S100 proteins are involved in control of processes which, are often altered in cancer i.e. $Ca^{2+}$ homeostasis, proliferation, differentiation, apoptosis, inflammation or migration. Contribution of GRHL2 and S100 proteins to carcinogenesis has been intensively examined, but never in the context of their mutual regulation. The regulatory mechanism of S100 protein expression is still unclear. Bioinformatic analysis of S100 gene promoters confirmed that there are highly conserved binding sites for GRHL2 transcription factor in the promoter of the S100A10 gene. Thus, the aim of this study was to check, whether GRHL2 regulates expression of S100A10 gene and how it affects S100A10 protein level. Several approaches have been applied to confirm this hypothesis. Among them were estimation of S100A10 mRNA and protein level in cells with altered level of GRHL2, verification whether GRHL2 directly binds to the promoter sequence of S100A10 and investigation of the promoter activity in cells with overexpressed GRHL2. Obtained results confirmed the influence of the GRHL2 transcription factor on S100A10 expression and inspired further research concerning the use of S100A10 as a cancer marker in diagnostic medicine. Acknowledgements: This work was supported by statutory funds from the Nencki Institute of Experimental Biology.

## P-35-057
## Effects of PC3 cells-derived exosomes on NE differentiation in LNCaP cells

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Neuroendocrine differentiation (NED) process contributes to castration-resistant prostate cancer (CRPCa) associated with an aggressive phenotype of poor prognosis. Neuroendocrine (NE) cells produce and secrete peptide hormones and growth factors in a paracrine/autocrine manner promoting the progression of the disease. In addition, exosomes are released by prostate cancer cells supporting the spread of prostate cancer progression via multiple mechanisms. In this study, we evaluated the phenotypic changes induced by PC3-exosomes in LNCaP cells with or without pre-treatment with growth hormone-releasing hormone (GHRH) which has been previously implicated in the progression of prostate cancer. Thus, human PC3 cells were grown in medium with exosome-free serum for 72 h in the presence or absence of the neuropeptide, (+) GHRH and (−) GHRH, respectively. Thereafter, the exosomes were isolated from culture media. LNCaP cells were treated with both types of exosomes derived from PC3 cells: (+) GHRH-PC3-exosomes or (−) GHRH-PC3-exosomes. After 24 h, LNCaP cells showed (32–37%) outgrown neurites in presence of all exosomes types. In addition, both types of exosomes derived from PC3 cells provoked an increase (26–38%) on the expression of neuron-specific enolase (NSE) levels as compared with those in control group, but not significant changes were observed between both treatments. Our studies demonstrate that CRPCa cells-derived exosomes are able to induce NED in androgen-responsive prostate cancer cells and this feature is not modified by pre-treatment with GHRH. More studies are needed to clarify whether the composition of exosomes derived from CRPCa cells determines a similar effect on the NED process.

## P-35-058
## Phospholipase D in prostate cancer tumor progression

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Prostate cancer (PCa) is the most frequent cancer in men 65 years and older, and the second most lethal. Phospholipase D (PLD) is an important enzyme, which regulates cancer cells development. PLD hydrolyzes phosphatidylcholine to yield phosphatidic acid. We hypothesize that PLD, and particularly its isoforms PLD1 and PLD2, regulates the tumor progression of PCa cells, and their ability to withstand nutrient starvation. To model diverse stages of PCa, we used prostatic human cell lines: WPMY-1 a non-tumoral cell line, used as our control, C4-2B a bone metastasis-derived cell line, and PC-3 bone metastasis cell line which is very aggressive. PLD1 was selectively inhibited by CAY10593, PLD2 by CAY10594, and Halopemide (a pan-inhibitor) was used to inhibit simultaneously the two isoforms. The gene and protein expression of PLD1 and PLD2, as well as the PLD activity, are 2–3 times more important in the tumor cell lines C4-2B and PC-3, compared to the control cell line WPMY-1. For C4-2B and PC-3 the pharmacologic inhibition of PLD leads to a viability drop of 25% after 72 h, and a proliferation decrease of 30% after 48 h. The migration of PC-3 cells is reduced by 25% after 24 h of treatment by Halopemide. Pharmacologic inhibition of PLD, does not have any effect on the control cell line WPMY-1, underlying the importance of PLD in cancer cells only. To model metabolic stress, PC-3 cells were serum starved. In this case, PLD activity and PLD2 protein expression rises 2.5 times compared to the non-starved cells. The increase in PLD activity is correlated to an augmentation of PC-3 tumor cells migration, which is resolved by the use of PLD1 or PLD2 selective inhibitors. PLD1 and PLD2 are important players of prostate tumor cell development. PLD also appears to be involved in the response to serum starvation, thus it may play a role in cancer cells resilience in restrictive growth conditions. PLD would though constitute a potential therapeutic target for PCa treatment. *The authors marked with an asterisk equally contributed to the work.


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## P-35-059
## Possible role of spliceosomal proteins in chemoresistance of cancer cells

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Ovarian cancer is one of the most deadly types of gynecological cancers. The problem of low patient survival is associated with fast cancer recurrence. In this regard, the aim of this work was to reveal potential molecular participants of cancer cell communication, that lead to chemotherapy resistance. Ascitic fluid is a symptom of late stages of ovarian cancer and consists of cancer cells, normal cells, their metabolites, and other soluble components. In our study, we showed that incubation of ovarian cancer cells with ascites after treatment promotes tumor resistance to chemotherapy and leads to epithelial-mesenchymal transition. To determine possible causes of these effects, we compared proteome profiles of paired ascites from patients before and after chemotherapy using the LC-MS/MS analysis. We found that chemotherapy increases the diversity of ascites proteome. Functional annotation of up-regulated proteins in ascites after treatment showed that the most pronounced cluster was associated with spliceosome. To investigate whether spliceosomal proteins can be internalized into recipient cancer cells, the Stable Isotope Labeling with Amino acids in Cell culture (SILAC) was performed. We isolated extracellular vesicles from labeled cancer cells after cisplatin treatment and incubated them with unlabeled recipient cells for 10 h. Further LC-MS/MS analysis showed that spliceosomal proteins and proteins responsible for translation regulation were detected among the labeled uptake proteins. Thus, intercellular communication with spliceosomal proteins could affect splicing programme of recipient cells and potentially promote their chemoresistance. The work was supported by the RSF 17-75-20205 (for LC-MS/MS analysis) and RFBR 17-00-00172.

## P-35-060
## Effects of bleomycin and *N*-acetyl-L-cysteine on the MAPK signalling pathway in testicular germ-cell tumor

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Bleomycin is used in chemotherapy regimens for the treatment of patients having testicular germ-cell tumor. Bleomycin generates oxygen radicals, induces oxidative cleavage of DNA strand. MAPKs are important mediators involved in the intracellular network of interacting proteins that transduce extracellular signals to intracellular responses. Antioxidants are widely used in dietary supplements to prevent excess free radical formation and reactions. There is no study in the literature investigating the effects of Bleomycin and/or *N*-Acetyl-L-Cysteine (NAC) as commonly used antioxidant adjuvants on MAPK signalling pathway in testicular germ cell tumors. Ntera-2 cells were incubated with bleomycin, NAC and bleomycin+NAC for 24 h. The levels of p38, p-MEK1/2, p-ERK1/2, and p-SAPK/JNK, members of MAPK signalling pathway, were determined using the PathScan MAPK Multi-Target Sandwich ELISA in NTera-2 testicular cancer cells. Bleomycin, led to the activation of JNK and p38 and inactivation of phospho-MEK and phospho-ERK. NAC decreased the activation of p38 and JNK which were upregulated by bleomycin and led to enhancement in phospho-MEK and phospho-ERK levels. Incubation with NAC alone did not cause any change in p38-MAPK and JNK levels. Any difference was seen in the dephosphorylated-JNK and dephosphorylated-MEK levels among the groups incubated with different agents. Bleomycin led to a decrease in phosphorylated-MEK and phosphorylated-ERK levels. In contrast, concurrent incubation of bleomycin with NAC caused an enhancement in p-MEK and p-ERK levels. Coincubation of bleomycin with NAC reversed effects caused by bleomycin. Our results highlight the important role of MAPK pathway occurring during the use of bleomycin and its concurrent use with antioxidants which can adjuvate the cytotoxic effects of the chemotherapeutic agents. This result evidences the impact on different cell metabolic pathways that is nowadays seen as a way to improve the responsivity to chemotherapy.

## P-35-061
## Elucidation of the mechanism underlying CD44v6-induced transformation of IEC-6 normal intestinal epithelial cells

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CD44 is a known surface marker of various cancer stem cells (CSC). Among these CD44 variants, CD44v6 has been shown not only to be upregulated but also associated with poor prognosis in a number of cancers. Together, while the crucial roles of CD44s and different CD44v in the malignant progression of a wide variety of tumors were well documented, little is known about the transformation abilities of CD44s and CD44v6 in normal intestinal epithelial cells. We established both CD44s- and CD44v6-overexpressing stable clones from IEC-6 cells and demonstrated that the CD44v6 clones have several features of transformation. In the meantime, significant increased Bcl-2 and reduced DNA damage were also detected. Moreover, inhibition of c-Met and VEGFR2 signalings only reduced the saturation densities of CD44v6 clones. Treatment with the exogenous HGF not only increased the saturation densities but also activated both AKT and ERK in all five clones. Interestingly, higher active AKT and ERK were detected in CD44v6 clones which might account for their saturation densities and anchorage-independent growth abilities were significantly reduced by lower doses of inhibitors. Surprisingly, the nuclear localization of YAP is present mainly in the nuclei in CD44v6 clones regardless of their cell densities. To delineate the role of YAP in CD44v6-induced transformation, we treated inhibitor or knockdown YAP and found that verteporfin only reduced the saturation densities of CD44v6 clones and YAP silencing also markedly diminished their transformation. By contrast, constitutively active YAP robustly increased their saturation densities and colony numbers. Collectively, our results suggest that upregulation of CD44v6, but not CD44s, induces the transformation of normal intestinal epithelial cells possibly via activating the c-Met/AKT/YAP pathway which might also explain the important role of CD44v6 in the initiation of various carcinomas.


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## P-35-062
## Histone deacetylase inhibitor differently modulates DNA repair in the oncogene-transformed and normal cells

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Histone deacetylase inhibitors (HDACi) suppress the growth of tumor cells due to the induction of cell cycle arrest, senescence or apoptosis. There are a few data concerning the reduction of DNA repair capability by HDACi in tumor cells. This data mostly relate to non-homologous end joining (NHEJ) protein machinery, since NHEJ is a principal repair mechanism of the double-strand breaks (DSB) whatever of the cell cycle phase. Previously we established that HDACi-induced cell cycle arrest of E1A+Ras-transformed mouse embryonic fibroblasts was accompanied with an accumulation of the marker of damaged DNA – γH2AX foci. The DNA breaks may accumulate due to the disruption of DNA repair machinery by HDACi in oncogene-transformed cells. Since HDACi are considered as promising anticancer agents it is reasonable to compare the influence of HDACi on the DNA repair system in transformed and normal cells. Using the host-cell reactivation assay we modeled the NHEJ repair mechanism. E1A+Ras-transformed fibroblasts and non-transformed fibroblasts (NIH3T3 and REF52) were transfected with luciferase reporter vector, damaged with endonuclease and etoposide, and incubated in the presence or absence of sodium butyrate. The efficiency of DNA repair was evaluated by measurement of the luciferase activity. Double-strand breaks, which were introduced by a damaging agent or an endonuclease into the plasmid DNA, decreased luciferase transcription. However, it reclaimed afterwards owing to DNA repair. We have shown that the recovery of DNA has occurred less efficiently in the presence of sodium butyrate in transformed cells, while in normal fibroblasts sodium butyrate did not affect the NHEJ-repair efficiency. Further, the HDACi suppressed the expression of repair proteins (Ku80, Ku70, Mre11) in transformed, but not in normal cells. We have thus demonstrated a fundamental difference between the influence of HDACi on DNA repair in oncogene-transformed and in normal cells.

## P-35-063
## Regulatory elements responsible for TGF-β-induced activation of CCL2 chemokine gene in breast cancer cells

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Transforming growth factor beta (TGF-β) is the main cytokine responsible for induction of epithelial-mesenchymal transition (EMT) of breast cancer cells that is a hallmark of tumor transformation to metastatic phenotype. This process is accompanied by activation of a range of pro-metastatic genes including several chemokines (CXCL1, CXCL5, CCL20). Recently it has been shown that chemokine CCL2 gene expression level directly correlates with TGF-β activity in breast cancer patients. CCL2 is able to attract tumor-associated macrophages and therefore is considered as an important inductor of breast cancer progression, but precise mechanisms underlying its regulation by TGF-β are unknown. The main aim of this project is identification of CCL2 gene promoter and enhancer regions that could be responsible for its activation by TGF-β in breast cancer cells. We identified CCL2 cis-regulatory elements (promoter and 2 enhancers located in the first and the second introns) using epigenetic features (histone modifications H3K4me1, H3K4me3, H3K27ac, genome segmentation patterns) available from published data. Deletion screening of these elements in luciferase reporter system using MCF-7 and MDA-MB-231 human breast cancer cell lines treated with recombinant TGF-β allowed us to identify their TGF-β-responsive fragments. Determination of specific transcription factors mediating TGF-β effects in our system is underway. The study was supported by grant 18-54-45020 from Russian Foundation for Basic Research and by the Program of fundamental research for state academies for 2013–2020, research topic 01201363823. *The authors marked with an asterisk equally contributed to the work.

## P-35-064
## Complexation of 4-thiazolidinone derivatives with PEG-containing polymeric nanocarrier increases their chemotherapeutic potential and reduces negative side effects in laboratory mice

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The aims of this study were: (i) providing water solubility of 4-thiazolidinone-based anticancer agents via their conjugation with PEG-containing polymeric nanocarrier; (ii) studying of anticancer activity of these agents in BALB/C mice grafted with NK/Ly lymphoma; (iii) measuring cytological and enzymatic indicators of general toxicity of studied compounds in the lymphoma-bearing mice. Novel PEG-containing polymeric nanocarrier was synthesized at Lviv Polytechnic National University and used for modulating their pro-apoptotic action towards tumor cells and treatment of BALB/C mice were implanted with the ascitic NK/Ly lymphoma. Les-3833 and Les-3288 compounds demonstrated a pronounced treatment effect towards the ascitic NK/Ly lymphoma grafted to BALB/C mice that were comparable with Doxorubicin's action. A distinct decrease in the amount of the ascite fluid with lymphoma cells was revealed in the treated mice, while a 1.5 times increase of its amount was detected in the untreated mice of the control group. The activity of aspartate and alanine aminotransferases in blood serum (biochemical indicators of liver damage) was elevated on the 14th day of animal treatment and returned to the normal level in the 21st day. Doxorubicin-induced severe anemia with a reduction of number of red blood cells, while the 4-thiazolidinone derivatives demonstrated much less toxicity, and erythrocytes count stayed normal after 21 days of animal treatment. The development of NK/Ly lymphoma in mice led to an increase in neutrophils number, while the applied anticancer compounds reduced it significantly. Besides, doxorubicin caused an increase in the number of lymphocytes in animals' blood, while the studied 4-thiazolidinone derivatives did not affect their count over the normal level. Concluding, a conjugation of studied derivatives with polymeric nanocarrier enhanced their anticancer effects *in vitro* and *in vivo* experimental models. *The authors marked with an asterisk equally contributed to the work.


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## P-35-065
## Human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) secreted by *Lactococcus lactis* bacteria demonstrates antitumor activity onto HCT116 colon cancer cells *in vitro* and *in vivo*

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Introduction: The Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) induces apoptosis in a variety of tumor cell lines *in vitro*, however was shown to be ineffective in cancer patients because of its short biological half-life after systemic administration. Another problem is resistance of many cancer cells to TRAIL-induced apoptosis. Here, we propose a TRAIL-based model therapy of colon cancer with the use of non-pathogenic *Lactococcus lactis* bacteria and metformin (MetF). The use of lactic acid bacteria as delivery system for TRAIL may enable production of TRAIL locally in the tumor site for a longer period of time, while MetF might restore sensitivity to TRAIL-induced apoptosis. Objective: To assess the antitumor activity of the hsTRAIL-producing *L. lactis* bacteria used alone and in combination with MetF on colon cancer cells *in vitro* and *in vivo*. Methods: Using codon-optimization and Nisin-Controlled Gene Expression System (NICE), we prepared *L. lactis* bacteria secreting human soluble TRAIL (*L. lactis* (hsTRAIL+)). By MTT-assay and subcutaneous NOD-SCID mice model we examined antitumor activity of *L. lactis* (hsTRAIL+) against HCT116 human colon cancer cells *in vitro* and *in vivo,* respectively, both as a single agent and in combination with MetF. Results: The supernatant of *L. lactis* (hsTRAIL+) showed significant cytotoxicity against HCT116 cells *in vitro* comparing to controls, while intratumoral injections of *L. lactis* (hsTRAIL+) markedly reduced the tumor growth in NOD-SCID mice during the time of the study. Moreover, combination of *L. lactis* (hsTRAIL+) with MetF showed its synergistic antitumor effect both *in vitro* and *in vivo*. Conclusions: Recombinant *L. lactis* (hsTRAIL+) produce hsTRAIL, which affects viability of HCT116 cancer cells *in vitro* and reduces the growth of subcutaneous tumor *in vivo,* acting synergistically with MetF. Acknowledgments: This study is supported by National Science Centre in Poland (Grant no. UMO2014/15/B/NZ5/03484).

## P-35-066
## The approach for selection optimal combinations of chemotherapy drugs with GD2-specific antibodies for the effective elimination of GD2-positive tumor cells

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Using of GD2-specific antibodies in combination therapy becoming the standard approach for treatment of high-risk neuroblastoma. Current issue is the selection of effective combinations of chemotherapy drugs and antibodies to enhance the antitumor effects and reduce side effects. Since one of the mechanisms of the antitumor activity of GD2-specific antibodies is direct induction of cell death, the analysis of signaling pathways triggered by antibodies and chemotherapy could help to select combinations with maximum antitumor effects, as well as to avoid combinations with the antagonistic effects. Based on previously studied signaling pathways involved in the triggering of cell death induced by GD2-specific antibodies, and signaling pathways of a number of well-known targeted chemotherapy drugs, combinations of drugs and antibodies potentially capable for synergistic, additive, and antagonistic actions against GD2-positive tumor cells were predicted using bioinformatic approaches. The experimental testing of the combined action of GD2-specific antibodies with different drugs was carried out in inhibitory analysis on GD2-positive cell lines. The obtained data allowed us to divide chemotherapy into groups according to the strength of cytotoxic effects in combination with chimeric GD2-specific antibodies. As an example, inhibitors of mTOR (temsirolimus, everolimus) showed an antagonistic effect, and multi-kinase inhibitors (sorafenib, sunitinib), an inhibitor of tubulin polymerization (monomethyl auristatin E), inhibitors of topoisomerase 1 (topotecan, irinotecan), and an inhibitor of actin microfilaments (cytochalasin D) has additive potency, an inhibitor of tyrosine kinases (imatinib) showed a slight synergistic effect, which is consistent with the effects predicted by bioinformatic methods. These results could be used for personalized therapy of patients with GD2-positive tumors. This work is supported by the Russian Science Foundation under grant 17-75-10211. *The authors marked with an asterisk equally contributed to the work.

## P-35-067
## Urinary exosomes as potential markers for prostate cancer

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New biomarkers should be discovered in order to establish a better diagnosis in prostate cancer. In this study, we isolated urinary exosomes and carried out a first approach to characterize such microvesicles. Urine samples were collected after prostatic massage to patients. Both ultracentrifugation and filtration were involved in the isolation method. The size of urinary exosomes was characterized by dynamic light scattering (DLS), UV-vis spectroscopy and transmission electron microscope (TEM). DLS measurements indicate that exosomes are dispersed with a broad size distribution (in intensity). The average hydrodynamic diameter was ca. 160 $\pm$ 79 nm. UV-visible spectrum in the 300–400 nm range presents specific characteristic that reveal differences in the composition of exosomes of the urine of a patient with prostate cancer. TEM shows the presence of numerous urinary vesicles immunolabelled with CD-63 antibody suggesting quantitative differences in the exosomes number between healthy and prostate cancer patients. Furthermore, the activity of gamma-glutamyltransferase (GGT), a cell-surface enzyme that regulates the catabolism of extracellular glutathione, was also assessed. We found that the activity of GGT was higher in the urine of patients with prostate cancer than in that of control patients. The results obtained shed more light on the characterization of exosomes


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derived from prostate cancer as well as help to discover specific biomarkers in the diagnosis of prostate cancer.

## P-35-068
## Resveratrol inhibit melanogenesis in B16F10 cells via PI-3K/Akt and ERK-1/-2 pathway

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Resveratrol (3,5,4′-trihydroxy-trans-stilbene, Res), a natural polyphenolic compound, is known as a promising anti-cancer agent and able to inhibit tyrosinase directly. However, the mechanism of action in all of those cases is not completely comprehended *in vitro* as well as *in vivo*. This study was aimed to examine the anti-melanogenic effect of resveratrol and its signal transduction pathways in mouse B16F10 melanoma cells and zebrafish model. The toxic effects of resveratrol on B16F10 cells were assessed using MTT assay. To understand the mechanism of the effect of resveratrol on melanogenesis in B16F10 cells, melanin production and tyrosinase activity were measured by using a melanin content assay, tyrosinase activity assay. Western blotting analysis was used to investigate the effects of resveratrol on the melanogenic protein (Tyrosinase, MITF) expression. Finally, zebrafish embryos at 10 h post-fertilization (hpf) were exposed to various concentrations of resveratrol, and its *in vivo* effect on pigmentation investigated at 72 hpf. The results showed that resveratrol inhibition of tyrosinase activity. Western blotting showed that resveratrol decreased tyrosinase and MITF protein levels slightly, in a dose- and time-dependent manner. In addition, resveratrol also induces phosphorylation of p38 kinase and reduced activation of phosphoinositide-3(PI-3)-kinase/Akt, ERK-1/-2. Moreover, resveratrol dose-dependently decreased zebrafish pigment formation. These results indicated that resveratrol inhibited melanogenesis by downregulating tyrosinase activity and MITF via PI-3K/Akt and ERK-1/-2 pathway. Other signaling pathways may also play a role in resveratrol-reduced melanogenesis in B16F10 cells and zebrafish.

## P-35-069
## Development of a new high-throughput assay of siRNA libraries for identification of genes inducing chromosome instability (CIN) in cancer cells

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Chromosome instability (CIN), involving the unequal distribution of chromosomes to daughter cells during mitosis leads to aneuploidy. CIN genes encode components that control the mitotic checkpoint and chromosome segregation. Presently, approximately 400 human genes that control proper transmission of chromosomes have been annotated with gene ontology terms, while systematic CIN gene screens in yeast have revealed 692 genes. Therefore, it may be supposed that many human CIN genes remain unidentified. The identification of new genes controlling chromosome segregation is impeded by the lack of a suitable experimental assay. None of the existing methods developed to assess CIN frequency are entirely satisfactory. Additional information about genes which control chromosome transmission will allow researchers to develop methods of early cancer diagnostic. In addition, study for identification of genes inducing chromosome instability in cancer cells might show specific information about the role of new genes in the development of CIN. Furthermore, the results of such screening will make possible identification of new targets for anti-cancer treatment. As a sensor for CIN we suggest to use Human Artificial Chromosome (HAC), which is a functional kinetochore. Usually, this HAC is mitotically stable. However, siRNA dependent depletion of genes that are essential for proper segregation of normal human chromosomes induces HAC missegregation. We have created a test-system based on specially modified short half-life green fluorescent protein (GFP) expression. The system is able to detect loss of GFP signal caused by chromosome instability almost 9 h after destabilization of HAC. This fast assay can be used in high-throughput siRNA screen to identify CIN genes in human. In our current study, we have optimized the test-system specifically for high-throughput siRNA-based screening of genes libraries and their effect on CIN. *The authors marked with an asterisk equally contributed to the work.

## P-35-070
## Leukemia-associated Nup98 fusion proteins impair nucleocytoplasmic transport through changing the localization of Nup98

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Nucleocytoplasmic transport is mediated by nuclear transport receptors (NTRs) through the nuclear pore complexes (NPCs). It has been reported that qualitative or quantitative changes of NPCs and NTRs commonly cause nucleocytoplasmic transport abnormalities which closely link to diseases. *Nup98* gene encoding an NPCs component is known to be fused to at least 28 different partner genes in hematopoietic malignancies. Although it has been reported that expressions of several *nup98* fusion genes lead to the onset of leukemia in mice, the detailed functions of these fusion proteins remain unclear. In particular, a function(s) of Nup98 in nucleocytoplasmic transport and how Nup98 fusion proteins affect nucleocytoplasmic transport are poorly understood. In this study, we have examined the effect of Nup98 fusion proteins on nucleocytoplasmic transport by focusing on the exportins, which are the members of NTRs and facilitate nuclear export of biomolecules. When Nup98 fusion proteins were overexpressed, several exportins and their cargoes were mis-localized and accumulated in the nucleus. Similarly, knockdown of *nup98* caused the accumulation of exportins and their cargoes in the nucleus. In addition, we found that Nup98 fusion proteins recruited Nup98 to their sites in the nucleus. These results suggest that Nup98 is required for the nuclear export of cargoes through the proper localization of exportins, and Nup98 fusion proteins may inhibit endogenous Nup98 function by changing Nup98 localization, leading to the impairment of nucleocytoplasmic transport.

## P-35-071
## The role of autophagy in the epithelial to mesenchymal transition and cancer metastasis

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In cancer, autophagy plays two opposite roles. It can protect cells by effectively removing the damaged materials derived from the genotoxic components. Autophagy also promote cancer cell survival by supplying minimal amounts of nutrients during cancer progression. However, the molecular mechanism underlying how


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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autophagy regulates the epithelial-to-mesenchymal transition (EMT) and cancer metastasis is still unclear. Here we showed that starvation stimulated the Snail degradation and consequently inhibited EMT and metastasis in cancer cells. Interestingly, Snail was physically associated with LC3 and SQSTM1 in cancer cells. Also autophagy-dependent Snail degradation was closely related to the decreased levels of EMT and metastatic proteins in starvation. Furthermore, the autophagy deficient conditions caused an increase of the cellular level of Snail. Moreover, cancer cell migration was significantly prohibited by starvation-induced autophagy. These findings suggest that autophagy-dependent Snail degradation could regulate EMT and cancer metastasis during tumorigenesis. Keywords: autophagy, cancer metastasis, EMT, Snail.

## P-35-072
## Mst1 is a potential biomarker for assessment of β-catenin-mediated colon cancer prognosis

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Mammalian STE20-like kinase 1 *(MST1/STK4)* encodes a serine threonine kinase that is the mammalian homolog of *Drosophila* Hippo. STK4 plays an important role for controlling cell growth, apoptosis and organ size. However, the role of STK4 defect in promotion of the progression of colon cancer is still understudied. Here, we found that STK4 was significantly downregulated in colon cancer and was associated with tumor size, distal metastasis, and poor survival. Furthermore, *STK4* knockdown enhanced sphere formation and metastasis *in vitro*. Moreover, the change of STK4 levels did not cause any effects on cell proliferation *in vitro*. However, *STK4* knockdown promoted tumor development *in vivo*. In addition, we found that STK4 colocalized with β-catenin and directly phosphorylated β-catenin that leads to its degradation via ubiquitin-mediated pathway. This may suggest that *STK4* knockdown causes the failure of β-catenin phosphorylation and subsequently β-catenin accumulation that consequently leads to anchorage-independent growth and metastasis in colon cancer. Our results support that STK4 may act as a potential candidate for assessment of β-catenin-mediated colon cancer prognosis.

## P-35-073
## Oxidative stress in the 7,12-dimethylbenz[a] anthracene rat model of breast cancer: suppression by a NO-synthase inhibitor $N^G$-nitro-L-arginine methyl ester

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Studies have shown that carcinomas involves disruption of tissue redox balance; in turn, this suggests that biochemical and pathophysiological disturbances may result from oxidative damage. Would treating the rats with NO-synthase (NOS) inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME) affect the oxidative responses? A total of 38 adult female Wistar rats weighing 90–120 g were used. All conditions were in accordance with the rules of the Ethical Committee of Armenia. Rats were divided into five groups (7 rats per group): group I was untreated and served as Control. Group II and III served as Saline and L-NAME, respectively. Rats in group IV (DMBA, 10 rats) and group V (DMBA+L-NAME) were administrated intragastrically by gavage each with a single dose of 20 mg/mL DMBA, dissolved in 0.5 mL olive oil and 0.5 mL saline. Rats in DMBA+L-NAME group were injected by L-NAME intraperitoneally for 5 weeks (after 10 days of DMBA administration, every 3rd day) in dose of 30 mg/kg body-weight in 0.25 mL saline. Histopathological alteration in DMBA group at 20th week (after DMBA administration) has revealed the ductal papillary carcinoma which has intraductal and papillary carcinoma, ductal solid carcinoma and comedocarcinoma. In DMBA+L-NAME group the examination has revealed only precancerous lesions with *in situ* ductal papillary carcinoma. Treatment with DMBA in group IV significantly increased blood MDA and $NO_2^-$ levels at 8th (98.7% and 34.9%, respectively), 13th (115.1% and 78.2%) and 20th (96.8% and 63.5%) weeks after DMBA administration comparing to Control group. Importantly, subsequent co-treatment with the NOS inhibitor L-NAME blocked these increases in group V, resulting in mean values close to those of Control group for MDA (increased 10.2% at 20th week) and leading to an even lower mean for $NO_2^-$ (decreased 11.1% at 20th week). We conclude that NOS inhibition by L-NAME *in vivo* can protect against oxidative stress and 7,12-DMBA-induced mammary cancer.

## P-35-074
## Ectodomain shedding of epidermal growth factor receptor by cysteine cathepsins

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Epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases, and play an important role in cell differentiation, migration, proliferation, and metabolism. This receptor is found to be overexpressed in a number of cancers. Moreover, cancers overexpressing EGFR showed to be more aggressive and resistant to the chemotherapeutics. Consequently, EGFR represents one of the important targets in anticancer therapy. Signaling through EGFR is commonly triggered by ligand binding, however, deletions in the extracellular region of EGFR can also cause constitutive activation. Such deletions can influence receptor activation and downstream signaling cascades such as phosphatidylinositol 3-kinase (PI3K) pathway. EGFR was also identified as a substrate of extracellularly present cysteine cathepsins, known to cleave ectodomains of membrane proteins including receptors, cytokines, and adhesion proteins. Using mass spectrometry-based proteomics, we have shown that cathepsin L proteolytically cleaves domain II of EGFR and that this cleavage causes its activation and influences its physiological function. We have shown that ectodomain deletion which emulates cathepsin cleavage affects phosphorylation profile of cellular kinases. Our results also indicate that the presence of truncated EGFR in breast cancer cells triggers an alteration in expression of profilin 1, actin-monomer binding protein, involved in several cellular processes, such as cell cycle and proliferation. Consistent with previous studies, the presence of truncated EGFR in breast cancer cells showed a similar effect on the phosphorylation of cellular kinases as in breast cancer cells overexpressing profilin 1. However, our findings open new questions about EGFR and


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could possibly lead to more effective strategies in anticancer therapy.

## P-35-075
## The change of polyamine metabolism in lymphocytes of glioma patients after mitogen stimulation

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Tumor-associated inflammation stimulates the growth and progression of malignant brain gliomas. Lymphocytes of peripheral blood play an important role in tumor-associated inflammation, migrating to the focus of inflammation and stimulating tumor cells to proliferation and invasion of surrounding healthy tissues. The enzymes of polyamine (PA) oxidation – polyamine oxidase (PAO) and diamine oxidase (DAO) are important factors in the regulation of the proliferative function of cells in inflammation, primarily lymphocytes. They oxidize PA into iminoaldehydes, which act as inhibitors of proliferation. The purpose of the study was to explore the activity of enzymes DAO and PAO in lymphocytes stimulated by different concentrations of phytohemagglutinin (PHA). PHA is also a well-known membrane modifier and transmembrane potential affecting agent. The objects of the study were blood samples taken from patients with brain gliomas of various tumor grade. PHA was diluted within a concentration gradient. Cells were cultured according to the standard technique of PHA stimulation with the subsequent evaluation of the number of blast cells. The activity of DAO and PAO was determined by a spectrophotometric method. The results showed that it was decreased in benign gliomas patients and increased in those with malignant gliomas. The change in the DAO and PAO activity in the culture medium excreted by blood cells during the blast-transformation may indicate their regulatory role in the proliferative activity of lymphocytes in neurosurgical pathology including brain gliomas of various grades of malignancy. The relationship between the level of the aggregation of blood cells and the activity of DAO and PAO enzymes under the influence of various dilutions of PHA underscores the important role of the transmembrane potential in proliferative activity of lymphocytes in inflammation and tumor growth. The study was supported by the "RUDN University Program 5-100".

## P-35-076
## Investigation of PI3K/AKT and EMT-targeted miRNA profiles in palbociclib-treated Panc-1 and MiaPaCa-2 pancreatic cancer cells

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The therapeutic strategies of pancreatic cancer (PC) are with the various combination treatment of anti-cancer drugs still in progress. However, the survival rate of PC is still under 6%, because of the limited therapeutics and no controllable prognosis. miRNAs have an important role in the regulation of metabolic cascades which are critical in the differentiation of PC progression. In the current study, we aimed to investigate the role of palbociclib (CDK4/6 inhibitor) on aberrantly activated pathways, PI3K/AKT, and EMT signaling axis through differently expressed miRNA profiles in Panc-1 and MiaPaCa-2 cells. The effect of palbociclib on cell viability was determined by MTT cell viability test in time and dose-dependent manner in PC cells. The expression profiles were analyzed by RT-PCR. We found that palbociclib effectively reduced cell viability and proliferation for following exposure of Panc-1 and MiaPaCa-2 cells for 24 h. Additively, Panc-1 and MiaPaCa-2 cells were sensitive to palbociclib with the significant blockade in the G1 phase of the cell cycle. Palbociclib decreased the expression of PI3K and p-AKT in PC cells. Moreover, palbociclib downregulated the levels of ß-catenin in Panc-1 cells, but not in MiaPaCa-2 cells. Palbociclib treatment led to increased levels of tumor suppressor miR-506, miR-100, and miR-141 in MiaPaCa-2 cells, while the only detectable increase in tumor suppressor miRNA was detected for miR-100 levels in Panc-1 cells. Additionally, the oncomir miR-208 increased after palbociclib treatment in MiaPaCa-2 cells. In conclusion, palbociclib induced cell cycle arrest and reduced cell viability. However, palbociclib had a various effect on the regulation of PI3K/AKT and EMT signaling in each PC cell line. Investigating the effect of palbociclib on the tumor suppressor and oncomir miRNA profiles is a new therapeutic strategy to reduce cell viability and metastatic process of pancreatic cancer.

## P-35-077
## Polyamines deprivation as a targeted therapy for mammary carcinoma in rats induced by 7,12-dimethylbenz[a]anthracene

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Recent investigations revealed that increased polyamine availability enhances the capability of cancer cells to invade and metastasize to new tissues while diminishing immune cells' anti-tumor immune functions. The goal of work was to study the influence of polyamines deprivation by $N^G$-hydroxy-nor-L-arginine (nor-NOHA) arginase inhibitor on breast cancer developing in rats induced by 7,12-dimethylbenz[a]anthracene (DMBA). Rats were divided into five groups (Control, Saline, nor-NOHA, DMBA (10 rats) and DMBA+nor-NOHA, 7 rats per group). A total of 38 adult female Wistar rats weighing 90–120 g were used (Ethical Committee of Armenia). Rats in DMBA and DMBA + nor-NOHA groups were administrated intragastrically each with a single dose of 20 mg/mL DMBA dissolved in 0.5 mL olive oil and 0.5 mL saline. Rats in nor-NOHA and DMBA+nor-NOHA groups were injected (i.p.) by nor-NOHA for 5 weeks (after 10 days of DMBA administration, every 3rd day) in dose of 3 mg/kg body-weight in 0.25 mL saline. Blood total polyamines quantity were determined by TLC (nmol/mL blood). At the end of 20th week, rats were sacrificed by cervical decapitation under anesthesia. Histopathological alteration in DMBA group has revealed the ductal papillary carcinoma by numerous intraductal proliferations, intraductal carcinoma (cribriform and comedo), papillary carcinoma, ductal solid carcinoma and ductal comedo-carcinoma. In DMBA+nor-NOHA group the histopathological examination has revealed only benign lesions and precancerous lesions. In DMBA group blood polyamines levels are increased at the 13th (21.9%), 16th (32.5%) and 20th (45.7%) weeks comparing to Control group. Subsequent co-treatment with nor-NOHA decreased blood total polyamines levels at the 13th (10.7%), 16th (8.3%) and 20th (6.7%) weeks comparing to Control group. It is concluded polyamines quantity downstream attenuated tumor growth and histopathological progression.


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Results may used in cancer treatment as a part of antitumor therapy.

## P-35-078
## Dendritic cells as carriers of oncolytic viruses

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Oncolytic virus therapy is a promising approach to cancer treatment. A major problem of virotherapy is the low efficiency of a systemic delivery to tumors associated with a rapid inactivation of viruses in the circulation. The potential of dendritic cells (DCs) as carriers of oncolytic viruses (OVs) was studied with a panel of three human enteroviruses: Coxsackievirus B5 (LEV14), Echovirus 12 (LEV7) and the Sabine strain of Polio Type 2. The DCs loaded with viruses and treated with neutralizing antibodies produced a complete cytopathic effect when transferred to RD cells, unlike the control samples of free viruses that were completely inactivated with antibodies. The delivery of OVs to tumor xenografts in mice was evaluated by immunostaining of tumor slides. We observed an efficient virus dissemination to the tumors following the intravenous delivery of OV-loaded DCs, while the injection of free virus produced few rather imperceptible sites of viral replication. To assess the therapeutic effect of DCs-assisted virus delivery on the growth and metastasizing of tumors we used the mouse model of tumors produced by injection of 4T1 breast carcinoma cells. A significant difference in tumor size between the experimental (injected virus-infected DCs) and control (injected saline) groups (16 mice in each group) was observed on Day 3 after the first injection (25 vs 4 $mm^3$), and it increased further (408 vs 34 $mm^3$). Tumor size in the groups treated with free viruses or uninfected DCs did not show any significant difference with control groups. The spreading of metastases was evaluated by seeding cells from disintegrated organs (liver, lungs, spleen and blood) into selective medium allowing growth of tumor cells. In the group treated with virus-loaded DCs the colony number was substantially reduced (one of the four mice in the group had a metastasis in one organ) compared with samples from free virus treated mice (each of four mice had metastases in 2–4 organs).

## P-35-079
## Regulation of FGFR1 spatial distribution and activity by extracellular galectins

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Fibroblast growth factor receptors (FGFRs) are integral membrane proteins that transmit signals through the plasma membrane. FGFR signaling needs to be precisely controlled as aberrant FGFR signaling is observed in numerous human cancer types. The function and trafficking of FGFRs relies on the formation of multiprotein complexes. Here we used mass spectrometry-based identification of FGFR1 interaction partners. We identified galectin-1 and galectin-3, lectins implicated in cancer development and progression as novel FGFR1 interactors. We demonstrated that galectin-1 and galectin-3 directly bind to sugar chains of the glycosylated extracellular region of FGFR1. Studied galectins regulate FGFR1 activity and distribution, but to different extent. Extracellular galectin-1 efficiently activates FGFR1 and receptor-downstream signaling pathways in the absence of the growth factors and as a result induces cell proliferation and protects cells from apoptosis. In contrast, galectin-3 causes extensive receptor clustering that results in only very weak activation of signaling cascades. Galectin-3 induced FGFR1 clusters are highly dynamic as that are efficiently disassembled by FGF2. Summarizing, our data demonstrate that galectin-1 and galectin-3 are novel FGFR1-binding proteins that differentially regulate receptor distribution on the plasma membrane and receptor function. Galectin-FGFR1 interplay may contribute to uncontrolled FGFR signaling in various cancers. This project was carried out within the First TEAM programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund, awarded to Ł.O. This work was supported by the National Science Centre, Poland (Sonata Bis grant 2015/18/E/NZ3/00501), awarded to MZ.

## P-35-080
## Screening polyoxometalates as aquaporin inhibitors for cancer therapeutics

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Aquaporins (AQPs) are transmembrane protein channels that facilitate the diffusion of water and glycerol across cell membranes, crucial for water and energy homeostasis. These proteins were found overexpressed in different cancer cells and tissues, being involved in cell proliferation and migration, tumor formation, and angiogenesis, suggesting their great potential as novel drug targets for cancer treatment. Identification of potent and selective aquaporin inhibitors to be used in cancer therapeutics is of utmost importance. Polyoxometalates (POMs) are transition metal complexes that exhibit a broad diversity of structures and properties. POMs are able to inhibit phosphatases, ecto-nucleotidases, and P-type ATPases thus affecting several biochemical pathways, rendering them promising for biological purposes. In this work, we screened POMs as inhibitors of aquaporin-mediated membrane permeability in human red blood cells (RBCs) and further validated their potency and selectivity in yeast cells transformed with human AQP1 and AQP3. Among the various compounds tested, we identified one polyoxotungstate (POT) as a potent inhibitor of glycerol permeability via AQP3 ($IC_{50} \approx 0.74 \pm 0.14$ μM) and lack of effect on water permeability via AQP1. Moreover, the effect of POT on tumor progression was investigated in pancreatic cancer cells (BxPC-3). The obtained marked decrease in cell proliferation ($IC_{50} \approx 9.15 \pm 0.65$ μM) and impairment of cell migration (20% reduction) revealed promising anti-cancer properties of this compound that correlate with its AQP3-inhibitory feature. Further studies are ongoing to fully characterize the selectivity, potency, and toxicity of this POT, establishing polyoxotungstates as novel AQP inhibitors with high potential for cancer therapeutics.


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## P-35-081
## HMGB1 regulates MNAT1 expression in the human prostate cancer cell line PC-3

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The CDK-activating kinase assembly factor MAT1 (MAT1) stabilizes the cyclin H-CDK7 complex which enables the activation of other cyclin-associated kinases and RNA polymerase II by phosphorylation, being involved in cell cycle control and in RNA transcription. This protein has been found to be upregulated in some cancers such as colorectal and breast cancer and it is related to pro-oncogenic features. Therefore, we decided to test if the gene *MNAT1* (encoding the protein MAT1) could be also dysregulated in human prostate cancer by comparing its mRNA levels between PC-3 and HPEpiC cancerous and healthy cell lines, respectively. RT-qPCR results turned out to match with those in the other cancer types at this extent. On the other hand, High mobility group protein B1 (HMGB1), a myriad-function protein including transcriptional regulation, is also dysregulated in many cancers, showing controversial and paradoxical functions on cancer onset and development. We have tested if HMGB1 controls the expression of the gene *MNAT1* via siRNA technology in PC-3 cells. The outcome of HMGB1 silencing was an additional increase in *MNAT1* gene expression and cellular levels of the protein, confirming a regulatory relationship. We discuss our results in reference to other previous high throughput prostate cancer studies in available online databases. *The authors marked with an asterisk equally contributed to the work.

## P-35-082
## Novel cytotoxic conjugates of peptibodies targeting fibroblast growth factor receptors (FGFR)

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Fibroblast growth factor receptors (FGFRs) are an emerging target for directed cancer therapy. The aim of our project is to develop a new conjugate aimed at FGFR-expressing cancer cells, based on FGFR-binding peptibody conjugated with a potent cytotoxic drug, monomethylauristatin E (MMAE). New peptides with FGFR-binding properties are being studied within the project and used to construct peptibodies, a promising alternative to monoclonal antibodies. In order to identify new peptides binding to FGFR, we plan to screen natural FGFR ligands – fibroblast growth factors (FGFs), to isolate binding regions from ligands interacting with receptor and minimize these regions down to linear peptides. For two peptibodies containing FGF2-derived FGFR-binding peptides we have shown specific internalization into FGFR1-expressing cells. FGFR-binding peptibodies will be then conjugated with MMAE, a cytotoxic drug well-studied for clinical applications, and used as selective delivery vehicles targeting FGFR-expressing cancer cells. These peptibody-drug conjugates may serve as a basis for development of therapy for tumors relying on overexpressed or malfunctioning FGFRs. *The authors marked with an asterisk equally contributed to the work.

## P-35-083
## Plant phenolic oleuropein inhibits HT-29 colon cancer cell proliferation through DNA damage, ROS generation and downregulation of xenobiotic metabolizing enzymes

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This study was aimed to identify the effects of oleuropein on colon cancer cell proliferation along with its impacts on apoptosis, reactive oxygen species (ROS) generation, DNA damage formation and protein expressions of CYP1A1 and GSTM1 enzymes. Oleuropein is one of the substantial phenolic compound in the fruit and leaves of olive tree. The cytotoxic effect of oleuropein on colon cancer cells was studied previously but its underlying action mechanism remains largely unknown. The studies on apoptosis, DNA damage, ROS generation and modulation of some xenobiotic metabolizing enzymes which take role in carcinogenesis may provide many significant results in regard to its mechanism of action. In the light of this information, in order to achieve goals of this study, HT-29 human colon cancer cells were grown and treated with increasing oleuropein doses (100–900 μM) and $IC_{50}$ value was determined as 600 μM for HT-29 cell line. Effects of oleuropein on apoptosis, DNA damage formation, ROS generation and CYP1A1 and GSTM1 protein expression levels were assessed by acridine orange double staining, Comet assay, DCFH-DA assay and Western Blotting, respectively. In conclusion, the results of this study showed that oleuropein inhibits the growth of colon carcinoma cells and induces apoptosis by increasing the DNA damage formation and ROS generation and also inhibits the protein expressions of CYP1A1, GSTM1 enzymes.

## P-35-084
## Comparative evaluation of growth inhibitory effect of phenolic derivatives on human cancer cell lines

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Phenolic compounds are the potent anticancer agents that can induce antiproliferative effect in various cancers. Several phenolic derivatives withstood successfully *in vivo* and phase clinical trials. Hence, study of the effect and mechanism of action of phenolic compounds on metastatic cancers is crucial to stop the growth of cancerous cells. Here, inhibitory effects and the underlying molecular mechanism of a series of phenolic derivatives on osteosarcoma and glioblastoma (GBM) were comparatively evaluated. We review the outcome of two important ability of phenolic derivatives on both cancers: (i) the antiproliferative effect (ii) the apoptosis-inducing activity. The results indicate that the antiproliferative effect of alkylaminophenols were stronger than all other tested phenolic derivatives with $IC_{50}$ values ranging from 26.5 to 74 μM. Among these alkylaminophenols, compound PD is the most potent inhibitors of these cell lines with the $IC_{50}$


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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of 36.6 and 26.5 μM on osteosarcoma and GBM cells, respectively. PD also induced the percentage of apoptotic cell death through the activation of ROS and caspase 3/7 signaling in these cancerous cells. Moreover, cell cycle analysis and transcriptome profiling are performed to understand the action of mechanism of PD on cancerous cells. The ability of targeting GBM stem cells (GSCs) and resistance GSCs of PD compared with temozolomide. Our study hopes to provide insight into mechanism of action of phenolic derivatives and the results suggest that alkylaminophenols may represent a new class of therapeutic agents in the management of bone and brain cancers.

## P-35-085
## The role of TKS5 in the invadopodia formation, endocytosis, plasma membrane remodeling and actin cytoskeleton reorganization

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TKS5 is a critical component of invadopodia as its absence results in the loss of cancer cells ability to form these invasive structures. This fact makes TKS5 a potential target for the cancer cure and one of the central proteins in the investigation of cancer cell invasion. Additionally, the question remains about the function of TKS5 in normal cells. Therefore, in order to extend knowledge about TKS5 role in healthy and invasive cells, we tested the TKS5 interaction with 5′-inositol-phosphatase SHIP2; the protein involved in plasma membrane remodeling: AMPH1, BIN1, CIN85, ITSN1, ITSN2; the protein involved in the actin cytoskeleton rearrangement: CR16, WIRE, CTTN; the proteins involved in signal transduction: PLCγ1, SRC, CRK, CSK; and another scaffold protein TKS4. We used the GST Pull-down assay to identify the protein-protein interaction. We revealed that TKS5 interacts with SHIP2. TKS5 near the plasma membrane can recruit SHIP2, which provides local increasing of phosphatidylinositol-3,4-bisphosphate with subsequently TKS5 secondary involvement that binds invadopodia initiation protein complex. We have shown the TKS5 interaction with TKS4 that may realize on a stage of invadopodia maturation. We identified the TKS5 interaction with the neuron-specific SH3A domain of ITSN1, ITSN2, AMPH1, BIN1, CIN85, CTTN, CR16, and WIRE, that may appoint its role in plasma membrane remodeling, endocytosis, and actin cytoskeleton rearrangement. It is significant that AMPH1 and BIN1 may act as negative regulators of invadopodia initiation. Their interaction with TKS5 proline motifs can prohibit the formation of the invadopodia initiation complex on the scaffold of TKS5. Noticeably that TKS5 establishes the triple complex with WIRE and β-actin, however, TKS5 interacts with CR16 without β-actin. Hence, TKS5 may modulate actin cytoskeleton rearrangement depending on the binding partner. *The authors marked with an asterisk equally contributed to the work.

## P-35-086
## Sera levels of homoarginine, homocysteine and ischemia modified albumin in patients with breast cancer

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Aim: L-homoarginine is formed from lysine by arginine glycine amidinotransferase (AGAT) and is mainly produced in the kidney. It has been shown to increase nitric oxide (NO) production by inhibiting either arginase or protein arginine methyltransferases (PRMTs). Thus, increased L-homoarginine concentrations might exert a positive effect on endothelial function. The aim of this study was to determine whether there was a change in serum levels of homoarginine, homocysteine and ischemia modified albumin in patients with breast cancer. Methods: Twenty-seven early stage breast cancer patients and 27 healthy controls were selected for this study. Blood samples were collected preoperatively and postoperatively from cancer patients. Participants with known systemic diseases, including cardiovascular disease, renal disease, gastrointestinal disease, pulmonary disease, acute infection, chronic inflammation were excluded. Serum homoarginine levels were analyzed with API 3200 AB SCIEX LC-MS/MS system. Asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively), NG monomethyl-L-arginine (L-NMMA), L-Arginine and L-Citrulline levels were measured with Liquid chromatography-tandem mass spectrometry. SDMA/ADMA and Arginine/ADMA ratios are calculated. Preoperative values were compared against healthy controls and postoperative values. Results: Serum homoarginine levels were significantly decreased but Serum IMA levels were increased and there was no difference for serum homocysteine levels in group 1 compared to group 2 ($P < 0.001$), respectively. Conclusions: Homoarginine and IMA are potentially useful diagnostic biomarkers for breast cancer diagnosis. Besides, Homoarginine and IMA can be useful biomarkers for breast cancer follow-up.

## P-35-087
## Alpha-catulin as a marker of a specific population of invasive breast cancer cells

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Breast cancer is the most commonly diagnosed cancer in women worldwide. The epithelial-to-mesenchymal transition (EMT) has been associated with breast cancer progression. However transient, reversible nature of EMT and the fact that only a small minority of carcinoma cells may undergo an EMT in primary tumours makes observing an EMT *in vivo* a great challenge. In our previous studies, we observed that the upregulation of catulin expression correlates with the transition of tumour cells from epithelial to mesenchymal morphology. Therefore we developed a reporter system, Catulin-GFP that allows us for the first time to isolate and characterize *in vivo* a small population of invasive breast cancer cells from the xenograft model and test their cancer initiating potential. We used two triple negative breast cancer cell lines, MDA231, and HCC1806 that show high expression of


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catulin to develop stable cell lines with reporter system. It is a reporter driving GFP expression from catulin gene promoter. *In vitro* 3D invasion assay in Matrigel confirmed specific localization of the GFP positive cells in invading cells. In addition, flow cytometry analysis of those reporter cell lines showed that GFP signal overlaps with known breast cancer stem cell markers CD44+ and CD24−. Therefore both cell lines were injected into a mammary fat pad of Nod.Scid mice. FACS analysis was performed on the tumours that formed after 8 weeks. GFP+/CD49f+ and GFP−/CD49f+ cells were collected and isolated RNA was subjected to RNAseq analysis. Part of the tumours was cut and stained with antibodies for known EMT markers (N-cadherin, Vimentin, CD44, and others) to determine the localization of specific populations within the tumour. Our data show that the Catulin-GFP reporter system marks invasive cells that localize highly outermost in spheres as well as in whole tumours and allows us to perform transcriptional profiling of those cells for characterization of early invasion markers of breast cancer.

## P-35-088

## Weighted gene co-expression network analysis reveals different gene profiles in lung cancer current and never smoker patients

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Although 85–90% of LUAD malignancies have been attributed directly to tobacco smoking, little is known regarding the epigenetic changes of the molecular mechanisms involved in epithelial to mesenchymal transition (EMT). LUAD patients with a smoking history have low E-cadherin levels with even further reduced levels in smokers, thus indicating a poor prognostic factor. Cigarette smoking plays a vital role in promoting EMT and is associated with poor survival, cell migration and invasion in non-small cell lung cancer (NSCLC) through the deregulation of E-cadherin. Almost 25% of all lung cancer cases worldwide have been found in never smokers (NS), with environmental tobacco being a relatively weak carcinogen. In our analyses we used mRNASeq expression and clinical data downloaded from The Cancer Genome Atlas (TCGA). Weighted gene correlation network analysis (WGCNA) was used for the association of constructed gene modules and clinical traits. Gene set enrichment analyses (GSEA) was used to analyse global gene expression differentiation between groups of current smokers (CS) and NS. The differentially expressed genes were analyzed by Gene Ontology (GO), then Cytoscape to construct a co-expression network of hub differentially expressed genes mediated pathways. LUAD in CS and NS patients shows clear differentiation of gene profiles. In addition, we found that differentially expressed genes are responsible for important functions such as replication and EMT with a higher expression in CS. Our results highlight the potential difference in biological mechanisms of cancer development due to tobacco smoking. According to our results, there are significant differences between the gene expression profiles in CS and NS. The overexpression of certain genes involved in cell cycle, EMT and replication in current smokers seems to be key drivers in LUAD progression. Acknowledgment: This work was supported by the Medical University of Lodz grant number 503/0-078-02/503-01-004. *The authors marked with an asterisk equally contributed to the work.

## P-35-089

## New oxidovanadium(IV) coordination complex containing 2-methylnitrilotriacetate ligands induces cell cycle arrest and autophagy in human pancreatic ductal adenocarcinoma cell lines

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Background: The pancreatic cancer is a relatively rare type of cancer, however, due to minimally effective of the current chemotherapeutic options it is the fourth leading cause of cancer-related death worldwide. Metals are involved in many biochemical processes including electron transfer and catalysis. Metal-based compounds form new shapes that can more effectively explore new chemical entity (NCE), which may be applied to rational design of new drugs including anticancer therapy. Methods: We investigated three newly synthesized oxidovanadium(IV) complexes with organic ligands: 2-methylnitrilotriacetate, N-(2-carbamoylethyl)iminodiacetate and N-(phosphonomethyl)-iminodiacetate. All studies were performed by using two human pancreas ductal adenocarcinoma cell lines (PANC-1 and MIA PaCa2) and non-tumor immortalized pancreas duct cells (hTERT-HPNE). We used MTT, neutral red and lactate dehydrogenase (LDH) assays to evaluate the cytotoxicity. The antiproliferative activity was measured by bromodeoxyuridine BrdU assay. We used flow cytometry technique to determine reactive oxygen species (ROS) generation as well as cell cycle analysis. Proteins level was analysed by western blot. Confocal laser scanning microscopy was used to evaluate morphology changes. Results: The results were shown that tested oxidovanadium(IV) complexes exhibited selective cytotoxicity on pancreatic cancer cells compare with non-tumor immortalized cells over the concentration range of 10–25 μM. Furthermore, cytotoxicity of selected oxidovanadium complex, containing 2-methylnitrilotriacetate ligands, was dependent on antiproliferative activity, increased ROS generation, cell cycle arrest in G2/M phase with simultaneous triggering of the p53/p21 pathway, binucleation, and induction of autophagy. Conclusion: Our study indicates that oxidovanadium(IV) coordination complexes may provide promising candidates for preclinical development of novel anticancer drugs targeting pancreatic cancer.

## P-35-090

## Investigation of microRNAs which regulate S6K2 may enlighten new paths for breast cancer therapy

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Recent research about S6K2 has revealed that the younger member of S6 Kinase family, S6K2, has important roles in lung and breast cancer cell survival and resistance to chemotherapeutic drugs. Previously, S6K2 had been displayed to be overexpressed in breast cancer when compared to normal breast tissue. Therefore, the regulation of cellular steady-state level of S6K2 is very crucial in maintenance of normal cellular physiology. There are post-translational modifications of S6K2 such as ubiquitination and acetylation which adjusts cellular S6K2 steady-state level but the details of these mechanisms are still not completely understood. Moreover, still very little is known about the post-transcriptional regulation of S6K2 expression. The only discovered microRNA which downregulates S6K2 expression is miR-193a-3p. Downregulation of S6K2 by this microRNA blocks the metastasis of human NSCLC (non-small-cell lung cancer) cell


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lines such as A549 and H1299. High throughput analysis has revealed that S6K2 might be one of the targets of miR-26b and miR-98-5p. High throughput immunoprecipitation in BT474 breast cancer cell line displayed that miR-98-5p might downregulate S6K2 expression. Also, microarray analysis showed the interaction between S6K2 mRNA and both of these microRNAs. Also, it had been shown before that miR-26b inhibited cellular proliferation of MCF-7 and MDA-MB-231 breast cancer cell lines by downregulating CDK8 (cyclin-dependent kinase 8) expression. Moreover, the invasion and metastasis of highly aggressive 4T1 cells were displayed to be blocked by miR-98-5p by downregulating ALK4 (activin receptor-like kinase-4) and MMP11 (matrix metalloproteinase-11). Thus, these microRNAs are suppressors of breast cancer. We aim to perform low throughput analysis such as luciferase assay, Western blotting (mimicking or inhibition experiments) or quantitative RT-PCR in breast cancer cell lines so that we ensure that S6K2 is a direct target of both miR-26b and miR-98-5p.

## P-35-091
## The new form of flavonoids delivery to cancer cells

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Tumours, along with cardiovascular diseases, are among the most serious diseases of civilization. Currently, the effectiveness of cancer treatment is seen in the early diagnosis and development of modern drugs, capable of eliminating single cancer cells, while reducing side effects on normal cells of the organism. Therefore, naturally occurring compounds or their modified derivatives are becoming more and more popular in research. Flavonoids belong to the group of phenolic compounds occurring in plants. Relatively low costs of obtaining those compounds, combined with their anti-cancer properties, make flavonoids an attractive research target for many scientific centres. It is known that some cancer cells overexpress certain receptors and deregulated redox balance. These features, which are unique to cancer cells, seem to be possible ways to develop targeted anti-cancer therapies. In this research, select cancer targeting molecules and natural hydroxyflavonoids, as an antioxidant and cytotoxic agents, are combined in conjugate molecules and evaluated for their activity to cancer and non-cancer cells. Bioconjugates with the highest anticancer potential, selected in the first stage, were next evaluated for their uptake and accumulation in selected cellular models. Acknowledgments: This study was supported by grant No. 2017/01/X/NZ9/00161 assigned by the National Science Centre, Poland, to M.S.

## P-35-092
## Increased PARP fragmentation and decreased p-Akt expression accompany coenzyme Q0-induced death of HL-60 and K562 leukemia cells

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Coenzyme Q0 (CoQ0) is a novel redox-active ubiquinone compound which has been shown to exert anti-inflammatory and cancer modulatory activities in different cell lines. This study was designed to investigate the antiproliferative and apoptotic activity of CoQ0 in human acute and chronic myeloid leukemia (HL-60 and K562) cell lines. HL-60 and K562 cells were treated with different concentrations of CoQ0 and cell proliferation, caspase-3 activity and phospho-Akt Ser473 and PARP-1 protein expressions were determined by colorimetric MTT assay, caspase-3 activity assay and western blot, respectively. CoQ0 inhibited proliferation of HL-60 and K562 cells between 2.5–5 μM and 10–12.5 μM concentrations. CoQ0 significantly led to the activation of caspase-3 after 24 h treatment in HL-60 cells. Full-length PARP-1 (116 kDa) protein expression was significantly downregulated and cleavage fragment of PARP-1 (85 kDa) was partially increased in the 5 μM CoQ0-treated group compared to the control group in HL-60 cells. Furthermore, the 89-kDa cleavage fragment of PARP-1 was increased at 10 and 12.5 μM concentration of CoQ0 in K562 human leukemic cells but the protein expression of full-length PARP-1 did not change by treatment. Treatment of HL-60 cells with 5 μM CoQ0 and K562 cells with 12.5 μM CoQ0 resulted in decreased phospho-Akt Ser473 protein expression. In conclusion, CoQ0 inhibits cell proliferation and enhances apoptosis which was confirmed by increased PARP fragmentation through the inhibition of AKT activation in human acute and chronic myeloid leukemia (HL-60 and K562) cells. Further research is needed to explain a detailed mechanism of action of CoQ0 in human leukemic cells.

## P-35-093
## Antiproliferative activity of selective ligands of membrane progesterone receptors in cancer cells

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Progesterone (P4) plays an important role in regulation of reproductive function and acts through classical nuclear receptors (nPRs) and membrane receptors (mPRs). Controversial effects of P4 on differentiation, proliferation and cell death in different tissues were described. For example, P4 shows carcinogenic activity in breast cancer, but we found its anti-proliferative effects in BxPC3 (pancreatic adenocarcinoma) cells. According to RT-PCR data these cells have high level of mPRs mRNA and lack of nPRs mRNA. Two selective ligands of mPRs – 19-hydroxypregn-4en-20-one (I) and 19- hydroxy-5β-pregn-3-en-20-one (II) were identified previously. In this study we have tested the action of selective ligands of mPRs and P4 on the proliferation of BxPC3 cell line in the presence or absence of two inhibitors of different MAPK (p38 and JNK) using XTT test (cell viability test). In the absence of MAPK inhibitors viability of BxPC3 cells decreased at the 5 μM, 20 μM P4 and 20 μM (I). The p38 inhibitor showed the same effect on cell viability at 1 μM P4, 1 μM (I) and 20 μM (II). In the presence of JNK inhibitor viability of BxPC3 cells decreased only when 20 μM P4 was used. These data suggested that (I) exerts anti-proliferative effect on BxPC3 cells by acting through mPRs especially in the presence of p38 inhibitor. In the presence of JNK inhibitor, (I) did not affect viability of BxPC3 cells. Thereby JNK seems to play a key role in the mPRs signaling pathway. The mPRs selective ligands can be used instead of P4 to prevent its side effects in tissues with classical receptors. This work was supported by the Russian Foundation for Basic Research (project 17-04-00234).


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## P-35-094

## Role of paraoxonase-2 in bladder cancer: effect of shRNA-mediated gene silencing on cell proliferation and tumourigenicity

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Bladder cancer (BC) is characterized by high recurrence and represents the 7th most common neoplasm in men and the 17th most common in women, worldwide. The identification of reliable biomarkers becomes essential for both early diagnosis and effective therapy of BC. Paraoxonase-2 (PON2) belongs to the paraoxonase protein family and exhibits anti-oxidant and anti-apoptotic activity. Enzyme upregulation was recently reported in association with BC. Therefore, the aim of this study was to explore the involvement of PON2 in BC cell metabolism, focusing on the effect induced by shRNA-mediated gene silencing on cell proliferation and tumourigenicity. First, human urinary bladder carcinoma cell line T24 was transfected with pLKO.1 vectors containing stem-loop cassettes encoding short hairpin RNA (shRNA) targeted to human PON2, and with empty pLKO.1, used as control. The efficiency of PON2 silencing was detected by Real-Time PCR, and Western blot analyses. Subsequently, T24 cells were treated with different concentrations of gemcitabine and cisplatin, drugs used for systemic chemotherapy of BC. Cell viability and susceptibility to oxidative stress induced by reactive oxygen species (ROS) were further evaluated by using MTT assay and 2′,7′-dichlorodihydrofluorescein diacetate ($H_2$DCFDA) probe, respectively. In addition, Real-time PCR and Western blot analyses were used to evaluate PON2 expression levels after treatment with drugs. Results obtained showed that shRNA-mediated gene silencing of PON2 led to a significant cell proliferation inhibition that was enhanced by the treatment with both drugs. Moreover, PON2 knockdown was significantly associated with an increase of intracellular ROS levels in T24 cells upon treatment with drugs. Our results showed that the downregulation of PON2 significantly reduced proliferation and *in vitro* tumorigenicity of T24 cells, suggesting that the enzyme could represent an interesting molecular target for BC therapy.

## P-35-095

## Expression and function of NFAT (nuclear factor of activated T cells) transcription factors in human gliomas

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The NFAT (Nuclear Factor of Activated T cells) transcription factors were initially described as regulators of T cell activation and differentiation. Emerging evidence points out that various NFAT isoforms perform important functions in cancer cells and other compartments of the tumor microenvironment, including fibroblasts, endothelial cells and infiltrating immune cells. Our previous studies demonstrated abnormal expression of NFAT proteins and activation of NFAT signaling in glioma cells. Malignant gliomas are invasive, fast growing brain tumors originating from neural stem or progenitor cells. Precise functions and transcriptional targets of NFAT proteins in glioma cells remain unknown. The analysis of TCGA datasets revealed significant upregulation of *NFATc1, NFATc2* and *NFATc3* mRNAs in human WHO grades II-IV gliomas. This was confirmed in the independent cohort, in which we evaluated the expression of *NFAT* genes by qPCR in human low and high grade gliomas in comparison to non-tumor brain tissues. Upregulated levels and activity of NFAT proteins have been detected in a number of established and primary human glioma cell lines. Using chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) we identified genes transcriptionally regulated by NFAT in a whole genome scale. Corroborating data were acquired from RNA-seq and qPCR gene expression analyses after selective gene knock-down using specific NFAT-targeting siRNA and treatment of glioma cells with a cell-permeable peptide inhibitor preventing NFAT activation. Our results provide new insights into the role of NFAT signaling pathway in glioma pathogenesis and indicate NFATs as a plausible target for future therapeutics. The study was supported by grant 2014/15/B/NZ3/04704 from The National Science Centre, Poland.

## P-35-096

## Inhibition of PAK1/2 kinases enhance imatinib mechanism of action in eradication of CML stem cells

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of hematopoietic stem cells. At the molecular level the disorder results from t(9;22)(q34;q11) reciprocal translocation between chromosomes, which leads to the formation of an oncogenic *BCR-ABL* gene fusion. Introduction of tyrosine kinase inhibitors (TKIs) revolutionized the treatment of patients especially in chronic phase of CML (CML-CP). Unfortunately, during disease progression further molecular and biological alterations appears. Therefore, some new treatment modalities are necessary to improve therapeutic outcomes. The aim of the study was to found out whether the inhibition of signaling pathways activated by BCR-ABL oncogenic tyrosine and PAK serine/threonine kineses may improve the treatment of CML. In our study PAK1 and PAK2 kinase were chosen because they are expressed in hematopoietic cells and may play an important role in the promotion of CML cells survival. For this purpose a series of *in vitro* experiments involving small molecule inhibitor IPA-3 (inhibitor of PAK1), shRNA construct for *PAK2* gen and TKI such as imatinib (IM) were conducted. The studies were carried on K562 and KCL-22 CML cell lines, $32Dcl^{BCR\text{-}ABL}$ cells and hematopoietic stem cells (HSC) isolated from the bone marrow of newly diagnosed CML-CP patients. The results indicate that inhibition of PAK kinases activity may improve IM mechanism of action. Our studies revealed that combination of IM with IPA-3 generates synergistic effects at the 50% of the cell kill level (Fa = 50). We observed that the combined use of IM with IPA-3 can increase growth inhibition and apoptosis of cancer cells. Moreover, targeting PAK1 and/or PAK2 in IM-treated CML cells downregulated the level of ROS responsible for oxidative DNA damage. This work was supported by the National Science Centre of Poland (grant no 2013/11/B/NZ7/02248; http://www.ncn.gov.pl/).


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## P-35-097
## FGF1 enhances the cytotoxic activity of honokiol in tumor cells expressing FGFR1

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Fibroblast growth factor 1 (FGF1) is a canonical member of the FGFs family. Through specific receptors (FGFR1-4), it activates signaling pathways (Ras/MAPK, PI3K/Akt/mTOR, PLCγ/PKC) leading to cell proliferation, migration, and survival in stress conditions. It has been shown, that FGF1 may induce resistance to chemotherapy in tumor cells expressing FGF receptors. Surprisingly, here we show that FGF1 may also increase the effect of a cytotoxic drug, honokiol. Honokiol is a natural component of *Magnolia* species, which induces apoptosis and suppresses growth in tumor cells without effect on non-malignant cells. We found, that simultaneous administration of honokiol and FGF1 reduces cell viability and increases caspases activity as compared to honokiol alone. This effect is dependent on FGFR1 activation since it was abolished by specific FGFR inhibitor. Using siRNA and inhibitors we also evaluated the impact of PI3K/Akt/mTOR and Ras/MAPK pathways on honokiol action. Our results show that growth factors can not only stimulate the survival of cancer cells but, in some cases, may also enhance the effect of anti-cancer drugs. Acknowledgments The work was supported by the National Science Centre, Poland (Sonata Bis 2015/18/E/NZ3/00501).

## P-35-098
## Anterior gradient 2 stabilizes the hypoxia-inducible factor-2α in a human clear cell renal carcinoma cell line Caki-1

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Hypoxia is a common characteristic of solid tumors. It affects cancer biology and increases resistance to treatment leading to poor prognosis for the patients. Hypoxia stimulates the activation of hypoxia-inducible factors, HIFs, that results in the expression of genes involved in angiogenesis, epithelial-mesenchymal transition or cellular metabolism. Anterior gradient 2 (AGR2), a disulfide isomerase, has been shown to play a key role in the stabilization of the HIF-1α in breast cancer cells. Here we aimed to determine whether AGR2 affects another protein of HIF family, HIF-2α, in a human clear cell renal carcinoma cell line (ccRCC), Caki-1. To address this question, Caki-1 cells were transduced with a Tet-On lentiviral vector to obtain a cell line with inducible gene expression of the AGR2. After induction of the AGR2 expression, cells were cultured in hypoxic condition (0.5% $O_2$) or in normoxic condition (21% $O_2$). Our results show that AGR2 upregulates HIF-2α at both the transcript and protein levels. We also found that AGR2 induces expression of CCND1, PGK1 and TGFα genes. Moreover, real-time PCR and ELISA analyses showed that AGR2 induces a pro-inflammatory phenotype and angiogenesis in Caki-1 cells by upregulation of IL-6 and VEGFA, respectively. Our results indicate that AGR2 affects cell signaling network by activation of NF-κB and suppression of STAT3 pathways. Taken together, these findings demonstrate that AGR2 plays an important role in ccRCC progression by upregulation of the HIF-2α and alteration of cell signaling and metabolism. *The authors marked with an asterisk equally contributed to the work.

## P-35-099
## Novel kinesin Eg5 inhibitors as potential drugs for gastric adenocarcinoma treatment

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The kinesins are motor proteins mediating different functions inside the cell, such as intracellular transport, cell division and microtubule movement. The key role of the mitosis is played by the mitotic spindle consisting of microtubules, formed by α/ß tubulin dimers, emerging from the spindle poles. Recently, kinesin Eg5 inhibitors received a great attention as new chemotherapeutic agents, due to the lack of side effects and resistance mechanisms commonly exhibited by Vinca Alkaloids and Taxanes. Our aim was to investigate the biological effects of new kinesin Eg5 inhibitors, synthetized in our laboratory, in an *in vitro* model of gastric adenocarcinoma (AGS cell line), in order to improve the design of anti-cancer therapies. Preliminary studies, performed on several kinesin Eg5 inhibitors, led us to select two of them (Cmp2 and Cmp41) for their low micromolar IC50, comparable to K858 IC50, a common kinesin Eg5 inhibitor. Next, kinesin inhibitors capability to promote the apoptotic event was evaluated by measuring Bax and Caspase-3 protein expression, finding that Cmp41 and K858 strongly increase Bax expression, whereas only K858 and its co-administration with Cmp2 and Cmp41 triggers caspase-3 activation. In parallel, to confirm the inhibition of mitotic spindle induced by kinesin Eg5 inhibitors, a β-tubulin immunofluorescence was carried out revealing, for the first time, the formation of monopolar spindles in AGS cells treated with Cmp2, Cmp41 and K858. To conclude kinesin inhibitors ability to counteract cancer cells migration was measured, evidencing a dramatic reduction of migrated cells when Cmp2, Cmp41 and K858 are administered compared to cells treated with DMSO. These data indicate that kinesin Eg5 is an interesting target for new therapeutic approaches for gastric adenocarcinoma. Moreover, it could be argued that Cmp2, Cmp41 and K858 are promising molecules to be included in an *in vivo* animal study to confirm their usefulness in gastric adenocarcinoma.

## P-35-100
## Induction of epithelial-mesenchymal transition in liver of mice fed high-fat diet

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Non-alcoholic fatty liver disease (NAFLD) is recognized as one of the most common liver diseases in the world. During NAFLD, hepatocytes accumulate excessive amount of lipids, which leads to its steatosis. Factors that cause the development of this disease are overweight and obesity. Steatosis can lead to the development of nonalcoholic fatty liver disease and even fibrosis. The epithelial to mesenchymal transition (EMT) is characterized by the loss of cellular connections and the change of cells polarity. Cells have also increased motility and acquisition of invasive ability. During EMT, there is an increase in the expression of mesenchymal markers such as transcription factors: Slug, Zeb1/2 or structural proteins such as fibronectin or N-cadherin, while the level of epithelial markers decreases. To check whether high-fat diet can induce EMT, C57BL/6J mice were fed for 12 weeks with control and high-fat diet, then the hepatocytes were isolated and the mesenchymal markers expression was checked. During *in vitro* studies hepatocarcinoma cells Huh7 were stimulated with sodium oleate for various time points. We also checked the level of mesenchymal markers and immunofluorescence staining were


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performed. *In vitro* studies have shown that the mesenchymal markers level is higher in cells stimulated with sodium oleate compared to control. These results have been confirmed *in vivo*. In mice fed a high-fat diet, the level of mesenchymal markers such as transcription factors: Slug, Zeb1/2 or structural proteins like vimentin or fibronectin increases markedly. It can therefore be concluded that a high-fat diet leads to the induction of epithelial-mesenchymal transition. Acknowledgment: SONATA BIS 2017/26/E/NZ5/00691.

## P-35-101
## Anticancer activity of a cationic Ru-based nanosystem in a human xenograft model of breast cancer

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Breast cancer is the second most common cancer worldwide and the leading cause of death in women. The global burden of breast cancers exceeds all other cancers and its incidence is increasing so that the search for novel chemotherapeutic options is nowadays an essential requirement to fight neoplasm subtypes. By exploring new effective metal-based chemotherapeutics, many ruthenium complexes have been recently proposed as anticancer drug candidates, showing ability to impact on diverse cellular targets. In addition, many nanomaterial Ru complexes have been designed and developed into anticancer drugs with interesting beneficial properties. In this context, we have demonstrated the efficacy of a ruthenium (III)-complex (AziRu) incorporated into a cationic nanosystem (HoThyRu/DOTAP), proved to be hitherto one of the most effective within the suite of nucleolipidic formulations we have developed for the *in vivo* transport of anticancer ruthenium (III)-based drugs. Based on very interesting results on breast cancer cells (BCC), to evaluate the animal biological response to systemic administration of HoThyRu/DOTAP nanosystem, as well as its effects on the progression of breast cancer *in vivo*, we performed an antitumour study using athymic nude mice bearing human BCC xenografts. In depth analysis of tumour growth shows that HoThyRu/DOTAP significantly inhibits breast cancer cell proliferation in mice, without any sign of toxicity in treated animal group. In addition, molecular biology experiments *ex vivo* are currently ongoing to deepen the cellular responses and/or resistance to treatments, as well as to support *in vivo* the activation of specific cell death pathways, i.e. apoptosis and autophagy. On this path, we have validated the efficacy and the safety *in vivo* of our ruthenium-based candidate drugs in the perspective of novel cancer therapeutic options.

## P-35-102
## Autophagy-mediated control of apolipoprotein A-I impacts lipid and cholesterol homeostasis

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Autophagy is involved in lipid metabolism as it is required for the breakdown of lipid droplets (LDs), and perturbations of its function contribute to the pathogenesis of fatty liver diseases and hepatocellular carcinoma (HCC). Apolipoprotein A-I (ApoA-I), the major protein constituent of high density lipoprotein, is synthesized mainly in the liver and plays a key role in reverse cholesterol transport. Deregulated ApoA-I levels have been associated with liver metabolic disorders such as fatty liver disease and various cancers, including colorectal and HCC. While the role of extracellular ApoA-I in lipid metabolism is well-established, its intracellular function and regulation of expression are poorly characterized. Our initial observation that ApoA-I levels are significantly increased in steatotic liver of high-fat fed mice, a condition that is accompanied by autophagic liver perturbations and increased lipid load, prompted us to examine the regulation of ApoA-I by autophagy in hepatocytes. We demonstrate that ApoA-I is catabolized by autophagy and co-localizes with autophagic markers LC3 and p62. Gene silencing of key players of the autophagic machinery shows that ApoA-I degradation is mediated by a BECLIN-1-dependent pathway. Further investigation of the intracellular role of ApoA-I in the regulation of liver lipid metabolism revealed differences in the number and size of LDs upon *ApoA1* knock-down. Mechanistic insight provided by *in vitro* studies revealed that ApoA-I may act as a break to lipid accumulation upon autophagic inhibition by modulating the expression of cholesterol and lipid synthesis genes. Collectively, our results highlight a link between autophagy and apolipoproteins in the intracellular regulation of lipid metabolism in the liver that could provide further understanding of the pathogenesis of metabolic syndrome disorders associated with liver diseases.

## P-35-103
## Determination of the WWOX gene function in AP-2 γ signaling pathway in metastatic bladder cancer

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The WWOX gene is one of the unusual tumor suppressor genes, whose molecular function has not been fully understood in bladder cancer. The gene encodes a protein containing two N-terminal WW domain and the centrally located short chain dehydrogenase domain (SDR). The first WW domain interacts with AP-2γ transcription factor (encoded by TFAP2C gene) and inhibits its oncogenic activity by inducing the redistribution of AP-2γ to the cytosol, modulate proliferation and tamoxifen response in breast cancer. Moreover, the most malignant type of breast tumour, the triple negative cancer, showed high expression of AP2γ and frequent loss of WWOX. In the present study, an *in vitro* model of CAL29 bladder cancer cell line (grade 4) with different level of WWOX and high TFAP2C was created. We used knock-in lentivirus and CRISPR-Cas9 system to overexpress and silence WWOX gene, respectively. The WWOX gene modifications induced higher expression of TFAP2C gene in compare to control. The aim of our study was to assess the effect of different WWOX expression level and high AP2γ on biological processes such as adhesion to extracellular matrix proteins, colony formation, apoptosis, growth in 3D, activity of metalloproteinases, mitochondrial redox potential. In a high WWOX/high TFAP2C variant, we observed lower the ability of a single cell to grow into a colony, decreased expression of metalloproteinase MMP2 and proliferation potential, but increased adhesion to collagen I, collagen IV, laminin, lower mitochondrial redox potential and forming smaller spheres in Matrigel, which can suggest a reduction of aggressive potential. However, in this model was noticed a decline number of caspase positive/death cells after induction of apoptosis by staurosporine. Our results suggest a relevant biological role of WWOX gene in metastatic bladder cancer with high expression of AP2γ transcription factor. This


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work was supported by the National Science Centre of Poland 2016/21/B/NZ2/01823. *The authors marked with an asterisk equally contributed to the work.

## P-35-104
## The effect of a new synthetic ferrocene containing camphor sulfonamide on breast cancer cell lines

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Background: Drug resistance is a major cause of cancer treatment failure. Most cancer therapies involve multiple agents, to overcome it. Compounds that exhibit strong anti-tumor effect without damaging normal cells are more and more in the focus of research. Chemotherapeutic drugs, combining several functional groups in one molecule, can modulate different regulatory pathways in the cell and thus reach the higher efficacy than the agents, which impact only one cellular process. Methods: We tested the effect of newly synthesized ferrocene-containing camphor sulfonamide on two breast cancer and one breast non-cancer cell lines. The cytotoxic effects were evaluated using the standard MTT-dye reduction and clonogenic assays. The apoptotic or autophagic effects were evaluated by annexin v binding or LC3 puncta formation assays respectively. Cell cycle arrest was determined using flow cytometry. Western blot and immunofluorescent analyses were used to estimate the localization and cellular distribution of key regulatory factors NFκB and p53. Results: The newly synthesized ferrocene-containing camphor sulfonamide has well pronounced cytotoxicity greater to cancer cells (MDA-MB-231 and MCF-7) compared to non-cancerous (MCF-10A). IC50 of the substance caused a cell cycle arrest in G1 phase and induced apoptosis up to 24 h in both tumor cells, although being more pronounced in MCF-7, a functional p53 cell line. Treatment with IC50 concentration of the compound provoked autophagy in both tumor lines being more pronounced in the more aggressive cancer line (MDA-MB-231). Conclusion: The tested newly synthesized ferrocene-containing camphor sulfonamide showed promising properties for a potential therapeutic agent. Keywords: Ferrocene-containing camphor sulfonamide, breast cancer cells, cytotoxicity, cell cycle arrest, apoptosis, autophagy, NFκB, p53. Acknowledgements: This work was fully supported by grant DN11/16 16.12.2017 of the Bulgarian National Science Fund.

## P-35-105
## Cysteine cathepsins in myeloid derived suppressor cells

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Cysteine cathepsins are lysosomal peptidases that besides terminal protein degradation control important cellular biological processes. They are implicated in various functions of innate and adaptive immune response, most notable examples being antigen presentation and immune cell cytotoxicity. Moreover, cysteine cathepsins are frequently overexpressed in cancer, where they contribute to tumor invasion, proliferation and metastasis. Levels and activities of cysteine cathepsins are known to be differentially regulated during immune cell development. Their function in macrophages, lymphocytes and dendritic cells has been frequently studied, however little is known about their role in myeloid derived suppressor cells (MDSC). MDSC represent heterogenous population of immature myeloid cells that expands in response to tumor cell derived inflammatory cytokines. Their hallmark is the ability to strongly suppress T cell-mediated immunity. Among cysteine cathepsins, monocarboxypeptidase cathepsin X is unique, because it is localized predominately to the cells of immune system and suggested to regulate adhesion and proliferation of monocytes and macrophages as well as migration of T cells. Based on its important role in cells of monocyte/macrophage lineage we hypothesized that cathepsin X impacts function of myeloid derived suppressor cell (MDSC) as well. We established an *in vitro* model that allowed us to evaluate induction of immunosuppressive properties in monocytes that were stimulated with selected cytokines or tumor cell lines. We correlated levels of cathepsin X and other cysteine cathepsins in stimulated myeloid cells to the degree of immunosuppression that was measured in a T cell proliferation assay. Next, we tested the impact of selective cathepsin X inhibitor AMS-36 and broad-spectrum cathepsin inhibitor E64-d on the development of immunosuppressive myeloid cells.

## P-35-106
## Identification of new secretory glycoprotein SPX-40 ligands

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A family of 40 kDa secretory glycoproteins (SPX-40) secreted during involution works as a protective signalling factor through its binding to viable cells. Recently, the role of SPX-40 proteins in the progression of breast cancer has been observed. To enrich the preliminary data on relationship of SPX-40 proteins and tumorigenesis we attempted to identify new ligands of SPX-40 by methods of molecular docking. A high resolution crystal structure of SPS-40 [PDB:5Z4V] was taken as the initial model of receptor protein for docking studies of a small library of heteroarene-fused anthraquinones. 3D structure of all the compounds were calculated individually using program Corina followed by energy minimization and were docked in the binding site with the help of molecular docking program AutoDock 4.0.6 using default parameters. The docked pose (positional conformer) with lowest binding energy among the largest cluster was taken as the most probable binding mode. The analysis yielded that naphto[2,3-*f*] isatine derivative LCTA-1655 can bind in the cavity of SPS-40 through hydrophobic interactions of anthraquinone moiety with resides of non-polar amino acids and hydrogen bonded interactions of heterocyclic ring with the environment of the binding site. *In silico* complexation of SPX-40 was further verified by isothermal titration calorimetry measurements to determine the binding affinity of LCTA-1655 for SPX-40. The protein was purified to homogeneity and ITC experiments with the best ligand indicated a dissociation constant $K_D$ of 13.6 mM revealing that LCTA-1655 has good affinity for the protein. Additionally, LCTA-1655 inhibits growth of MCF-7 breast cancer cell line at micromolar concentration. In summary, we identified a new scaffold of secretory glycoprotein SPS-40 ligands which could be helpful for further investigation of this protein and its role in progression of breast cancer. This study was jointly funded in part by RFBR (17-53-45105) and DST (INT/RUS/RFBR/P-291).


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## P-35-107
## LOX-1 activation by oxLDL promotes enzalutamide resistance on C4-2B prostate cancer cells

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The androgen receptor antagonist, enzalutamide, is a drug used for castration-resistant prostate cancer (CRPC) treatment; however, a high percentage of patients develop resistance. This resistance is induced by pro-inflammatory cytokines, the activation of STAT3 and NF-kB signaling pathways, and the overexpression of androgen receptor (AR) or its splicing variant AR-V7. On the other hand, oxidized low-density lipoprotein receptor 1 (LOX-1) and its ligand, the oxidized low-density lipoprotein (oxLDL), are increased in patients with advanced prostate cancer. LOX-1 activation by oxLDL promotes an inflammatory process through the STAT3 and NF-kB activation in several chronic diseases. Our aim was to analyze the role of LOX-1/oxLDL on enzalutamide resistance in CRPC cells. LOX-1 expression was analyzed by real-time PCR and Western blot in androgen-dependent prostate cancer cells (LNCaP), CRPC cells (C4-2B), and CRPC-enzalutamide resistant cells (C4-2B/ENZ-R). Then, C4-2B and C4-2B/LOX-1 knockdown cells were treated with oxLDL to analyze the expression of IL-6, IL-1β, and IL-8, AR, and AR-V7 by real-time PCR and Western blot. STAT3 and NF-kB activation were analyzed by Western blot. Finally, the effect of LOX-1/oxLDL in the $IC_{50}$ of enzalutamide on C4-2B cells was evaluated by clonogenic assays. The results showed that LOX-1 was overexpressed in C4-2B cells compared with LNCaP cells and was overexpressed in C4-2B/ENZ-R compared with parental cells. Moreover, oxLDL treatments promote the expression of IL-6, IL1B, IL-8, AR, AR-V7, PSA and induce the phosphorylation of NF-kB p65 (Ser 311) and STAT-3 (Tyr 705); however, LOX-1 knockdown suppresses the effects induced by oxLDL on C4-2B cells. Furthermore, LOX-1 activation by oxLDL reduced the enzalutamide cytotoxic effects, increasing its $IC_{50}$ in C4-2B cells. Our results suggest that LOX-1 and oxLDL could be new players in the promotion of mechanisms that lead to enzalutamide resistance in CRPC cells. Grant: Fondecyt 11181105.

## P-35-108
## Regulation of endogenous formaldehyde metabolism to induce cancer cell death in humans

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The main aim of most cancer treatment methods is to induce the death of tumour cells. Therefore, the identification of factors that restore the capability to induce apoptosis or necroptosis in tumour cells will contribute to both the prevention of cancer and the improvement of its treatment. One of these factors may be metabolic formaldehyde (FA), which is associated with increases in the general metabolism of amino acids and nucleotides in cancer cells. The accumulation of FA in cancer cells and within the bodies of patients results in changes in the activity of the aldehyde dehydrogenase 1 and 2 (ALDH1 and ALDH2) genes, which are involved in the oxidation of FA. It can be assumed that the suppression of the oxidation of endogenous FA and the promotion of FA accumulation within cancer cells may contribute to their death. To verify this assumption, we used the Her2/neu-positive human breast cancer cell line BT474. First, we modified and optimized two methods that could be used to measure FA concentrations in biological samples such as blood serum, culture medium or cell lysates. Both methods are based on FA derivatization followed by the measurement of absorbance or fluorescence. We found that, when the cancer cells were treated with the Her2/neu-specific monoclonal antibody trastuzumab, cell death was accompanied by the suppression of ALDH2 activity and the accumulation of FA. We confirmed this result in experiments that utilized disulfiram, which is an inhibitor of ALDH1 and ALDH2. In the presence of disulfiram, the effect of trastuzumab on cancer cells was sharply increased, and it also induced the death of cancer cells via its own activity, which was accompanied by the increased production of endogenous formaldehyde. By studying the mechanisms underlying the induction of cell death, we found that disulfiram and FA likely resulted in necroptosis in BT474 cancer cells. This study was performed with the financial support of the RFBR (project No. 18-34-20062).

## P-35-109
## SNAIL-dependent microRNAs are key regulators of rhabdomyosarcoma development and progression

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Rhabdomyosarcoma (RMS) is a frequent mesenchymal tumor of soft tissue in children. It originates from an impaired myogenic differentiation of satellite cells/myoblasts or mesenchymal stem cells. Previously, we demonstrated that SNAIL, a transcription factor regulating the epithelial to mesenchymal transition in tumors of epithelial origin, is also a key regulator of RMS growth and myogenic differentiation. In our current studies we hypothesized that SNAIL-dependent miRNAs are crucial regulators of RMS growth and differentiation. Analysis of miRNA transcriptome revealed that SNAIL inhibits myogenic differentiation and metastasis by regulation of not only myogenic factors, but it also regulates expression of miRNAs associated with muscle and actin cytoskeleton structure. Moreover, we discovered SNAIL-dependent small RNAs and putative novel miRNAs. We selected several miRNA candidates that were upregulated by SNAIL silencing for further analysis. Overexpression of two candidates induced formation of fibrotic structures and abnormal blood vessels in RMS xenografts in immunodeficient mice. They diminished proliferation and arrested the cells in G0/G1 cell cycle phase *in vitro*, but tumor growth *in vivo* was inhibited only when both of them were overexpressed at the same time. Moreover, those two miRNAs inhibited migration in a scratch assay and chemotaxis to SDF1. They induced also myogenic differentiation of RMS cells, but they affected expression of different myogenic factors crucial for RMS growth. After transfection of miRNA precursors into myoblasts, one miRNA upregulated early myogenic factors, and the second induced expression of later differentiation markers. To conclude, we discovered SNAIL-dependent miRNAs that may be new diagnostic and therapeutic targets in RMS after validation of precise mechanism of their action. The project was supported by grants from the National Science Centre in Poland 2015/17/D/NZ5/02202; 2013/09/B/NZ5/00769 and K/DSC/005268 from UJCM.


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## P-35-110
## Anti-metastatic potential of *Citrus unshiu* peel in murine melanoma B16F10 *in vitro* and *in vivo*

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The peel of *Citrus unshiu* (CU), called "Jinpi" in Korea, and "Chenpi" in China, has long been used as a traditional medicine that has therapeutic effects against pathogenic diseases, including asthma, vomiting, dyspepsia, blood circulation disorders, and various type of cancer. In this study, we investigated the effect of CU peel on metastatic melanoma in B16F10 melanoma cells, and in B16F10 cell inoculated-C57BL/6 mice. Our results show that ethanol extracts of CU (EECU) inhibited cell growth, and increased the apoptotic cells in B16F10 cells. EECU also stimulated the induction of mitochondria-mediated intrinsic pathway, with reduced mitochondrial membrane potential (MMP, $\Delta\psi_m$), and increased generation of intracellular reactive oxygen species (ROS). Furthermore, EECU suppressed the migration, invasion, and colony formation of B16F10 cells. EECU also induced the down-regulated expression of matrix metalloproteinase-2 (MMP-2) and MMP-9, as well as the up-regulated expression of their tissue inhibitor. In addition, the oral administration of EECU reduced serum lactate dehydrogenase activity without weight loss, hepatotoxicity, or nephrotoxicity in B16F10 cell inoculated-mice. Moreover, EECU markedly suppressed lung hypertrophy, increasing the number and expression of metastatic tumor nodules, and the expression of inflammatory tumor necrosis factor-alpha in lung tissue. Additionally, we identified that EECU contains bioactive flavonoids, including naringin, hesperidin, and neohesperidin by HPLC analysis. Our findings suggest that the inhibitory effect of EECU on the metastasis of melanoma indicates that it may be regarded as a potential therapeutic herbal drug for melanoma.

## P-35-111
## Expression of PD-1 protein on the surface of myeloid-derived suppressor cells (MDSCs) derived from tumor-bearing mice

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous cell population frequently observed in cancer, infection, and autoimmune disease that are able to suppress T cell function. Immune checkpoint molecules, such as programmed death 1 (PD-1) expressed on T cells and its ligand (PD-L1) expressed on tumor cells or antigen-presenting cells, have received extensive attention in the past decade due to the dramatic effects of their inhibitors in patients with various types of cancer. While checkpoint molecules were initially associated with T cell functions, recent evidence suggests a broader expression and function in innate myeloid cells including MDSCs that are induced during tumor progression. In particular, the significance and regulation of PD-1 expression on MDSCs in a tumor environment remain poorly understood. In the present study, we investigated the expression of PD-1 on MDSCs in bone marrow, spleen, and tumor tissue derived from breast tumor-bearing mice. Our studies demonstrate that PD-1 expression is markedly increased in tumor-infiltrating MDSCs compared to expression in bone marrow and spleens and that it can be induced by LPS that is able to mediate NF-κB signaling. Moreover, expression of PD-L1 and CD80 on PD-1$^+$ MDSCs was higher than on PD-1$^-$ MDSCs and proliferation of MDSCs in a tumor microenvironment was more strongly induced in PD-1$^+$ MDSCs than in PD-1$^-$ MDSCs. Although we could not characterize the inducer of PD-1 expression derived from cancer cells, our findings indicate that the study on the mechanism of PD-1 induction in MDSCs is important and necessary for the control of MDSC activity; our results suggest that PD-1$^+$ MDSCs in a tumor microenvironment may induce tumor development and relapse through the modulation of their proliferation and suppressive molecules.

## P-35-112
## Roles of basal transcription factor 3 (BTF3) on etoposide-induced cell death in cervical cancer cells

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Basal Transcription Factor 3 (BTF3) was first identified as one of general transcription factors (TFIIs) and also confirmed as the identical protein with β-nascent polypeptide-associated complex (β-NAC). Most of BTF3 researches have been done on the protein targeting. BTF3 is involved in the control of protein targeting to ribosome and mitochondria. We examined the roles of BTF3 and its molecular mechanisms on chemotherapeutic drug-induced cell death in cancer cell lines. Hela (cervical cancer cell line) cells were transfected with BTF3 siRNA (siBTF3) and cytotoxicity assays (MTT) were performed with various chemotherapeutic drugs. siBTF3-transfected (BTF3 K/D) cells showed significant resistance against cytotoxicity in cells treated with etoposide, compared with that of control cells. The resistance in the BTF3 K/D cells was blocked with pretreatment of Erk1/2 inhibitor (SCH772984). Increase of Erk1/2 phosphorylation was shown in BTF3 K/D cells in immunoblot analysis. Assays with co-transfection of siBTF3 and siErk1 showed the corresponding findings in MTT and immunoblot analyses. Baseline Bcl-xL protein level increased in BTF3 K/D cells and a higher Bcl-xL was maintained in the cells treated with etoposide. The resistance in etoposide-treated BTF3 K/D cells was blocked in cells pretreated with Bcl-xL inhibitor (A1155483) and in the cells co-transfected with siBcl-xL. Immunoblot analysis showed that Erk1/2 phosphorylation in BTF3 K/D cells was dependent on the Bcl-xL. Our data suggest that BTF3 has an inductive role on etoposide-induced cell death via down regulation of Bcl-xL expression and Erk1/2 activity in cervical cancer cells.

## P-35-113
## Hematopoietic- and neurologic-expressed sequence1 modulates tumorigenesis of hepatocellular carcinoma

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Despite the hematopoietic- and neurologic-expressed sequence1 (HN1) gene is expressed a high expression level in various cancers, its biological significance of HN1 in hepatocellular carcinoma (HCC) remains unclear. Thus, we explored the biological role of HN1 in HCC using HepG2 and SNU449 cells. Silencing of HN1 significantly diminished the viability and induced apoptosis of HCC cells whereas overexpression of HN1 stimulated the viability of HCC cells. In addition, silencing of HN1 inhibited the invasion and metastasis of HCC cells whereas overexpression of HN1 promoted the invasion and metastasis of HCC cells. In gene expression profiling, we identified 130 upregulated genes and 379 downregulated genes after HN1 silencing in HCC cells.


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Putative gene networks revealed suppressed expression of proteins associated with lipogenic signaling pathway. Silencing of HN1 significantly inhibited the expression levels of lipid genes of HCC cells whereas overexpression of HN1 increased the expression levels of lipid genes of HCC cells. Taken together, HN1 encourages the proliferation, invasion and metastasis of HCC cells, therefore, our results suggest that targeting HN1 may constitute a potential therapeutic strategy for HCC.

## P-35-114
## Frequency of somatic TP53 mutations in non–small cell lung cancer in Kazakhstan

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Lung cancer is one of the leading types of cancer in Kazakhstan, whose relevance in most countries of the world has not declined in recent years. In Kazakhstan 2016, it was 9.9% of the total number of new cases of cancer. In 2016, approximately 3635 new cases of lung cancer were detected. The 5-year survival rate for 2016 was 48.0%. The *TP53* protein is the most frequently mutated tumor suppressor in cancer, responsible for a range of critical cellular functions that are damaged by the presence of a mutation. Abnormality of the *TP53* gene is one of the most significant events in lung cancers and plays an important role in the tumorigenesis of lung epithelial cells. The aim of the present research was to identify mutations of *TP53* gene at patients with lung cancer living in Almaty, which is located in a seismically active area with a high radon emanation. The objects of research were peripheral blood samples and histological material obtained from patients with lung cancer after the surgery. Our cohort consisted of 44 paraffin-embedded NSCLC tumor specimens, where 4–10 exons of the TP53 gene were analyzed for mutations by direct sequencing. The mean age of the patients (male 42, female-24) was 62.7 years. It is also clear that smoking is a major risk factor for the lung cancer. According to a histological type, all cancer patients were distributed in the following way: a squamous cell of lung cancer – 63.64%, adenocarcinoma – 33.33%, a small cell lung cancer – 3.03%. All tumors were categorized by the stages using TNM criteria: stage I – 15 cases (22.73%); stage II – 22 cases (33.33%); stage III – 22 cases (33.33%) and stage IV – 6 cases (10.61%). In general, the results of sequencing revealed 21(31.82%) patients with any molecular genetic changes. These mutations were in 20 different codons, which there were not any correlations between mutation of *TP53* and sex, age, smoking status, stage of cancer development.

## P-35-115
## Attenuation of p53-alpha isoform transactivation by inverted repeat sequences in p53 target sites

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p53 is one of the most studied tumour suppressor proteins, playing important roles in regulating basic biological processes including cell cycle, apoptosis, senescence and metabolism. The human Tp53 gene contains alternative promoters and thanks to alternative splicing can produce several isoforms. p53 protein function is realized by binding to specific DNA response elements resulting in the transactivation of target genes. Here we present results of p53alpha isoform obtained using a yeast isogenic system for *in vivo* transactivation studies in chromosomal context to specifically evaluate the influence of secondary DNA structures on transactivation. We used a panel of *S. cerevisiae* haploid strains that are isogenic except for different p53 DNA binding sites positioned upstream of a luciferase reporter gene and chosen based on different propensities to form DNA structures. The targeting of the chosen p53 binding site was achieved by the *Delitto Perfetto* oligonucleotide targeting technique by the replacement of a double reporter ICORE cassette, facilitated by the induction of a single site-specific DNA double strand break. The obtained yeast reporter strains differing in the p53 target site (with and without propensity to form cruciform structure) were transformed with a plasmid for the expression of p53alpha. Our results show that transactivation is correlated better with the relative propensity of a response element to form cruciform structure than to its predicted DNA binding affinity. These results point to the fact that structural features of DNAs are an important determinant to its DNA-binding and transactivation function. In the follow-up experiments we would compare DNA-binding and transactivation potential of other p53 isoforms relevant in cancer development, expressed alone or co-expressed with p53alpha.

## P-35-116
## Thioredoxin-interacting protein (TXNIP) inhibits TLR4/NF-κB-induced survival of acute myeloid leukemia cells under fludarabine treatment

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The Toll-like receptor 4 (TLR4) signaling pathway is involved in many aspects of biological functions of acute myeloid leukemia (AML) cells, including the regulation of pro-inflammatory cytokine products, myeloid differentiation, and survival of AML cells. Thus, targeting TLR4 of AML patients for therapeutic purposes should be carefully addressed. In this report, we investigated the possible role of TLR4 as a regulatory factor against fludarabine (FA) cytotoxicity activity. We identified the differential expression of TLR4 and CD14 receptors in AML cell lines and examined their relationship to FA sensitivity. We found that the stimulation of TLR4 with lipopolysaccharide (LPS) in a TLR4-expressing cell line, THP-1, increased cell viability under FA treatment condition and showed that TLR4 stimulation overcame FA sensitivity through the activation of NF-κB, which subsequently upregulated several anti-apoptotic genes. The inhibition of TLR4/NF-κB signaling could partially or completely reverse LPS-induced cell survival under FA treatment conditions. Interestingly, we found that the expression of thioredoxin-interacting protein (TXNIP), a well-known tumor suppressor, was induced by FA treatment; however, it was suppressed by LPS treatment. Furthermore, the expression level of TXNIP was critical for FA-induced cytotoxicity or LPS-induced FA resistance of THP-1 cells. Our data suggest that TXNIP plays an important role in FA-induced cytotoxicity and TLR4/NF-κB-mediated FA resistance of AML cells. Therefore, TXNIP may be a potential therapeutic target for AML treatment.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-35-117
## Prostaglandin E2 produced by thyroid cancer suppresses NK cell functions by inhibiting NF-κB and MAPK/ERK pathways

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Natural killer (NK) cells play crucial roles in immune surveillance, however tumor microenvironment suppresses NK cell function and allows cancer cells to evade immune surveillance. In this study, we investigated the role of tumor microenvironment in thyroid cancer cells on NK cells. We found that prostaglandin (PG) E2 produced by thyroid cancer cells suppressed the cytolytic activity of NK cells by inhibiting the expression of the natural cytotoxicity receptors NKp44 and NKp30 and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). PGE2 and cyclooxygenase-2 were highly expressed in thyroid cancer cells; moreover, anaplastic thyroid cancer cells released higher amounts of PGE2 than papillary subtype. Suppression of NK cell functions by PGE2 was associated with the inhibition of nuclear factor (NF)-κB and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways via PGE2 receptor (EP)2 and EP4 on NK cells. In addition, PGE2 also inhibited the differentiation of NK cells and reduced their cytotoxicity against target cells. These results suggest that PGE2 promotes thyroid cancer progression by inhibiting NK cell differentiation and cytotoxicity. Thus, therapeutic strategies targeting PGE2 in thyroid cancer could potentiate immune response and improve patient's prognosis.

## P-35-118
## Knockdown of TFII-I enhances chemosensitivity of human myelogenous leukemia K562 cells to doxorubicin

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Multidrug resistance is one of the major reasons for human chronic myelogenous leukemia (CML) chemotherapy failure. MDR is mainly associated with the overexpression of drug efflux transporters of the ATP-binding cassette (ABC) proteins. Transcription factor TFII-I has been shown to regulate genes involved in cell cycle regulation, DNA repair, cellular differentiation, and in the response to cellular stress signals. Our previous studies showed that TFII-I deficiency reduced the proliferation of human chronic myelogenous leukemia (K562) cells two to three fold and increased the sensitivity toward doxorubicin mediated cell death at lower doxorubicin concentrations. The present study was aimed at investigating the effects of TFII-I knockdown on multidrug resistance and expression of the MDR-1 and MRP-1 genes. To reduce expression of TFII-I, K562 cells were transfected with the plasmid pGIPZ-shTFII-I. Control cells were transfected with a plasmid expressing a scrambled control shRNA (pGIPZ-shSc). The results of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis showed that the mRNA and protein expression of TFII-I was markedly suppressed. SC or TFII-I KD cells were incubated with different concentrations of doxorubicin (5 and 50 μM) for 24 h. MRP-1 gene expression wasn't change compare to the SC control cells in all groups but TFII-I KD cells expressed MDR-1 at higher levels in all groups. These results indicate that knockdown of TFII-I increased the sensitivity toward doxorubicin toxicity by a mechanism that does not involve multidrug resistance.

## P-35-119
## Prognostic markers in locally advanced TMPRSS2-ERG-positive prostate cancer

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Making decision whether to administrate adjuvant therapy after radical surgical treatment of locally advanced prostate cancer (LAPC) remains an unanswered problem. About 40% of prostate cancers are characterized with the expression of *TMPRSS2-ERG* fusion transcript and specific transcriptomic phenotype. In this work, we analyzed TCGA RNA-Seq data on LAPC and identified genes that are differentially expressed between groups of favorable and poor prognosis (82 patients). We focused only on *TMPRSS2-ERG*-positive tumors. Second, we tested if these genes maintain their prognostic significance on an independent sampling of Russian population (15 individuals). According to TCGA data, we identified at least 27 genes that statistically significantly discriminate two prognostic groups (Mann-Whitney test $P$-value < 0.05). Among them, there are several genes participating in the immune response*: PRKACB, ITPR2, TLR3, AZGP1, APOBEC3C, AOC1*, and other. Their expression is predominantly elevated in the group of favorable prognosis suggesting that immune response (possibly, tumor infiltrating lymphocytes) contribute to the better prostate cancer survival. Such trend is much more pronounced in the case of lymph-node negative LAPC (LAPC-N0). At least 12 genes that participate immune response are upregulated in the group of LAPC-N0 with better prognosis, including *IFNG, IDO1, MUC6, CXCL11, GBP1, UGT2B15*. The results derived on the independent sampling of Russian population did not repeat the findings based on TCGA: in the group of worse prognosis, we observed an increased expression of several genes participating microtubule cytoskeleton organization (including *TUBA4B, DNAI1, TEKT2, ROPN1L, CCDC114, SPEF1*). Additionally, we observed an elevated expression of many neuron-specific genes, and this indicates that neuroendocrine differentiation is an important factor of adverse LAPC prognosis. This work was funded by the Russian Science Foundation grant N 18-75-10127.

## P-35-120
## CDK6 is an essential signaling node in JAK2V617F-mutant MPN

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Myeloproliferative neoplasms (MPNs) summarize a disease group in which the myeloid precursors produce too many blood cells and frequently progress to acute myeloid leukaemia (AML). More than 80% of Philadelphia chromosome-negative MPN


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patients harbour the acquired somatic *JAK2*$^{V617F}$ mutation. This activates the transcription factor STAT5 by tyrosine phosphorylation (pYSTAT5), which initiates the transcription of target genes leading to increased production of megakaryocytes, erythrocytes or thrombocytes. Furthermore, the cell division protein kinase *CDK6* gene is frequently amplified or overexpressed in AML. CDK6 does not only function in G1/S phase transition, but also modulates transcription in a kinase-independent as well as kinase-dependent manner. In MPN diseases, inhibition of JAK2 is not curative and fails to induce a persistent response in most patients, emphasizing the need for the development of novel therapeutic approaches. We found that in the absence of *Cdk6*, JAK2$^{V617F}$ knock-in mice have a prolonged survival and disease symptoms are ameliorated. We describe a critical role for CDK6 in MPN evolution, as it interferes with disease hallmarks: besides regulating malignant stem cell quiescence, it promotes NFκB signaling and contributes to cytokine production while inhibiting apoptosis. We could not mirror the described effects on MPN cells with the CDK6-kinase inhibitor palbociclib, indicating that the functions of CDK6 in MPN pathogenesis are largely kinase-independent. In addition, pYSTAT5 levels are altered in JAK2$^{V617F}$-MPN cells lacking CDK6. In the next steps, we aim to explore how CDK6 influences STAT5 signaling in MPN cells. Our findings provide a rationale for targeting CDK6 levels in MPN to improve the quality of life of MPN patients.

## P-35-121
## Nrf2 modulates pancreatic tumor growth in the orthotopic mouse model

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Pancreatic ductal adenocarcinoma (PDAC) is a type of aggressive cancer characterized by a strong resistance to chemotherapy. PDAC remains one of the leading causes of cancer-related deaths, with a very low 5-year survival rate post diagnosis. A growing body of evidence suggests that novel therapeutic modalities against PDAC should target not only cancerous cells, but also specific tumor microenvironment, which could account for even up to 80% of the tumor mass. The aim of our study was to determine the role of cytoprotective transcription factor Nrf2 as a modulator of PDAC growth, as well as its response to chemotherapy. We used an orthotopic PDAC model in which Panc02 cancer cells were injected into the pancreata of the syngenic C57BL/6J mouse hosts, either wild type (WT; n = 30) or Nfr2 transcriptional knockout (tKO; n = 30) animals. This approach allowed to study PDAC in its natural pancreatic niche, also in the context of pancreatic cancer cell-normal cell interactions. To achieve this goal, we first introduced luciferase and green fluorescent protein (GFP) expression in Panc02 cells via lentiviral vectors. Panc02-Luc-GFP cells were then injected orthotopically into the pancreata of WT or Nrf2 tKO mice, and the tumor growth was monitored by noninvasive chemiluminescence measurements (IVIS). On the 12th day post cell injection, the mice were treated with the chemotherapeutic drug gemcitabine (50 mg/kg) alone, or together with the hypoxia-activated prodrug tarloxotinib (30 mg/kg); and the control mice received placebo. Our results suggest that microenvironment of the Nrf2 tKO pancreata is significantly less permissive for tumor growth compared to the WT organ, as assessed by the IVIS measurements and post mortem analyses. Also, tKO mice respond to the therapy better than the WT tumor hosts. This opens up a novel therapeutic avenue of Nrf2 modulation in order to improve the effectiveness of chemotherapeutic strategies against PDAC. *The authors marked with an asterisk equally contributed to the work.

## P-35-122
## Expression of nicotinamide N-methyltransferase in non-melanoma skin cancers

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Nicotinamide N-methyltransferase (NNMT) is a cytosolic enzyme which catalyzes the reaction of N-methylation of nicotinamide and other pyridine compounds. Several studies described enzyme upregulation in association with different solid tumors including melanoma, but there are no data regarding non-melanoma skin cancers (NMSCs). The most common forms of NMSCs are cutaneous squamous cell carcinoma (cSCC) and cutaneous basal cell carcinoma (cBCC), whose incidence continues to increase worldwide. Although surgery excision represents a successful strategy for the treatment of these neoplasms, an increasing number of cBCC cases relapse and many cSCCs display high rates of recurrence and metastasis. The aim of this study was to evaluate the potential role of NNMT in NMSCs. In particular, 40 cases of cBCC and 39 cases of cSCC were examined by immunohistochemistry to evaluate NNMT expression. Sections obtained from formalin-fixed and paraffin-embedded blocks were mounted on poly-L-lysine coated glass slides and incubated for 1 h at room temperature with rabbit polyclonal anti-NNMT antibody (1:1500). For each sample, cell staining was evaluated in at least ten fields and enzyme levels were expressed as percentage of positive cells. NNMT was significantly overexpressed in cBCCs compared with control samples, while in cSCCs enzyme levels were significantly lower than in normal samples. Moreover, NNMT expression was significantly lower in more aggressive forms compared with that detected in less aggressive ones, both for cBCC (infiltrative versus nodular) and cSCC (head-neck versus rest of the body). Results obtained demonstrated that NNMT dysregulation detected in NMSCs compared with control tissues is strictly related to the different histologic origin between cBCC and cSCC. In addition, enzyme levels seem to be inversely correlated with tumor aggressiveness, thus suggesting the potential role of NNMT as a prognostic biomarker.

## P-35-123
## Modulation of the Nrf2 signaling pathway by selected phytochemicals and their combinations in human pancreatic cancer cells

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Pancreatic cancer is characterized by a lower incidence compared to other gastrointestinal cancers, while the mortality rate remains significantly higher. Therefore, the search for new


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chemopreventive and/or therapeutic strategies is still required. One of the proposed strategies is modulation of Nrf2-ARE signaling pathway acting as a double-edged sword: Nrf2 activity protects cells and makes the cell resistant to oxidative and electrophilic stresses, whereas elevated Nrf2 activity may allow cancer cell survival and proliferation. Series of naturally or synthetic compounds can act as inducers or inhibitors of Nrf2-ARE signaling pathways. The aim of this preliminary study was to evaluate the effect of selected phytochemicals and their combinations on the expression and activation of Nrf2 transcription factor in human pancreatic cancer cell line PSN-1. The cells were maintained in standard conditions and incubated for 24 h with indole-3-carbinol (I3C), phenethyl isothiocyanate (PEITC), xanthohumol (X) and resveratrol (RES) at the dose of 5 μM, which was selected based on the results of the MTT test. The expression of Nrf2 and SOD controlled by this transcription factor was assessed by both r-t-PCR and Western blot. The lowest transcript and protein levels of both genes were observed as the result of treatment with X and PEITC used as single compounds as well as in combinations. The combinatorial treatment with I3C+X, X+RES, PEITC+RES exerted a similar effect. The reduced expression of Nrf2 accompanied its decreased activity, assessed by the measurement of its binding to an oligonucleotide containing the ARE consensus sequence. The inhibition of Nrf2-ARE pathway by naturally occurring phytochemicals in pancreatic cancer cells, suggest their possible role in protection against the multiple drug resistance phenotype resulting from Nrf2 up-regulation. Acknowledgements: This work was supported by grant no 2016/21/B/NZ5/01390 from the National Science Centre, Poland.

## P-35-124
## Differential Notch signaling as predictive values of colon cancer

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Colon cancer is one the most common and fatal malignancies worldwide. Novel approaches targeting cancer-related signaling pathways are of great importance in combating these disease. Notch signaling is an evolutionary-conserved pathway regulating essential cellular processes. However, the association between disease recurrence and Notch signaling in colon cancer remains unclear. Our study aimed to investigate the significance of differential expression of the Notch members on cancer-free recurrence in colon cancer. mRNAseq expression and clinical data of 297 patients of Colon Adenocarcinoma (COAD) cohort from The Cancer Genome Atlas (TCGA) were analyzed. Disease-free survival (DFS) analysis using Kaplan-Meier was also performed. Global biological differences between cancer-free recurrence groups were analyzed with Gene Set Enrichment Analysis (GSEA). To find differences in gene expression profiles between favorable/unfavorable groups, we used Weighted Gene Co-expression Network Analysis (WGCNA). Our results revealed that low expression of most of the Notch signaling members was associated with favorable prognosis. Amongst the overexpressed gene sets, within the unfavorable prognosis group; included functions related to epithelial-to-mesenchymal transition (EMT) such as focal adhesion, gap junction, adherens junction, Wnt signaling pathway, MAPK signaling pathway. WGCNA performed on adhesion and EMT markers showed that their expression clearly differentiates the groups. This research aims to highlight the potential of Notch members that could possibly serve as targets and prognostic markers to indicate the risk of disease recurrence. Moreover, downstream Notch signaling tends to affect cell cycle, tissue remodeling and architecture, thus indicating its potential to trigger EMT. Acknowledgements: The study was founded by the National Science Centre, Poland nr 2016/23/N/NZ5/02575.

## P-35-125
## Blocking osteopontin signaling inhibits glioma invasiveness and reduces tumor growth of intracranial human gliomas in nude mice

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Glioblastoma (WHO grade IV, GBM) is the most frequent primary malignant brain tumor in adults which despite many years of research and some advancement in GBM therapy remains incurable. We and others demonstrated that microglia and peripheral macrophages infiltrating tumor microenvironment play an supportive role in GBM growth, invasion and poor responses to treatment. Microglia are the brain resident macrophages and constitute the first line of immune defense. These cells respond to pathogens or injury by polarizing to pro-inflammatory cells, which also carry on antitumor activity in benign gliomas. However in GBMs, instead of fighting the tumor, glioma-associated microglia and macrophages (GAMs) acquire a pro-tumorigenic phenotype, express factors that support invasion and angiogenesis, while inducing immunosuppression. We found that osteopontin (secreted phosphoprotein 1, SPP1) secreted by glioma cells is a key factor involved in microglia polarization and supporting glioma invasiveness. SPP1 is known to interact with αVβ3 and αVβ5 integrins via RGD motif. We developed short interfering RGD peptides that block the ligand-integrin signaling and in the Matrigel Matrix cell invasion assay inhibited human glioma cell invasion stimulated by human or mouse microglia. Similar effect was observed after stable genetic SPP1 depletion of osteopontin in U87MG glioma. Moreover, implantation of U87MG cells with silenced expression of *SPP1* into the striatum of Athymic nude mice reduced tumor growth *in vivo*. Immunohistochemical analysis was performed to verify the phenotype profile of tumor infiltrating microglia in SPP1-depleted and control tumors. Our findings display a vital role of osteopontin/SPP1- integrin signaling in shaping the phenotype of tumor-associated immune cells and supporting glioma growth.

## P-35-126
## Apoptotic effects of progesterone and its analogues mediated by membrane receptors in human tumor cells

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Progesterone (P4) plays an important role in female reproductive functions. It is involved in regulation of proliferation and apoptosis as well. P4 action is mediated by two types of receptors – nuclear receptors (nPRs) and membrane receptors (mPRs). The effects of P4 mediated by different types of receptors can vary considerably. In our laboratory, two mPRs selective ligands were identified: 19-hydroxypregn-4-en-20-one (I) and 19-hydroxy-5β-pregn-3-en-20-one (II). The purpose of this work is to study the mechanisms of action of P4 and mPRs selective ligands on the expression of genes associated with apoptosis in pancreatic adenocarcinoma BxPC3 cells. These cells have high level of mPRs


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mRNA and lack of nPRs mRNA. First of all we tested the effect of different concentrations of P4 and its analogues on transcription of the genes coding for apoptosis-related factors in BxPC3 cells. Secondly we use specific protein kinase inhibitors to determine the involvement of p38 MAPK and JNK in hormone-dependent changes on expression of apoptosis-associated genes. All three compounds did not affect the expression of Apaf1, bcl-2-alpha, BIK, Casp8, FAS and TP53 genes. In the absence of inhibitors, only compound II significantly increased the expression of proapoptotic DAPK and HRK genes at the maximal concentration used. In the presence of the p38 MAPK inhibitor, the compound II did not influence DAPK expression, but the expression of the HRK gene was still increased. The JNK inhibitor did not change the effects of compound II on DAPK and HRK genes expression. At the same time, P4 and compound I also increased the level of HRK mRNA at the maximal concentration used. Thus, compound II enhances the expression of two pro-apoptotic factors out of nine studied, and these changes are not dependent on JNK pathway. The effect of compound II on the level of DAPK mRNA is mediated by p38 MAPK. The work was supported by Russian Foundation for Basic Research (project 17-04-00234).

## P-35-127
## Melanoma cells resistant to vemurafenib exhibited changes in expression profile of growth factor receptors and elevated invasive abilities

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The therapy of melanoma poses a substantial challenge, mainly due to the increased incidence of metastasis and mortality rate among cancer patients. Malignant cells are often characterized by overexpression and hyperactivation of growth factor receptors and their signaling cascades, as well as by mutations present in proteins involved in regulation of cell proliferation and motility. The V600E mutation found in Braf protein, the part of Ras/MAPK cascade, is the most common aberration discovered in melanoma patients. However, patients treated with vemurafenib (Braf V600E inhibitor) often develop resistance to the treatment. In order to study resistance mechanisms of melanoma cells and offer new targets for therapy, we generated vemurafenib-resistant cell lines (A375 and WM9). Obtained cells exhibited increased expression of EGFR and MET (epidermal and hepatocyte growth factor receptors, respectively), with simultaneous loss of ErbB3 and ErbB4 expression. Additionally, even in the absence of growth factors, resistant cells demonstrated activation of upstream effectors. We also performed migration and invasion assays in 2D and 3D conditions, and observed that resistant cell lines exhibit elevated motile activities. In our previous studies in A375 and WM9 cell lines treated with EGFR and MET inhibitors (lapatinib and foretinib, respectively), we observed drugs' synergistic cytotoxic effect and their impact on invasive abilities. Here, we show that resistant cell lines differ in their sensitivity to inhibitors' treatment compared to the parental lines, and this occurrence can result from the changed expression profile of EGFR and MET. To conclude, further research concerning the resistance mechanisms in melanoma treatment is essential to improve anti-cancer therapy, and growth factor receptors show promise as potential targets. This project was funded by National Science Centre – OPUS 8 (No. 2014/15/B/NZ5/01467) and 15 (No. 2018/29/B/NZ5/00967).

## P-35-128
## Transcriptional competence of HSF1 activated by 17β-estradiol or heat shock

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Heat Shock Transcription Factor 1 (HSF1) is strongly activated under stress conditions, and after binding to heat shock elements (HSEs), it primarily induces the expression of heat shock genes. The HSF1 function as the major the heat shock response transcriptional activator is well described in experiments utilizing severe hyperthermia (HS) or other acute stresses. However, there is limited information about HSF1 activity when the stress signal is mild but chronic. We found that 17β-estradiol (E2) stimulated HSF1 phosphorylation on Ser326 in estrogen receptor-positive MCF7 cells. Compared to the strongly HS-induced phosphorylation of HSF1 on S326 (about 10-fold), E2-induced phosphorylation (about 2-fold) appears to be moderate. Thus, we analyzed whether HSF1 is transcriptionally active in response to E2 treatment, i.e. if it is able to bind to DNA (ChIP-seq) and regulate the expression of target genes (RNA-seq). In response to E2 or HS, HSF1 binds to regulatory regions of 358 or nearly 9000 genes, respectively (294 common genes for both treatments). Only 63 genes from 358 showed expression changes after E2 treatment (only 9 genes were downregulated). HSEs were detected in 28 of 63 regulatory regions. Validation experiments using qPCR confirmed E2-induced HSF1 binding and expression changes in case of HSPB8, LHX4, NBPF1, PRKCE, WWC1, which suggest potentially direct regulation of these genes by HSF1. It should be noted, that HSF1 enrichment in the same loci was much higher after HS treatment than after E2 stimulation (which was consistent with higher HSF1 phosphorylation level) but did not translate into higher expression level of these genes. We conclude that the ability of HSF1 to bind to DNA depends on its phosphorylation level, but the binding strength does not determine changes in the expression level of regulated genes. This work was supported by Polish National Science Centre, Grants 2014/13/B/NZ7/02341 and 2015/17/B/NZ3/03760.

## P-35-129
## KRAS status-dependent sorting of lung cancer-associated long noncoding RNAs (lncRNAs) in exosomes released by A549 lung adenocarcinoma cells

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Exosomes are membrane-bound nanovesicles secreted by both normal and cancer cells into the peripheral circulation. As a result of their biogenesis, exosomes harbor genetic material reflective of their cell of origin, and are capable of shuttling these contents into recipient cells to modulate various biological functions. As such, identification of cancer-associated cargo may help elucidate the mechanisms underlying cancer development and progression, with translational application as minimally invasive biomarkers. This is particularly beneficial for lung cancer, the leading cause of cancer mortality worldwide, which is often diagnosed at the late stage and accompanied by chemoresistance and metastasis. In this study, exosomes from the A549 lung adenocarcinoma cell line were isolated, and characterized by transmission


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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electron microscopy and western blot analysis. Semi-quantitative and real time PCR analysis show that certain long noncoding RNAs (lncRNAs) are either preferentially retained in the cells or packaged into exosomes. KRAS mutations, the most common molecular aberration in lung cancer, strongly influenced cargo loading. Exosomes derived from KRAS G12D-transfected cells exhibited differentially expressed levels of lncRNAs responsible for the regulation of cell migration and proliferation, consistent with the reported phenotype of KRAS G12D-driven lung adenocarcinoma. In line with this, transcriptomics analysis of KRAS-expressing cell lines is currently underway to identify oncogenic RNAs as well as potential novel biomarkers. To further demonstrate the role of exosomes as intercellular messengers, the internalization of A549-derived exosomes by normal lung fibroblasts, a frequent site of metastasis, was visualized. Taken together, these results indicate that cancer cells utilize exosomes via their molecular cargo to promote an oncogenic state by influencing recipient cells, supporting a form of cell to cell communication for mutant KRAS in lung cancer.

## P-35-130
## Transcriptome analysis of acromegaly patients with and without medical therapy

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The pituitary is one of the most important endocrine glands in the human body. It consists of two anatomically and functionally diverse parts – anterior and posterior pituitary. The main function of anterior pituitary includes hormone secretion, such as growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Pituitary adenomas are benign intracranial endocrine tumors with potentially high prevalence in population. As they arise, patients may develop a wide range of health problems. Somatotroph adenomas, which develops from anterior pituitary somatotroph cells, are characterized by increased synthesis and secretion of GH. They constitute 10–15% of all pituitary adenomas and usually cause serious disorders, like acromegaly or gigantism. In clinical practice, a number of adenomas treatment tactics are used, like resection or somatostatin (SSA) or dopamine (DA) analogue therapy, but they are not always effective. The main purpose of this study was transcriptome analysis of acromegaly patients with and without SST/DA analogue therapy. Total RNA was extracted from somatotroph adenoma tissue of acromegaly patients with and without SST/DA analogue therapy before surgery. Transcriptome libraries were processed and sequenced using IonProton System for Next-Generation Sequencing. After that, obtained data were analyzed using the Galaxy platform. Final results will be presented in the poster session. Work has been developed within the framework of the ERDF project no. 1.1.1.1/16/A/066 "Molecular markers of pituitary tumor development, progression and therapy response".

## P-35-131
## Dissecting the molecular mechanism of CDK8 and cyclin C in NK cells

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Cyclin-dependent kinases (CDKs) are serine/threonine kinases that are important regulators of the cell cycle and transcription. CDK8, whose kinase activity is regulated by cyclin C, acts by associating with the mediator complex or by directly phosphorylating transcription factors, thereby repressing or activating transcription of certain genes. Oncogenic activity of CDK8 has been reported in several cancer types and targeting CDK8 represents a potential therapeutic strategy. We have recently shown that loss of CDK8 in NK cells leads to increased cytotoxicity and improved tumor surveillance. These findings suggest that targeting CDK8 in cancer could hit two birds with one stone – enhancing NK cell activity while simultaneously blocking tumor cell proliferation. We now aim to dissect the mechanism underlying the inhibitory effect of CDK8 in NK cells. Therefore, we will analyse effects of cyclin C deficiency, which impairs CDK8 kinase activity, in parallel to CDK8 deficiency in NK cells making use of *Cdk8*$^{fl/fl}$ and *cyclin C*$^{fl/fl}$ *Ncr1-Cre* mice, lacking the indicated genes in NKp46$^{+}$ cells. This will allow us to characterize NK cell functionality *in vitro* as well as *in vivo*. The results will elucidate whether CDK8-mediated suppression of NK cell activity is kinase-dependent or not. Importantly, we aim to extend the research to human NK cells and investigate the effects of CDK8/Cyclin C deficiency and CDK8 inhibitors on NK cell activity.

## P-35-132
## Polyamine analogues are capable to inhibit the prostate cancer cells proliferation

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Polyamines (PA) are important for cell growth. It is known about the high concentration of PA in tumor cells compared to normal cells, which contributes to the proliferation and invasion of tumor cells. PA metabolism is an important target for anticancer therapy. The purpose of the study was to evaluate the significance of the structural PA analogue N,N,N',N'-tetrabutylpropan-1,3-diamine (TBP) and other PA analogues for chemotherapy of human prostate cancer. The effect of TBP on prostate cancer culture cells LNCAP clone of FSK (ECACC 89110211) and the basic mechanism of this effect were studied. LNCAP cells (Sigma-Aldrich) were grown in RPMI-1640 medium containing 10% calf serum, 100 μg/mL streptomycin and 100 U/mL penicillin in wet 5% CO2 at 37 °C. The cell proliferation was determined by MTT test. The activity of polyamine oxidase (PAO), a key enzyme involved in PA decomposition, was assayed by the formation of hydrogen peroxide during spermine oxidation by PAO. TBP has been shown to inhibit the growth of prostate cancer cells of the LNCAP line by activating a key enzyme in PA catabolism and has potential value for clinical


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therapy of human prostate cancer. Synthetic analogues of PA N, N'(1,3)-dibutylpropan-1,3-diamine and N,N(1,1)-dibutylpropan-1,3-diamine are capable to inhibit the cell growth of prostate cancer lines LNCAP and activate PAO in different degrees. PAO is known to be an important factor in the induction of cell apoptosis. And N,N(1,1)dibutylpropan-1,3-diamine exceeds its antiproliferative activity of TBP, causing a stronger activation of the PAO. The disadvantage of this analogue is the need for higher concentrations. It is necessary to continue the search for effective analogues of PA, as well as search for combinations of PA analogues with other anticancer drugs. This work was financially supported by the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008) and by the "RUDN University Program 5-100".

## P-35-133
## Potential role of p53/MDM2 and PTEN/PI3k pathways in shaping the activity of ECs in hypoxia

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Hypoxia, the condition of lower-than-physiological oxygen level in tissue, is responsible for the modulation of distinct cell populations activity and shapes the tumor microenvironment. One of the key players of this phenomenon are endothelial cells (ECs) that achieve angiogenesis. ECs are regulated by hypoxia which, among other effects, regulates the PTEN/PI3k axis. ECs in response to hypoxia, activate a multilevel proangiogenic response. Mdm2–p53 pathway is a master regulator of cell activity and growth. Aim of the experiment was to establish if hypoxia affects this pathway in ECs in relation to PTEN activity and to characterize how this pathway is affected in ECs, according to their organo-specificity and their biological state i.e. mainly in relation to their pathologic state in the tumor site. Established cell lines from the endothelium of healthy tissue and primary tumor isolated from the same patient with breast cancer, were cultured *in vitro* in hypoxia (~ 1% $pO_2$) and normoxia (~ 19% $pO_2$) for 48 h. Western blots were used to measure PTEN, Mdm2, p53 in cell lysates. Secretion of VEGF into the medium was detected by ELISA and cell proliferation was studied by Alamar blue assay. ECs from healthy breast tissue in response to hypoxia are characterized by the down regulation of p53 and Mdm2 but not PTEN. Although the proangiogenic response was activated in hypoxic conditions (VEGF was increased), a slower cell proliferation was observed. Tumor derived ECs cultured in low $pO_2$ did not undergo a modification in the growth rate, Mdm2 amounts, whereas p53 and PTEN were down regulated comparing to normoxic conditions. These results suggest that cancer tissue ECs expressed disturbed phenotype what may mediate pathologic angiogenesis in hypoxic tumor microenvironment (The research was funded by Kościuszko I, CRU/WIM/1435/2016).

## P-35-134
## Integrated cytogenetic and biological analysis of selected cell culture models of urothelial bladder malignancies

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Urothelial carcinoma is one of the most frequent cancer types with a pronounced molecular and clinical heterogeneity. Urothelial carcinoma cell lines are useful experimental systems to model various aspects of cellular heterogeneity and clinical progression. Among others, urothelial carcinoma cell line RT112 have been extensively used to model clonal evolution and therapy resistance, and we were able to follow both of these processes by thorough cytogenetic analysis of RT112-derived sublines. We could demonstrate a separate, hitherto undescribed clonal evolution pathway, with a highly transformed RT112-derivative (RT112-Heidelberg) as mother cell line finally leading to the doxorubicin resistant subline RT112/D21. On the functional level, RT112-Heidelberg feature pronounced density dependent plasticity, reversibly switching between epithelial phenotype in sparse cultures to EMT-like phenotype in confluent cultures. Strikingly, only the epithelial phenotype is clonogenic in anchorage-independent assay, and highly dependent on oxidative phosphorylation. Besides this clonogenic and metabolic plasticity, we see complex changes in expression of various stemness genes. Bladder sarcoma represents a dramatically rarer tumour type. The most frequent mesenchymal bladder tumour is the inflammatory myofibroblastic tumour (IMT), which, too, represents a range of phenotypes, from indolent myofibroblastic proliferation to overt sarcoma. There is also a pronounced heterogeneity at the molecular and karyotypic level, frequently involving the gene coding for ALK kinase (chr 2). We were able to establish (to the best of our knowledge the first) IMT tumour cell line. Detailed cytogenetic analysis revealed a hitherto undescribed translocation t(3;18). Possible molecular mechanisms of myofibroblastic transformation will be discussed. Supported by the Czech Science Foundation Project No 17-17636S and Charles University Specific Student Research Projects Nr. 260394/2017, Nr. 260393/2017.

## P-35-135
## Modulation of α7 nicotinic acetylcholine receptors by human prototoxin Lynx1 induces apoptosis in lung cancer cells

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Lynx1 is the GPI-anchored modulator of nicotinic acetylcholine receptors (nAChRs) found in the mammalian neuronal and non-neuronal cells. Previously we showed that Lynx1 structural homologs, secreted proteins SLURPs control the growth of epithelial cancer cells via interactions with nAChRs. Here we studied a Lynx1 expression and activity in human non-neuronal cells. Lynx1 is expressed both on mRNA and protein levels in normal oral keratinocytes, and lung, colon, epidermal, and breast cancer cells, but not in embryonic kidney cells. Lynx1 co-localizes with α7-nAChRs on the cell membrane and in intracellular


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compartments in all types of cells studied. Treatment of non-small lung cancer A549 cells with water-soluble variant of Lynx1 (ws-Lynx1) affects the cells proliferation. ws-Lynx1 interacts with α7-nAChRs and activates different intracellular signaling pathways subsequently resulted in the phosphorylation of proapoptotic tumor suppressor protein p53 and different kinases participated in the regulation of gene transcription, cell growth, adhesion and differentiation. We also revealed externalization of phosphatidylserine, the early apoptosis marker, upon the ws-Lynx1 treatment. Our data revealed that endogenous prototoxins which modulate α7-nAChRs activity are the promising tools for the treatment of cancers of epithelial origin. The study was supported by the Russian Science Foundation (Project No. 17-74-20161).

## P-35-136
## Novel method for intravital investigation of anticancer drug pharmacokinetics and metabolite detection in mice tumors

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Antitumor drugs demonstrate different effectiveness for the specific tumors therapy. In this regard, method that allows to rapidly and precisely assess the effectiveness of a drug for a particular tumor in a living model is required. In this study, a novel highly sensitive electrochemical method using nanoelectrodes with different chemical modifications is offered for intravital investigation of antitumor drugs pharmacokinetics directly in tumor at a single cell level. This approach allows to detect changes in the metabolites levels, such as oxygen, hydrogen and reactive oxygen species (ROS), inside the tumor in response to tumor therapy. In the framework of the project we first developed the technique for building nanoelectrodes, tumors preparation and data analysis. Using cisplatin as an antitumor drug the dynamic of its accumulation in 4T1 murine breast cancer after intravenous injection was investigated. Its concentration in the tumor was shown to be low during first hour after administration and reached maximum level 17 h later. Besides, the ability to examine metabolites in tumor and healthy tissues using the developed model was confirmed. We demonstrated lower pH values inside the tumor compared to normal tissue. Wherein, acidity level varied from tumor to tumor and within a single tumor depending on the specific area. The decrease in the oxygen level was found while moving deep into the tumor. Finally, ROS changes were revealed in different tumor parts. Thus, the suggested approach can be used for drug delivery *in vivo* tracking and studying tumors metabolites changes as well as investigation of tumors heterogeneity. The work was supported by the Ministry of Education and Science of the Russian Federation, Agreement No. 14.575.21.0147 (Unique identifier RFMEFI57517X0147).

## P-35-137
## Remote apoptosis resulted by external magnetic field in cancer cells loaded with magnetic nanoparticles as potential therapeutic method

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Modern cell biology is mainly focusing on optimization of cell targeting methods, intracellular uptake mechanism, drug and gene delivery and developing suitable transport agents for these purposes nowadays. Magnetic nanoparticles, SPIONS (Superparamagnetic iron oxide nanoparticles) are known as potential delivery agents by virtue of its relatively low cytotoxicity, its paramagnetic properties and overall effectiveness. Previous studies prove, that application of MF (magnetic field) significantly enhance nanoparticles uptake by living cells and cause no cell membrane damage. Moreover, it was reported recently that nanoparticle agglomerates are internalized with lysosomes in SPIONs loaded cancer cells. Together with application of MF these findings could be used as tentative method for the remotely controlled induction of cell death. Our work propose proof of concept in remote apoptosis induced by external pulsed MF in SPIONs-loaded cancerous cells. The uptake rate were enhanced by high intensity (7 T) short pulse width (~ 15 μs) magnetic field application on two hepatocellular carcinoma cell lines (Huh7 and Alexander) and one blastoma (HepG2) loaded with SPIONs of different concentrations. Viability rate, relative fluorescence measurement and confocal imaging reported that cells loaded with the highest concentration of nanoparticles (100 μg/mL) significantly induces cell death. For relatively big nanoparticle clusters magnetic force reaches already critical value for lysosomal rupture ~ 500 pN. To summarize, nanoparticle clusters colocalized with lysosomes induce lysosomal membrane permeabilization and following release the lysosomal content into the cell cytosol and cause apoptosis. Our proof of concept displays potential methods for effective intracellular uptake and cell labelling within pulsed MF and provide promising tool for further remote apoptosis induction in biomedicine and cell signalling research.

## P-35-138
## Age-related benign prostatic hyperplasia in rat is linked with the presence of polysaccharide deposits rich in O-glucosidic bonds

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Age-related benign prostatic hyperplasia (BPH) may lead to inflammation of the prostatic gland and can contribute to the development of prostate tumours. Mucopolysaccharide is a characteristic biomolecule present in amyloid deposits that are identified to be present regularly in BPH. However, the exact role and


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composition of these amyloid deposits are not known. Taking this into account, the main objective of this work is to determine in prostatic rat samples the molecular features of PAS-positive amyloid deposits in BPH. For this purpose, prostatic tissues from 12-month-old male Wistar rats were sectioned and stained by Periodic Acid-Schiff (PAS) technique. Using this method, we have corroborated carbohydrate presence in rat PAS-positive prostatic tissues that confirmed the existence of a rich carbohydrate secretion. Subsequently, 400 mg of prostatic samples were homogenized with 1 mL of buffer using a basic homogenizer. Homogenates were then centrifuged at 13,000 ***g*** for 10 min at 4 °C and the supernatant was stored at −80 °C. Later on, 30 μL of supernatant were incubated at 37 °C with either a-amylase (8 UI) or amyloglycosidase (8 UI) or N-glycosidase (4 UI). After different times of incubation, 1 and 2 h, the enzymatic reaction was stopped by submerging the sample in boiling water. Finally, glucose concentration was measured in each sample, these results show a significant increase of glucose concentration in samples incubated 1 and 2 h with α-amylase or amyloglycosidase. On the contrary, glucose does not increase in samples incubated with N-glycosidase at the same times. In conclusion, our data suggest that amyloid deposits linked to rat BPH are composed by a substance with high molecular weight, highly ramified polysaccharide with a predominance of O-glucosidic bonds. More in-depth analyses are required to identify the exact nature of this polysaccharide present in rat prostatic hyperplasia. *The authors marked with an asterisk equally contributed to the work.

## P-35-139
## Cell-free oxidized DNA: an activator of DNA repair in human glioma cells

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Astrocytoma is a malignant glioma and is one of the deadliest forms of human cancer. Patients with glioma are treated with radiation therapy despite the fact that these types of tumor are fairly resistant to radiation. It is important to understand the mechanisms of glioma's resistance to chemotherapy and radiotherapy. Ionizing radiation used in radiotherapy induces double-strand breaks in the DNA of cell nuclei, dead cells release oxidized DNA into extracellular matrix. As we have shown earlier, oxidized cell-free DNA can induce DNA reparation and DNA Damage Response (DDR) in human MSC leading to resistance of MSC to subsequent damage. In this work we investigated the effect of oxidized cell-free DNA on human astrocytoma cells. Oxidized cell-free DNA was added in concentration 50–100 ng/mL to the cultivated astrocytoma cells 1321NI *in vitro*. The level of DSB in the cells was assessed with flow cytometry, gene expression level – with real-time PCR (RT-PCR). Oxidized cell-free DNA (50–100 ng/mL) causes oxidative damage to the nuclear DNA of astrocytoma cells 15–180 min after exposure: 8-oxodG level increases 2–3- fold. After 3 h of incubation the amount of cells with DSB increases 1,5-fold. A simultaneous increase in expression of CDKI (cyclin-dependent kinase inhibitors) – CDKN1A 2–3-fold and CDKN2 5–7-fold was observed. This indicates arrest of G1/S transition and reparation activation. Expression of DNA reparation genes BRCA1 and BRCA2 increases 3–5-fold. Thus, oxidized cell-free DNA may act as a stress signalling molecule activating DNA reparation in astrocytoma cells exposed to radiation therapy. The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation, supported in part by RFBR (project No. 17-29-06017ofi_m and No. 16-04-00576_A).

## P-35-140
## Mechanism of glioma progression and its correction by verapamil, a calcium channel blocker

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It is very important to understand mechanisms of glioma progression promoting patients death. The uncompleted third stage of inflammation (proliferation and reparation) may be considered a precondition for tumor associated inflammation (TAI) and offers one of the basic causes of epithelial-mesenchymal transition (EMT). The polyamine catabolic enzymes diamine oxidase (DAO) and polyamine oxidase (PAO) are known to be involved in control of immune cells proliferation, as well as inflammation and EMT. The decrease of PAO activity is observed when activated of cellular division. The lymphocyte TMP lowering over a long period of time leads to peripheral blood lymphoblasts appearing in large quantities and their migrating to gliomas necrotic center. In this work we evaluated the genome stability and Snail-gene expression in lymphocytes taken from glioma patients after phytohemagglutinin (PHA) stimulation. The blast genome was shown to contain substantial number of chromosomal aberrations that can prevent from normal reparation processes and influence the Snail expression. We hypothesize that lymphoblasts with a decreased transmembrane potential (TMP) can influence the reprogramming of stem cells from multipotent to pluripotent and even totipotent phenotypes. Subsequent proliferation of blast cells with large regenerative potential may help to expound of glioblastoma multiforme morphological phenomenon. We explored the action of verapamil that is a well-known calcium-channel blocker on PHA-stimulated lymphocytes. The results showed that verapamil application causes TMP level increase (blood cells aggregation decrease), lymphocyte proliferative activity decrease, increase of DAO and PAO activity in their cultural liquid and suppression of gene Snail expression. Such pathogenic approach in clinical conditions may lead to substantial suppression of further glioma progression in remote postoperative period. This study was supported by the "RUDN University Program 5-100".

## P-35-141
## Nutrient deprivation induces lipid alterations and reduction of invasivity in triple negative breast cancer cells

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Tumor cells exhibit an altered metabolism compared with non-transformed cells, consuming glucose, glutamine and lipids as nutrients. One consequence of deregulated tumor metabolism is that cells can become dependent on the supply of metabolic


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substrates such as glucose or glutamine for viability and migration. A growing body of evidence *in vitro* and *in vivo* has demonstrated that various kinds of dietary restrictions induce an increase of life expectancy and a reduction of cell proliferation, ensuring well-being. On the contrary, there is a positive association between obesity and breast cancer mortality. Breast cancer is very intricate disease due to its heterogeneous nature. One subtype is Triple Negative Breast Cancer (TNBC), which is deficient in the estrogen receptor (ERα), progesterone receptor (PR), and human epidermal growth factor receptor (HER2) expression. Since there is a complex network that exogenous nutrients and cancer abnormal metabolism, we have evaluated the effects of nutrient deprivation on cell migration and lipid metabolism in TNBC cell line. The results obtained indicate that the different medium restrictions reduce cell viability and migration greatly influencing the lipid pattern of MDA-MB-231 cells. We measured total, phospholipid and neutral lipid fatty acids, especially arachidonic (AA) and eicosapentaenoic (EPA) acids which are precursors of eicosanoids involved in the cross-talk between cancer cells and immune cells. The data suggest significant changes in lipid composition, especially in omega-6/omega-3 ratio. Moreover, we have observed alterations of triglyceride and sterol content. In conclusion, nutrient deprivation influences lipid phenotype and invasiveness of TNBC cells suggesting a possible future approach for the prevention and treatment of breast adenocarcinoma, although further studies are needed to demonstrate its efficacy.

## P-35-142
## Lemon peel polyphenols affect invasiveness of human gastric AGS and MKN28 cells by inhibition of IL-6 induced matrix metalloproteinase-9/2

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Polyphenols are very abundant in food of mediterranean diet and, in the last years, they are subjects of increasing scientific interest for their potential health benefits against development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. In this study, we investigated the effects of lemon peel extracts (LPE) on human gastric adenocarcinoma MKN28 and AGS cell lines. First, we evaluated the effect of LPE on the invasive ability of AGS and MKN28 cells stimulated with IL-6, a pro-inflammatory factor. Migration and Matrigel invasion assays demonstrated that the pre-treatment with LPE (0.5 or 1 μg/mL gallic acid equivalent) provoked an inhibition of cell invasiveness induced by IL-6. In addition, LPE preincubation prevented the increase of Matrix Metalloproteinases (MMP)-9/2 mRNA and protein levels, whose expression is up-regulated by IL-6. Our results indicated that the pre-treatment with LPE was able to reduce MMP-9/2 expression at both protein and enzyme activity levels in the conditioned media of IL-6 stimulated MKN-28 and AGS cells. Finally, we have analyzed the effect of LPE on STAT3 levels, a transcriptional factor that becomes activated after phosphorylation by IL-6. In conclusion, our results suggest that LPE reduces the invasiveness of gastric MKN-28 and AGS cancer cells through the reduction of IL-6 induced MMP-9/2 up-regulation. Therefore, our data confirm the protective effects that LPE could exert against the metastatic process in gastric cancer.

## P-35-143
## L-canavanine enhances protein misfolding under arginine deprivation in human glioblastoma cells

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Arginine deprivation is being increasingly recognized as a promising approach for controlling malignant tumor growth. One of the currently unsolved problem in this anti-tumor strategy is ability of cancer cells to restore their growth after the treatment. One of the drugs that could be potentially used in combination with arginine deprivation is its analogue, L-canavanine. It has been shown that L-canavanine can effectively incorporate into proteins instead of L-arginine, causing protein dysfunction, resulting possibly from activation of unfolded protein response (UPR). Our recent data indicate that L-canavanine under arginine deprivation substantially decreases cell viability of human U251MG and U87MG glioblastoma cells but not of primary rat glia cells, used as control. In order to confirm whether these effects are indeed associated with protein misfolding, we analyzed the effects of L-canavanine on the endoplasmic reticulum (ER) and mitochondria functions. We observed that L-canavanine under arginine deprivation caused accumulation of glucose-regulated protein (GRP78), thus indicating an increase in the level of misfolded proteins within the ER lumen. Also, we showed that under arginine starvation L-canavanine could lead to inhibition of protein synthesis. We demonstrated that L-canavanine under arginine deprivation evoked substantial changes in the level of molecular chaperones such as mtHsp70 and Hsp60. Moreover, in response to the proteotoxic stress, we observed significant changes in mitochondria metabolism and induction of cell death. Thus our findings show that L-canavanine under arginine starvation promoted significant changes in the UPR in human glioblastoma cells, that might be potentially used for the development of an effective anticancer therapy. This work was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 665735 granted to the Nencki Institute.

## P-35-144
## Triptolide overcame autocrine growth hormone (GH) mediated resistance in a dose-dependent manner in MDA-MB-453 breast cancer cells via acting on EMT pathway

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Breast cancer is one of the most common cancer types among women. Beyond environmental and genetic risk factors such as age and genetic background, many growth factors (e.g. VEGF) and hormones (estrogen) are known to affect the development of breast cancer. Recently, autocrine growth hormone (GH), an essential hormone affecting post-natal growth, has been shown to trigger cell proliferation, invasion, metastasis, and also lead to drug resistance. Upregulation of GH and its receptor (GHR) expression was determined in mammary carcinoma cells. Forced GH expression induced drug resistance against tamoxifen, doxorubicin, mitomycin and curcumin in MCF-7, T47D and BT-474 breast cancer cells. Triptolide is a diterpenoid triepoxide which have been demonstrated to have multiple biological activities and also triggers apoptotic cell death in breast cancer cells. In this


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study, our aim was to investigate the role of epithelial-mesenchymal transition (EMT) signalling during triptolide-induced apoptotic cell death in MDA-MB-453 wt and GH+ breast cancer cells. Triptolide decreased cell viability in a dose-dependent manner, mitochondrial membrane potential loss and nuclear fragmentation were observed. 20 nM triptolide decreased cell viability by 25% and 15% in MDA-MB-453 wt and GH+ cells, respectively. Although autocrine GH expression triggered cell proliferation and colony formation in MDA-MB-453 cells, triptolide (20 nM) treatment suppressed forced GH-mediated invasion and metastasis. Furthermore, autocrine GH mediated aggressive profile was prevented by triptolide treatment through Slug, N-cadherin, Vimentin downregulation within 24 h. Triptolide induced caspase-dependent apoptotic cell death via modulating Bcl-2 family member's expression profile in MDA-MB-453. In consequence, autocrine GH overexpression increased aggressive phenotype of MDA-MD-453 breast cancer cells, however triptolide treatment overcame this resistance mechanism and induced apoptotic cell death.

## P-35-145
## EpCAM is a vital mediator of radioresistance and metastasis in breast cancer cells

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Radioresistance and metastasis are major challenges for breast cancer (BC) treatment. However the underlying mechanisms behind radioresistance is still not fully understood. Our study shows that cancer stem cells (CSCs) isolated from BC cells have 2-fold higher survival capabilities on exposure to γ-radiation, indicating its role in BC radioresistance. By exposing MCF-7 and ZR-75-1 cells to sublethal fractionated radiation (2 Gy for 10 and 15 fractions respectively), the radioresistant cell lines (FR) were established. FR cells were found to be CSC enriched, validating the role of CSCs in BC radioresistance. However it remains unclear of whether the radioresistance is imparted by the CSC itself or through a specific marker/regulator expressed in it. Here we report that Epithelial cell adhesion molecule (EpCAM), a known CSC marker, is upregulated by 1.5–2 folds (both at mRNA and protein level) in this radioresistant CSCs. In concurrence to this, EpCAM overexpression in ZR-75-1 cells (ZR-75-1EpCAM) resulted in 1.5–2-fold increased survival against γ-radiation. In addition, patients from METABRIC, TCGA dataset having high expression of EpCAM show significantly ($P = 0.0307$) poor response to radiotherapy, further confirming the role of EpCAM in BC radioresistance. This EpCAM mediated radioresistance can be attributed to faster ($P = 0.011$) and enhanced DNA repair capability in ZR-75-1EpCAM cells. It was also observed that EpCAM imparts radioresistance by activating ERK. We report that EpCAM overexpression also causes increased stemness (*in vitro* and *in vivo*). Further we found that EpCAM induces stemness by regulating pAKT. Additionally, EpCAM significantly ($P < 0.01$) increased migration and distant metastasis in orthotopic BC model. Taken together, this study shows that EpCAM expression in BC cells not only imparts tolerance to high doses of γ-radiation, but also aggravates the disease by promoting metastasis. Thus EpCAM can be a promising candidate for BC therapeutic study.

## P-35-146
## The functional comparison of HSPA1 and HSPA2 impact on growth and chemoresistance of non-small cell lung carcinoma cells (NSCLC)

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Heat shock protein (HSP) family is a large group of chaperone proteins. Their overexpression is observed in many types of cancer, however their functional significance in cancer cells remain unclear. Previously, we have shown an opposite prognostic significance of high expression of HSPA1 and HSPA2 proteins in tumor tissue in patients with primary non-small cell lung cancer (NSCLC). Although these two proteins have highly homologous structure, HSPA1 is stress-inducible, while HSPA2 is mostly known as a testis-enriched protein. In this work, we have compared, for the first time, the functions of these two proteins in NSCLC. We analyzed the impact of HSPA1 and HSPA2 on NSCLC cells growth and chemoresistance. We found that there is no relationship between the endogenous levels of HSPA1 or HSPA2 and susceptibility of NSCLC cells to platinum derivatives and bortezomib. Paralog-selective depletion of either HSPA1 or HSPA2 in NSCLC cells had negligible effect on proliferation, clonogenic ability and resistance to cisplatin, carboplatin and bortezomib. Instead, blocking of HSPA/HSP70 proteins activity by VER-155008, a specific pan-HSPAs inhibitor, caused potent antiproliferative effect and/or cell death. Moreover, pan-HSPA inhibition sensitized NSCLC cells to bortezomib, but not to platinum derivatives. It is worth mentioning that normal bronchial epithelial Beas-2B cells were insensitive to VER-155008 treatment. Our data suggest that HSPA proteins in NSCLC cells cooperate as a group of redundant, growth-promoting factors. A strategy aimed at simultaneous inhibition of HSPA family members may be regarded as potential new treatment option for NSCLC, especially in combination with proteasome inhibition. This work has been supported by National Science Centre, Poland research grants: DEC-2013/09/B/NZ5/01815 and 2016/21/N/NZ5/01917.

## P-35-147
## Modulation of breast cancer cells growth rate via the inhibitory effect of different chemical substances

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Breast cancer is heterogeneous in its genetic, clinical and molecular profile. Our aim was to develop cancer treatment approaches especially for human breast cancer patients. Cells were cultured with 40 and 50 μM curcumin, and 250 and 350 μg/mL bevacizumab separately or in combination for 24, 48, 72 h respectively. WST1 test was used to measure cytotoxic effect. IC50 values were calculated, synergy and combination index in drug combinations were investigated. Cellular growth and migration in response to the treatment was achieved via scratch assay. Combination groups which have both synergistic effect and <50% migration value were selected. Gene expressions were analysed with Real Time PCR, protein expression were analysed with Western blot. For MCF-7, MBA-MB-231 and MCF-10A IC50


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values for curcumin application determined were 46.96, 53.56 and 39.4 μM respectively, while, the IC50 values of bevacizumab were (232.8, 335.7, and 380.5) μg/mL, respectively. In cell viability assay two different doses of curcumin and bevacizumab executed cytotoxicity in MCF7 and MDA-MB-231 which was missing in noncancer MCF-10A cells during either 48 and 72 h application. VEGF, EPO, HIF-1α gene expression analyses revealed that, the concentration of 40 μM curcumin alone down regulate gene expression in MCF7 and MDA-MB-231 cells while it was up-regulated in MCF10A. Finally, we can say that *In vitro* application of curcumin to breast cancer cell lines MCF-10A, MCF-7, MDA-MB-231 as an alternative medical agent alone or in combination with Bevacizumab resulted in the modulation of the expression of the marker genes VEGF, EPO, HIF-1α. Curcumin have pro-angiogenic activation effect on MCF10A cell line by effecting VEGF gene expression while it displayed an inhibitory function in MCF7 and MDA-MB-231 cells. This work was supported by TUBITAK, Turkey (Project number 215S169). *The authors marked with an asterisk equally contributed to the work.

## P-35-148
## Adenosine signaling in stemness of proneural and mesenchymal glioblastoma stem-like cells

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Glioblastoma stem-like cells (GSCs) have been proposed as the main responsible for glioblastoma treatment failure due their enhanced chemoresistance and elevated tumorigenicity. Two GSC subtypes have been described, proneural (PN) and mesenchymal (Mes), which have characteristic growth patterns and response differently to chemotherapeutic treatments. Therefore, identification of GSC subtypes is an attractive approach for the elaboration of focused and personalized therapies. We have previously observed that adenosine signaling through low affinity receptors, A3AR and A2BAR is increased in GSCs compared to differentiated cancer cells, and its activation has been related to stemness-dependent chemoresistance. However, the role of adenosine signaling in PN and Mes subtypes has not been described. Here, we standardized *in vitro* identification of PN-GSCs and Mes-GSCs from different patients and evaluate adenosine-dependent stemness maintenance and chemoresistance. Gene expression and protein levels were evaluated by RT-qPCR and western blot, respectively. Adenosine accumulation was measured by HPLC. Stemness was evaluated by self-renewal and *in vitro* tumorigenicity assays. Adenosine signaling was studied by blocking A3AR and A2BAR with MRS1220 and MRS1754, respectively. Here we show for the first time that Mes-GSCs have enhanced extracellular adenosine accumulation and express higher A3AR and A2BAR protein levels compared to PN-GSCs. Pharmacological blockade of A3AR decreased spheres number and colony formation in both GSCs subtypes by 90%. A2BAR blockade but not A3AR, chemosensitized PN-GSCs and Mes-GSCs to taxol treatment. In conclusion, adenosine signaling is active in both GSC subtypes, thereby enhancing stemness through A3AR and promoting chemoresistance through A2BAR. Funding: FONDECYT 1160777.

## P-35-149
## NR4A3 is a novel target of p53 that promotes apoptosis

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Major tumor suppressor and transcription factor p53 is a key tumor suppressor. It regulates the expression of genes whose products induce cell cycle arrest, autophagy, and apoptosis in response to various stress stimuli. Orphan receptors represent a large group of nuclear receptors for which specific ligands are not known. Members of the NR4A family of orphan receptors (NR4A1, NR4A2 and NR4A3) are implicated in regulation of the genes involved in energy balance, metabolism, angiogenesis, thrombosis, proliferation, cell migration, and apoptosis. We demonstrated by various assays that p53 directly bound the promoter of NR4A3 gene and induced its transcription. We also showed that overexpression of NR4A3 attenuated proliferation of cancer cells and induced their apoptosis by augmenting the expression of pro-apoptotic genes. We also demonstrated potential mechanism of apoptosis caused by NR4A3 via its physical interaction with anti-apoptotic protein Bcl-2, which sequesters the latter into a non-functional complex. Moreover, the bioinformatics analysis demonstrated that high levels of NR4A3 expression correlated with better survival of breast and lung cancer patients. Our results showed that NR4A3 is a novel transcriptional target of p53, which is involved in apoptosis. Thus, we hypothesize that NR4A3 plays tumor suppressive role. This work was supported by RSF research project #18-75-10076.

## P-35-150
## Methyltransferase Set7/9 determines the tumorigenic potential and sensitivity to genotoxic drugs of lung cancer cells

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Lysine-specific methyl transferase Set7/9 was originally described as a histone-specific methyltransferase that methylates the 4th lysine of the canonical histone H3. Later it was shown that Set7/9 is capable to methylate up to 30 non-histone targets involved in such cellular processes as regulation of gene expression, differentiation, response to DNA damage including p53 tumor suppressor. As is known, p53 is a regulator of such cellular processes as cell cycle progression, autophagy, apoptosis, and the response of a cell to various stress stimuli. Earlier, our group showed that Set7/9 protein is able to influence p53 activity and cell sensitivity to genotoxic treatment. In this work, we focused on the study of such an urgent problem as lung cancer therapy. We hypothesized that lung cancer response to genotoxic chemotherapy may depend on the Set7/9 status. By the means of the CRISPR/Cas9 genomic editing system, we created the human lung cancer cell lines with different status of Set7/9 expression. Using the obtained cellular models and primary cell lines obtained from patients' samples, we showed that knockout of Set7/9 increases the sensitivity of lung cancer cells to genotoxic drugs doxorubicin and cisplatin, and also leads to the increase in the level of apoptosis in cells treated with etoposide and doxorubicin. We also demonstrated that Set7/9 knockout causes a decrease in the formation of pH2A.X repair foci in A549 cells. Based on our findings, we assume that Set7/9 methyltransferase is involved in DNA damage response and can be considered as a


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potential marker of the genotoxic chemotherapy effectiveness for lung cancer. We also tested Set7/9-specific small molecular inhibitor in combination with doxorubicin and cisplatin on primary lung cancer cell lines with high Set7/9 levels to demonstrate that Set7/9 suppression may contribute to increase the effectiveness of standard chemotherapy. This work was funded by RSF (project No 19-45-02011).

## P-35-151
## Cancer-testis antigens semenogelins 1 and 2 up-regulate energy metabolism of cancer cells

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Semenogelin 1 and 2 (Sg1 and 2, or Sgs) are two autosome cancer-testis antigens (CTAs). Normally, they are main proteins of human semen which regulate sperm motility and maturation. Besides reproductive tissues, Sgs are expressed in several tumors including lung, renal carcinoma and hemoblastosis. However, their biological role in cancer cells is not currently understood. We have shown that Sgs are expressed at the protein level in human cancer cell lines of different origin. However, Sg1 and Sg2 were observed with different frequencies. To identify interactants of Sgs, we have carried out GST pull-down assay coupled with LC-MS/MS of recombinant Sg1 and Sg2 with the extract of MCF7 breast carcinoma cells. When only 34 proteins were identified as common interactants of Sg1 and Sg2, 86 were associated only with Sg1 whereas only 4 were associated only with Sg2. So, both quantitative and functional diversity of interactants indicates a potentially more diverse functional role of Sg1 in comparison with Sg2. Among all functional groups of proteins associated with Sgs, we have focused on two key enzymes of cancer-related metabolism – LDHA and PKM2. These glycolytic enzymes are "hallmarks" of cancer-related metabolism and are usually associated with aggressive, metastatic tumors, resistance against chemo- and radiotherapy and shortened patients survival. We have established lung, breast and pancreatic cancer cell lines with stable overexpression and knockdown of Sgs and have demonstrated that both Sgs increased the activity of LDHA and PKM. Using MitoTracker staining, we have shown that both Sgs increased mitochondrial membrane potential. Moreover, by Sea-Horse profiling, we have demonstrated that both Sgs up-regulate glycolysis, respiration and superoxide production. By applying bioinformatics software, we have demonstrated that the expression levels of Sg1 and Sg2 are negatively associated with outcome of cancer patients. This work was supported by RSF grant # 19-45-02011.

## P-35-152
## Regulation of IL33 gene, encoding an IL-1 family cytokine, in human cancer cells

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Interleukin-33 (IL-33), a member of IL-1 family of cytokines, is an alarmin released upon tissue stress or damage and exerting pleiotropic activities in innate and adaptive immune responses. In cancer context, IL-33 can mediate both pro- and anti-tumorigenic effects, depending on the immune status of the tumor, and in certain cases may be a promising target for cancer immunotherapy. However, specific data on human *IL33* transcriptional regulation in various cancer cell types remain rather limited. The purpose of this study is to functionally map the regulatory areas of human *IL33* locus responsible for high *IL33* expression and to identify transcriptions factors involved in IL-33 regulation in lung carcinoma and breast cancer cells. We predicted *IL33* promoter and enhancer regulatory elements using data on relevant epigenetic marks available from published bioinformatic resources. We then assessed the role of these elements in *IL33* transcription by deletion screening of an *IL33* luciferase reporter in human NCIH-196 lung carcinoma and MCF-7 breast cancer cell lines. Identification of key transcription factors participating in IL-33-inducing cascades is underway. The study was supported by grant 18-34-01004 from Russian Foundation for Basic Research.

## P-35-153
## Small-molecule antagonists of the PD-1/PD-L1 pathway: a new possibility for cancer immunotherapy?

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Cancer immunotherapy has revolutionized cancer treatment during the last decade. This powerful strategy, of mobilizing the immune system to attack tumor cells, has shown impressive results with durable clinical antitumor responses and comprises a breakthrough in fight against cancer, even forcing formerly untreatable late stage tumors into complete remission. Current cancer immunotherapies involve the use of monoclonal antibodies (mAb) that selectively block the immune checkpoint receptors, particularly the programed cell death protein-1 (PD-1) and the programed cell death protein ligand-1 (PD-L1) protein-protein interaction. PD-1 is expressed on T lymphocyte cells and PD-L1 on tumor cells. The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells. Blocking the binding of PD-L1 to PD-1 with an antagonist allows the T cells to kill tumor cells. Although, antibodies exhibit very promising outcomes in cancer treatment, this mAb therapy is related to several limitations including: dangerous autoimmune reactions, resistance and no oral administration. Non-antibodies inhibitors can overcome drawbacks of mAb drugs leading to a highly efficient and less toxic anticancer therapy. We characterized small molecule inhibitors of the PD-1/PD-L1 interaction. We show that the compounds act by binding to the PD-L1 protein and evaluated their biological activity in cellular studies. Structural characterization shows that the compounds bind at the site that coincides with the PD-1 binding site of PD-L1, rationalizing the mechanism of the inhibition.

## P-35-154
## Contribution of heat shock factor 1 to epithelial–mesenchymal transition in epithelial breast cells

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HSF1 is a primary transcription factor responsible for stress-induced activation of HSP genes. Additionally, HSF1 is involved in the regulation of many other genes associated with multiple


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cellular processes including cell signaling, development, fertility, cell death, and metabolism. Clinical studies implicate that HSF1 is a risk factor for breast and endometrial tumors. The aim of the study was to check whether HSF1 may contribute to the acquisition of mesenchymal cell features by the human mammary epithelial cells. As an experimental model, we used non-tumorigenic MCF10A cell line, which exhibits expression of epithelial cell marker (E-cadherin) and protein markers characteristic for glandular epithelial cells with a basal and luminal phenotype (CD10, CDK14, and CK8 respectively). In these cells, the HSF1 level was stably decreased or increased using a lentiviral system. Overexpression of HSF1 correlated with decreased expression of E-cadherin and with increased expression of vimentin and Slug, mesenchymal cells protein markers. In contrast to wild-type cells, cells with elevated HSF1 level lost cell-cell contact in 2D culture and created less differentiated vesicle structures with decreased lateral E-cadherin location in 3D culture, while cells with silenced HSF1 had reduced ability to grow in 3D. Our results suggest, that HSF1 can support the transition of breast epithelial cells to the mesenchymal phenotype, which may facilitate the creation of metastases of tumors. This work was supported by Polish National Science Centre, Grants 2014/13/B/NZ7/02341 and 2015/17/B/NZ3/03760.

## P-35-155
## Type 1 calreticulin mutation in a chronic HCV-infected patient with progressive splenomegaly – NGS as a game changer

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Somatic frameshift mutations of the calreticulin (*CALR*) gene are driver mutations in *BCR-ABL1* negative myeloproliferative neoplasms (MPN) exhibiting essential thrombocythemia or primary myelofibrosis (PMF) phenotype. A case of a 68-year old patient with post-transfusional chronic hepatitis C (HCV) for 10 years, resistance to interferon and ribavirin and advanced liver fibrosis is reported. She exhibited a sustained virological response to direct-acting antiviral therapy, but also a rapidly progressing painful splenomegaly. Although splenomegaly could reflect liver circulatory problems with the patient exhibiting mild anemia, we also tested for MPN drivers. Both Sanger sequencing and GeneScan fragment analysis identified a type 1 *CALR* mutation (del 52) with an allelic burden of 50.2%. A targeted next-generation sequencing (NGS) panel comprising 54 genes relevant for myeloid disorders was employed at this chronic phase. Two additional mutations in *NRAS* (c.190T>A) and *PTPN11* (c.1504T>A) genes were identified with a VAF of 44.8% and 21.4%, respectively. These mutations displaying a high pathogenic score (0.99) were described in rare cases of myeloid neoplasms. Eight months later the patient was admitted to the hematology clinic with severe anemia and massive splenomegaly. Bone marrow biopsy identified the fibrotic PMF stage. After 1 month, rapid progression to secondary acute myeloid leukemia was observed. We show that targeted NGS detects additional somatic mutations that might negatively impact *CALR*-mutated PMF, otherwise characterized by a better prognosis compared to other molecular MPN subtypes. We discuss how NGS generated information could guide future targeted therapy, and how HCV and anti-HCV treatment could influence MPN. Acknowledgments: Funding from Competitiveness Operational Programme A1.1.4. P-37-798 MyeloAL-EdiaProT 149/26.10.2016, SMIS 106774 and infrastructure funded from Sectorial Operational Programme POSCCE O2.2.1, ONCOIVN 433/21.12.2012.

## P-35-156
## Novel chalcone thioderivative inhibits the NRF2 expression and activity in colorectal cancer cells

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Colorectal cancer is one of the leading causes of mortality and morbidity in the world. Nrf2 signaling pathway is involved in cell defense against ROS and electrophiles. In cancer cells up-regulation of Nrf2 leads to chemo- and radioresistance. Recent studies revealed that chalcone (1,3-diphenylprop-2-en-1-on) derivatives may modulate the Nrf2 pathway in cancer cells. Herein we present the preliminary studies on the effect of new chalcone derivatives and combretastatin A-4 (CA-4) on the activation of Nrf2 and the level of expression *SOD* gene in the colorectal cancer cells DLD-1. Structural modification of chalcone scaffold involved the implementation of different substituents in the 3,4,5-position of phenyl A-ring or 3,4-position of phenyl B-ring and bioisosteric replacement of the functional methoxy with the methylthio group. DLD-1 cells were incubated with tested compounds 2,3,5–8,10 and CA-4 at the doses of 5 and 15 μM, while 1 and 4 at the doses of 1 and 10 μM for 48 h. The results showed that compound 4 with 3-bromo-4,5-dimethoxy group in ring A and 4-methylthio group in ring B significantly decreased the expression of *Nrf2*, nuclear accumulation Nrf2 binding to DNA, and ultimately the expression of *SOD* gene. Induction of the Nrf2 expression and activation was observed after treatment with compound 7 possessing the bioisosteric methylthio group instead the functional methoxy group in *p*-position in ring A as well as compound 8 with 3,5-dimethoxy-4-methylthio group in ring A and 4-methoxy-3-methylthio group in ring B. As the consequence of the activation of Nrf2 enhanced level SOD protein after incubation with compounds 7 and 8 was observed. These results suggest that compound 4 may be considered as a novel inhibitor of Nrf2 in colorectal cancer cells. The project was supported by research grants: UMO-2015/17/B/ST4/03701 from the National Science Centre, Poland and 502-14-33024030-11106 from the Poznan University of Medical Sciences, Poznań, Poland.


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## P-35-157
## HIV-1 Tat induces cell type-specific expression of host genes in B-cells

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In European countries and North America, high rates of Burkitt lymphoma are found preferentially among patients infected with HIV. Interestingly, HIV is mostly associated with B-cell lymphomas although HIV does not infect B-cells. Oncogenesis in HIV-infected may be connected with the action of a small viral protein Tat, which is able to exit infected T-cells and enter other cells *via* its cell-penetrating domain. As Tat affects expression of host cell genes, we hypothesized that Tat protein could affect expression of B-cell genes; this could be a key event of lymphomagenesis in HIV-infected patients. To analyze the effect of HIV-1 Tat, we developed B-cell lines ectopically expressing Tat protein. To discover genes that are regulated by Tat, total RNA was collected and RNA-seq was performed. We identified six pathways regulated by Tat including virus response, cytokine-cytokine receptor interaction pathway, ubiquitin mediated proteolysis pathway. Unexpectedly, when comparing genes modulated by Tat in B-cells with those deregulated by Tat in T-cells, we observed just a small overlap between the two sets. Thus Tat protein appears to behave differently in B-cells and T-cells, exploiting distinct mechanisms to generate a permissive environment for virus in different tissues. The work was supported by Russian science foundation (project No. 17-75-20199). *The authors marked with an asterisk equally contributed to the work.

## P-35-158
## Pan-cancer tumor suppressor genes and oncogenes suggested on the basis of TCGA data analysis

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Tumor suppressor genes and oncogenes are the key genes responsible for the transformation of a normal epithelial cell into a malignant one. A characteristic feature of a significant part of such genes is a differential expression in tumor cells – downregulation of tumor suppressor genes and upregulation of oncogenes. Using the previously developed CrossHub tool, we analyzed the transcriptomic data from The Cancer Genome Atlas (TCGA) project for 10 cancer types with representative sample sets of primary tumors and matched histologically normal tissues. To search for potential pan-cancer tumor suppressor genes and oncogenes, a scoring system was developed that takes into account the frequency and extent of gene expression alterations in each examined cancer type. Based on the calculated score values and taking into account RefSeqGene, Gene Ontology, and PubMed data, 5 pan-cancer tumor suppressor genes and oncogenes were suggested: *CA4*, *ADH1B*, *ABCA8*, *SCARA5*, *PI16* and *KIF4A*, *TROAP*, *IQGAP3*, *KIF18B*, *NCAPH* respectively. The mRNA level of the selected genes was then evaluated by quantitative PCR on representative sets of lung, breast, and colon cancer samples. *CA4*, *ADH1B*, *ABCA8*, *SCARA5*, and *PI16* genes, selected as candidates for pan-cancer tumor suppressor genes, showed a significant expression decrease in the majority of samples of three cancer types. The most significant and frequent expression decrease in each examined cancer was found for the *CA4* gene – 20-fold (median) downregulation in more than 90% of samples. *KIF4A*, *TROAP*, *IQGAP3*, *KIF18B*, and *NCAPH* genes, selected as candidates for pan-cancer oncogenes, were characterized by a significant expression increase in all three cancer types. Thus, we suggested a number of novel pan-cancer tumor suppressor genes and oncogenes, whose functions in tumor cells are yet to be found out. This work was financially supported by the Russian Science Foundation, grant 17-74-20064.

## P-35-159
## Profiling of fatty acids in plasma and selected tissues in mice model of breast cancer (4T1) using GC/MS/MS

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Introduction: Fatty acids are present in all organisms as triglycerides and lipid components of cell membranes. They are found in the phospholipids and cholesterol esters which create the membrane structures in cells, e.g. antineoplastic and pro-cancer role of PUFA in the body is associated with their physiological role and location in phospholipids of cell membranes. All changes in the structure of the molecule or the ratio of n-3 PUFA to n-6 PUFA affect cellular dysfunction and impairment of its integrity, and thus enable the development of various diseases. Material and methods: The aim of the study was to assess the profile of saturated, monounsaturated and polyunsaturated fatty acids in plasma and selected tissues in mice in model of breast cancer (4T1) using GC-MS/MS. In the study Balb/c mice with orthotopically inoculated 4T1 tumour cells to the mice mammary glans were used. This animal model was tested in various weeks of disease development. Gas chromatography-tandem mass spectrometry (GCMS-TQ8040, Shimadzu, Japan) was applied for targeted profiling of fatty acids in mice plasma, tumors, lungs and livers. Results and conclusions: In the study of about forty compounds of saturated (e.g. methyl laurate, methyl tricosanoate, methyl behenate) and unsaturated (e.g. methyl petroselinate, methyl elaidate, methyl nervonate) fatty acids were determined using GC/MS/MS method. The varied changes in concentration of saturated and unsaturated fatty acids in the following weeks of tumor development was observed. Analysis of the lipid profile in mice plasma and selected tissues have shown changes in the concentrations of saturated, mono- and polyunsaturated fatty acids. The obtained results provide a better visualization and novel insights in advance understanding of the relationship between rapid progression of tumor growth and fatty acids metabolism.


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## P-35-160
## Ionizing radiation activates the atypical NF-κB pathway in RKO cells derived from human colorectal cancer

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The cellular response to DNA damage imposed by ionizing radiation (IR) includes activation of the so-called atypical NF-κB pathway. The aim of the work was to analyze activation of NF-κB pathway in irradiated RKO human colorectal carcinoma cell line. The parental line (RKO-wt) and its strain expressing E6 protein from HPV16 virus (RKO-E6), which reduces the level and activity of the p53 protein (along with the corresponding RKO-neo control) were used. Cells were irradiated with a single IR dose (in the range of 0.5–10 Gy), or incubated with the TNFα cytokine that activates the classical pro-inflammatory NF-κB pathway. At various time intervals after either stimulation levels of selected proteins associated with the activation of the NF-κB pathway were analyzed by Western blot and expression of genes regulated by the NF-κB transcription factor was analyzed by qRT-PCR. We observed that high doses of radiation (≥4 Gy) activated the NF-κB pathway in the dose-dependent pattern. Moreover, kinetics of NF-κB activation was slower in irradiated cells than in cytokine-stimulated cells. The expression of CXCL8 gene, a classical NF-κB-dependent gene, was comparable in cells stimulated with the TNFα cytokine and cells irradiated with a 8 Gy dose, yet its maximum level was observed at different time points. In general, so-called early genes (e.g., CXCL8, NFKBIA) were activated with the same kinetics as the so-called late genes (e.g., NFKB2, BIRC3) in irradiated cells; the term early and late genes referred to their activation in the classical pro-inflammatory response. Moreover, differences in kinetics of the NF-κB pathway activation and expression of NF-κB-dependent genes were observed between irradiated RKO-E6 and RKO-neo cells. This indicated a role of p53 in activation of the atypical NF-κB pathway in cell exposed to IR. The work was supported by the National Science Centre grant no. 2016/23/B/ST6/03455.

## P-35-161
## $P2X_7$ receptor role in biology of rat and human glioma cell lines

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$P2X_7$ is widely spread purinergic ionotropic receptor, forming cation permeable channel in plasma membrane. In certain circumstances the channel may open further, forming large, cation conducting, transmembrane pore. However, $P2X_7$ is also able to perform transmembrane signaling without forming any channel, the feature usually attributed to redox signaling pathways. Among numerous functions of $P2X_7$ receptors there is determination of cancer cell invasiveness. In the present work, we have screened several glioma cell lines for the presence and function of $P2X_7$ receptor. One rat (C6) and two human (U138 and U251) lines were examined. Very profound differences, both in gene expression and in the protein level were found. We have studied also the basic effects of receptor activation by BzATP, artificial, non-hydrolyzing agonist: the ability to evoke calcium signal and to form highly permeable pore. What interesting, there is no clear dependency between $P2X_7$ receptor level, calcium signal and pore formation ability in human glioma lines. We have also checked the influence of the receptor activation on oxidoreductase activity measured using MTS assay. We have not noticed any effects of $P2X_7$ receptor activation nor inhibition by brilliant blue G. Summarizing, presented results suggest that there is no common role of $P2X_7$ receptor activity in glioma biology and every case and cell line have to be analyzed separately. Acknowledgements: This work has been supported by National Science Centre research grant no. 2015/17/B/NZ3/03771.

## P-35-162
## Determination of the activity of antitumor enzyme L-lysine-α-oxidase

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Our study focuses on improving the method for the determination of the activity of L-lysine-α-oxidase EC 1.4.3.2. (LO) from Trichoderma. Preliminarily, ortho-dianisidine hydrochloride, a carcinogen, was used in the reaction medium. The aim of this work was to develop a more sensitive, less toxic, more stable and simplified method for the determination of the enzyme. The culture fluid Trichoderma harzianum Rifai F-180 was used as the test material. Carcinogenic reagent ortho-dianisidine hydrochloride was replaced by tetramethylbenzidine (TMB) in a chromogenic mixture of 1 mL having the following composition: citrate buffer *p*H-5.0 in the amount of 0.7 mL, TMB 0.05 mL, horseradish peroxidase RZ 0.05 mL and L-lysine 0.5 mM 0.1 mL and finally the culture liquid in the amount of 0.1 mL. The influence of some factors on the enzymatic activity in the presence of a chromogenic mixture in the reaction medium was studied: the optimum *p*H was 5.0, T-37 °C; the calibration curves for peroxide were constructed. This study was performed at a wavelength of 450 and 620 nm. We have observed that the proposed method provides an increase in the accuracy of the enzyme determination by 10 times while eliminating the use of the toxic substance ortho-dianisidine hydrochloride. In comparison to the ortho-dianisidine hydrochloride, the solution of TMB is more stable and can be stored for up to 1 year. Temperature control is excluded while determining the enzyme, (Detection of the enzyme activity occurs at room temperature). The proposed method provides the ability to determine the activity of the enzyme in the producer strains not only with a pronounced L-lysine-α-oxidase activity, but also in those where this activity has not previously been detected.

## P-35-163
## ssDNA aptamers against PD-1 and PD-L1 immune checkpoints

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Immune checkpoint blockade is one of the most promising strategies in fighting cancer. The clinical successes of targeting the programmed cell death protein 1 (PD-1) and its ligand, programmed death ligand 1 (PD-L1) are hallmarks in immunotherapy. Yet, PD-1 and PD-L1 may still find additional application in diagnosis and imaging. Herein, specific ssDNA aptamers against PD-1 and PD-L1 were developed. Selection of aptamers was performed using SELEX methodology. An initial random pool of ssDNA sequences was selected for PD-1 and PD-L1 binders. Identified binders were optimized and characterized in detail in terms of specificity and selectivity. Selected aptamers were further validated *in vitro* against


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PD-1 and PD-L1 overexpressing cells. The results indicate, that the obtained molecules showed specific responses to target cells, but little or no response to negative control cells. We also demonstrate that fluorescent-labelled aptamers allow for efficient recognition of PD-1/PD-L1 in super-resolution imaging. Thus, the aptamers may find use in diagnostic, clinical and research imaging as small probes for *in vitro* and *in vivo* detection. Acknowledgements: This work was supported by the NCN grants no 2017/25/B/NZ1/00827 and 2018/02/X/NZ1/02015.

## P-35-164
## Components of seminal plasma as factors regulating apoptosis of male gametes

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Polyamines (PA) – spermine (Spm) and spermidine (Spd) – play an important role in reproductive processes and, are poorly studied components of seminal plasma. The study of their role in the regulation of gamete apoptosis and fertility formation is contemporary. The aim of this study was to compare the content of (Spm) and (Spd) in the seminal plasma of fertile (n = 34) and infertile men (n = 40) as well as to identify the connection with the presence of apoptosis markers in gametes. Determination of PA in seminal plasma was performed by electrophoretic fractionation in agar gel and quantitative assessment using a special computer program "PN 5108". An early marker of apoptosis – phosphatidylserine (PS) expression was detected by staining gametes conjugated with fluorochrome Annexin-V and propidium iodide (BD, USA), using fluorescence microscopy. It has been revealed that infertile men have 2.5 times more spermatozoa that have entered apoptosis in the ejaculate and PA content as compared to fertile donors ($P < 0.001$): the level of Spm is reduced by almost 2 times, and the level of Spd almost 7 times. However, in infertile men, there is a change in the Spm/Spd ratio in seminal fluid with spm predominating over Spd by 4 times, which is the initiating factor for the initiation of apoptosis in gametes. The correlation between externalization of PS in gametes and Cn concentration in seminal plasma ($r = 0.5$; $P < 0.01$) was confirmed. Thus, the impaired of male fertility may be associated with changes in the level of PA, which are not only a necessary factor in the fertilization of gametes, but also a mechanism for regulating their viability. Determination of the content of PA in seminal plasma can be used to increase the amount of information of information for the study of causes impaired fertility in ejaculate. The study was prepared with the support of the "RUDN University Program 5-100".

## P-35-165
## Influence of plant polyphenolic-polysaccharide complex on ionizing radiation-induced cellular responses in normal and cancer cell lines

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Ionizing radiation is used for destruction of cancer cells and tumor shrinkage, however the increase in radioresistance of cancer cells and radiation toxicity to normal tissues remain severe concerns. Our previous study have shown that the polyphenolic-polysaccharide complex isolated from the inflorescence of *Sanguisorba officinalis (So)*, efficiently protected peripheral blood mononuclear cells against radiation damage. We hypothesize that this natural polyphenolic-polysaccharide complex might be able to selectively protect normal cells while maintaining the radiosensitivity of cancer cells. In the present study, human skin fibroblasts Hs68, non-tumorigenic mammary epithelial MCF-10, and breast adenocarcinoma MCF-7 cells grown in cell culture were treated with *So* (12.5–50 μg/mL) for 24 h prior to exposing the cells to 30 Gy of radiation. Cell survival was monitored with the Cell counting kit-8 (WST-8) 48 h after irradiation. Proliferation of cells was monitored based on the measurement of BrdU incorporation during DNA synthesis, and DNA damage was assessed by the alkaline comet assay. The results showed that pre-incubation with *So* increased the post-radiation viability of fibroblasts Hs68, *So* (12.5 and 25 μg/mL) had no significant effect on MCF-10A cell viability. On the other hand, approximately 2-fold lower percentage of MCF-7 breast cancer cells were observed after irradiation, when they were pre-incubated with *So*. The proliferation assay showed similar different responses of cells to radiation after pre-incubation with *So*. Moreover, the presence of *So* during irradiation significantly decreased the DNA damage in normal cells while it was not reduced in case of MCF-7 breast cancer cells. The results suggest that the plant polyphenolic-polysaccharide complex meets the criteria of the radioprotectors for potential use in radiotherapy. Presented research was supported by grant B1713500000/748000 from Inkubator of Innovation+. *The authors marked with an asterisk equally contributed to the work.

## P-35-166
## Antitumor activity of organic selenium compounds in human hepatocellular carcinoma-derived cell line

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Our study aimed to analyse the potential antitumor effect of an organic selenium compound, called selol in the hepatic cancer cell line. Selol is a mixture of seleninotriglycerides, which are unique selenium-containing compounds in the +4 oxidation state. The research model was represented by human hepatocellular carcinoma cells – HepG2, their subclone C3A cell line and modified by us genetically C3A cells to restore the urea cycle (C3A AO). Human lung A579 cancer cell line, which has well-documented sensitivity to selol was positive control, while negative control was represented by human embryonic kidney cells (HEK293). The flow cytometry analysis using propidium iodide was used to assess cell survival, while the MTT test method was used to study the metabolic activity of cells. The fluorescence activated cell sorter (FACS) analysis was used to determine the level of albumin, which lower expression is recognised as a marker of decreased metabolic function of these cells. We have shown that selol in a concentration-dependent manner increases the percentage of dead cells in all hepatic tumour lines, remaining without a statistically significant effect on the HEK293 line at concentrations of 5–50 μg Se/mL. In higher doses, selol causes a marked decrease in the metabolic activity of the above tumour lines. The albumin level in both C3A and C3A AO cells was decreased by selol. On the other hand, cell viability analysis of C3A AO cells indicated that selol abolish the lethal effect of C2-ceramide, giving the metabolic activity even higher


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than observed in control (untreated) cells. We have also demonstrated the cytoprotective effect of selol under conditions of oxidative stress and acetaminophen (APAP)-induced toxicity. Therefore, selol exerts its dual action in hepatic cells, expressed as an ameliorating effect on the C3A_AO cells in stress conditions, simultaneously handling the harmful impact on various cancer-derived cells in the same therapeutic dose.

## Bionanotechnology

### P-36-001
### Evaluation of the adaptive potential reducing model within *in vivo* experiment: approach to bionanotechnologies safety assessment

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The use of adaptive potential reducing models is considered to be the promising course of preclinical studies of potentially toxic objects, in particular, products of nanobiotechnologies. The model of rats' adaptation potential reducing is based on the modification of diets' vitamin-salt mixtures composition. It was tested earlier in subchronic studies, where herbicide glyphosate was used as a toxic factor. The purpose of this experiment was to assess the effectiveness of the model in reprotoxicological study on rats. The Wistar rats of the control groups received AIN-93 diet with different content of vitamins B1, B2, B3, B6 and minerals Fe and Mg: 18–19% content (C-18 group), 75% content (C-75). Animals of the test groups got the similar diets (T-18 and T-75) and also received glyphosate *per os*. Analysis of the generative function results revealed a definite correlation of the glyphosate effect with the supplying of essential substances: C-75 and T-75 showed no difference, whereas C-18 and T-18 demonstrated the differences of mating indicators: in the test group the mating efficiency was lower by ~ 35%. The pre- and postnatal development showed a similar pattern of the reaction: C-75 and T-75 didn't have differences, whereas T-18 expressed the decrease of the number of ovarian corpora-lutea and alive fetuses and the increase in post-implantation loss compared with C-18, as soon as in T-18 the total number of pregnant females, pups, the average litter size was by 71, 95, 35% lower than in C-18. Thus, the introduction of toxic factor on the background of reduced supplying with vitamins and salts led to significant changes in reproductive function, whereas within the normal availability of these substances the toxic effect of glyphosate was not observed. The obtained data allow to recommend this model for the study of reprotoxicity, e.g. during the safety assessment of nanobiotechnology products. This work was supported by Russian Science Foundation grant 16-16-00124.

### P-36-002
### Multiplex PCR protocol development for detection and quantification of GM potato event AV43-6-G7 in raw material and food

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According to various estimates, there presently exist from 21 to 51 commercial lines (transformational events) of GM potatoes on the world food market (ISAAA, 2018; BCH, 2018; FAO GM Foods Platform, 2018). The most of the GM potato lines registered for food and feed applications or for technical purposes account for the USA (43 events), Canada (27), Mexico (16), Australia (17), New Zealand (17), South Korea (8), Philippines (8), Japan (9) (ISAAA, 2018). Up to the moment, there has been registered no single GM potato line within the Eurasian Economic Union (EEU). Nevertheless, it is possible that the unregistered GM potato lines can enter the EEU food market considering the volume of potato consumption in the EEU and an increasing reliance on biotechnology in the agriculture of China as the world's largest potato exporter. In order to ensure the effective GMO control, there should be developed and approved the methods providing not only for events identification, but also allowing for quantification of GM potato in food. Real-time multiplex (duplex) PCR was developed for quantitative analysis of GM potato event AV43-6-G7 in raw material and food. The design of duplex was based on event- specific AV43-6-G7 DNA sequence detection and as soon as taxon-specific potato gene *Stp23* identification. Oligonucleotide primers and fluorescent probes were selected; composition of reaction mixtures, temperature and time parameters of PCR was optimized: 2.5× PCR-RT buffer in the presence of ROX, primers specific for the GM potato (AV43-6-G7-f/AV43-6-G7-r) and target taxon (GPF3/GPR3) in the amount of 300 nM/300 nM and 100 nM/100 nM, probes – 200 and 200 nM, respectively; BSA 0.04%; $MgCl_2$ – 3.5 mM, dNTP – 0.3 mM), as well as the temperature and time reaction profile (initial denaturation of 95 °C – 5 min, the next 45 cycles: 95 °C – 20 s, 58 °C – 20 s, 62 °C – 40 s. This work was supported by Russian Science Foundation grant No. 16-16-04123.

### P-36-003
### Duplex PCR protocol elaboration for detection and quantification of GM potato event EH92-527-1 in raw material and food

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The existing system of control over genetically modified organisms of plant origin (GMO) in the Russian food market is based on the polymerase chain reaction (PCR) method. The aspects of GMO legislative regulation such as the labelling threshold as well as the threshold for attribution to adventitious or technically unavoidable GMO presence is 0.9% determine application of quantitative methods of analysis including real-time PCR (PCR-RT). The multiplex PCR-RT method is the specific detection of two or more genetic sequences in the same reaction, that allows to significantly reduce the time of analysis, reagent consumption, risk of errors during pipetting, etc. The protocol for the quantitative detection of GM potato event EH92-527-1 in the format of duplex PCR-RT with TaqMan® PCR technology has been elaborated. The duplex system included two types of specific DNA primers and fluorescent probes: the 1st was for identifying of the event- specific EH92-527-1 DNA, the 2nd was for identifying of the taxon-specific potato gene Stp23. The selection of PCR parameters was carried out by empirical assortment of primers and probes, $Mg^{2+}$ ions, deoxyribonucleotides and polymerase stabilizing agent, primers annealing temperature and incubation time for the each cycle stage. The result of these studies was the optimized reaction mixture composition for the identifying of EH92-527-1 event and *Stp23* gene fragment in the duplex system: 2.5× PCR-RT buffer in the presence of ROX, primers specific


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for the GM potato (EH92-f / EH92-r) and target taxon (GPF3/GPR3) in the amount of 250/250 nM and 100/100 nM, probes – 200 and 200 nM, respectively; BSA 0.04%; $MgCl_2$ – 3.5 mM, dNTP – 0.3 mM), as well as the temperature-time reaction profile (initial denaturation of 95 °C – 5 min, the next 45 cycles: 95 °C – 20 s, 58 °C – 20 s, 62 °C – 40 s. This work was supported by Russian Science Foundation grant No. 16-16-04123.

## P-36-004
## Duplex PCR protocol elaboration for detection and quantification of GM potato event PH05-026-0048 in raw material and food

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A new duplex system for PCR detection and quantification of GM potato event PH05-026-0048 in food raw materials and products has been developed. This system allows to quantify the GM potato relative to the total potato DNA in the sample in the same reaction. Development of the duplex PCR method involved selection of a taxon-specific gene, optimization of reaction mixture, and verification of method efficiency and validation. According to the data analysis, for the role of taxon-specific potato genes were selected sus4, Stp23 and fru genes since they are monocopy and their sequences are highly specific for the potato genome. But for the further research was selected *stp23* gene since the primers for this gene and for event did not form dimers during amplification in the same reaction mixture. Using this primer combination the similar efficiency of taxon and GMO targets amplification was observed. The composition of the reaction mixture [concentration of primers, probes, Mg2+ ions, dNTP, bovine serum albumin (BSA)] and the real-time PCR protocol has been optimized. Designed amplification mixture for identification PH05-026-0048 event and Stp23 gene included 2.5× reaction buffer for PCR-RT in the presence of ROX (NPK Sintol, Russia), the primers specific for the event (PH05F/PH05R) and the target taxon (GPF3/GPR3) in the amount of 250 and 150 nm, respectively; the probes in the amount of 250 and 200 nM, respectively; BSA 0.02%; $MgCl_2$ – 3.0 mM; dNTP – 0.3 mM. The time-temperature profile of the reaction: the initial denaturation of 95 °C is 5 min, the next 45 cycles: 95 °C – 20 s, 58 °C – 20 s, 62 °C – 40 s. The conducted *in vitro* studies confirmed the method's linearity, precision, accuracy, limit of detection and limit of quantification the method and proved its reliability. This method shall be recommended for use in GM potato monitoring in the Russian Federation. This work was supported by Russian Science Foundation grant No. 16-16-04123.

## P-36-005
## Targeting brain metastases with a peptide conjugate

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Therapeutic peptides are among the most promising biopharmaceutical agents towards different malignancies, such as infectious and metabolic diseases, as well as in cancer. They are attracting increasing attention owing to their low toxicity, target specificity, potency, a broad range of targets, and low drug-drug interaction development. Here, we elaborate on the therapeutic potential of PepH3-vCPP2319, a conjugate combining an anticancer peptide (vCPP2319) with a sequence capable of blood-brain barrier (BBB) translocation (PepH3). The main goal of this therapeutic strategy is the translocation of the BBB and the elimination of brain metastases derived from triple-negative breast cancer (TNBC) cells, the most important cause of brain metastases in breast cancer patients. Our data shows that the conjugate behaves as expected from its component moieties. Thus, the translocation percentage of PepH3-vCPP2319 in our *in vitro* BBB model is comparable to PepH3, while no toxicity against brain endothelial cells is observed. With regard to antitumor activity, $IC_{50}$s of similar magnitude than vCPP2319 were found against different TNBC cell lines. Moreover, the conjugate demonstrated specificity towards cancer cells. Biophysical and biological techniques are currently applied to elucidate the mechanism of action. In contrast to the majority of anticancer peptides, which act at the cell membrane causing its direct disruption, neither PepH3-vCPP2319 nor vCPP2319 alone showed any membrane interaction. These results are a first step towards validating the applicability of the conjugate in the treatment of brain metastases.

## P-36-006
## Development of miRNA specific nanobiosensor systems and investigating their usability in the early diagnosis of breast cancer

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Breast cancer corresponds to 22.7% of total cases, with approximately 1.15 million new cases in women, among 23 different types of cancer. Its early diagnosis is the key to easy, inexpensive and effective treatment. Therefore, studies have been accelerated in order to determine biomarkers that will allow the diagnosis of breast cancer and even to identify the cancer, and to develop methods for determination of these markers. Various studies in breast cancer have supported expression of miRNA-155 at a different level than at different stages of the disease. The use of miR-155 as a potential biomarker in breast cancer enables the possibility of a simple serological test for breast cancer prognosis/diagnosis and follow-up. In this study, synthesized nano-sized nanopolymers to be used as biologically active layer on the sensor surface with thiol affinity technique based miRNA detection. The nanopolymers containing functional groups for use as bioactive layers in nanobiosensor system was synthesized using the mini emulsion polymerization method. Nanopolymers and nanobiosensor surface were characterized with advanced characterization method. The developed surfaces were used for the detection of target molecules for early detection of breast cancer, and the potential to be used in the form of medical diagnostic kit for early diagnosis will be elucidated. In the final part of study, the working conditions of the nanobiosensor systems were optimized. After analytical measurement characteristics determined, validation studies were done and the analysis were carried out in the real sample. With these new generation nanobiosensors, the aim of the clinic is to develop domestic products with a potential to become a commercialist, which will contribute to international literature and academic staff, reliable, real-time measurement, which responds quickly to early diagnosis of breast cancer. Keywords: Nanobiosensor, breast cancer, miRNA, miR-155. *The authors marked with an asterisk equally contributed to the work.


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## P-36-007
## Novel dimethylaminoethyl methacrylate-containing carriers for efficient gene delivery possess low cytotoxicity and lack mutagenicity

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Nanoscale cationic polymers are attractive systems for gene delivery in biotechnology and gene therapy, since their bio-functionalization can provide a possibility for addressed delivery of genes. However, their toxicity can be an obstacle for effective application. Here we evaluated a transfection potential of 8 novel poly (2-dimethylamino)ethyl methacrylate (DMAEMA)-based polymers, namely poly(fluorine-alkyl methacrylate(FMA)-*block*-poly (DMAEMA), poly(lauryl acrylate)-*block*-poly(DMAEMA), poly (butyl acrylate)-*block*-poly(DMAEMA), and poly(PEG-MA)-*block*-poly(DMAEMA) for gene delivery to mammalian cells, as well as their cyto- and genotoxicity. The poly-DMAEMA carriers (0.001% final concentration) possessed similar or higher gene delivery efficiency in HEK293, MCF-7 and HeLa cells compared to the polyethyleneimine (PEI). 82–95% of cells of these lines stayed viable after such treatment and 71–84% – after treatment with 0.01% polymers. A double staining of HEK293 cells with Hoechst 33342 and Propidium Iodide did not reveal an increase in number of cells with condensed chromatin, fragmented nucleus and membrane blebbing after 24 h cell treatment (0.001%). The poly-DMAEMA carriers caused less DNA single-strand breaks, as the olive tail moment equaled 2.6–3.3%, compared to 5.3% induced by PEI and the un-treated cells (2.2%). The level of fragmented DNA in HEK293 cells treated with polymers reached 1.5–2.1% compared to 3.2% induced by PEI, and 1.2% in control cells. *Allium cepa* ana-telophase chromosomal aberration test did not reveal a genotoxic effect of polymers. Higher retardation of mammalian cells' growth was found under the effect of PEI and synthesized PEG-modified carriers, compared to that under lauryl acrylate-, butyl acrylate-, and fluorine-containing polymers. Thus, low cytotoxicity and absence of mutagenic action of the poly-DMAEMA carriers allow considering them as promising carriers for gene delivery into targeted mammalian cells.

## P-36-008
## Cocktail of non-coding RNAs against uveal melanoma delivered by magnetic and gold nanoparticles

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Uveal melanoma (UM) is the most common primary intraocular malignant tumor in adults with a high metastatic risk. For this reason, new therapeutic approaches for this disease are needed. In this regard, microRNAs have shown to play a crucial role in neoplasia progression and might be used as a potent therapeutic tools. It has been observed in cancers, including UM, that microRNAs implicated in tumor suppression are downregulated, whereas elements involved in tumorigenesis are upregulated, such as some long non-coding RNAs (lncRNAs). Thus, restoring the normal levels of selected microRNAs can restore the normal behavior of cells. In this study, a cocktail of non-coding RNAs has been chosen to reprogram cancer cells into normal cells. In addition to regulating canonical oncogenes the microRNAs can also work against oncogenic lncRNAs. Particularly, magnetic nanoparticles (MNP) were evaluated in MEL202 UM cell line as hyperthermia agents and as a delivery system. First, the cells were treated with a non-toxic amount of MNPs (0.1 mg Fe/mL) for 24 h. Then, an electromagnetic field of 202 kHz and 30 mT was applied for 5 min, leading to a 40% reduction in cell viability. Currently, we are assessing the effect of the hyperthermia with a cocktail of non-coding RNAs previously evaluated in gold nanoparticles (AuNPs), which afforded remarkable results. In summary, nanoparticles can be used to deliver non-coding RNAs and reduce cell viability in the cancer cells. The use of magnetic hyperthermia might enhance the therapeutic effect of the microRNA cocktail employed.

## P-36-009
## Passive targeting therapy for brain delivery nanocarriers using natural dipeptide antioxidant L-carnosine-loaded niosomes

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Neurological diseases treatments are often limited by the low penetration of agents proposed because of the blood-brain barrier. Therefore, nanocarriers such as niosomes not only are able to protect the content from degradation and prolong their circulation, they also modulate their distribution and metabolic stability. On the other hand, natural dipeptide antioxidant (L-carnosine) received a great interest due to its antioxidant and anti-aging potential. Thus, L-carnosine-loaded niosomes represent a good non-invasive strategy for brain delivery. In this work, we formulated L-carnosine-loaded niosomes that are either PEG-coated or not, and their physiochemical and drug delivery potentials were assessed subsequently. Size and zeta potential were performed using dynamic light scattering reaching a size of 200 nm. The encapsulation efficiency of the different formulations reached a level of 30%. The stability and drug release characteristics were also followed. Due to the "anti-aging" effect of carnosine, an *in vitro* protein aggregation model was prepared where carnosine was tested for its inhibition of the beta-sheets formation using spectrofluorometric methods and Thioflavin T assay. U87-MG glioblastoma cells were used for testing the niosomes effects and their modulation of carnosine action. The results demonstrate that non-invasive treatment with PEG-modified surface of niosomes appear to be significantly useful to improve neurological diseases treatment. *The authors marked with an asterisk equally contributed to the work.

## P-36-010
## Long-term *in vivo* exposure to polyelectrolyte nanocapsules stimulates immune response

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Nanoparticles are powerful tool for nanomedicine applications such as drug delivery systems, vaccines or diagnostic agents. Despite of many researches, only few nanoparticles-based drug


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formulations are now in clinical use and toxicity of nanomaterials is considered as one of the most important factors limiting their medical application. Consequently, biodegradable nanomaterials which are believed to be safer than inorganic are now studied. However, the current knowledge of the possible side effects of biodegradable nanomaterials is still limited. We have developed biodegradable nanocapsules with polyelectrolyte shell consist of poly-L-lysine and poly-L-glutamic acid which were prepared by layer-by-layer technique. Our previous studies showed that polyelectrolyte nanocapsules could be used as carrier for different anticancer drugs. Here we present detailed *in vitro* and *in vivo* toxicity analysis of produced nanocapsules functionalized with different polymers poly-L-glutamic acid or copolymer poly(L-lysine)-graft-poly(ethylene glycol). Nanocapsules were tested *in vitro* in contest of their cytotoxicity, hemolytic, proinflammatory properties and genotoxicity. We also perform *in vivo* studies of biodistribution and toxicity of tested nanocapsules on mouse model. Analysis of blood biochemical parameters and histopathological evaluation of long-term toxicity performed after intravenous injections of nanomaterials demonstrated that tested nanocapsules had any undesirable effects on liver, kidney and spleen. However, after administration of nanocapsules terminated with polyethylene glycol increased serum levels of several cytokines were observed. We present for the first time that totally neutral *in vitro* nanocapsules may modulate immune response when exposed *in vivo* for long-term.

## P-36-011
## Lab-on-a-chip based biosensor for the real-time multiplex analysis of alpha thalassemia mutations from cell-free DNA

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α-Thalassemia is one of the most monogenic autosomal recessive disorder characterized by defective production of the hemoglobins α-chain. Definition of the α-globin genotype is necessary for genetic counselling in the carriers, and for predicting prognosis and management options in the patients with thalassemia. Genetic analysis of β-thalassemias routinely relies on polymerase chain reaction (PCR) and gel electrophoresis. The aim of this study is to develop a new procedure, a nanopolymer-based Lab-on-a-chip based biosensor for the real-time multiplex analysis of alpha thalassemia mutations from Cell-Free DNA. In this study, biospecific interaction analysis (BIA) employing quartz-crystal microbalance (QCM) and biosensor technologies was applied to the analysis of multiple mutations of the human alpha-globin gene. To this aim, large target polymerase chain reaction (PCR) products were immobilized on sensor chips and then probes detecting 3.7, 4.2, 20.5, MED-I, SEA α-thalassemia mutations were sequentially injected. The results obtained allow to conclude that discrimination between normal subjects, heterozygous, and homozygous patients is readily achieved for all the five mutations by PCR amplification of genomic DNA containing all the regions corresponding to the same mutations, immobilization of the same PCR products, and hybridization. The developed biosensor serves as a specific result for all the five mutations. It could accurately discriminate the mutations. Because of low costs, fast results, specificity and high detection/information effectiveness as compared with conventional prenatal diagnosis methods.

## P-36-012
## Short- and long-term effects of SWCNT-DNA complexes on glioma cells

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SWCNT-DNA complexes at concentration of 1.5–3.0 μg/mL stimulate slightly cell proliferation. SWCNTs do not affect the cell viability at concentrations of 10 μg/mL and lower. Using Raman microscopy it is shown that SWCNTs are accumulated in the cytoplasm as small agglomerates, do not permeate nuclei and remain in cells throughout 5–8 passages. SWCNTs agglomerates are detected in cells after 2 h of SWCNT-DNA complexes addition to cell culture and nanotube accumulation reaches the saturation point after 18 h of the exposure of cells to SWCNTs. As saturation has been reached the SWCNT distribution becomes more uniform. Specific method for SWCNTs concentration determination in a single living cell based on Raman spectra of SWCNTs has been developed. The average number of SWCNTs per an agglomerate in a living cell that depends on both the phase of nanotube accumulation and passage number ranges from 10 to 1000. Simultaneous application of Raman microspectroscopy and confocal fluorescent microscopy shows that the process of SWCNT-DNA complexes penetration into cells is accompanied by the modification of actin cytoskeleton. During SWCNT accumulation F-actin in the cytoplasm forms a coating over SWCNT agglomerates. The cortical actin cytoskeleton contributes mainly into the parameters of the mechanical properties of the cell surface measured with AFM. Fractal analysis of the microscale areas of the cell surface shows the significant change in the distribution of their geometrical and mechanical properties after 2 h incubation of glioma cells with SWCNT-DNA complexes that evidences the increase of the density of actin filament network and stiffness of the cell surface. SWCNTs are localized near mitochondria leading to the two times decrease of mitochondria membrane potential compared to that of control cells, initiate the increase in the production of superoxide, cause the changes in electron transfer in complexes I and III.

## P-36-013
## The role of self-assembled Congo red complexed with doxorubicin in reducing the dose of the drug in anticancer therapy

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Nanotechnology and supramolecular chemistry are rapidly growing fields of science. Recent progress in supramolecular chemistry has raised interest to biomedical application of self-assembled molecules of Congo red (CR) type which create supramolecular structures with ribbon-like shapes. This structure of such ligands allows for interaction with foreign particles, including drugs. This phenomenon is particularly interesting due to the selective binding of these ligands to antibodies, but only those engaged in the antigen-antibody complexes. Chemotherapy, which is widely used in the treatment of cancer, often proves effective but involves significant risks due to the poor selectivity of chemotherapeutic agents. The undesirable side effects of chemotherapy upon healthy tissues have led researchers to seek new solutions which limit toxicity of drugs. The aim of this study was to analyse the


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effect of the presence of CR in complex with doxorubicin (Dox) on the delivery and release of the drug in bladder cancer cell line (T24). The presence of doxorubicin in cells was evaluated using Raman spectroscopy (excitation wavelength 785 nm for CR and 532 nm for DOX). MTT and LDH assays were used to demonstrate the effect of tested compounds on T24 cell viability. We have chosen the CR dose, at which no changes in viability of T24 cells was observed. Results shown that the presence of CR complexed with Dox allows to reduce the dose of drug to the level at which free Dox does not penetrate the cells, whereas in the presence of CR we observe Dox presence in cells. We do not observe CR inside cells. Combination of supramolecular, ribbon-like structures with doxorubicin results in receiving an interesting hybrid system increasing delivery of chemotherapeutic drug to cancer cells. The advantages of the proposed system include its ability to reducing the dose of the drug. We acknowledge the financial support from the National Science Centre, Poland (grant no. 2016/21/D/NZ1/02763). *The authors marked with an asterisk equally contributed to the work.

## P-36-014
## Comparative characteristics of native cysteine proteases and those immobilized on chitosan matrix

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Ficin (EC 3.4.22.3), papain (EC 3.4.22.2) and bromelain (EC 3.4.22.32) are cysteine proteases from tropical plants. These enzymes have a clinical effect: they enhance the action of antibiotics and antimicrobials, and can be used in wound healing. Immobilized enzymes have several advantages compared with their native form. For example, sorbed biocatalysts are stable under conditions of high temperatures and extreme pH values of the medium, less susceptible to the action of microorganisms and fungi. Chitosan has antimicrobial and antiviral properties, promotes immunomodulation. Chitosan is used in medical practice a topical dressing in wound management. As a matrix to immobilize cysteine proteolytic enzymes in our studies, we used medium molecular weight (200 kDa) and high molecular weight (350 kDa) acid soluble chitosans. The aim of this study was investigation of the comparative characteristics of native and immobilized cysteine proteolytic enzymes on an acid soluble chitosan matrix. Soluble ficin loses its activity at 70 °C and pH 9.0, but in immobilized state, it is more thermostable and active in alkaline pH range. The activity of sorbed papain is 3.5 times higher than that of the native biocatalyst at 80 °C. Papain, both soluble and sorbed on chitosan, shows same activity throughout the pH range under study. When immobilized on chitosan, bromelain is more stable than that in soluble form: e.g., at 80 °C the former retain 3.3 times higher activity as compared to the latter, and it is not susceptible to inactivation at pH 9.0. Immobilization of ficin, papain, and bromelain on chitosan leads to decrease in the maximal rate of the enzymatic reaction and changes the degree of affinity of the enzymes to the substrate. We acknowledge the support by the Russian Ministry of Science and Higher Education under Grant No. 3.1761.2017/4.6.

## P-36-015
## An investigation to understand structural flexibility and salt tolerance mechanism of *Thermotoga maritima* ferritin

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Ferritin is a cage forming protein, whose normal physiological function is to store iron. It is found abundantly in all kingdoms of life. It has external and internal diameters of 12 and 8 nm respectively and typically has octahedral symmetry. The hollow cavity has been successfully utilized by filling with numerous materials for different applications but is hampered by the difficulties inherent in controlling the spontaneous assembly of the cage. Recently an interesting class of ferritin was discovered from archaea which shows more controllable assembly compared to existing ferritins and show promise as a model system to study ferritin assembly. *Thermotoga maritime* ferritin (TmFtn) is the only known octahedral ferritin which requires salt for its assembly. To understand the assembly process and the role of salt, we have analyzed the crystal structure of wild type TmFtn (with and without magnesium). We also carried out further analyses using complementary techniques, namely Native PAGE, Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). We observed that the arrangement of particular residues at subunit-subunit interfaces is crucial for cage formation. To further test this hypothesis, we have designed a number of TmFtn mutants and assessed their assembly properties. One designed mutant shows the salt-independent assembly, mimicking the assembly characteristics of "standard" octahedral ferritins. The crystal structure of this mutant offers useful insights into the potential assembly mechanisms of ferritin which may be beneficial in designing more controllable ferritin structures in the future.

## P-36-016
## Silicon nanowire sensor for simultaneous detection of various cancer biomarkers

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In recent years, a marked upward trend in oncological diseases has been noted. Early diagnosis in the initial stages of the disease and monitoring the effectiveness of the treatment greatly increase the chances of success. In this work we present a label-free highly sensitive biosensor based on a silicon nanowire field effect transistor (Si NW FET) with independent channels, allowing detection of biological active compounds. The Si structures with 70–90 nm width and 3–5 μm long NW were fabricated on silicon-on-insulator (SOI) substrates using e-beam lithography and reactive-ion etching in a fluorine plasma. Afterward, the titanium metallic contact leads were formed and additionally insulated by a sputtered silicon oxide layer to avoid a current leakage into analyte. Local field sensor was fabricated using similar technology. For the nanowire surface modification we used an original method of antibodies immobilization on gold nanoparticles. Theoretical calculations of the effect of gold nanoparticles on the sensitivity of the sensor were carried out. The developed biosensor was successfully applied for detection of a prostate specific


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antigen (PSA) in real serum samples. Limit of PSA detection was estimated as low as 23 fg/mL and it was two orders of magnitude better than for well-established ELISA methods. The construction of novel sensor allows combining several independent nanowires on one chip. It was shown that the sensor has the ability to measure independent signals from at least two nanowires simultaneously. We tested the sensor with independent channels towards determination of thyroglobulin (Tg). The low detection limit of the sensor (6 pg/mL) allows the measurement of analyte even in diluted serum samples of patients, which is very promising for practical applications. The work was supported by the Russian Foundation for Basic Research (RFBR grant ofi_m No. 16-29-03266) and Foundation for the Advancement of Theoretical Physics and Mathematics "BASIS".

## P-36-017
## Preparation of highly-aligned electrospun PLA mats by stretching in plasticizer for contact guidance investigations

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Electrospinning is a method of nano- and microfibers fabrication from polymer solutions or melts using a strong (~ 10 kV) electric field. The mats prepared via electrospinning consist of fibers and can be used as filters, scaffolds for tissue engineering, sorbents and in many other applications. Aligned electrospun mats are suitable for cell contact guidance investigations – studies of substrate influence on cell shape, adhesion and migration. Aligned electrospun mats are usually prepared using gap (two parallel blades) or rotating drum as a collecting electrode. In the present work, we obtained highly-aligned polylactide (PLA) electrospun mats by stretching randomly oriented mats in ethanol which served as a plasticizer for PLA. When the mats were stretched in air, the fibers demonstrated multiple necking, however, the deformation was uniform in ethanol. For the cultivation of HaCaT human keratinocytes we used electrospun aligned mats stretched in ethanol with different mean fiber diameters ($1.8 \pm 0.8$ μm and $0.5 \pm 0.2$ μm) and randomly oriented PLA mats as a control. The aspect ratio (the ratio of cell size along the fibers to the cell size across the fibers) of HaCaT cells grown on the aligned mats ($3.3 \pm 1.7$ and $3.0 \pm 1.5$) exceeded about two times the same value for cells grown on randomly oriented mats ($1.5 \pm 0.6$) regardless of fibers diameters. Our research was supported by the Russian science foundation (grant No. 17-75-30064).

## P-36-018
## Comparison of blend and coaxial electrospinning of PLA-BSA-HFIP system

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Electrospinning is a widely used method for producing polymer nanofibers using strong electric field. Electrospun mats can be used as filters, sorbents, scaffolds for tissue engineering and drug delivery. Recently protein-based electrospinning became popular for biomedical application because the addition of protein improves biocompatibility, hydrophilicity, and functionality of electrospun materials. Such multicomponent systems can be prepared by blend or coaxial electrospinning. In the first case, components are distributed throughout the fiber, in the other case the two components form a core-shell structure. In the present work, we investigated the properties of fibrous mats of polylactide (PLA) and bovine serum albumin (BSA) prepared by blend and coaxial electrospinning. PLA and BSA were dissolved in hexafluoroisopropanol (HFIP). The ternary phase diagram for PLA-BSA-HFIP system indicated that PLA-BSA blends had a phase separation in a broad range of concentration. The morphology of the both blended and coaxial fibers was examined by scanning electron microscopy. Transmission electron microscopy demonstrated the core-shell structure of the fibers prepared by coaxial electrospinning. Also, we examined the kinetics of BSA release from both types of mats when incubated in water. The coaxially electrospun mats dissolved faster than the blended ones because among the core-shell structure single-component fibers could be formed. However, both types of mats show the prolonged dissolution of the protein. The mats lost approximately 50% of BSA after 7 days of their incubation in water. The CD spectroscopy presented that secondary structure of BSA released from the mats was similar to original structure of the protein. Taken together, electrospun multicomponent mats could be used for prolonged protein-based drug release. Our research was supported by the Russian science foundation (grant No. 17-75-30064).

## P-36-019
## Modified halloysite nanotubes in targeted drug delivery

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It is from the earliest times that clay, being highly available material, is used for drug delivery. Nanotubes of the natural clay mineral halloysite are non-toxic and biocompatible nanomaterials and therefore is suitable as a biomaterial. Moreover, the chemical composition and structure of halloysite nanotubes determine the success of the directed modification of their surface, their internal loading and the prolonged release of various compounds. It is known that halloysite nanotubes in their natural state do not possess paramagnetic properties. The artificial magnetic properties of halloysite nanotubes were weaved by the method of deposition of iron oxide salts on the surface of nanotubes. During this reaction, an even magnetite layer is formed on the surface of the nanomaterial. So, these nanomaterials through its external magnetic field are delivered directly to accurate target localization, such as cancer cells, for example. While working on a unicellular sample object, the viability of a microorganism (*S. cerevisiae* yeast) after short-term interaction with modified nanotubes was evaluated. This assessment showed that 90% of the cells retain their viability and enzymatic activity at the physiological level necessary for the functioning of the cell. In our subsequent study we plan to estimate the toxic effect of modified nanotubes on mammalian cells (A549 and CaCo2) and do research on the delivery of drug compounds directly to target cells. Since modified halloysite nanotubes possesses sustained and prolonged therapeutic effect on target cells, we assume that they would be highly-demanded in clinical application and human use. The study is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project 16.2822.2017/4.6) and by RFBR according to the research project No. 18-29-25057. *The authors marked with an asterisk equally contributed to the work.


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## P-36-020
## Development and *in vitro* evaluation of epirubicin loaded polymeric nanoparticular systems for cancer treatment

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Epirubicin (Epi), an analog of doxorubicin, is widely used anthracycline in chemotherapy of various types of cancers. Epi is a newer and safer alternative to doxorubicin because of its lower side effects at an equivalent dose. In order to overcome the clinical problems of Epi and improve the therapeutic efficiency, in the present study, it was aimed to prepare and *in vitro* evaluate PLGA nanoparticles for loading Epi. Double emulsion solvent evaporation (DESE) and nanoprecipitation methods were used to prepare PLGA nanoparticles. The mean particle size, polydispersity index (PDI) and zeta potential were measured by Malvern Zetasizer Nano ZS before and after lyophilization. The morphological structure of the PLGA nanoparticles was investigated using scanning electron microscopy (SEM). Encapsulation efficiency and drug loading efficiency were calculated by collecting supernatant after centrifugation of nanoparticles at 13,500 rpm. The *in vitro* release studies were performed in phosphate buffered saline (pH 7.4) and citrate buffer (pH 5.5) at 37 °C. Cellular cytotoxicity was assessed by tetrazolium dye-based XTT assay on A549 cells. Each experiment was repeated at least three times. To evaluate the uptake of EPI-loaded PLGA nanoparticles by A549 human lung adenocarcinoma cells, fluorescence microscopy was used. It was prepared nanoparticular carrier systems with high epirubicin encapsulation efficiency, optimum stability and efficacy-affecting properties such as particle size, PDI and zeta potential. The EPI-loaded PLGA nanoparticles were showed biphasic release profile, initial burst release followed by sustained release. These nanoparticles can internalize into the lung cancer cells. While the Epi-loaded nanoparticles showed a cytotoxic effect, blank nanoparticles showed a non-cytotoxic effect on the lung cancer cells. This study was supported by a grant of Hacettepe University Scientific Research Programs (Project number: TSA-2018-16747). *The authors marked with an asterisk equally contributed to the work.

## P-36-021
## Vaccinia virus genome-editing which changes its immunogenic and pathogenic properties

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The most effective method to prevent virus diseases is provided by vaccination. The object of current investigation is the LIVP strain of vaccinia virus (VACV), which is a live smallpox vaccine used in Russian Federation for human vaccination (first generation vaccine). Its use was effective for preventing the spread of smallpox, but was also associated with adverse events, some severe. A reasonable strategy of selection of attenuated but still highly immunogenic and replicating VACV variants is to create an expanded set of the virus strains with either single or multiple deletions (or mutations) of selected individual genes and subsequent comparative study of their virulence and immunogenicity in several biological model systems both *in vitro* and *in vivo*. With genetic engineering approach a number of modified strains of VACV LIVP with target deleted genes had been created. They have been used for experiments in different *in vitro* and *in vivo* systems to find out a set of optimal genetic modifications of parent LIVP virus leading to creation of more safe but still highly immunogenic (and also protective) live fourth generation smallpox vaccines, which can be used as a vector platform for creation of a new multivalent vaccines against other infectious agents. The safety profiles of developed candidate vaccine strains have been evaluated by studying the infection process in sensitive animals such as mice, rabbits and guinea pigs. Different infection routes were used, such as intranasal, subcutaneous, and intradermal injections, allowing study the influence of virus entrance on primary and secondary organ targets, on viral spread and on the overall infection process. This work is supported by grant of Russian Science Foundation No. 19-04-00006.

## P-36-022
## Quantum dots dilemma – to PET or to FRET?

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Colloidal quantum dots (QDs), as fluorescent nanoparticles, may become energy donors or acceptors by fluorescence resonance energy transfer (FRET). The role in FRET is simply defined by QDs emission maximum. The same nanoparticles may also be a source of photoexcited electrons and may participate in photoinduced electron transfer (PET). In PET, the probability of electron transfer depends on midpoint potentials of redox pair. Introduction of QDs to biological assays opens it for both mechanisms of energy transfer. Natural proteins and other biomolecules usually have the conformational features, which help in recognition of proper partner of interaction. QDs, being pseudo-spherical, equally charged nanoparticle, does not contain such attributes. But, is the energy transfer just simply a matter of mass low in the mixture? Here we are trying to answer this question using assays composed of cadmium telluride QDs of various emission maxima, phycocyanin and other fluorescent proteins as FRET donors or acceptors, and quinones as electron acceptors. We used steady-state and time-resolved fluorescence spectroscopy to obtain FRET characteristics, and spectrophotometry and oxygen consumption measurement to describe PET. The comparison of reaction rates obtained for several test systems indicates that the QD dilemma is more over the simple law of mass action. Research financed by the National Science Centre, Poland, grant no. UMO-2016/22/E/NZ1/00673.

## P-36-023
## DNA origami and lipid nanodiscs for controlling the orientation of membrane protein

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Membrane proteins play important roles in various cellular processes and are extremely important in life and human health. However, membrane proteins are difficult to isolate and manipulate. The development of methods that would allow such proteins to retain their native structure during characterization is highly desirable, not least for structural studies. In this work, I have used the tools of DNA nanotechnology and lipid nanodiscs for developing a "bionano" device which aims to capture and control the orientation of a membrane protein. Here I show that lipid


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nanodiscs made of a single domain of lipids, encircled by two layers of Membrane Scaffold Protein, can be used as a native-like environment for the target membrane protein, with bacteriorhodopsin being used as a model protein. In order to further extend the applications of the lipid nanodisc, we have used DNA origami, a widely used technique in the field of DNA nanotechnology, to interface with the lipid nanodiscs. The stability of such lipid-DNA origami-protein arrangements is being further studied *in silico* using molecular dynamics. The envisaged device, if successful, will have a potential for development as a general tool allowing capture and manipulation of membrane proteins. *The authors marked with an asterisk equally contributed to the work.

## P-36-024
## Chimeric protein joining E protein domain III of tick-borne encephalitis virus and OmpF porin of *Yersinia pseudotuberculosis* as the antigen of TI-complexes

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Tick-borne encephalitis (TBE) is a widespread dangerous disease of the nervous system caused by the TBE virus (TBEV). Whole inactivated viral vaccines prevent TBE infection most effectively. However, they have side effects, and their production is associated with the processing of large amounts of pathogenic material. Subunit vaccines based on nanoparticulate antigen delivery system named tubular immunostimulating complex (TI-complex) self-organized from the mixture of cucumarioside $A_2$-2 from *Cucumaria japonica*, glycolipid monogalactosyldiacylglycerol (MGDG) from different marine macrophytes and cholesterol allow to avoid these difficulties. The present work was aimed to test immunogenicity of the recombinant fusion protein combining E protein domain III (EIII) of TBEV and OmpF porin of *Yersinia pseudotuberculosis* incorporated into TI-complexes. EIII contains the main antigenic determinants of TBEV, and OmpF porin promotes incorporation of a chimeric antigen into the glycolipid matrix of TI-complexes. It was shown that an individual antigen exhibits its own immunogenicity and induces a 3.5-fold increase in the level of antibodies against EIII-OmpF compared with the control group of animals. Incorporation of the antigen into TI-complexes resulted in the increase the production of antibodies against EIII-OmpF by 6–8 times compared with a group of animals immunized with an individual antigen. The intrinsic fluorescence of the protein showed that changes in the spatial structure of the antigen under the influence of the glycolipid environment lead to greater exposure of the EIII to the external environment and its greater accessibility for the immune system. The maximum protective effect was provided by the individual antigen and the antigen incorporated into TI-complexes based on MGDG from *Laminaria japonica* or *Zostera marina*. The last TI-complex also showed the greatest adjuvant effect. This work was supported by Russian Science Foundation (grant 15-15-00035-P). *The authors marked with an asterisk equally contributed to the work.

## P-36-025
## Investigation of bacterial polysaccharides molecular properties for radionuclides and heavy metals bindings

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Using the methods of Raman and infrared (IR) spectroscopy, the molecular structure of levan and alginate synthesized by Azotobacter vinelandii D-08 (levan) and A. vinelandii D-05 (alginate) on a sugar-containing medium and molasses were investigated. This polysaccharides are promising sorbents of heavy metals and radionuclides. The IR spectra included typical for the polysaccharides characteristic bands. Also the levan IR spectrum included bands, caused by ν(CO) fluctuations of the fructose furanose ring groups (monomeric unit of the molecule) and the typical molecules band at 1030 $cm^{-1}$ and at 940 $cm^{-1}$. Analysis of the alginate IR spectra allowed to record the skeletal vibrations of mannuronic acid, skeletal vibrations –C-O-C, deformation vibrations of carbon atoms. The band at 1290 $cm^{-1}$, which is caused by vibrations of guluronic acid, is expressed in the form of the shoulder in IR spectra. Since in the alginate IR spectrum the peak of 1630 $cm^{-1}$ (COO-) shifts to the 1730 $cm^{-1}$ (COOH), it can be concluded that the alginate is in the non-ionized alginic acid form. Raman spectra of the levan is characterized by bands at 1400–1650 $cm^{-1}$, due to planar vibrations of C–C bonds and deformation vibrations of C–H, as well as bands of 1455 and 1517 $cm^{-1}$, characterizing the polysaccharide crystallinity degree. Analysis of the IR and Raman spectra characteristic bands indicates the identity molecular structure of the levan, obtained by cultivating bacteria on a sucrose medium and on molasses. Using X-ray fluorescence analysis, data on changes in the adsorption of metals (copper and cadmium ions) by levan and alginate were obtained. Natural mesoporous expanded clay with immobilized polysaccharide was used as an adsorbent, which effectively adsorbed radionuclides. According to the authors, the obtained biocomposite materials can be used in processing of biotechnological and radiochemical wastes. This work was supported by the RFBR project 18-29-05054\18. *The authors marked with an asterisk equally contributed to the work.

## P-36-026
## Cell viability, hemotoxicity and photoacoustic properties of HSA modified MelaSil_Ag nanoparticles

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The use of nanoparticles (NPs) in therapeutic and diagnostic applications is one of the most interesting fields in medical biotechnologies, indeed NPs can be used to diagnose and treat different cancer diseases, to enhance drugs efficiency and to avoid acquired drug resistance phenomena. NPs, besides to improve drugs and contrast agents delivery to cancer tissues, have to be biocompatible and to show low hemotoxicity to prevent side effects during treatments. In this work, silicon-melanin nanoparticles (MelaSil_Ag NPs) constituted of a metallic silver core and an external shell constituted by silicon and low percentages of a melanin-like compound (5,6-dihydroxyindole-2-carboxylic acid, DHICA) are presented. These NPs can be used for early


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diagnosis by using photoacoustic (PA) properties of the couple DHICA-Ag. Previously, it was shown that MelaSil_Ag NPs presented slight biocompatibility, when used *in vitro*, and high hemotoxicity when incubated with red blood cells (RBCs). To overcome these side effects, human serum albumin (HSA) was conjugated to NPs and the outcomes of this biomodification on cell viability, hemotoxicity and PA properties were evaluated. Viability assessments using bare and HSA functionalized NPs with PANC-1 and BxPC-3 cell lines were performed. Results showed total absence of cytotoxicity using HSA-MelaSil Ag NPs (up to 100 μg/mL for 72 h of incubation) compared with bare NPs (about 60% of toxicity at same conditions). Same results were obtained in hemotoxicity assay, in fact bare NPs caused the lysis of RBCs already after 1 h of incubation at 100 μg/mL while HSA MelaSil_Ag NPs showed total absence of hemotoxicity up to incubation at 100 μg/mL for 72 h. Furthermore, despite HSA modified MelaSil_Ag NPs lost about 20% of PA signal intensity, they showed an increase in signal stability.

## P-36-027

## Enzyme prodrug therapy: *in vivo* antibacterial efficacy of the pharmacological pair containing methionine γ-lyase loaded PICsomes and S-alk(en)yl-L-cysteine sulfoxides

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Lung disease caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and other pathogens is the principal reason of mortality in patients with cystic fibrosis. The increasing frequency of multi-resistant bacteria encourages the researches for new anti-infective agents with new principles of action. Objectives: *In vivo* evaluation of the pharmacological pairs naked/encapsulated C115H MGL with S-alk(en)yl-L-cysteine sulfoxides in inhibiting multi-resistant *Achromabacter ruhlandii* 44B, *P. aeruginosa* 203-2 and *S. aureus* 129B strains from patients with cystic fibrosis. C115H MGL was loaded in polyionic polymeric vesicles (C115H-PICsomes). The *in vivo* efficacy of the pharmacological pairs naked/encapsulated C115H MGL with S-alk(en)yl-L-cysteine sulfoxides were tested in outbred mice infected intraperitoneally (IP) with bacterial strains. Naked enzyme (300 U/kg body weight) was injected IP four times simultaneously with the S-alk(en)yl-L-cysteine sulfoxides after 1, 24, 48 and 72 h after infection. C115H-PICsomes (75 U/kg body weight) was administrated single-time after 1 h after infection. Treatment by pharmacological pairs C115H-PICsomes with alliin/methiin provides 100% survival of animals infected by *A. ruhlandii* 44B and *S. aureus* 129B strains. For infections caused by *P. aeruginosa* 203-2 the therapeutic efficacy was shown only by the pair of C115H-PICsomes/methiin. Usage of naked C115H MGL as an enzyme component of the pharmacological pair did not show *in vivo* effectiveness of these drugs. The C115H-PICsomes as enzyme component of antimicrobial binary system against pathogenic bacteria is promising for a development of new antibacterial drugs that is not due (based on the known mechanism of thiosulfinates action) to cause a resistance. Acknowledgements The work was supported by the Russian Science Foundation (project No. 15-14-00009).

## P-36-028

## One protein, different ion cofactors, huge difference: interactions between metal-substituted cytochromes c and quantum dots

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Molecular systems composed of biomolecules and nanoparticles have a wide potential for application in the energetics, the biosensing and various therapies. Fluorescent semiconductor nanocrystals called quantum dots (QDs) are particularly exploited in this area. They are possible donors of electrons and excitation in the processes of photoreduction and fluorescence resonance energy transfer (FRET), respectively. As previous research indicated, QDs are capable of reducing ferredoxin and cytochrome c (Cyt c), and simultaneously their fluorescence is quenched by those redox proteins. In this study, we compared impact of Cyt c and its fluorescent derivatives – iron-free porphyrin Cyt c, zinc substituted-Cyt c and tin substituted-Cyt c on QDs fluorescence. Steady-state and time resolved fluorescence measurements showed that the different metal occupancy of Cyt c heme moiety affects interactions between QDs and proteins, especially in terms of fluorescence quenching mechanism. Quenching properties do not seem to depend on direct binding of proteins to QDs, as determined by gel filtration. Substituted Cyt c derivatives suppressed photoinduced QD dependent native Cyt c reduction, what suggests the competitive processes at QD-protein interface. Our data provides insight into the mechanisms of formation and photochemical reactions ongoing in the bio-nano-hybrid systems. Research financed by National Science Centre, Poland, grant no. UMO-016/22/E/NZ1/00673.

## P-36-029

## The effect of polyvinylpyrrolidone on sulfonated aluminum phthalocyanine photodynamic activity

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The main side effect of phthalocyanine (PcS) derivatives in photodynamic therapy is prolonged skin photosensitivity. Photosensitivity is caused by the accumulation and retention of PcS in the skin in over a long period of time, because of which the patient has to comply with limited light mode for 4–6 duration weeks. The simultaneous administration of high-molecular compounds, such as polyvinylpyrrolidone (PVP), increases the photodynamic activity of PcS due to the primary disaggregation of the drug to nanoparticles in the tumor. The possibility of reducing the skin phototoxicity of sulfonated aluminum phthalocyanine (AlPcS) by reducing the therapeutic dose with co-administration of PVP has been studied. Studies were performed on female mice lines CBA. The solid form of transplantable sarcoma S-37 was used. Photodynamic activity of AlPcS in doses of 2 mg/kg (without PVP) and 0.5 mg/kg (without PVP and with PVP 600–1800 mg/kg) were compared. The irradiation session (675 nm, 120 mJ/cm$^2$) was performed 5 h after the intravenous administration of the tested samples. The effectiveness of photodynamic therapy was evaluated by the values of tumor growth inhibition rate (TGI) out for 20 days after medication. Intravenous injection of AlPcS (0.5 mg/kg) is not resulted in significant inhibition of tumor growth (TGI ≤40%). Photodynamic activity with AlPcS in a dose


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of 0.5 mg/kg with the simultaneous injection of PVP was significantly higher. The values of TGI rate reached 60–80% depending on PVP dose. The results are comparable with the effectiveness of the AlPcS without PVP (2 mg/kg). The use of AlPcS in combination with PVP can reduce the injected dose of drug by 2–4 times in comparison with the recommended dose without losing the effectiveness of treatment, which in turn may lead to a decrease in the severity and duration of phototoxicity caused by the accumulation of drug in the skin. The publication was prepared with the support of the RUDN University Program 5-100.

## P-36-030
## Binding of silver nanoparticles with proteins

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Silver nanoparticles (NP) due to tunable optical properties, surface plasmon resonance, surface-enhanced Raman scattering, light scattering as well as bigger sizes, higher coefficient of extinction and lower prices compared to other noble metal NP can be used in immunoassays. Our aim was to determine specificity and sensitivity limits of binding of AgNP with proteins in comparison with immunofluorescence assay. Recovery of $Ag^+$ from its complex $[Ag(NH_3)_2]^+$ resulted in AgNP of different shapes, sizes and UV-visible light spectra. The stable AgNP were rapidly dissolved in the presence of EDTA and salts. However, binding with immunoglobulins (Ig) of different origin and types, fibrinogen, albumins and *Staphylococcus aureus* protein A significantly increased the colloids stability. Neither aggregation, nor oxidative dissolvement was observed during the nanoconjugate storage for several years at +4 °C. The structures consisting of Ag core 20–140 nm and protein shells up to 10 nm were visualized by transmission electron microscopy. The nanoconjugates may be used for immunoassays based on light scattering or optical density measurement using the crossed polarizers. Protein solutions were loaded onto glass modified with polyallylamines or organosilanes through the air as droplets ~ 500 pL or by contact microdispensers with dots ~ 60 nL. Binding occured from a laminar flow into the microfluidic channel. Specificity of immunodetection using AgNP was restricted by their selective binding with the main blood proteins (albumins, fibrinogen, Ig). The sensitivity threshold of AgNP binding was ~ 10 pg per dot for direct binding with attached IgG and ~ 100 pg for immune complexes of attached antigens and specific antibodies. For comparison both ELISA and immunofluorescent assays with their sensitivity limit near 1 pg/mL possess proven specificity for their laboratory and clinical use with blood samples. The research was supported by the Russian science foundation (grant No. 17-75-30064).

## P-36-031
## Synthesis and biological activity evaluation of novel photoswitchable dibenzo[b,f]oxepines derivatives as tubulin polymerization inhibitors with potential anticancer activity

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One of the most attractive anticancer drugs target are microtubules which play crucial roles in a diverse array of cellular processes such as morphogenesis, motility, organelle and vesicle trafficking, and chromosome segregation during mitosis. Microtubules are highly dynamic polymers formed in process that consist of polymerization and depolymerization of αβ-tubulin heterodimers. Microtubule targeting agents (MTAs) interacting with tubulin interfere this dynamic equilibrium which can result in cell arrest during interphase and as a consequence apoptosis. Microtubule targeting agents can be classified into two groups: microtubule stabilizing agents e.g. taxanes and microtubule destabilizing agents such as colchicine or vinca alkaloids. In recent decades some of those compounds have been successfully applied in the clinical treatment of different human cancers. Although MTAs have shown effectiveness in cancer treatment, their usefulness in clinical applications is limited due to complex and burdensome synthesis or isolation procedure as well as due to the adverse side effects associated with their long- and short-term use, mainly potent toxicity to normal cells and primary or acquired resistance. Photopharmacology in this context offers a promising alternative. Dynamic control is achieved through addressing a small, photoresponsive molecule and is comparable to classical pharmacotherapy, making this method potentially interesting for medical applications. In this work, we present studies on synthesis and biological activity evaluation of novel photoswitchable dibenzo[*b,f*]oxepines derivatives as tubulin polymerization inhibitors with potential anticancer activity. Results from organic synthesis as well as investigation of anticancer activity and mechanism of action of photoswitchable dibenzo[*b, f*]oxepines derivatives are presented. *The authors marked with an asterisk equally contributed to the work.

## P-36-032
## Prokaryotic contribution to biodegradation of petroleum hydrocarbons in the coastal zone of the Baltic Sea

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Hydrocarbon degrading bacteria play an important role in biodegradation of components of crude oil in aquatic ecosystems. The purpose of this study was to isolate, identify and culture microorganisms, naturally occurring in the Baltic Sea, capable of degrading petroleum hydrocarbons. Release of petroleum hydrocarbons is mainly caused by spillage from shipping industry. Sampling was carried out near the port of Baltiysk in May 2018, at a water temperature of 14 °C, salinity of 6.2‰, and pH ≈ 7.1. On the day of sampling, bacterial biomass was recovered by filtration of the water samples and the biomass recovered on the filters was taken for 16S rRNA gene sequencing. Remaining parts of the samples were cultivated on a selective medium containing paraffin as the sole carbon source. Paraffin, was chosen for this study since it represents a significant proportion of all hydrocarbon compounds in crude oil. On the basis of the molecular and microbiological methods used, we have identified the paraffin-degrading bacterial species in this study as belonging to the genus *Acinetobacter*. Our results contribute both to advancing the understanding of the Baltic Sea microbiota and point to its potential as a tool for developing bioremediation approaches to clean sea water from hydrocarbon oil spills.


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## P-36-033
## Matrix lipid polyene nanocomplexes interact with model membranes

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Polyene antibiotics are highly effective antifungal drugs for the treatment of surface and systemic mycoses Polyenes used to form ion channels in the membranes of pathogenic cells. Despite the high fungicidal activity of polyenes are toxic to the human cells. Earlier we found that flavonoids may increase the activity of membrane targeting drugs in a number of model lipid bilayer systems. Within the framework of the present project we developed the matrix lipid nanocomplexes of polyene with flavonoids (MLNPF) in favor to increase the efficiency and reduce the toxicity of polyene antibiotics. Generally, equimolar mixture of polyene, phloretin, and phospholipid-cholesterol mix was dissolved in the chloroform-methanol solution. Then organic solvent was removed under vacuum and the resulted film was dissolved in the aqueous buffer solution. Resulted nanocomplexes has been tested in several model membrane systems. It has been shown that MLNPF induce up to 20% leakage of fluorescent marker calcein out of cholesterol or ergosterol containing large unilamellar vesicles without their disruption. MLNPF showed higher affinity to the ergosterol containing liposomes compared to the cholesterol ones. The presence of phloretin in the MLNPF drastically increased release efficiency of the nanocomplexes. MLNPF induced transmembrane current in planar lipid bilayers with symmetrical volt-ampere characteristic while addition of monomeric polyene causes formation of single-length channel with asymmetric voltage-current plot. One may conclude that MLNPF contribute to the formation of double-length channels even when added to the one side of membrane. Also, MLNPFs did not induce lateral segregation in phosphocholine membranes of giant unilamellar vesicles while monomeric polyene form induces macroscopic solid ordered domains. The study was supported by grant of the President of RF # MD-2711.2019.4.

## P-36-034
## Construction of the chimeric antibody against E-protein of the tick-borne encephalitis virus for diagnostic and therapeutic applications

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Human immunoglobulin against tick-borne encephalitis virus (TBEV) is used in addition to vaccine for the prevention and treatment of the infection. Production of the human TBEV immunoglobulins is associated with a number of obstacles due to deficiency of raw materials and/or its contamination with bacteria, viruses, prions or other pathogens. In this study we aimed to develop chimeric neutralizing antibodies (chAbs) against TBEV E-protein. For this purpose we generated hybridoma line secreting mouse monoclonal antibody (MAb) with strong neutralizing activity against TBEV *in vitro*. In addition, it has been shown, that MAb specifically reacts with TBEV E-protein and do not recognize other Flaviviruses, such as WNV, JEV, DENV and YFV, which allows to use of this MAb for diagnostic purposes. The nucleotide sequences of the light (VL) and heavy (VH) variable region chains were isolated from the hybridoma mRNA by PCR. Mouse VH and VL kappa chains were cloned separately into vectors, coding the constant region of the human immunoglobulin G1 heavy chain (chHG-TBE) and the human constant light kappa chain (chLK-TBE), respectively. The CHO cells were co-transfected with chHG-TBE and chLK-TBE vectors. On the 4th day after transfection, cell lysate and culture media were analyzed by ELISA for the presence of chAbs targeting TBEV E-protein. As a result, specific chAbs were detected both in the culture media and in the cell lysate. Two general-purpose vectors including nucleotide sequences encoding under the eukaryotic CMV promoter the light and heavy chain genes of the anti-TBEV chAb were constructed. Moreover, each of the fragments of chimeric genes (leader sequence, variable and constant regions) flanked by unique restriction sites, which allows to change the specificity, class or species of the antibodies by replacing the corresponding gene fragments. Supported by RSF 17-15-01525.

## P-36-035
## Green synthesis of silver nanoparticles for the targeted delivery to HER2-overexpressing cancer cells

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A considerable attention of researchers in the field of biomedicine is being directed toward the development of new strategies of effective anticancer therapy. It has been shown for many times that different nanoparticle-based multifunctional structures are promising agents for this challenge. However, common methods used for nanoparticle synthesis are very expensive, time consuming, and non-environmentally friendly. To meet the challenge, we developed the method of green synthesis of silver nanoparticles possessing by surface plasmon resonance (SPR) and capable of selective HER2-overexpressing cancer cells killing. *HER2* gene is amplified in 30% of human breast carcinomas and in many other types of malignant tumors. Overexpression of the HER2 receptor often correlates with tumor resistance to chemotherapy and poor prognosis. Thus, a highly selective HER-overexpressing cancer cells targeting with therapeutic nanostructures has important clinical implications. Here we describe the synthesis and characterization of silver nanoparticles with a size of $13 \pm 5$ nm (determined by SEM) using an aqueous extract of aseptic plant *Lavandula angustifolia* Mill. These nanoparticles were modified with HER2/neu-directed affibody through the formation of a coordination bond between –SH group at the N-terminus of the protein and the surface of silver nanoparticles. The effective binding of these nanoparticles to HER2-overexpressing cells was confirmed by fluorescence microscopy and flow cytometry. We showed that after irradiation with 488 nm light, particles were able to generate heat and to selectively kill HER2-overexpressing cells being non-toxic without irradiation. The developed cost-effective method of the targeted silver nanoparticle synthesis for HER2-overexpressing cells targeting and killing can be considered as an effective tool for designing new cancer treatment strategies *in vivo*. The work was supported by the Russian Science Foundation (grant No. 17-74-20146).


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## P-36-036
## Co-encapsulated resveratrol and paclitaxel in liposomes for drug resistance reversal in breast cancer

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Multidrug resistance (MDR) is a major impediment to cancer treatment. A promising strategy for treating MDR is the joint delivery of combined anticancer agents to tumor cells in a single nanocarrier. Here, for the first time, Resveratrol (Res) was co-encapsulated with paclitaxel (PTX) in a PEGylated liposome to construct a carrier-delivered form of combination therapy for drug-resistant tumors. The composite liposome had an average diameter of 50 nm with encapsulated efficiencies of above 50%. The studies demonstrated that the composite liposome could generate potent cytotoxicity against the drug-resistant MCF-7/Adr tumor cells *in vitro* and enhance the bioavailability and the tumor-retention of the drugs *in vivo*. Moreover, systemic therapy with the composite liposome effectively inhibited drug-resistant tumor in mice ($P < 0.01$), without any notable increase in the toxicity. These results suggested that the co-delivery of Res and a cytotoxic agent in a nanocarrier may potentially improve the treatment of drug-resistant tumors.

## P-36-037
## Terplex with RAGE-binding ligand, cationic polymer, and adiponectin gene for the treatment of acute lung injury

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Acute lung injury (ALI) is characterized by acute inflammation and tissue injury disease. ALI is induced by various causes including infections, traumas, and ischemia-reperfusion damages. Receptor for advanced glycation end products (RAGE) is overexpressed in the lung epithelial cells of ALI. The previous reports demonstrated that a RAGE-antagonist peptide (RAP) from high-mobility group box-1 inhibited RAGE-mediated signal and reduced pro-inflammatory cytokines. In another report, dexamethasone-conjugated polyamidoamine dendrimer (PAM-Dexa) was used as a gene delivery carrier of the Adiponectin (APN) gene. In this study, the terplex was prepared with RAP, PAM-Dexa, and the adiponectin plasmid (pAPN). Gel retardation study showed that RBP, PAM-Dexa, and plasmid DNA (pDNA) formed stable terplex. The size and zeta potential of the terplex was similar to those of PAM-Dexa/pDNA complex. In L2 cell, the terplex had less cytotoxicity that the PEI25k/pDNA complexes. Furthermore, transfection efficiency of terplex was higher than PAM-Dexa/pDNA and PEI25k/pDNA complexes. The terplex of RAP, PAM-Dexa, and pAPN was administered to ALI animal models via intratracheal injection. The terplex decreased the pro-inflammatory cytokines such as TNF-a and IL-1b in BAL fluid and tissues. Hematoxylin and eosin staining showed that inflammation reaction was reduced by delivery of the terplex in the lungs of ALI models. Therefore, delivery of pAPN using the terplex system is useful for anti-inflammatory therapy of ALI. *The authors marked with an asterisk equally contributed to the work.

## P-36-038
## Systemic delivery of HMGB1 siRNA using brain-targeting ligand decorated exosomes for ischemic stroke therapy

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Stroke is a major cause of mortality and disability worldwide. Ischemic stroke, which is caused by the lack of blood flow in the brain, occurs more often than hemorrhagic stroke. Tissue plasminogen activator (tPA) is commonly used for reperfusion of occluded brain. However, the ischemia-reperfusion damages such as neuroinflammation persist, increasing cell death and infarct volume. In this ischemia-reperfusion damage, high mobility group box 1 (HMGB1), which is a non-histone nuclear protein, is translocated into extracellular milieu and acts as pro-inflammatory cytokines. In this study, HMGB1 siRNA was loaded into exosomes via electroporation and delivered to rat middle cerebral artery occlusion (MCAO) model systemically. To generate brain targeting exosomes, the exosomes were decorated with rabies virus glycoprotein (RVG) peptide by recombinant DNA technology, producing RVG decorated exosome (RVG-Exo). RVG-Exo showed higher delivery efficiency to Neuro2A cells than unmodified exosomes (Unmod-Exo) *in vitro* by facilitating receptor mediated transcytosis. Furthermore, RVG-Exo/HMGB1 siRNA reduced the HMGB1 level effectively than Unmod-Exo/HMGB1 siRNA both *in vitro* and *in vivo*. TTC staining results demonstrated that RVG-Exo/HMGB1 siRNA significantly suppressed infarct size. Taken together, RVG-Exo delivered HMGB1 siRNA to the brain effectively and may be useful for treatment of ischemic stroke. *The authors marked with an asterisk equally contributed to the work.

## P-36-039
## Modification of histone H2A for delivery of DNA into the cells of tumor stroma

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It was previously shown that histone H2A delivers DNA into different human cells. The cells of tumor stroma, such as tumor-associated fibroblasts (TAFs), are a promising target for gene therapy. Beta-type platelet-derived growth factor receptor PDGFRb is one of the surface markers of TAFs. The peptide ligand for PDGFRb, yG2, is able to bind to and internalize into PDGFRb+ cells. Thus, we assumed that fusion of histone H2A with yG2 could improve the efficiency of DNA delivery into PDGFRb+ cells of tumor stroma. The purpose of this work was to produce recombinant histone H2A fused to YG2 and to study its transfecting properties. Gene encoding H2A-YG2 was cloned into a pET30a vector. The target protein was produced in the E. coli, isolated and purified. The yield of purified protein was about 8.5 mg per liter, purity was at least 90%. As shown by agarose gel-mobility assay the ability of binding H2A-YG2 to plasmid DNA to be comparable with H2A. Therefore, the efficiency of delivery of plasmid DNA encoding a double reporter (CMV-EGFP-P2A-luc2) into Pdgfrb+ cells was estimated. The mice NIH3T3 fibroblast was chosen as an appropriate cell line, since they were shown to express Pdgfrb mRNA (confirmed by RT-PCR) and the Pdgfrb protein (confirmed by flow cytometry). Cells were transfected using histone H2A and H2A-YG2 complexes with plasmid DNA in various N/P ratios, then the amount of GFP+ cells was estimated and luciferase activity was


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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measured. The amount of GFP+ cells after the transfection by H2A and H2A-YG2 in the same N/P ratios was close. The maximum luciferase activity in the cells after transfection by H2A-YG2 was several times higher than for H2A, which may indicate Pdgfrb-dependent improvement of delivery. Thus, new recombinant histone H2A-YG2 was obtained. Introduced peptide modification leads to an improvement transfecting properties of histone H2A in Pdgfrb+ cells. The reported study was funded by RFBR according to the research project No. 18-34-00852. *The authors marked with an asterisk equally contributed to the work.

## P-36-040
## Viability of polyelectrolytes (PDADMAC and PAA) layer-by-layer coated *Escherichia coli* bacteria

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The layer-by-layer modification of cells and nanomaterials with polyelectrolytes is one of the most productive, effective, and biocompatible methods used in biomaterials research. In particular, polyelectrolyte coatings are employed to create hybrid systems for bioremediation, tissue engineering and biophotovoltaics. Here we coated *Escherichia coli* OP-50 strain cells with poly(diallyldimethylammonium chloride) (PDADMAC, Mw 100–200 kDa) and poly(acrylic acid) (PAA, Mw 100 kDa) polyelectrolytes. We have evaluated the polymer-coated microbial cells viability using viability fluorescence dyes (3,3′-dihexyloxacarbocyanine iodide and propidium iodide). The growth dynamics of modified microorganisms was determined using a microplate photometer for 48 h. We also evaluated the efficiency of polyelectrolyte coating measuring the zeta-potential of cells in water. The layer-by-layer deposition of polyelectrolytes resulted in consecutive inversion of bacterial surface potential. We found that PDADMAC/PAA coatings prolong the lag-phase duration by 10 h, thus inhibiting the growth of nanocoated bacteria. Staining with vital dyes showed 28% of dead *E. coli* cells after coating with PDADMAC/PAA polyelectrolytes. Microscopic observations have confirmed the aggregation degree in polymer-coated cells was similar to that in intact cells. We found that PDADMAC/PAA coating is effectively immobilized on the surface of *E. coli* cells and does not cause their aggregation, however it moderately affects the growth rate and cell viability. The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project 16.2822.2017/4.6), by RFBR according to the research project No. 18-29-25057 and project No. 18-34-20126.

## P-36-041
## Immobilization and characterization of arginase onto electrospun cellulose acetate/polyvinylpyrrolidone as a high-performance membrane platform for efficiency and reusability

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Arginase (EC 3.5.3.1) is a manganese-metalloenzyme which irreversibly catalyzes the hydrolysis of L-arginine into L-ornithine and urea. It has recently been considered not only as an analytical tool for L-arginine assays but also as a prospective pharmaceutical in enzyme therapy of cancers auxotrophic for arginine. Tumor growth requires a plentiful supply of arginine, which is essential for cancer cell growth. The restriction of arginine concentration to a low level could have a devastating effect on many types of cancer. Thus, the administration of arginine catabolizing enzymes, such as arginase or arginine deiminase, is a promising approach to control cancer. Enzyme immobilization has a wide working area in clinical and industrial aspects. Immobilized enzymes have most advantages such as repeated use of the enzyme, inhibition of the enzyme by matrix, inhibition of contaminating with the product, resistance to effects such as pH and temperature, and the possibility of achieving more than one reaction at the same time. In this submitted work, arginase was immobilized onto cellulose acetate (CA)/polyvinylpyrrolidone (PVP) nanofibers which were synthesized by electrospinning technique. Arginase was immobilized on to the nanofibers by adsorption and crosslinking methods. For the optimization of arginase immobilization, the amount of nanofiber (10 mg), the adsorption time (20 min), the unit of arginase (1 U/mL) and the amount of glutaraldehyde (4%) were determined as basic parameters. The optimum temperature, optimum pH, thermal stability, pH stability, kinetic parameters and reusability parameters were investigated in the characterization of arginase immobilized nanofibers prepared under optimum conditions. The significance of this study is improving of stability properties of arginase for various biotechnological applications such as the production of L-ornithine and promising approach to control cancer.

## P-36-042
## Cytotoxicity and apoptotic effects of green nanoparticles – biomedical applications

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Green synthesis of nanoparticles (NP) is a more efficient and environmentally-friendly method that relies on a variety of plant extracts to produce metal NP. The effects of NP obtained by green synthesis (Ag, Au, Pd, Se metals and plant extracts from *Levisticum officinale, Origanum vulgare*) were evaluated on several cell types: monocytes (CRL9855), primary epidermal keratinocytes (HEKs), dysplastic oral keratinocyte (DOKs) and aggressive tumor cells-glioblastoma cell line (U87). Our methods were MTS assays for viability measurements, LDH assay for cytotoxicity, xCELLigence – electrical impedance measurements and xMAP array for apoptosis evaluation in cell lysates. The results of *MTS and LDH assays,* on monocytes cell lines, showed that metal salts alone have higher cytotoxicity than green NP, because the plant extract reduces toxicity; the vegetal extracts alone had proliferative effects. *xCELLigence* measurements showed a dose-dependent proliferative effect for some of the studied NP. xMAP analysis revealed characteristic results for apoptosis in tumoral cells: activation of caspase-9 during treatment with Ag-based NP, correlated with the results for p53, suggesting that Au and Ag-based NP induced p53-mediated apoptosis in tumor cells by DNA fragmentation, which correlates with decreased Bcl-2 expression. Our results confirmed that these NP could induce apoptosis in tumoral cells through caspase activation. Green technology is rising as a nontoxic and safe option


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in biomedical applications, thus improving NP in terms of biodegradability, functionalization, and biocompatibility. Acknowledgement: Partially supported by the grant COP A 1.2.3., ID: P_40_197/2016, Ctr. 52/2016 and by Ministry of Research and Innovation in Romania, under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018.

## P-36-043
## Structural modifications of adenoviral dodecahedron virus-like particle provide a universal platform for tumor-associated antigen delivery

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One of the critical steps that provide a strong immune response against the tumor is the effective delivery of tumor-derived antigens to antigen presenting cells (APCs). Virus-like particles (VLP) allow for the incorporation of immunogenic peptides that can be efficiently delivered to APCs, which results in the antigen epitope processing, presentation, and induction of the adaptive immunity. The aim of our research is to use the nano-size VLP, adenoviral dodecahedron (Dd), as an antigen delivery platform in the model of therapeutic cancer vaccine. This proteinaceous particle, composed of twelve self-assembling pentameric penton base proteins (Pb), derived from the capsid of the human adenovirus type 3, serves both as a carrier of the selected epitopes and as an adjuvant that enhances the immune response. Here, we demonstrate that through a genetic manipulation it is possible to obtain a recombinant Dd VLP carrying 60 copies of antigen epitope. We designed several variants of Dds carrying different tumor-derived epitopes of various length ranged from 10 to 43 amino acids inserted at the N-terminal end or in the variable loop of the Pb monomer. Since the choice of a proper epitope is critical for the therapeutic outcome, we selected, for the needs of the preliminary study in the animal model, the ovalbumin (OVA)-derived peptide OVA242–284, which covers CD8+ (OVA257–264) and CD4+ (OVA265–280) epitopes. The modification in which the long OVA242–284 peptide replaced 42 N-terminal amino acids in each monomer of the Pb protein did not disturb the integrity of Dd structure. We demonstrated that the incorporation of OVA242–284 peptides into the Dd particle provided efficient delivery of epitopes to APCs. The results of the IFNγ enzyme-linked immunospot assay performed on splenocytes isolated from spleens of the C57BL/6 mice immunized with DdOVA242–284 variant indicated that the vaccine without any additional adjuvants strongly induces peptide-specific CD8+ T cells response.

## P-36-044
## Diagnosis of the thyroid gland diseases: nanotechnology in the development of diagnostic test systems

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The most common thyroid problem is the abnormal production of thyroglobulin hormones. The release of large amounts of thyroid hormones leads to a condition known as hyperthyroidism. Such a disease can be controlled if it is diagnosed in time and properly treated. This study describes the development of diagnostic hyperthyroidism and the effect of nanoparticles on the biological activity of the protein. The method of detecting thyroglobulin autoantibodies in serum is carried out by incorporating silver and gold nanoparticles into the diagnostic test system. This interaction leads to the formation of a dynamic nanoparticle-protein corona. Protein crown can affect the absorption, inflammation, accumulation, degradation and purification of nanoparticles. In addition, the surface of the nanoparticles can cause conformational changes in adsorbed protein molecules. To study the effect of gold and silver nanoparticles on the biological activity of the protein, an enzyme immunoassay (ELISA) was performed using autoantibody conjugates with nanoparticles. According to the results of the experiment, it was seen that in the control experiments, the signal intensity in ELISA, proportional to the degree of binding of monoclonal antibodies to the antigen, is stable and amounts to 0.43 absorbance units (AU), Polymeric microspheres (PS-49) with a diameter of 49 nm gold) have no effect on the binding of monoclonal antibodies to the antigen. However, with the use of silver nanoparticles, a dose-dependent effect of amplification of the ELISA signal is observed at 100% to 0.8 AU with a maximum at a concentration of nanoparticles of 32 μg/mL. For gold nanoparticles, the opposite effect was observed: at a concentration of nanoparticles of 32 μg/mL, a decrease in the signal by 50% was observed, and at a concentration of 62 μg/mL, a complete suppression of the binding of monoclonal antibodies was observed.

## P-36-045
## Development of enhanced lateral flow assay for potato virus X based on recombinase polymerase amplification

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Potato virus X (PVX) is one of priority potato pathogens causing significant yield losses. Development of a sensitive and rapid technique for PVX detection is an important actual task. We have proposed and developed PVX assay based on recombinase polymerase amplification with reverse transcription (RT-RPA) and lateral flow test. A fragment of coat protein gene (gp5) of PVX with length of 146 bp was chosen for specific recognition after reverse transcription. RT RPA was performed using RPA Twist Dx basic kit to obtain target DNA flanked by biotin and fluorescein (FAM) for lateral flow assay (LFA). We compared two LFA formats: (i) antibodies against FAM were immobilized


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at the test strip and conjugate of gold nanoparticles (GNPs) and streptavidin bound the target DNA via its biotin end; (ii) immobilized streptavidin and anti-FAM antibody – GNP conjugate were used. GNPs with a diameter of 17.4 ± 1.0 nm and their conjugates with anti-FAM antibody and streptavidin were synthesized. The first LFA format was found to be more sensitive; the limit of target DNA detection was 20 pM. However, the reaction media for RT-RPA contains dithiothreitol that aggregates GNP-streptavidin conjugate. So the first format in not compatible with the amplification stages. For integration with the second LFA format, buffer components, stabilizing molecules and RPA mode were optimized. Total RNA from infected and non-infected potato plants was extracted. For the obtained samples RT RPA was performed under the chosen conditions during 20 min and at 37 °C. As a result, the developed RT-RPA enhanced LFA reached a detection limit of PVX equal to 0.2 ng/g of potato leaf, being the same as for commercial RT-PCR kit. However, the proposed assay excludes the use of complex equipment and provides simply registered results. This study was financially supported by the RF President Grants for State Support of Young Russian Scientists – PhD (award MK-6712.2018.4).

## P-36-046
## DNA origami nanocapsules for controllable cargo accessibility

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DNA nanotechnology is a bottom-up nanotechnology taking advantage of DNA as either a biologically or synthetically produced polymer with self-assembling properties. The DNA origami methodology [1] folds a single-stranded phage DNA (scaffold) via hundreds of synthesised oligo DNAs (staples). It offers the feasible assembly of arbitrarily addressable structures at a size of around a hundred nanometres. Using this method, a number of encapsulating structures have been produced to date including a nanoscale box with controllable lid [2], a clamshell shaped nanorobot able to expose cargo in response to target signal [3] and a nanovault aiming to cage an active enzyme [4]. However, the encapsulation capacity and cargo isolation efficiency of such structures are still limited. Here we report the design of a two component capsule-shaped DNA origami nanostructure with each component having a cavity of ca 20 × 20 × 10 nm. The design offers full accessibility to anchored cargos when the shell is open while the closed form contains a cavity of approximately 11 attolitres. We evaluated cargo accessibility and the loading capacity of the DNA origami capsule as well as its ability to protect cargo using split GFP system as proof of principle. Initial results suggest that capture of the protein cargo within the nanocapsule system is possible. References: 1. Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. Nature 440, 297–302, https://doi.org/10.1038/nature 04586 (2006). 2. Andersen, E. S. et al. Self-assembly of a nanoscale DNA box with a controllable lid. Nature 459, 73–76, https://doi.org/10.1038/nature07971 (2009). 3. Douglas, S. M., Bachelet, I. & Church, G. M. A logic-gated nanorobot for targeted transport of molecular payloads. Science 335, 831–834, https://doi.org/10.1126/science.1214081 (2012). 4. Grossi, G., Dalgaard Ebbesen Jepsen, M., Kjems, J. & Andersen, E. S. Control of enzyme reactions by a reconfigurable DNA nanovault. Nat Commun 8, 992, https://doi.org/10.1038/s41467-017-01072-8 (2017).

## P-36-047
## O-acetylhomoserine sulfhydrylases from Clostridia pathogenic bacteria

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Bacteria of Clostridia class cause a variety of human diseases, such as gas gangrene, tetanus, pseudomembranous colitis. *O*-Acetylhomoserine sulfhydrylase (OAHS, EC 2.5.1.49) involved in bacterial methionine biosynthesis may be potential drug target in pathogenic bacteria. There are two pathways of methionine biosynthesis in microorganisms: direct sulfhydrylation, in which OAHS is involved, and transsulfuration, involving cystathionine γ-synthase and cysteine-S-conjugate β-lyase. Analysis of pathogenic bacteria genomes of the genus *Clostridium* and pathogenic bacterium *Clostridioides difficile* revealed that probably direct sulfhydrylation is the only way of methionine biosynthesis in *C. novyi*, *C. tetani* and *C. difficile*. The genes of *O*-acetylhomoserine sulfhydrylases from *C. difficile*, *C. novyi* and *C. tetani* were cloned and expressed in *E. coli* cells. Recombinant enzymes were purified and characterized. Their molecules are tetramers with molecular weight of about 200 kDa and possess absorption spectra in the region of 300–500 nm typical for pyridoxal 5′-phosphate-dependent enzymes. The enzymes catalyze the synthesis of L-homocysteine *via* sulfhydrylation reaction of *O*-acetylhomoserine with $Na_2S$. They have pH-optimum of the activity at pH 7.5. OAHS from *C. difficile* possesses the highest activity of 50 U/mg, OAHSes from *C. novyi* and *C. tetani* exhibit activity of 1.1 and 0.3 U/mg, respectively. The enzymes are quite stable in the temperature range from 25 to 60 °C. OAHS activity is inhibited by some substrate analogues including metabolic end product L-methionine. The data may provide the basis for further study of OAHS role in pathogenic bacteria and potential inhibitors development. The work was supported by the Russian Foundation for Basic Research (project No. 18-34-00669-mol-a).

## P-36-048
## Development and characterisation of novel polymeric nanocapsules for oral delivery of biomolecules

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Clinical applications of oral protein therapy for the treatment of various diseases are limited due to the harsh conditions encounter the proteins during their journey in the Gastrointestinal Tract. Although nanotechnology forms a platform for the development of novel formulations, obtaining physicochemically stable formulations able to deliver active therapeutic proteins is still challenging because of harsh preparation and delivery conditions. This study proposes the use of poly (D, L-lactic-co-caprolactone)-based nanocapsules at different monomers' ratios for protein loading and oral delivery by utilising the Design of Experiment as a methodology for saving time, materials, and effort. Lysozyme was used as a model protein and the approach was then validated by evaluated its ability to load therapeutic protein (DNase). This is the first report on the investigation of trehalose quantity role in protecting proteins during the polymeric nanoencapsulation processes. All formulations had a spherical shape


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and nano-scale size, and the encapsulation efficiency of Insulin reached 80% and significantly affected by monomers' ratio. Trehalose and physical state of lysozyme had a significant effect on its biological activity ($P < 0.05$). Less than 10% of the protein was released in simulated gastric fluid, and 73% was the highest recorded accumulative release percentage in simulated intestinal fluid (SIF) over 24 h. The higher caprolactone, the higher encapsulation efficiency (EE) and the lower SIF release recorded. The utilisation of the DoE helped in obtaining further quantitative details about the significant factors and was able to optimise these factors to attain the desired qualities and attributes. Therefore, the formulation factors were optimised and the obtained system was PEGylated wisely to attain EE 80%, 81% SIF release within 24 h, and 98% lysozyme biological activity. The optimum formulation was prepared to deliver DNase, and similar attributes were obtained.

## P-36-049
## Novel artificial protein cage as delivery vehicle to mammalian cells

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Protein nanocages have emerged as a new class of potential drug-carriers or diagnostic agents. Their genetic and chemical plasticity render them amenable to carry cargo molecules in their interior but also to display cell-specific targeting ligands externally. We have recently described production of a synthetic nanocage built of multiple copies of a bacterial TRAP protein. Importantly, its assembly can be controlled via metal coordination at the protein-protein interfaces. The TRAP cage displays extreme chemical and thermal stability but also a unique geometry. Here we show TRAP cage loaded with GFP and modified with cell targeting moieties and transduction studies. Using Fluorescence-activated Cell Sorting and laser scanning confocal microscopy we demonstrate that the fluorescent protein can be successfully delivered and released within HeLa cells. We have also initially tested toxicity of the carrier in different cell lines. Currently we are aiming to develop methods to track the cage protein in cells and to load with diverse cargoes. These studies constitute a proof –of-concept for developing a versatile nanodevice that could find application in diagnostics and/or therapeutics. *The authors marked with an asterisk equally contributed to the work.

## P-36-050
## Fluorescent polarization aptamer assay for ochratoxin A with gold nanoparticles as enhancing anchors

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The combination of oligonucleotide receptors, aptamers, with measurements of fluorescence polarization (FP) for registration of binding, provides simple, sensitive, and rapid assay technique for low molecular weight targets. However, efficient assays need in large changes of fluorophore-labelled ligand motions. The inclusion of aptamer in complexes with high molecular weight carriers seems to be the right solution to increase differences of sizes (and, correspondingly, rotation motions) for bound and unbound state of the labeled ligand. The proposed FP assay is based on the competition between free ochratoxin A (OTA) and OTA coupled with 4′-(aminomethyl)fluorescein (OTA-AMF) for aptamer binding. Gold nanoparticles (GNPs) were used in the assay as anchors. The diameter of the synthesized GNPs was $23.7 \pm 2.5$ nm according to transmission electron microscopy measurements. The GNP surface was modified with streptavidin by physical adsorption, and then the biotinylated OTA-specific aptamer (5′-GAT-CGG-GTG-TGG-GTG-GCG-TAA-AGG-GAG-CAT-CGG-ACA-3′) was specifically attached. The limit of detection of the competitive GNP-enhanced FP aptamer assay was 4.7 nM of OTA in buffer solution. The assay applied to control OTA in wine was characterized by the detection limit equal to 5.6 nM (2.3 μg/kg) being 26-fold lower as compared with the FP assay based on free aptamer. The assay duration was 15 min. The maximal residue limit for OTA in wine in European Union is 2 μg/kg. Thus, the proposed GNPs-anchor technique allows achieving lower sensitivity which makes it a promising analytical tool. This study was partially supported by the Russian Foundation for Basic Research (grant No. 18-08-01397).

## P-36-051
## Development of therapeutic transdermal systems for microbial biofilm destruction

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Conventional antifungal agents encounter difficulty in treating and fully eradicating biofilm-related infections. We investigated the interactions between Farnesol and Lyticase as antifungal agents. The combined effects against C. albicans biofilms were assessed *in vitro*. Farnesol is a natural 15-carbon organic compound which is an acyclic sesquiterpene alcohol that represents the first quorum sensing molecule identified in eukaryotic microorganisms where it causes a range of physiological effects. In C. albicans farnesol inhibits the yeast to hyphal switch to prevent colonization of different niche environments, it has also antioxidant and antibacterial effects. Lyticase is an enzyme produced by the Micrococcus luteus that breaks down the cell wall of the fungi, which transform into a spheroplast that sensitive to changes in osmotic pressure, resulting in limited viability of cells. Substances that deplete the biofilm and facilitate the access of antimicrobial agents currently are being monitored in the world. Innovative therapeutic transdermal delivery systems make it possible to prescribe drugs with large molecular sizes as Farnesol and Lyticase, and reduce the volume and dose of the substance. Non-toxic quick-dissolving medicinal film preparation Sedatin 0.2 mg prepared on a gelatin based. It can be applied sublingually, intranasally, rectally, vaginally. The adhesive effect of gelatin is manifested, which ensures a strong fixation of the film in a given zone. The targeted delivery of drugs to the pathological focus is realized, which allows to achieve effect in doses of 1/10 or 1/20 of the average therapeutic dose. As increasingly isolated strains present resistance to currently used antifungals, it is necessary to develop novel and safe antimycotics. Multiple medicinal substances applied to the gelatin based film may be the most effective way of eliminating fungi biofilms. This publication was prepared with the support of the RUDN University program 5-100.


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## P-36-052
## Electron coupling between retinal and Y185 lead to red shift and more stable excited state of bacteriorhodopsin

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The role of a conservative amino acid residue of tyrosine in the active center of retinal-containing proteins (Y185 in bacteriorhodopsin) in the regulation of the initial stages of the microbial rhodopsins photocycle is discussed. It is shown that the replacement of this residue leads to a significant change in the spectral-kinetic characteristics — a blue shift occurs in the wavelengths of the absorption maximum of the ground state, as well as the primary intermediates I, J, and K by 10–15 nm. This shift is accompanied by a more rapid decay of the excited state (intermediate I) and its transition to the main electronic level (intermediate J). The calculation of the electronic structure of the retinal and the nearest amino acid residues shows that these effects can be associated with electronic conjugation between the chromophore (retinal covalently associated with K216) and the aromatic residues Y185, W86 and W182. *The authors marked with an asterisk equally contributed to the work.

## P-36-053
## PLGA-nanoparticles modified with anti-HER-2 affibody for enhance anti-tumor drug delivery

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In the field of cancer treatment, the need for a personalized approach to the patient is becoming ever more acute. The use of multifunctional nanoparticles is a promising solution for this problem due to their ability to perform several functions simultaneously, including targeting, drug delivery, imaging. We have designed doxorubicin-loaded PLGA-nanoparticles that recognize HER-2 positive cancer cells. Overexpression of HER-2 (human epidermal growth factor receptor 2, ERBB-2) receptor is found among a wide range of human cancers, including up to 30% of breast cancer cases, and it is an indicator of poor prognosis. We have developed methods for conjugation of nanoparticles with HER-2 recognizing affibody, and methods of doxorubicin encapsulation in nanoparticles. These nanoconstructions have a high biocompatibility and biodegradability due to use of FDA-approved poly(lactide-co-glycolide) polymer. The PLGA-affibody-doxorubicin conjugates selectively target HER-2-positive cells and have no binding with cells possessing normal level of HER-2 (or without expression of that receptor). It has been revealed that the targeted nanoparticles have a higher level of cytotoxicity for HER-2 cells compared to non-targeted ones. The dependence was opposite in the case of cells without overexpression of this receptor. Thus, this study is an important step towards the creation of therapeutic nanoparticles. The work was supported by the Russian Science Foundation (grant No. 17-74-20146).

## P-36-054
## Electrochemical nanoprobe for label-free and real-time analyses of 3D tumor spheroids

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Tumor spheroids are a complex 3D cell culture model that reproduce *in vitro* some features of solid tumors *in vivo*, including oxygen supply limitations, the composition of extracellular matrix, secretion of soluble mediators, and drug resistance mechanisms. As a result, tumor spheroids are often used as an advanced *in vitro* model for tumor biology research and screening novel anticancer drugs. However, there is a need in techniques that allow monitoring the microenvironment within the spheroids, especially in the spheroids core. In the current research, we propose a novel highly sensitive electrochemical technique based on nanoelectrodes for label-free and real-time analyses of 3D tumor spheroids. For this purpose, tumor spheroids from U-87 MG human glioblastoma cells were generated, and the nanoelectrodes (about 100 nm in diameter) were carefully introduced into the spheroids using the precise micromanipulator under optical control. We detected the changes in the oxygen and pH levels inside the spheroids as a function of the distance from the spheroids surface. Thus, the oxygen level dramatically decreased from the $240 \pm 20$ μM to $120 \pm 20$ μM when the nanoelectrode was introduced to a depth of 100 μm (n = 6). The pH level decreased in a similar manner and changed from pH 7.4 to 6.5–6.6. This is in a good correlation with previously described data for spheroids internal structure. However, contrary to the classical approaches, the proposed technique avoids significant damage to cells due to the nanosize of electrodes. As a result, it could be promising for real-time monitoring of 3D cell culture metabolites, both for cancer biology study and screening antitumor drugs. The work was carried out with financial support from the Ministry of Education and Science of the Russian Federation in the framework of Increase Competitiveness Program of NUST MISiS (No. K4-2018-052) and was also supported by RFBR, research project No. 18-04-01087 (in part of spheroids formation).

## P-36-055
## Synthesis and photodynamic antimicrobial activity of cationic meso-arylporphyrins

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Photodynamic therapy (PDT) is being actively investigated for the inactivation of microorganisms. PDT kills bacteria, causing


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oxidative stress in several cellular targets. Synthetic porphyrins are used as photosensitizing agents in antimicrobial PDT due to their high extinction coefficients, ease of synthesis, low cost, and ease of modification of the macrocycle to produce compounds with desired properties. For the target compounds synthesis, we used a synthetic strategy consisting in condensation of functionalized benzaldehydes and pyrrole under mild conditions according to the method of Lindsay and subsequent chemical modification of terminal groups in long-chain substituents. Thus, pyridile-containing porphyrins and their Zn complexes were received. Further, dark toxicity and photodynamic activity of obtained compounds were studied against E. coli and S. aureus bacteria. It was found that porphyrins with spacer containing 5 and 10 methylene groups between the macrocycle and cationic residue have been shown to quite strongly inhibit the growth of gram-positive S. aureus bacteria in aqueous solutions of compounds containing the Pluronic F-127 solubilizer without irradiation with light. Light exposure leads to an increase in S. aureus inhibition compared with the dark control only in the case of compound with 5 methylene group. In the case of the use of solutions in Pluronic, all compounds inhibit the growth of E. coli bacteria at high concentrations without irradiation with light; however, the minimal inhibitory concentration was not achieved in the investigated concentration range of 0.3–40 μM. The photodynamic activity of the compounds against the biofilms of the E. coli and S. aureus bacteria was also investigated. For all compounds 100% inhibition of viable bacteria was achieved at concentrations of 10–20 μM. This work was supported by the Russian Science Foundation, project No. 17-73-10470.

## P-36-056
## Magnetic nanoparticle-assisted drug use in the reduction of multiple drug resistance in the acute promyelocytic leukemia cell line (HL60)

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Chemotherapy is one of the most preferred methods for the treatment of cancer. Anthracyclines are among the most effective and widely used chemotherapeutic drugs. These drugs are frequently used in the treatment of solid tumors and leukemias. However, their use is limited due to their influence on the whole organism, their cardiotoxic effects and the development of multiple drug resistance. An important way to improve the efficacy of chemotherapeutic agents and to overcome MDR is drug targeting. Drug targeting is a principle in which the distribution of the drug within the organism and the major fraction are directed to interact only with the target tissue at the cellular or lower cellular level. In this study, we used $FE_3O_4$ magnetic nanoparticles (MNP) to overcome drug resistance in HL60 acute promyelocytic cell line. Firstly, MNP synthesized in the range of 100–200 nm and then glutaraldehyde (GA) and 3-aminopropyltriethoxysilane (3-ABDES) intermediate arms were ligated. Then, different concentrations of idarubicin (IDA) (10.0; 5.0; 1.0; 0.5; 0.1; 0.05 and 0.01 μM) were linked to the MNP-GA and MNP-ABDES complex. The prepared MNP-IDA complexes and free IDA solutions were added to the HL60 cell line and incubated at 37 °C, 95% humidity and 5% $CO_2$ for 24 and 48 h. MTT and ATP viability tests were performed at the end of each incubation period. Finally, $IC_{50}$ values were determined 2.5 μM (24 h) and 0.25 μM (48 h) for free IDA, 0.95 μM (24 h) and 0.06 μM (48 h) for MNP-IDA-GA complex and 1.0 μM (24 h) and 0.06 μM (48 h) for MNP-IDA-ABDES complex. It was observed that MNP-IDA complex was 3–4 times more active than free IDA. Since IDA reduced multiple drug resistance when attached to MNP, its effect on cells increased and thus showed the same effect at much lower doses. If this method is used for treatment in patients, it is expected that IDA will be given in lower doses and therefore side effects of drug and the cost of the drug will decrease.

## P-36-057
## The study of interaction between blood proteins and peroxidase-like iron oxide nanoparticles

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Magnetically targeted nanosystems with protein coatings are considered to be applicable in different areas of biology and medicine including theranostics and biosensing techniques. Proteins represent extremely susceptible targets for reactive oxygen species (ROS). Earlier we have developed the free radical approach to immobilizing of protein on magnetic nanoparticles (MNPs) in sols that allowed obtaining biocompatible multifunctional coatings on MNPs. The aim of the investigation was to study the oxidative modification of the proteins immobilized (human serum albumin (HSA), immunoglobulin G (IgG) and hemoglobin (Hb)) on the surface of peroxidase-like iron oxide MNPs under conditions of induced ROS generation and without them. Dynamic light scattering measurements and UV-Visible spectrophotometry demonstrated aggregation in the systems containing MNPs and HSA, IgG, Hb and fibrinogen. A pronounced change of thermodynamic parameters of denaturation for the proteins in solutions containing MNPs under hydrogen peroxide action has been detected. The influence of oxidative modification and adsorption on MNPs surface on the chemical structure of proteins has been studied for the first time by HPLC-MS/MS analysis and the sites of the oxidative modification have been revealed. The work was funded by the Russian Science Foundation project No. 18-73-00350 (obtaining stable albumin coatings on MNPs) and by RFBR, according to the research project No. 16-34-60244 mol_a_dk (IgG and hemoglobin study on the surface of MNPs). The part of research devoted to the development of DSC technique and other methods applications to the competitive adsorption processes was carried out within the framework of the government task (themes 0084-2014-0001, State reg. No 01201253311, and 0084-2014-0005, State reg. No 01201253307). For HPLC-MS/MS analysis we used devices of Core Facility of the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences "New Materials and Technologies".

## P-36-058
## Optimization of genetically-encoded nanocages for protein transfer between cells

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The mechanisms of cell to cell protein delivery are widely probed in both basic studies and drug development. Improvement of protein delivery will significantly enhance the efficiency of developed therapeutics. Previously group of Neil King and colleagues designed proteins that were able to self-assemble into precisely


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specified, highly ordered enveloped protein nanocages (EPN). In conjunction with the vesicular stomatitis virus glycoprotein (VSV-G) described nanocages were able to release from eukaryotic cells and deliver desired recombinant proteins. Unfortunately, the technology of production of these nanocages was rather complicated and required simultaneous co-transfection with three vectors, which made it difficult to apply for therapy. We solved this problem by construction of lentiviral vector, combining all necessary elements for protein delivery utilizing nanocage-containing extracellular vesicles. We developed and optimized multicistronic vectors with two or three Internal Ribosome Entry Site (IRES) sequences. It allows you to get in one step stable cell line, producing genetically encoded extracellular vesicles loaded with nanocages and desired recombinant protein. Second main disadvantage of previous system was its cytotoxicity, what is manifested in harmful influence of utilizing binding domain (Vpr) to the cycle of mammalian cells. It does not allow its use for human therapy. To solve this problem, we replaced Vpr protein in our vectors with Jun-Fos complex and optimized its arrangement in order not to break self-assembly of nanocages. Complexes formed with Fos and Jun are not toxic to humans and gives us the opportunity to use our system for drug delivery. This study was supported by Russian Science Foundation grant # 18-74-10079.

## P-36-059
## Gold, cadmium and composite nanoclusters: formation, structure and fluorescence

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Metal nanoclusters (NC) up to hundred atoms exhibit unique properties due to size-dependent electronic structure. Our research was aimed at structure-functional analysis of Au and Cd NC fabricated in the presence of bovine serum albumin (BSA). Fluorescent Au, Cd and mixed Au-Cd NC were prepared by recovery of metal ions with BSA as the only reducing agent. NC sizes were less than 1.6 nm as shown by transmission electron microscopy. According to mass spectrometry and energy-dispersive X-ray (EDX) analysis NC contained ~ 15 Au atoms and ~ 35 Cd atoms. Excitation of NC fluorescence in broad range resulted in emission with maximum at 650 nm for Au NC and at 500 nm for Cd NC. The EDX analysis of composite NC (molar ratio of ions 1:1) confirmed the presence of both Au and Cd atoms at ratio ~ 1:3, respectively. Their emission demonstrated stable peak at 630 nm and tunable peak at 420–520 nm depending on excitation wavelengths. Tryptophan emission of BSA-NC at 345 nm was decreased in comparison with pure BSA of the same concentrations. This effect could be explained by the Förster resonance energy transfer between tryptophan and metal NC. Circular dichroism spectra of BSA with the metal NC differed from native protein due to partial denaturation at pH ~ 11 during NC fabrication. The denatured BSA retained after NC formation and dialysis. Fluorescent nanoparticles (NP) ~ 100 nm in average were fabricated by nanoprecipitation from BSA-NC. Metal NC did not aggregate in the NP and maintained their original optical properties. Thus, we have obtained fluorescent Au, Cd and Au-Cd NC stabilized with BSA. The protein protects metal NC from aggregation in solution and after NP formation. Fluorescent properties of the metal NC can be tuned by mixing of Au and Cd ions. Our research was supported by the Russian science foundation (grant No. 17-75-30064).

## P-36-060
## Antibody-decorated superparamagnetic iron oxide nanoparticles for prostate cancer diagnosis

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Tumor metastasis is the most common cause of death in cancer diseases. Metastasis is a complicated and poorly understood process in which circulating tumor cells (CTCs) play an important role. CTCs released from the primary tumor penetrate into the blood or lymph vessels, travelling to the most distant sites, and giving rise to secondary tumors. The detection and analysis of CTCs can significantly help in the early diagnosis of cancer and may help to establish an individual treatment plan for the patient. The aim of our research is to develop a new method of separation of CTCs from the bloodstream using magnetic surface-modified nanoparticles with a tumor-specific antibody. Superparamagnetic nanoparticles (SPIONs) were obtained by co-precipitation of iron oxides II and III in a solution of cationic chitosan derivative and then modified by introducing tosyl groups to their surface. The presence of tosyl groups enables attachment of the carefully selected antibodies. The obtained nanoparticles were characterized physicochemically, magnetically, and visualized using STEM. Biological tests were performed for prostate cancer cell lines: LNCaP and PC-3, and for prostate normal cell lines: RWPE-1 and PZHPV-7. The cytotoxicity of the particles was examined by the MTT assay, expression of the selected proteins and MMPs activity were verified by the Western Blot method and zymography technique respectively. The selected antibody against N-Cadherin was tested for its specificity toward surface proteins of tumor cells. Preliminary tests of separation using nanoparticles with attached antibody were also performed. Karolina Karnas acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16.

## P-36-061
## Development of artificial yeast strain with gene clusters for biosynthesis of antimicrobial protein

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The growth of infectious strains of microorganisms with multiple resistance became a serious problem of modern medicine. This requires the creation of innovative methods and approaches for screening of novel antibiotic drugs. These approaches include the search for antibiotics among "nonclassical" natural sources of biodiversity (microbiota of humans and animals, microorganisms-extremophiles) and creation of artificial libraries of antimicrobial substances. Our work project is focused on the development of libraries of gene clusters for biosynthesis of antimicrobial agents. Here, we transfer the gene cluster of lysostaphin – a Staphylococcus metalloendopeptidase – into yeast *Pichia pastoris* genome. Finally, we develop the recombinant


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yeast strain producing lysostaphin, which possess anti *Staphylococcus aureus* activity. Thus, the combination of modern methods of genomic editing, synthetic biology and the microfluidic method of high-performance screening of cell libraries developed previously by this group opens new prospects to produce antimicrobials of new generation. This work is supported by RFBR grant 18-29-08054.

## P-36-062
## Production of recombinant human transferrin in yeast

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At present time, a mammalian cell cultures are widely used to obtain a spectrum of modern pharmacological drugs based on recombinant proteins (vaccines, blood clotting factors, therapeutic antibodies, etc.). In this regard, one of the most important branches of biotechnology is the development and production of serum-free culture media, which allow significantly the cost of the products obtained and ensure the standardization of biotechnological production. Replacing the protein components of serum-free nutrient media with their recombinant counterparts will eliminate the risk of contamination by various infectious agents. Human transferrin is used as one of the protein components of serum-free media responsible for the transport of Fe3+ iron ions into the cell. We obtained the *P. pastoris* strain-producer secreting human transferrin into the culture medium. It is shown that the maximum expression level of transferrin 50 mg/L is achieved by using the constitutive GAP promoter and maintaining the pH of the medium at 6.5. This work is supported by grant of Ministry of Science and High Education of Russia RFMEFI60717X0177.

## P-36-063
## Ferritin-based nanocarriers for the delivery of metal compounds with interest in cancer therapy

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Human ferritin is a nanocage protein composed of 24 subunits that self-assemble giving rise to an almost spherical shell. Its characteristic architecture provides an internal cavity of 12 nm in diameter that can host thousands of Fe atoms. The biological role of ferritin is sequestering harmful free iron ions preventing adverse reactions inside cells. The peculiarity of human ferritins is the possibility to be internalized inside cells by receptor-mediated endocytosis: Scavenger receptor class A type 5 (SCARA5) and Transferrin receptor 1 (Tfr1) recognize L-richer and H-richer ferritins, respectively. This feature prospects kind of targeted drug delivery in the development of ferritins loaded with metal compounds interesting for cancer therapy. Bionanotechnology inspired us to study the interactions of human H-ferritin with some interesting metal compounds with anticancer applications that contain metal centers like Pt (cisplatin), Ru (NAMI-A) and Fe (as naked ions or hemin). In the case of cisplatin and NAMI-A, only the metal species enter the protein after the loss of their ligands through the uptake along ferritin channels. Otherwise, when the simple diffusion is not permitted, like in the case of hemin, the encapsulation method consisting in the disassembly/assembly of ferritin due to pH was applied. UV-vis spectroscopy, Circular dichroism analysis, fluorescence assays combined with X-ray crystallography gave us the possibility to characterize the interactions of ferritin with the compounds cited below. Interestingly, even Fe(III)-loaded ferritins can be toxic if administered to cancer cells as we observed with HeLa cell lines. Exogenous iron-loaded ferritins incorporated in cancer cells by endocytosis along the endosome-lysosome maturation pathway released iron ions that triggered ROS production causing cell death. In conclusion, those presented are some methods to obtain ferritin adducts with metal compounds to be exploited in targeted delivery in biomedicine.

## P-36-064
## Functional reconstitution of CYP3A4 with NADPH-cytochrome P450 reductase and microsomal cytochrome b5 in peptide-based lipid nanodiscs

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Cytochrome P450 3A4 (CYP3A4) is the most important P450 enzyme in human liver that plays a significant role in the metabolism of a wide range of drugs. For catalysis, CYP3A4 requires interaction with the redox partner, NADPH-cytochrome P450 reductase (CPR), which is an electron donor for microsomal monooxygenases. While the ability of P450's to oxidize substrates requires interaction with the redox partner, catalysis can be also modulated by the interaction with microsomal cytochrome $b_5$ ($b_5$). The role of $b_5$ in reaction catalyzed by CYP3A4 still not fully understood. Experimental *in vitro* studies of the monooxygenase system by its incorporation into a model membrane, for example to peptide-based nanodiscs (ND), will bring it closer to the conditions in the cell. In this work, we prepared $b_5$-ND, CYP3A4-CPR-ND and CYP3A4-CPR-$b_5$-ND complexes. The sizes and homogeneity of the complexes were determined using DLS, gel-filtration and DOSY NMR. When $b_5$-ND is added to the ND-free form of CYP3A4 and CPR at the final ratio 1:1:1, the yield of 6β-hydroxytestosterone (6T) is increased by 60% as compared to the system with ND-free $b_5$. In the case of CYP3A4-CPR-ND complex with CYP3A4:CPR ratio of 1:1 the system also has hydroxylation activity and the addition of $b_5$ leads to the increase of 6T yield, but the kinetic time constant does not changed. This may be indication of the presence of CYP3A4 form that is not able to bind the substrate. Therefore, we assume that interaction of $b_5$ with CYP3A4 leads to a conformational changes resulting in the "opening" of the substrate access channel without affecting the electron transfer from CPR to CYP3A4. The CYP3A4-CPR-$b_5$-ND complex was obtained with the ratio of proteins 1:1:3. This system also has hydroxylation activity, but the yield of 6T is less than in the case of the CYP3A4-CPR-ND system with the same protein ratio. Elucidation of these differences requires further more detailed comparative study.


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## P-36-065

### Supramolecular complexes of Congo red and Titan yellow with drugs (doxorubicin and imatinib) and their interactions with proteins (immunoglobulin light chain and albumin) for targeted drug delivery

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Targeted drug delivery to the tumor tissue is one of the major nowadays challenges. The aim of study was to analyze the possibility of targeted delivering drugs via two compounds forming supramolecular assemblies: Congo red (CR, ribbon like structures), Titan yellow (TY) and proteins. Both CR and TY form strong complexes with model drugs: doxorubicin (Dox) and imatinib (Im) and can also interact with proteins – albumin and immunoglobulins (antigen-complexed antibodies, Ab). In Ab the binding site forms due to local destabilization of the domain due to strains accompanying antigen binding. Plasticity of supramolecular CR, which allows to adapt to the binding site, is vital for CR binding to Ab. Immunoglobulin chain λ (Lλ) was used as a model protein because conformational changes observed in subdenaturing conditions in Lλ mimic those in Ab. Methods used: agarose gel electrophoresis, gel filtration, DLS, molecular modeling. Modeling by fuzzy oil drop method used to analyze the crystal structure of proteins: Lλ (monomer and dimer) and albumin, aimed to determine the structural possibilities and obstacles in the analyzed proteins, affecting differences in the binding of supramolecular assemblies and their complexes with drugs. Supramolecular forms of both CR and TY bind to Lλ while their mixed structures with Dox or Im are not capable to form such complexes. Albumin binds both CR or TY and their complexes with drugs. DLS analyses showed that interaction with Dox or Im markedly increases the dimensions of both CR and TY supramolecular structures. This suggests increased stability of intermolecular interactions resulting in decreased plasticity of the supramolecular structure – which can still bind to albumin but cannot adapt to the binding site created within the beta-sheet of the partly destabilized Lλ domain. We acknowledge the financial support from the National Science Centre, Poland (grant no. 2016/21/D/NZ1/02763). *The authors marked with an asterisk equally contributed to the work.

## Epigenetics and protein glycosylation

## P-37-001

Upgraded to Short Talk ShT-37-2

## P-37-002

### Overcoming bortezomib resistance by HDAC6-selective inhibitor in multiple myeloma

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Although multiple myeloma (MM) patients benefit from standard bortezomib (BTZ) chemotherapy, many relapse, developing drug resistance. We investigated whether histone deacetylase 6 (HDAC6) inhibitor A452 overcomes bortezomib resistance in MM. We show here that HDAC6-selective inhibitor A452 significantly decreases the activation of BTZ-resistant markers, such as NF-kB and ERK, in acquired BTZ-resistant MM cells. Combination treatment of A452 and BTZ synergistically reduces BTZ-resistant markers. In addition, the combination of A452 and BTZ inhibits the activation of NF-κB, translocation to the nucleus, and its binding to the promoter of LMP2, resulting in a decreased expression of LMP2. Furthermore, combining A452 with BTZ leads to synergistic inhibition of cell growth and decreases in the viability of BTZ-resistant cells, as well as increased levels of apoptosis. Furthermore, enhanced cell death is associated inactivation of AKT and ERK1/2. Our findings reveal HDAC6-selective inhibitor as a promising therapy for BTZ-chemoresistant MM.

## P-37-003

### Modulation of poly(ADP-ribose) polymerase 1 activity and chromatin condensation in thymocyte and liver nuclei after treatment of rats with tannic acid

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Poly(ADP-ribose) polymers (PAR) play significant role in basal biological processes, determining cell live or die decisions. PAR polymer catabolism is maintained by complex interplay of enzymes responsible for PAR synthesis and degradation-poly (ADP-ribose)polymerase (PARP 1) and poly(ADP-ribose) glycohydrolase (PARG). PARP 1 inhibitors entered into clinical treatment of cancer patients and the search of more effective and less toxic ones is in progress. Tannic acid (TA), a cocktail of plant-derived polyphenols inhibits PARG and modulates PAR catabolism in *in vitro* experimental settings. It was hypothesized, that slowing down of PAR polymer catabolism could inhibit PARP 1 activity in cells. Herein, we examine whether i.p. injection of TA to rats could influence PARP 1 activity in thymocyte and liver nuclei. Our data come to show, that in 1 h after i.p. injection of TA (25 mg/1 kg) to rats, PARP 1 activity was suppressed by 30% and 12% in thymocyte and liver nuclei correspondingly. In contrast, when 100 mg/1 kg TA was administered to rats, we observed PARP 1 stimulation (by 12% in thymocyte and 50% in liver nuclei) in time-dependent manner. Considering the role of PARP 1 in maintaining chromatin structure, we investigated the effect of TA on chromatin accessibility to artificially activated endogenous $Ca^{+2}/Mg^{+2}$-dependent endonuclease. We observed augmentation of liver chromatin resistance to DNA internucleosomal fragmentation in 2 h after TA i.p. injection to rats. This data indicate on chromatin condensation in liver nuclei after treatment of rats with TA. TA administered to rats affects PARP 1 activity in thymocyte and liver nuclei in dose- and organ-specific manner. TA-induced modulations in PARP 1 activity were paralleled with chromatin condensation in liver nuclei. This work was supported by the RA MES State Committee of Science, in the frames of the research project No. 18T-1F011.

## P-37-004

### Rad6 is critical for the translation of ubiquitylated H2B signal to H3K4 trimethylating activity of Set1/COMPASS

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The methylation of the fourth lysine on histone H3 is a well-known mark of transcription activation, and the ubiquitination of the 123rd lysine of on histone H2B monoubiquitination (H2Bub1) is


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the prerequisite for H3K4 trimethylation (H3K4me3) in *Saccharomyces cerevisiae*. All three types of H3K4 methylation, including mono, di, and tri-methylation, occur by sole H3K4 methyltransferase, Set1 in *S. cerevisiae*. Set1 exists as a complex, called Set1/COMPASS, by interacting with seven other polypeptides containing Swd2. Rad6 is an E2 enzyme for the H2Bub1, and we have created two strains that contain different H3K4me3 levels depending on the epitope position of Rad6 under tagged Swd2, which has been known as a key regulator of histone crosstalk between H2Bub1 and H3K4me3. One of two stains has a defective bulk level of H3K4me3 even when H2Bub1 signal is normal without any deletion or mutation of proteins. Our result can suggest that Rad6 can translate the H2Bub1 signal to H3K4 trimethylating activity of Set1/COMPASS. Our data suggest a functional interaction between Rad6 and Swd2 for the H3K4me3. We also found that some oxidation-reduction related genes are less-expressed in H3K4me3 defective strain comparing the strains bearing normal level of H3K4me3 by RNA-seq analysis.

## P-37-005
## Glycation of fibrinogen affects FXIII-induced crosslinking and shear flow response of fibrin networks

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Glycation of fibrinogen, and subsequently fibrin is a natural process taking place under normal physiological conditions and giving rise to fibrin networks with characteristic structural and mechanical properties. However, excess glycation and the accompanied elevated levels of fibrinogen, as observed in diabetes states, yield formation of modified fibrin clots with more compact and difficult to lyse structure. One possible mechanism underlying these differences may be related to a modified rigidity of individual fibrin fibers resulting from altered FXIII-induced α- and γ-chain crosslinking. Here, using biochemical tools we investigate the effect of glycation on FXIII-induced crosslinking in fibrin networks. Additionally, we use combined mechanical (parallel-plate flow chamber) and optical (confocal microscopy) methods to follow *in situ* changes in the spatial organization of individual glycosylated fibrin fibers in the presence of shear flow. Our results show that fully crosslinked fibrin networks show similar patterns of ligation, regardless of the presence or absence of glycation. Surprisingly however, in FXIII-inhibited glycated fibrin networks, the level of α- and γ-chain crosslinking for a given inhibitor concentration varies compared to control clots. This suggests differences in FXIII activity and/or changes in the overall number of covalent bonds formed across fibrin fibers. Interestingly, glycated fibrin fibers show different distribution within fibrin networks in the absence and presence of shear force, while the distribution of non-glycated fibers does not change upon flow application. Thus, by competing with FXIII crosslinks sites, glucose may interfere with covalent bond formation, influencing fibrin fibers mechanical stability and consequently the response of fibrin networks under shear flow.

## P-37-006
## A continuous activity assay for HDAC11 enabling re-evaluation of HDAC inhibitors

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Histone deacetylase 11 (HDAC11) is the member of HDAC subfamily, yet very low deacetylation activity has been ascribed to the protein. Recently we found HDAC11 to exhibit robust defatty acylase activity, a finding independently and simultaneously discovered by the laboratories of Lin and Olsen. On the one hand, this finding provides insights into so far described HDAC11 functions. On the other hand, it shows that the use of acetylated substrates in the search for HDAC11 inhibitors was not befitting. Here we report the development and validation of a continuous fluorescence-based activity assay using internally quenched myristoylated TNFα-derived peptide as a substrate. Re-evaluation of a set of hydroxamate-based HDAC11 inhibitors by this activity assay revealed their limited potency and selectivity. Consequently, we suggest re-evaluation of biological experiments focused on HDAC11 function using certain HDAC11 inhibitors. Furthermore, we believe our data lay a foundation for the screening campaigns aimed at the identification of future HDAC11-selective compounds.

## P-37-007
## Different mechanisms of vitamin D receptor gene expression or silencing in blood cells of mice and man

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Hematopoiesis is a complex process by which all blood cellular components are formed. Blood cells develop from hematopoietic stem cells (HSC). It has been shown that vitamin D receptor (VDR) is present in several types of blood cells, and that active form of vitamin D, 1,25-dihydroxyvitamin D (1,25D) is important for the proper function of the immune system. Activities of retinoic acid receptor (RAR) also play an important role in hematopoietic differentiation. Both receptors acts as transcription factors, and RAR takes part in regulation of VDR expression. We decided to compare the effects of 1,25D and all-trans-retinoic acid (ATRA) stimulation on *VDR* gene expression in normal human and murine blood cells at various steps of their development. We demonstrated that regulation of *VDR* expression in humans is different from in mice. We showed that in human hematopoietic stem and progenitor cells (HSPCs) ATRA upregulates the expression of *VDR*. However, in murine blood cells only auto-regulation takes place, and 1,25D upregulates the expression of *VDR*. Both receptors, VDR and RAR are transcriptionally active in human and murine blood cells at early steps of hematopoiesis, but less at the later steps. Thus, we decided to examine if epigenetic mechanisms, such as DNA methylation, take part in


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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VDR silencing at late steps of blood development. In mice, methylation of VDR appeared not to be a key to maintaining the different level of VDR expression throughout blood development. In human blood cells isolated from healthy donors, main and alternative VDR gene promoters were not methylated. However, in leukaemia cells alternative VDR gene promoter was often methylated. Supported by grant OPUS 2015/17/B/NZ4/02632 from the National Science Centre of Poland. *The authors marked with an asterisk equally contributed to the work.

## P-37-008
## Folic acid modifies DNA methylation machinery and remodels promoter methylation profile of selected tumor suppressor genes in normal-like mammary epithelial MCF10A cells

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Folate, one of the most studied dietary compounds, has been still the main topic of debate on food fortification. Although low folate levels may be associated with increased risk of cancer development, simultaneously several reports indicate detrimental effects mediated by high folate concentrations. Folate is a water-soluble form of vitamin B9, which is important for DNA synthesis, repair, and methylation. Folate as a crucial nutrient involved in one-carbon metabolism has a direct effect on the level of a methyl donor, S-adenosyl-L-methionine. Our previous *in vitro* studies in human breast cancer cells demonstrated that the increasing concentrations of folic acid lead to a dose-dependent down-regulation of the selected tumor suppressor genes, which may be linked to the increased DNA methylation detected within their promoter regions. The effects of folic acid exposure were more remarkable in non-invasive MCF7 cells, where we also observed robust *DNMT1* up-regulation. In the present study, using methylation-sensitive restriction analysis and qPCR, we tested the impact of folic acid on promoter methylation and expression of *PTEN* and *RARB* tumor suppressor genes in normal-like mammary epithelial MCF10A cells, used as an experimental *in vitro* model of human normal cells. The results show that folic acid exposure modifies DNA methylation machinery (*DNMT1* and *CDKN1A* expression) and remodels DNA methylation profile of selected tumor suppressor genes in MCF10A cells. Moreover, bioinformatic analysis of publicly available data from the GEO DataSets was carried out in order to evaluate the effects of folic acid supplementation on *PTEN* and *RARB* methylation in human blood DNA. Our findings show that caution needs to be used when introducing folic acid supplementation, and designing *in vitro* and *in vivo* studies on folic acid exposure. The Medical University of Lodz financed the study, grants #503/6-099-01/503-61-001 and #502-03/6-099-01/502-64-133-18.

## P-37-009
## Piperine inhibits adipocyte differentiation via dynamic regulation of histone modifications

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Previously, we reported that piperine, one of the major pungent components in black pepper, attenuates adipogenesis by repressing PPARγ activity in 3T3-L1 preadipocytes. However, the epigenetic mechanisms underlying this activity remain unexplored. Here, gene set enrichment analysis using microarray data indicated that there was significant downregulation of adipogenesis-associated and PPARγ target genes, and upregulation of genes bound with H3K27me3, in response to piperine. As shown by Gene Ontology analysis, the upregulated genes are related to lipid oxidation and polycomb repressive complex 2 (PRC2). Chromatin immunoprecipitation assays revealed that PPARγ (and its coactivators), H3K4me3, and H3K9ac were less enriched at the PPAR response element of three adipogenic genes, whereas increased accumulation of H3K9me2, H3K27me3, and Ezh2 was found, which likely led to the reduced gene expression. Further analysis using three lipolytic genes revealed the opposite enrichment pattern of H3K4me3 and H3K27me3 at the Ezh2 binding site. Treatment with GSK343, an Ezh2 inhibitor, elevated lipolytic gene expression by decreasing the enrichment of H3K27me3 during adipogenesis, which confirms that Ezh2 plays a repressive role in lipolysis. Overall, these results suggest that piperine regulates the expression of adipogenic and lipolytic genes by dynamic regulation of histone modifications, leading to the repression of adipocyte differentiation.

## P-37-010
## Construction and analysis of an interactome between nucleosomes and chromatin proteins

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At the core of regulating and maintaining the epigenetic state of a eukaryotic cell lies the nucleosome – a stretch of DNA wrapped around an octamer of histone proteins. Nucleosomes participate in dynamic interactions with many chromatin proteins enabling the functioning of the cell's genome. Nucleosomes themselves undergo compositional changes through histone turnover, incorporation of histone variant genes, splice-isoforms and post-translational modifications. In the course of evolution, chromatin proteins evolved to distinguish slight variations in the nucleosome structure. Studying the network of these interactions is important to understand the function of the eukaryotic cells. However, the network of nucleosomal interactions is currently not well resolved because the high throughput approaches usually are unable to distinguish between the multitude of histone genes and their variants. Hence we set forth to reexamine the publicly available datasets of protein interactions and to construct an interactome focused on understanding the details of various nucleosome and chromatin proteins interactions. First of all, we constructed the comprehensive list of histone genes, pseudogenes and proteins with splice-isoforms in humans. For initial data from STRING and BioGRID databases, we designed pipeline to process, analyse and visualize nucleosome interactions. We conducted a quantitative analysis to understand how the interactome depends on the data sources and score thresholds. As the result of this work we constructed the nucleosome interactome with both experimental and predicted interactions. To qualitatively analyze the interactome, we designed a functional hierarchical classification for chromatin proteins. Overall, our findings reveal a complex network of interactions between nucleosomes and chromatin proteins, many of which are specific to the histone composition of the nucleosome. This work was supported by Russian Science Foundation Grant No. 18-74-10006.


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## P-37-011
## Accurate and high-throughput, targeted multiplex quantification of CpG methylation without DNA extraction and bisulfite treatment

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DNA methylations at specific CpG loci act as biomarkers for cancer diagnostics. Targeted quantification of CpG methylation currently requires tedious DNA extraction and complex multistep procedures that prevented clinical applications. Accurate quantification of CpG methylation is often difficult using bisulfite-based quantitative assays due to inconsistent C to U conversion and template DNA degradation. We described a quantitative and high throughput CpG methylation assay that does not involve DNA extraction and bisulfite conversion. After cell lysis target DNAs are captured to the bottom of the 96-well plate via sandwich hybridization with multiple contiguous probes having defined 5′- or 3′-end tail sequences. After wash and enzymatic ligation of the bound probes, the ligation products spanning each target CpG site are treated either with or without a methylation-sensitive restriction endonuclease, which will cleave at specific unmethylated-cytosine residues while leaving the methylated ones intact. Afterwards PCR amplification is performed in the same well with a universal primer pair. Quantification of the CpG methylation is obtained by comparing between restriction-treated and untreated groups the amount of each amplified products. For single CpG measurement this is achieved via real-time PCR; and for multiple CpG determination this can be achieved via MOLDI-TOF mass spectrometry. We measured the degree of methylation using our assay with HpaII on test DNA samples ranging from 0% to 100% methylation. A linear regression revealed quantitative and reproducible recovery across the entire methylation range, with a slope of 1.016 and $R^2$ of 0.993. With a significantly simplified procedure and a much higher throughput, our assay efficiently offered better accuracy than the bisulfite-based pyrosequencing and MethLight. The robustness and sensitivity of our assay in quantitative CpG methylation assessment in cancer as well as in human aging will be presented.

## P-37-012
## The effect of Suv39H1 histone methyltransferase knock-out in keratinocytes on epidermal differentiation process

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The epidermis is a dynamic, continually renewing tissue that constitutes a life-sustaining interface separating the organism from the environment. It is established upon a terminal differentiation process during which basal keratinocytes sequentially switch gene expression program. The regulation of keratinocyte gene expression may involve epigenetic factors. Here, by means of CRISPR/Cas9 method, we obtained a model of a stable keratinocyte cell line, HaCaT, with a knock-out of Suv39H1 histone methyltransferase, a key enzyme that trimethylates lysine 9 in histone H3 (H3K9me3). H3K9me3 is a known hallmark of inactive chromatin. Suv39H1 KO cells reveal a 50% drop in the level of H3K9me3, which leads to local chromatin decondensation in the nucleus as shown on immunostaining images. Our studies indicate that SUV39H1 knock-out profoundly affects the expression of differentiation markers (loricrin, involucrin) and other proteins crucial for epidermal formation such as, for example, the late cornified envelope genes – *LCE1A-F* and, in consequence, the process of keratinocyte differentiation The cells also exhibit a lower proliferation rate in comparison to a wild type cells. Moreover, we found that HaCaT Suv39H1-KO cells prefer to grow aggregated in clumps, which may be explained by altered cell adhesion. In short, we present a new, Suv39H1-KO HaCaT keratinocyte model to study effects of the repressive H3K9me3 modification on the process of terminal differentiation in keratinocytes.

## P-37-013
## Clofarabine inhibits DNA methylation machinery, upregulates tumor suppressor genes and induces apoptosis of ALL cells

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Clofarabine (2-chloro-2′-fluoro-2′-deoxyarabinosyladenine, ClF), a second-generation 2′-deoxyadenosine analogue, possesses manifold anti-cancer activities. Our previous reports and some others demonstrate potential capacity of ClF to regulate epigenetic machinery. Our present study is the first to investigate the influence of ClF on the modulators of DNA methylation machinery, including *DNMT1* and *CDKN1A* in acute lymphoblastic leukemia (ALL) cells. ClF-mediated effects on promoter methylation and transcriptional activity of hypermethylated and silenced tumor suppressor genes (TSGs), including *APC*, *CDKN2A PTEN* and *RARB*, have been tested as well. Methylation level of proximal promoter region of *APC*, *CDKN2A*, *PTEN* and *RARB* as well as expression of those TSGs, *DNMT1* and *CDKN1A*, were estimated using methylation-sensitive restriction analysis and qPCR, respectively. The NALM6 cell line was used as an experimental *in vitro* model of ALL cells. We observed ClF-mediated inhibition of cellular viability and apoptosis induction of NALM6 cells with increased percentage of cells positive for active Caspase-3. Interestingly, exposure of NALM6 cells to CIF at 20 nM concentration for 3 days led to significant *DNMT1* downregulation accompanied by robust *CDKN1A* upregulation. ClF caused hypomethylation of *APC*, *CDKN2A*, *PTEN* and *RARB* with concomitant increase in their transcript levels. Taken together, our results demonstrate the ability of ClF to reactivate DNA methylation-silenced TSG in ALL cells. It may implicate translational significance of our findings and support ClF application as a new epigenetic modulator in anti-leukemic therapy. The Medical University of Lodz financed the study, grant no. 503/6-099-01/503-61-001.

## P-37-014
## Identification and analysis of super-enhancers as novel biomarkers and potential therapeutic targets for age-associated diseases

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A modern epigenomic approach, which has already shown high efficiency, is the analysis of super-enhancers—large domains consisting of multiple closely located enhancers. Currently, super-enhancers as biotargets have already demonstrated their significant potential in the studies of cancer and a wide range of other diseases. Our research is devoted to identification and analysis of such super-enhancer regions specific for age-associated diseases. Using epigenomic profiling of cell lines and clinical samples, we


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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search for and identify super-enhancer elements. It was shown earlier that some super-enhancers play an important role in regulating transcriptional gene activity in many diseases, and their deletion or suppression is effective against the disease progression. Here, we have developed a universal method for rapid analysis and identification of super-enhancers based on genome-wide studies of H3K27ac and H3K4me1 by the ChIP-Seq, taking into account the distribution profile of the mediator complex (Med1) of bromodomain-containing protein 4 (BRD4). Several hundred super-enhancers were identified and categorized according to such parameters as length; binding to Med1 and BRD4; saturation H3K27ac and H3K4me1 histone modifications; and presence of DNase I hypersensitive sites. The developed method can be used to quickly analyze the data from various patients in order to search for, identify and categorize super-enhancers. Such analysis is promising for identifying statistically significant differences in the patterns correlated with disease stages. It will enable identifying targets that are promising for further studies based on the use of specific inhibitors and CRISPR/Cas genome-editing. Such methods could play a significant role in combating age-associated diseases as corrective and adjunct to conventional systemic therapy. The research was supported by Russian Foundation for Basic Research (grant 16-33-60228).

## P-37-015
## Fc-linked N-glycosylation of murine IgG1 variants

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The structure of N-glycans attached to the Asn297 in fragment crystallizable (Fc) region of immunoglobulin G (IgG) affects the affinity of IgG to its ligands and consequent immune responses. Great inter-individual variation in Fc-linked N-glycome of IgG is observed both in human populations and between mouse strains. Mice have four IgG subclasses (IgG1, IgG2a/c, IgG2b and IgG3) each with distinct immune functions and specific N-glycosylation profile. In our recent study we identified a single nucleotide change (rs51376262) in the *Ighg1* gene as one of the candidate genetic variants influencing total IgG N-glycosylation in mice. Allelic state of rs51376262 defines the amino acid residue (phenylalanine or isoleucine) preceding the N-glycosylated Asn297 in the Fc of mouse IgG1 subclass. The aim of our study was to test if this mutation is associated with the changes in IgG1 Fc-linked N-glycome. We analyzed IgG Fc-glycopeptides with liquid chromatography-mass-spectrometry in 95 strains of the powerful Collaborative Cross (CC) resource of recombinant inbred mouse strains (Geniad Pty Ltd, Animal Resources Centre, Murdoch, WA, Australia). The CC strains were genotyped using the MegaMUGA platform (GeneSeek; Lincoln, NE). We performed quantitative trait loci mapping of subclass-specific IgG Fc-linked glycosylation traits using online tools (www.sysgen.org/GeneMiner). We observed that lower levels of galactosylated, mono- and disialylated N-glycans in the IgG1 Fc-linked glycome were associated with the 296Ile IgG1 variant. Functional studies would be needed to obtain further proof of the effect of amino acid composition of mouse IgG1 heavy chain on its N-glycosylation.

## P-37-016
## Altered expression of T cell glycosyltransferases in Graves' disease

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The increased number of circulating CD4+ T cells is characteristic for Graves' disease (GD), one of autoimmune thyroid diseases (AITD). N-glycans are essential for proper functioning of immune system. Altered glycosylation of immune proteins is well documented for multiple sclerosis and rheumatoid arthritis while glycosylation in GD has been studied to a limited extent. The aim of our study was to assess the mRNA expression of glycosyltransferases, the enzymes that catalyse synthesis of oligosaccharides, in CD4+ T cells from GD patients and control group. T cells were isolated from GD donors before thyrostatic drug treatment and 3 months after stabilization of TSH blood level. Age-matched healthy individuals were enrolled to the control group based on TSH level and the absence or low level of anti-thyroid antibodies (thyroperoxidase, TPO; thyroglobulin, Tg; and thyrotropin receptor, TSHR). Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood samples by the density gradient centrifugation method. CD4+ T cells were purified from PBMCs using immunomagnetic cell sorter and finally the pool of activated CD25+ cells were separated from CD4+ lymphocytes. The mRNA level of MGAT5, ST6Gal1, and FUT8 was quantified by qRT-PCR using TaqMan gene expression assays and normalized to the expression of RPL13A endogenous gene. The relative quantification of gene expression was performed using the $2-\Delta\Delta Ct$ method. The results showed a down-regulation of MGAT5 and ST6Gal1 expression in GD patients in comparison to healthy donors. In contrast we observed an elevated expression of FUT8 in GD. Considering the crucial role of N-glycans in immune system, the obtained results might contribute to the better understanding of glycosylation changes in immune cell dysregulation and thyroid autoimmunity. Acknowledgments: This research project is founded by the National Science Center (grant No. 2015/18/E/NZ6/00602).

## P-37-017
## Epigenetic mechanism involved in regulation of two immunity factors in cervical cancer

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Cervical cancer (CC), the third leading malignancy among women in the world and is caused by persistent infection with high-risk human papillomavirus types (hrHPV). A key role in cervical oncogenesis is played by the two viral oncogenes (E6 and E7) of hrHPV types. E6 oncoprotein is supposed to target IRF3 (IFN regulatory factor 3) and to inactivate p53 activity. IRF3 is a transcription regulator of cellular responses and is involved in innate immune system's response to viral infection by promoting type I IFN (IFN-α, -β). Currently, the molecular mechanism of IRF3 inactivation has not been yet elucidated. Therefore, the present study aims to evaluate IRF3 gene promoter methylation status and its expression levels in CC. We also intend to evaluate the expression levels of IFN-β, IRF3 target. For this, 43 patients were investigated: 19 SCC (cervical squamous cell carcinoma)


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(median age = 38 years) and 24 control group (negative for cytology and HPV infection) (median age 31 years). DNA isolation was performed and samples were modified with sodium bisulfate. Total RNA was extracted from investigated samples and, then was used for cDNA synthesis. In order to quantify expression levels of IRF3 and IFN-β, qRT-PCR was performed. Statistical analysis was performed using GraphPad Prism 5.0. Our data revealed that the IRF3 gene promoter is significantly methylated ($P = 0.0040$) in tumors samples (median = 36.02%; range: 29.14–99.95) versus control (median = 1.671%; range: 0.01848–8.099). Regarding investigated genes expression levels we noted that both IRF3 and IFN-β display a down-regulated expression pattern in tumour samples. Moreover, an inverse correlation between IRF3 promoter's methylation and expression levels was noted ($r^2 = -0.8$; $P = 0.043$). We suggest that the viral mechanism of IRF3 and IFN-β expression deregulation is at epigenetic levels. This represents a new insight on hrHPVs role in deregulated immune cellular response and CC development.

## P-37-018
## Role of direct MLE–CLAMP interaction in recruiting of dosage compensation complex on the male X chromosome in Drosophila

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In Drosophila, a multisubunit dosage compensation complex (DCC) is responsible for an increase in the expression of genes on the X-chromosome in males. DCC consists of five proteins, MSL1, MSL2, MSL3, MOF, and MLE, and includes two non-coding RNAs, roX1 (3.7 kb) and roX2 (0.6 kb) that perform similar functions. The MLE protein, an ATP-dependent RNA/DNA helicase of the DEAD subfamily, interacts with either roX1 or roX2, and induces their unwinding and, as a result, making both roX RNAs capable of binding MSL2. Thus, roX RNAs link the MLE and MSL2 proteins into a single complex. One of the key questions is how the DCC specifically binds to the X-chromosome of males. It was shown recently that simultaneous interaction of MSL2 with specific DNA motif and DNA bound Zinc-finger protein CLAMP is critical for recruitment of DCC to the X chromosome. Here we mapped a 20 bp region in MLE that interacts with two zing fingers that are not needed for CLAMP binding to DNA. The deletion of this region in MLE is lethal at pupae stage of development. GAL4-induced transcription of a mutant MLE with a deletion of the Clamp-binding region leads to the selective death of male flies. The latest results demonstrating the role of the CLAMP-MLE interaction for recruiting DCC will be presented. The work was supported by the Russian Scientific Foundation grant 17-74-20155.

## P-37-019
## Insulator binding sites can modulate PREs activity in *Drosophila melanogaster*

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The proteins of the PcG group (Polycomb group) are conserved regulators of gene transcription. Mutations in the genes encoding these proteins can cause cancer. In *Drosophila melanogaster* PcG proteins are recruited to specific loci called PREs (Polycomb response elements). PREs participate in maintaining the silent state of many genes. At the same time, the repressing activity of PREs can be switched off allowing activation of genes. Such modulation of PREs activity is required for establishing the correct pattern of gene expression during development and its mechanisms are still poorly understood. In this work we hypothesized that the PREs activity can depend on a chromatin status surrounding the PRE-containing locus. To test this idea, we have created a model system allowing to study the influence of various elements on PRE activity in the same place in the genome. In this system the activity of PRE is monitored by its ability to repress the *white* gene reporter. Using this approach, we have shown that the addition of binding sites for dCTCF, Su(Hw), Pita to the PRE sequence leads to a dramatic increase in the binding of PcG proteins and to repression of the *white* gene reporter. We assume that the above DNA regulatory elements change the chromatin status of the locus to stabilize the binding of the PcG repressors to chromatin.

# Genome editing (CRISPR)

## P-38-001
## Nuclear receptor RORC as a link between cholesterol synthesis and the circadian clock

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Cholesterol synthesis is a complex process composed of more than 20 reactions and is, as many other processes in the body, regulated by the circadian clock. We focus on the post-lanosterol cholesterol synthesis. Non-polar sterols that arise as intermediates during cholesterol synthesis pathway can bind to RAR Related Orphan Receptor C (RORC), which is nuclear receptor and a transcription factor. Within this work we test the hypothesis that activation of RORC with non-polar sterols modulates the expression of core clock genes that, in turn, influences the clock-dependent lipid metabolism. We propose a new molecular link between metabolism and the circadian clock that we named the sterol-clock network. We obtained mouse embryonic fibroblasts (MEF) with partial or full knockout in the lanosterol 14 alpha-demethylase gene (Cyp51), enzyme from late part of cholesterol synthesis. 24-h circadian experiments were performed in MEFCyp51−/− knock-out and wild type MEFCyp5+/+cells, followed by gene expression and protein expression analyses. The MEFCyp51−/− cells cannot synthesize cholesterol due to blockade at the CYP51 enzymatic step. Consequently, the concentration of sterol intermediates before and after the blockade is altered. Initial data support the RORC hypothesis, since we observed modulated amplitudes and phases of the core clock gene in MEFCyp51−/− cells. To investigate if the lipid metabolism-clock network is a more general phenomenon operating outside the embryonic cells, we applied human immortal hepatocyte HepG2 cells and with CRISPR-Cas9 knocked-out four genes from the late (sterol) part of cholesterol synthesis (CYP51, DHCR24, SC5DL and HSD17B). Every HepG2 cell line with knockout in one of the late cholesterol synthesis genes has a different collection sterols intermediates. These unique cell models will permit identification of crucial sterols of the RORC signaling and provide mechanistic insights into the novel sterol-clock molecular network.


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## P-38-002
## DNA lesions against genome editing: how Cas9 cleaves the substrates with damages

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One of the methods that used in genome editing is a site-specific generation of double-strand break in DNA and following DNA repair. A Cas9 protein – a nuclease associated with CRISPR – together with a single guide RNA (sgRNA) recognizes a protospacer adjacent motive (PAM, 5′-TGG-3′) in DNA, then forms heteroduplex with a protospacer and cleaves DNA. Previously it was shown, that mismatches in the protospacer decrease efficiency of the DNA binding and cleavage by the Cas9/sgRNA complex. However, how DNA lesions impact on cleavage by the Cas9/sgRNA previously wasn't studied. Therefore we decided to study the Cas9/sgRNA cleavage of duplexes and plasmids with point DNA lesions in PAM and the protospacer. As substrate for Cas9 nuclease, we used plasmids and radiolabelled duplexes with substitutions such as 8-oxoguanine (8-oxoGua), stable AP-site (dSpacer) and uracil (Ura). These substitutions were in PAM and the protospacer. As a result of biochemical screening *in vitro*, it was shown that any substitutions in PAM sequence of guanine (Gua) by 8-oxoguanine (8-oxoGua) or stable AP-site (dSpacer) abrogate the cleavage of duplex substrate. But plasmids with the same substitutions were cleaved by the Cas9/sgRNA complex. There are several differences between substrate specificity in the protospacer. They are located at distal area of protospacer, that included in a check point before cleavage stage. Sum up, the DNA cleavage by Cas9 depends on the type of DNA lesion, its position in the protospacer or PAM, and the type of substrate.

## P-38-003
## Modulation of snoRNA activity in human cells using CRISPR/Cas9 tools

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Small nucleolar RNAs (snoRNAs) are encoded in the introns of various host genes and guide 2′-O-methylation of rRNA nucleotides in eukaryotic cells. There are several main elements in the structure of snoRNAs: conserved boxes C and D and a guide sequence complementary to a region within the target rRNA. We have constructed and obtained plasmids expressing CRISPR/Cas9 system elements targeted at various sites within the snoRNA structure. A series of snoRNAs encoded in the introns of *gas5* gene were chosen for the study. The obtained plasmids were transfected into 293FT cells, and modified cell lines expressing only snoRNAs with altered structure and lacking wild-type snoRNAs were generated. Analysis of snoRNA expression by RNA-Seq and qRT-PCR demonstrated the presence of mutant snoRNA forms among transcripts in the obtained cells. Analysis of 2′-O-methylation of the target rRNA showed decreased modification level of the target nucleotide. The studies also revealed the presence of snoRNA with altered targeted specificity. Mutation in the target snoRNA gene resulted in altered expression of the host gene and snoRNAs encoded in other introns of the host gene. Altered splicing pattern and intron retention were noted for the obtained mutant cells. The obtained results demonstrate that snoRNA can be edited with further generation of viable cell lines stably expressing mutant snoRNAs. However, the structure of the host gene should be considered in such cases since mutations in snoRNA can affect splicing of the nost gene. The study is supported by RFBR grant No. 18-29-07073.

## P-38-004
## Mild hypothermia and hypoxia increase the frequency of homologous recombination directed DNA repair in HEK cell line

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Genome editing allows for introducing deliberate and specific changes into the genome of different organisms with the use of designer nucleases and a DNA template if necessary. This is an extremely useful tool in many areas of biotechnology, such as plant engineering, disease modeling, and importantly, in gene therapies in humans. Precise genome editing requires the involvement of homologous recombination (HR) pathway in the repair of double-strand breaks (DSBs) introduced by designer nucleases. An alternative pathway – non-homologous end-joining (NHEJ) occurs more often in cells, however, it frequently introduces small mutations into the DNA sequence. In our research, we have investigated environmental factors which increase the frequency of HR events in cells in order to improve efficiency of precise genome editing. For this purpose, we have used a fluorescence-based molecular reporter allowing for monitoring DSBs repair in cells. The DSBs in the reporter were introduced by I-SceI meganuclease. The repair of the breaks with the contribution of HR pathway was reported by green fluorescence, coming from enhanced green fluorescent protein (EGFP), whereas restoration of DNA sequence by NHEJ pathway was indicated by red fluorescence coming from mCherry protein. We have exposed human embryonic kidney (HEK) cell line with transiently expressed reporter to different environmental conditions, such as various temperatures and oxygen concentrations. We have shown that transient cells treatment with mild hypothermia (32 °C) and hypoxia (5% $O_2$) increases usage of HR pathway in the repair of DSBs, when compared to standard cell culture conditions (37 °C, 21% $O_2$). This result will be used to elaborate a new protocol of efficient and precise genome editing in cells. We conclude, that environmental conditions affect DNA repair mechanisms in cells, which may be implicated in improving precise genome editing methodology.

## P-38-005
## F508del editing in CFTE29o- cell line and iPSCs from patient with cystic fibrosis by CRISPR/Cas9

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Treatment of cystic fibrosis (CF), most common monogenic hereditary disease, remain the problem even with the existence of


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pathogenesis-based therapy. Genome editing allows to correct mutation and develop cause-specific treatment of the disorders. The aim of this work is editing the F508del mutation in *CFTR* gene in CFTE29o- cell line and iPSCs using CRISPR/Cas9. Work was performed in CFTE29o- cell – immortalized cell line of tracheal epithelium from a patient with CF (F508del/F508del) and iPSCs obtained from skin fibroblasts from a patient with CF (F508del/F508del). Three nucleases (eSpCas9(1.1), SpCas9(HF4), SaCas9) in combination with four sgRNAs were used. Two sgRNAs were targeted specifically to F508del (sgCFTR#1, sa_sgCFTR#3); the other two were to downstream region (sgCFTR#2, sgCFTR#3). In addition, we used a plasmid with an insert of *CFTR* fragment with F508del to study potential influence of the genomic context on the editing efficiency. Four single-stranded oligodeoxynucleotides (ssODNs) were designed to repair double-strand DNA breaks. Cas9+sgRNA plasmids were co-transfected with model plasmid and ssODN into CFTE29o- or iPSCs by lipofection. The editing efficacy was evaluated by TIDE and TIDER methods. In iPSCs indel formation efficacy varied from 1.4% to 9.6%. The average cut rate in pGEM-CFTR was 9.3%, while in genomic locus – only 4.3%. The best combination was eSpCas9(1.1)/sgCFTR#3. CTT knock-in efficacy using different ssODNs was low (0–0.5%). In CFTE29o- indel formation efficacy was 2.1–7.5% in the plasmid and 1.4–7.9% in the genomic locus. Then we co-transfected cells with the most effective combination eSpCas9(1.1)/sgCFTR#3 together with ssODN, homologous repair (CTT insertion) in genomic locus was 8.7%. This level of efficiency makes reasonable development of treatment of CF by genome editing. Additional studies are necessary to confirm these results and increase efficacy. Work was supported by Russian Science Foundation (Agreement No. 17-75-20095).

## P-38-006
## *Pasteurella pneumotropica* and *Demequina sediminicola* Cas9 orthologs characterization

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SpCas9 from *Streptococcus pyogenes* is the most investigated Cas protein which is widely used for genome editing in eukaryotes due to its efficiency and usability. SpCas9 gene size doesn't allow its packaging into AAV particles with all components of CRISPR Cas systems. Several smaller orthologs including SaCas9 and CjCas9 were found but they require the presence of long complicated PAM (protospacer adjacent motif) sequence on DNA target, which limits the application of these nucleases. We characterized new Cas9 orthologs from *Pasteurella pneumotropica* (rodent pathogen) and *Demequina sediminicola* (bacteria from sea sediment) which have a relatively small size. RNA sequencing analysis showed that all components of these CRISPR Cas systems are actively transcribed. Reconstruction of Cas9-crRNA-tracrRNA complexes *in vitro* showed that these proteins efficiently cleave DNA targets at 37 °C, which makes them suitable candidates for genome editing in human cells. Moreover, PpCas9 and DsCas9 nucleases can protect *Escherichia coli* from plasmid invaders. Bacterial interference PAM screens showed that both proteins have relatively short "AC"-rich PAM sequences, different from known to date to our knowledge. Overall, our results demonstrate that PpCas9 from *Pasteurella pneumotropica* and DsCas9 from *Demequina sediminicola* are active small size nucleases, which potentially could be used for genome editing in human cells. This research is supported by the Ministry of Education and Science of the Russian Federation Subsidy Agreement No. 14.606.21.0006. The unique project identifier – RFMEFI60617X0006.

## P-38-007
## CRISPR Cas9 system from *Clostridium cellulolyticum*

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*Clostridium cellulolyticum* is a mesophilic cellulolytic bacterium originally isolated from decayed grass compost. The capability of fermentation of plant materials with yielding ethanol and H 2 makes this organism an attractive instrument for biofuels production. Improvements of Clostridium cellulolyticum fermentation features require fast and efficient methods to modify the bacteria's genome. Clostridium cellulolyticum possesses CRISPR Cas system class IIC which is presented by CcCas9 effector protein, adaptation module, CRISPR cassette and sequence coding for tracrRNA. This domestic CRISPR Cas can serve as a convenient instrument for Clostridium cellulolyticum genome manipulation and screens in order to improve cellulose fermentation. To study *Clostridium cellulolyticum* CRISPR Cas system we created the heterologous CRISPR loci expression system in E. coli. RNA sequencing showed robust expression of all significant CRISPR Cas system components including CcCas9 effector gene, tracrRNA and crRNAs. Using bacterial interference experiments and *in vitro* DNA cleavage screens we showed that CcCas9 recognizes short pyrin-rich PAM sequences. Thus, we biochemically characterized CcCas9 CRISPR system and developed *in vitro* DNA cleavage system based on this nuclease. These results could enable fast and efficient genome editing of Clostridium cellulolyticum by transformation the bacteria by short crRNA instead of using long plasmids coding for heterogonous SpCas9 system from Streptococcus pyogenes. This research is supported by the Ministry of Education and Science of the Russian Federation Subsidy Agreement No. 14.606.21.0006. The unique project identifier – RFMEFI60617X0006.

## P-38-008
## Manganese-dependent Cas9 ortholog from *Defluviimonas* sp. *20V17*

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Initially found in bacteria CRISPR Cas9 systems became a powerful tool in genome engineering. Complement pairing between guide crRNA and DNA sequence allows Cas9 proteins to recognize target site and introduce double-stranded break within it. Although all Cas9 nucleases have a common domain organization, orthologous Cas9 proteins can possess specific features due to different habitats of their bacterial hosts. *Defluviimonas 20V17* is a marine bacterium, which was isolated from a deep-sea hydrothermal vent chimney collected from the South-west Indian Ridge (Jiang et al, 2014). Bioinformatics search revealed the presence of CRISPR Cas9 locus in its genome (DfCas9 further). We cloned DfCas9 loci into pACYC184 to perform heterologous expression in E. coli. RNA sequencing analysis along with bacterial interference tests showed that CRISPR DfCas9 loci is an


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active bacterial defence system. We purified recombinant DfCas9, determined its PAM sequence and set up *in vitro* DfCas9 DNA cleavage system. Surprisingly we found that DfCas9 displays a low level of nuclease activity in presence of Mg2+ ions but efficiently cleaves DNA in presence of Mn2+. The nuclease activity of the majority of known Cas9 proteins requires Mg2+ binding in active sites of the proteins. DfCas9 preference to manganese ions and almost absence of activity with magnesium could be an evolution outcome: *Defluviimonas 20V17* habitat is Mn-salts rich sedimentation of hydrothermal vent chimneys. This unusual environmental salt composition could shift DfCas9 active center to Mn2+ preference. Thus, despite on extensive studying of SpCas9 nuclease and numerous newly developed Cas-studying approaches, biochemical characterization of each Cas9 ortholog still remains challenging due to unique features of these evolving in different environments proteins. This research is supported by the Ministry of Education and Science of the Russian Federation Subsidy Agreement No. 14.606.21.0006. The project identifier – RFMEFI60617X0006.

## P-38-009
## A novel approach to discovering new molecular mechanisms in autoimmunity

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Currently only a small fraction of human genome serve as pharmaceutical targets, and broadening of our knowledge on the druggable genome is a high priority goal for basic research and industry. Autoimmune diseases are complex diseases with considerable, but not fully uncovered heritability, for which treatment options are still unsatisfactory and limited. We suggest a new approach to searching for therapy targets for these conditions. After identification of functional regulatory autoimmunity-associated single-nucleotide polymorphisms (SNPs) with unknown gene targets, alternative alleles should be introduced into a relevant cell line using CRISPR/Cas9 system and affected genes could then be found through RNA sequencing and quantitative real-time PCR validation. To find such candidate SNPs, we have used four parameters: (i) Association data from Immunochip-based Genome-wide association studies (GWASes); (ii) Overlap with H3K27Ac and DNase I hypersensitivity sites in disease-relevant primary human cells according to the ENCODE Project data; (iii) Relative probability of being causative according to the mathematical algorithm called Probabilistic Identification of Causal single-nucleotide polymorphisms (PICS); and (iv) Predicted interference with a transcription factor binding motif according to Jaspar and HOCOMOCO11 databases and PERFECTOS-APE software. We found five most likely causative regulatory SNPs associated with autoimmune diseases, for which target genes are not currently known: rs2163226, rs4772201, rs61972489, rs6832151, and rs2387397. Their target genes are expected to participate in immune system regulation and may become new therapy targets. This work is supported by the Russian Science Foundation (http://rscf.ru/en), grant #18-75-00072.

## P-38-010
## Development of constructs based on the CRISPR/Cas9 system for studying the role of non-canonical mismatch repair in editing the epigenome

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The recent evolution of genome editing tools based on the CRISPR/Cas9 system is driving biomedical and molecular biology research into a new era. Methods for epigenetic editing are just only developing and design improvement could provide a high level of control over the state of cells and their functions. In mammals epigenetic active DNA demethylation occurs during cell differentiation processes and leads to altering expression specific genes via hydroxylation of 5-methylcytosine by TET and/or deamination by AID/APOBEC family enzymes. The resulting 5-methylcytosine derivatives are removed by DNA repair processes. At the previous work we developed an approach for assessment the contribution of the individual repair pathway into the total DNA repair using specially constructed circular DNA substrate and cell-free extracts from mammalian cell lines for reconstruction active DNA demethylation. We discovered cooperation between the base excision repair and non-canonical mismatch repair pathways for the removal of multiple mismatches and methylated regions. Using data from previous work, we developed new tool based on the CRISPR-Cas9 system which initiates the non-canonical DNA mismatch repair for epigenetic active DNA demethylation. These findings provide the development of possible techniques for epigenetic editing through active DNA demethylation of regulatory areas individual genes. This study has been supported by RFBR according to the research project No. 18-29-07059\18.

## P-38-011
## A diagnostic panel of HEK-293T cell sublines with knockouts of the genes for virus-entry receptors and its use for the development of sets of oncolytic enteroviruses

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For more than a century, oncolytic virotherapy is considered as a promising treatment modality for a wide range of malignancies, including lung cancer, malignant melanoma, pancreatic cancer, and prostate cancer. Today there has been tested a number of low- or non-pathogenic naturally occurring or attenuated viruses, as well as artificially modified virus strains for their ability to target selectively tumor cells for destruction and spread throughout the tumor mass. However, tumor cells vary considerably in their sensitivity to individual therapeutic viral strains. We assume that each virus entry pathway initiated by a specific host-cell receptor might have specific bottle-necks that limit virus sensitivity of tumor cells from individual cancer patients. Therefore, our goal was to develop a virus panel composed of viruses that utilize different host-cell receptors. Therapeutic strains effective for an individual patient can be selected from the panel. Human enteroviruses are known to use several different cell surface receptors, such as ICAM-1, PVR, CAR, integrins, scavenger receptor SCARB2, and adhesion


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molecule CD55/DAF. In order to obtain a diagnostic panel of cells for testing the requirement of newly isolated viruses for host cell receptors we performed genomic knockouts of the appropriate genes in HEK293T cells using CRISPR/Cas9 technology. We obtained knockout cell sublines with indels within the PVR, CXADR, CD55, ITGA2, SCARB2 and ICAM1 genes, which have been verified by DNA sequencing, flow cytometry, and western blotting. Subsequently, the obtained cell panel has been used to determine receptors required for 11 strains of non-pathogenic enteroviruses isolated from the intestines of healthy children. Based on the results obtained, we established a panel of viruses that use the different receptors for penetration into the cells. The viruses will be further tested for oncolytic properties in experiments in cell culture and laboratory animals.

## P-38-012
## Ago nucleases from *Clostridium butyricum* and *Limnothrix rosea* can process DNA substrates at moderate temperatures

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Prokaryotic Argonaute proteins (pAgos) are diverse homologs of eukaryotic Argonautes (eAgos) involved in RNA interference. In contrast to eAgos, which are RNA-guided RNA nucleases, several pAgos were reported to act as DNA nucleases suggesting that they may be used as an alternative to CRISPR-Cas nucleases for genome editing. However, all previously studied pAgos were isolated from thermophilic bacteria or archaea thus limiting their potential use in genomic applications. We describe two pAgo nucleases from mesophilic bacteria, *Clostridium butyricum* (CbAgo) and *Limnothrix rosea* (LrAgo). Both CbAgo and LrAgo use small DNA guides to cleave complementary DNA targets and are active at physiological temperatures. At the same time, the two proteins reveal significant variations in DNA processing depending on the reaction conditions and guide structure, including changes in the guide 5′-end and the presence of mismatches. We show that CbAgo is highly active under a wide range of conditions and can precisely cleave single-stranded and double-stranded DNA at moderate temperatures suggesting that it may be used for DNA manipulations both *in vitro* and *in vivo*. This work was supported by the grant 14.W03.31.0007 of the Ministry of Science and Higher Education of the Russian Federation.

## P-38-013
## Efficient genome editing of the non-conventional yeast *Debaryomyces hansenii*

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CRISPR/Cas systems used by prokaryotes to combat with foreign genetic material are repurposed for genomic editing of all model and some non-model organisms. CRISPR/SpyCas9-based genomic editing systems are developed for various yeasts that have importance for biotechnology, food industry, and medicine. *Debaryomyces hansenii* – the osmo- and halotolerant non-conventional yeast – has an application in food industry, biotechnology, as well as in academic research as a model for investigation of halo- and osmotolerance. However, the lack of efficient methods for manipulation with *D. hansenii* genome limits the research of this yeast and uncovering its biotechnological potential. We have developed optimized all-in-one vectors of CRISPR/SpyCas9 system utilizing one or two spacers for efficient genome editing of *D. hansenii*. Previously we characterized DhRpn4 as the transcriptional regulator of *D. hansenii* proteasomal genes. Here, using CRISPR/SpyCas9 system we obtained mutant D. hansenii strains with impaired DhRpn4-dependent regulation of DhPRE1 and DhRPT4 genes encoding essential subunits of 20S proteolytic and 19S regulatory proteasome subcomplexes. DhPRE1 mutant strains display sensitivity to various proteotoxic and genotoxic agents including high salinity, while DhRPT4 mutant shows sensitivity only to oxidising agents. Thus, for the first time, we have developed an efficient molecular tool for manipulation with *D. hansenii* genome and find that 20S proteasome is important for *D. hansenii* halotolerance. This work was supported by the Russian Foundation for Basic Research (project no. 18-29-07021) and the Programm of Fundamental Research for State Academies for the years 2013–2020 (No. 01201363822).

## P-38-014
## Photocleavable guide RNAs for controllable genome editing with CRISPR/Cas9 system

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Using of the CRISPR/Cas9 system for therapeutic purposes is the issue of a day. We proposed to apply this system for treatment of diseases caused by increased numbers of gene copies, for example Charcot-Marie-Tooth disease. The controllable decrease of copies numbers of multiplied gene using light switchable gene editing is nontrivial approach for the treatment of such diseases. The main idea of our researches is design of photosensitive guide RNAs containing 1-(2-nitrophenyl)-1,2-ethandiol modification and their usage for controllable gene editing using CRISPR/Cas9 system. sgRNA and crRNA/tracrRNA and their analogues containing one or two photocleavable linkers both in the region of targeted DNA binding and in the region of Cas9 protein interaction were synthesized by solid phase phosphoramidite method. The phosphoramidite of 1-(2-nitrophenyl)-1,2-ethandiol was specially synthetized for this purpose. The cleavage assays of photosensitive RNAs demonstrated 75% cleavage degree after 10 min of UV-irradiation. The possibility of DNA cleavage *in vitro* by CRISPR/Cas9 system in the presence of all synthesized guide RNAs was confirmed. The system containing photocleavable crRNA was more effective among the all modified guide RNAs. This system permits to reach 80% cleavage degree of DNA *in vitro* during 20 min. The UV-irradiation provoked the modified RNAs cleavage and stopped the gene editing. The parameters of UV-treatment of gene editing reaction mixture were optimized to obtain the 50% cleavage degree. The possibility of controllable switching off the gene editing system using photocleavable guide RNAs was demonstrated. Proposed strategy is prospective as the basis for the development of unique approach for deleting "unnecessary" gene copies from genome. The work was supported by RFBR grant N19-04-00838 and the project of the PFSR SAS VI.62.1.4, 0309-2016-0004 "Smart material for biomedicine" (2017–2020).


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## P-38-015
## Synthetical CRISPR array with dual gRNAs for efficient knockout of mycobacterial genes

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*Mycobacterium tuberculosis* (MTB) remains a main cause of deaths from infectious diseases. At the same time, almost a quarter of 4000 MTB genes are annotated as unknown, though about 5% of them are essential for MTB vitality, and, therefore, may be potential targets for the development of anti-tuberculosis drugs. The search for such targets is hampered by the existence of just few tools for genomic engineering of mycobacteria and restricted capabilities of the tools. The difficulties are due to the low homologous recombination frequencies in this group of microorganisms primarily, which could be improved by applying of CRISPR system. Recently, CRISPR/Cas systems have been developed to manipulate with mycobacterial genome sequences including CRISPR/Cas12a (FnCpf1). Gene knockout using single DNA double strand break (DSB) followed by action of DNA repair by non-homologous end joining (NHEJ) or making of small indels several nucleotides long using oligonucleotides are very effective. However, deletion of larger regions are highly ineffective (less than 1%). We have created a CRISPR/Cas12a system with synthetic dual gRNAs array for highly efficient targeted deletion of large fragments in the genomes in mycobacteria. Using this system we have quickly deleted 1700 bp-fragment of *ureC* gene in *Mycobacterium smegmatis* with efficiency 10–30%. The system shows low toxicity to the cells. Thus, we have created an efficient tool that will help studying of mycobacterial physiology and identification of new targets for the development of novel anti-tuberculosis drugs. The work was partially supported by RFBR 19-015-00149-A. *The authors marked with an asterisk equally contributed to the work.

## P-38-016
## Development of CRISPR-assisted genome editing tool to study phenotypic bacterial resistance to antimicrobial peptides

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Dramatically growing number of drug-resistant microbes is public health problem. In addition to the classic drug resistance strategies, microorganisms have developed mechanism of persistence allowing bacterial survival under stress conditions. Key activators of this phenomenon are Quorum Sensing alarmones, toxin-antitoxin (TA) system components and transcriptional factors. How these systems are working in relationship with antimicrobial peptides is largely unknown. We have observed that morphological changes occurring in *E. coli* cells following exposure to antimicrobial peptide 4 (p4), such as chromatin condensation, membrane damage and depolarization, look similar to effects of TA system activation. Differential resistance to p4 action during stationary phase and membrane permeabilization pattern suggests activation of bacterial persistence. The sigma factor rpoS that is global stress-induced gene expression regulator seems to be involved in this mechanism. Level of rpoS transcript fluctuates in p4- treated *E. coli* and is slight different in stationary and logarithmic growth phase. More deep investigation of the role of p4 in direct activation of rpoS-mediated persistence requires developing *E. coli* strains with impaired *rpoS* gene. Microbial genome editing method using CRISPR-associated nuclease Cas9 allows site-specific, chromosome modification including deletion, insertion or gene replacement. We designed *rpoS* gene silencing method by implementation of CRISPRi system targeting rpoS sequence and inducible expression of catalytically dead Cas9 (dCas9). Here we demonstrate how CRISPR-Cas system can be used to precise gene editing of genes conferring phenotypic microbial tolerance to stress condition.

## P-38-017
## Copper metabolism shift in H1299 cells with CTR1 and DMT1 genes knockout

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Copper (Cu) is an essential trace element that performs two independent functions: it is catalytic cofactor for vital cuproenzymes, and serves as a secondary messenger. We hypothesized, that Cu possibly was recruited for these roles through different copper transport pathways. Because two proteins can import copper in mammalian cells: CTR1, high affinity Cu importer 1, and DMT1, divalent metal transporter 1 for Fe, Cu, Zn, Cd, Mn, gene silencing of either CTR1 or DMT1, was done using CRISP/Cas9 technology. From H1299 cell line, cell clones with CTR1 or DMT1 single knockout (KO) and CTR1/DMT1 double knockout (DKO) were obtained. To confirm KOs of the genes qRT-PCR and Western blot (WB) technologies were used. In CTR1-KO cells, DMT1 gene expression was significantly increased, but not vice versa. In subcellular compartments (nuclei, mitochondria, intracellular membranes, and cytosol) Cu concentration, measured by atomic-absorption spectrometry, decreased, and dramatically dropped in DKO cells. CTR1-KO cells, but not DMT1-KO, demonstrated reduced sensitivity to cisplatin and silver ions, agents that enter the cell most through CTR1. The bulk of the silver entering the cells was accumulated by organelles, co-sedimenting with the nuclei. The mitochondrial nature of these organelles was established by the presence of mtDNA that was identified by PCR on D-loop region of mtDNA. The expression of genes, whose protein products require copper for catalytic functions (ceruloplasmin and Cu,Zn-SOD1) or signaling (HIF1α, XIAP, COMMD1 and NF-kB), changed their level in KO clones according data of RT-PCR and WB. We assume these data will help us understand how the disturbances of copper homeodynamics lead to the development of neurodegenerative and oncological disorders. The Possible usage of CTR1 KO and DMT1 KO cells for homeodynamics study of catalytic and signaling copper selectively is discussed. The work was supported by RFBR grants 18-015-00481, 18-515-7811, and MK2718.2018.4.


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## P-38-018
## High-throughput functional screening for PTEN-cooperating tumour suppressor networks in prostate cancer

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Prostate cancer remains a leading cause of cancer death worldwide. Nearly 50% of primary and 100% of metastatic prostate tumours have genetic alterations in the PI3K/AKT oncogenic pathway, mostly through loss of the tumour suppressor gene *PTEN*. Yet the mechanisms driving *PTEN*-deficient prostate cancer progression remain poorly understood, which ultimately limits design of new therapeutic strategies. Using a unique model designed to require genetic collaboration in the same cell, we have conducted a transposon-based screen and identified over a hundred *PTEN*-cooperating genes in murine prostate tumours. These include clinically relevant known and new candidate tumour suppressors frequently altered in human prostate cancer, most of which had gone unnoticed to date. Our current work seeks to understand the cooperative roles of these genes and *PTEN* in prostate cancer. For this purpose, we are developing novel genetic tools for high-throughput *in-vivo* interrogation of these collaborating events. Combining the use of CRISPR-Cas9 knockout libraries with intra-prostate gene delivery in mice, we aim to induce autochthonous prostate tumours whose growth and dissemination could be tracked *in vivo*. These approaches will shed light on the mechanisms driving *PTEN*-deficient prostate cancer progression and will hopefully pave the way for new therapeutic strategies.

## P-38-019
## Revealing the functional role of Bag-1 in MCF-7 breast cancer cells through CRISPR/Cas9-mediated gene knockout

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Bag-1 is a multifunctional protein target which has interactions with a number of cellular proteins. Therefore, Bag-1 participates in several significant biological processes such as cell proliferation, survival, and apoptosis. Elevated expression levels of Bag-1 are associated with progression of cancer and resistance mechanism against cancer. In our previous studies, we obtained results showing Bag-1 silencing enhancing the apoptotic potentials of cisplatin or paclitaxel through modulating PI3K/Akt/mTOR pathways in breast cancer cells, however, the function of Bag-1 knockout in these cells has not been fully explored. Here, we performed the CRISPR/Cas9 technique to knockout Bag-1 gene and successfully produced Bag-1 knockout cell line (Bag-1 KO). Sanger sequencing was used to confirm the gene knockout mediated insertions or deletions (indels) in MCF-7 cells. Correspondingly, the mRNA and protein expressions of Bag-1 were markedly reduced. We determined the effect of Bag-1 KO on cell survival by MTT and trypan blue dye exclusion assay. Alternations of total protein expression profiles between wild-type and Bag-1 KO cells were determined through PathScan analysis and immunoblotting assays. Our results showed that knockout of Bag-1 suppressed the proliferation and growth potential of MCF-7 cells by decreasing the number of colony formations. According to the immunoblotting results, knockout of Bag-1 significantly upregulated Akt phosphorylation and b-actin downregulation. We concluded that Bag-1 influenced actin disorganization through stress-induced Akt activation and alternations on expression profiles of focal adhesion kinases.

# Proteomic technologies

## P-39-001
## Yeast alcohol dehydrogenase production in *Vibrio natriegens* – alternative to production in *Escherichia coli*

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*Vibrio natriegens* is a Gram-negative marine non-pathogenic bacterium that is being considered as a potentially new host for fast and effective recombinant protein production. With its doubling time of 10 min under ideal conditions represents a competitive tool for protein production to currently widely used *E. coli*. For this purpose, a plasmid containing ADH1 gene from *Saccharomyces cerevisiae* under the control of T7 promoter was designed. For the expression in *E. coli*, a well-established BL21(DE3) strain has been used and compared to the thus far only commercially available *V. natriegens* strain, carrying T7 RNA polymerase, termed Vmax Express. This work compares small scale production of alcohol dehydrogenase under variety of conditions *in V. natriegens* and *E. coli*. This work is supported by APVV-14-0375 – Synthetic biology and production of peroxidases de novo; APVV-15-0466 – Preparation of natural flavors by biotransformation using the comprehensive analytical methods; APVV-17-0333 – Research and development of effective processes for the preparation of vanillin and other natural flavors using the oxidative and protective effect of recombinant catalase and peroxidase.

## P-39-002
## Investigating the peptide profile of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): application of MALDI mass spectrometry imaging

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The lack of papillary structures and faint and/or unclear core features of follicular variant of papillary thyroid carcinoma (FV-PTC) may hamper the definitive fine needle aspiration biopsy (FNAB) based diagnosis. Recently, the nomenclature of noninvasive encapsulated FV-PTC was revised as "noninvasive follicular thyroid neoplasms with papillary-like nuclear features" (NIFTP). However it remains inconclusive whether or not the peptide patterns differ between NIFTP and FV-PTC. The main objectives of the study were to investigate the viability of matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) in the pathological assessment of NIFTP and evaluate discriminatory power of MALDI MSI for the classification of classical variant of PTC (CV-PTC), FV-PTC, and NIFTP. MALDI MSI was employed to investigate the changes in peptide profiles from 21 formalin-fixed paraffin-embedded (FFPE) tissue samples (n = 7 from each group of CV-PTC, FV-PTC, and NIFTP). Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the identification of the peptide signals detected in MALDI MSI. Using ROC analysis, 10 peptide signals distinguished NIFTP from normal thyroid parenchyma (AUC > 0.80).To evaluate the discriminatory power of MALDI MSI, statistically significant peptide


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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signals (n = 88) within three groups were used for hierarchical clustering. The method had high discriminatory power for distinguishing CV-PTC from NIFTP and FV-PTC. However, no visual clusters obtained within FV-PTC and NIFTP. High signal intensities of S100-A6, vimentin and cytoplasmic actin 1 were detected in FV-PTC, prelamin A/C in CV-PTC, and 60S ribosomal protein L6 and L8 in NIFTP tissues. MALDI MSI, a powerful tool combining histological and mass spectrometric data, enabled the detection of NIFTP from normal thyroid parenchyma. Although NIFTP is a recent definition emerged from noninvasive encapsulated FV-PTC, the peptide profiles of NIFTP and FV-PTC were found to be similar.

## P-39-003
## Feasibility of studying the changes in relative distribution of allergen specific antibody isotypes overtime

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Allergen specific immunotherapy is the only available disease-modifying treatment for allergic patients. Proteomics offers a novel approach to study potentially hundreds of proteins and peptides using little amounts of a single biological sample, which can provide an informative measure of multifactorial diseases. Recently, several strategies using mass spectrometry (MS) have been developed, especially for the relative and absolute quantification of proteins such as the novel tandem mass tag (TMT). This technique allows distinct labelling of multiple peptide samples with isobaric chemical tags with same molecular structure and mass. Nevertheless, each tag releases a unique signal ion when fragmented in a mass spectrometer. $MS^2$ intensities from each tag represent the relative abundances of the peptide in each sample. All allergen specific antibodies from patient serum were enriched through the binding to biotin-conjugated allergens captured by magnetic beads. The protein eluates was digested to peptides and labelled with TMT. Labelled peptides from several time points and conditions were mixed and subjected to LC-MS/MS for the identification and quantification of antibody isotype distribution overtime. We successfully developed the protocol (with low serum amounts, 100–200 μL), repeatedly identified and quantified of broad range of allergen specific immunoglobulins IgG1-IgG4, IgE, IgA1 and IgM. Finally, with success, applied the TMT method, obtaining the proper peptide ratios according to the samples used. This methodology optimization can be an important improvement in the study of the changes in distribution of several allergen specific antibody isotypes simultaneously during different pathological or treatment situations and studies overtime. The strongholds are that small amounts of plasma or serum are used to obtain measurements of several allergen specific antibody isotypes simultaneously indicating the antibody response changes during immunotherapy.

## P-39-004
## Deciphering sexual dimorphism in type 2 diabetes by redox proteomics

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Despite the prevalence of obesity and type 2 diabetes (T2DM) is rising globally, little is known about the role for adipose tissue mitochondria in this context. We have recently validated that antioxidant response is one of the gender-specific hallmarks of sexual dimorphism in adipose tissue. Since mitochondrion is recognized as the main source of oxidative stress, in this work we aimed to better understand the impact of gender on adipocyte mitochondria. For this purpose, mitochondrial samples were obtained from adipocytes previously isolated from visceral adipose tissue biopsies collected from morbidly obese patients (BMI > 35 $kg/m^2$) suffering T2DM. Protein abundance and redox differences between women and men suffering T2DM were assessed following a high-throughput approach encompassing differential alkylation of reduced and reversible oxidised Cys (FASILOX protocol), isobaric labelling (iTRAQ) and high-performance liquid chromatography (HPLC). LC-MS/MS analysis allowed the identification of 15,763 peptides at 1% FDR, corresponding to 2895 proteins in mitochondrial extracts. Of note, 754 proteins were annotated in the current version of Human MitoCarta v.2 (68% coverage). FASILOX technology allowed the identification of 277 oxidized and 1990 reduced Cys-containing peptides. According to the WSPP model, Cys oxidation was globally increased in diabetic men compared to women. Enrichment analyses showed that oxidation was focalized on the oxidative phosphorylation (OXPHOS) system, especially in Complex I ($P < 0.001$, FDR 1%). These results suggest male patients displayed higher oxidation than females in adipocyte mitochondria, most likely revealing a substantial oxidative stress on OXPHOS machinery and providing the first evidence for a decreased efficiency in the antioxidant response of men as compared to women. The identification of gender-matched oxidation targets in T2DM may help to improve the therapeutic approaches currently available for these patients.

## P-39-005
## The components of *Bombina variegata* skin secretions affect some parameters of hemostasis

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Amphibian skin secretions contain various bioactive molecules and seem to be a perfect raw material to search for new pharmaceutical agents. Since our previous experiments had proven that the crude skin secretions of *B. variegata* affect some parameters of hemostasis, the aim of this study was to fractionate the crude skin secretions of *B. variegata* and analyze the effects of different protein fractions on some parameters of hemostasis system. The nine protein fractions were collected in the result of size-exclusion chromatography on the Superdex G200 PG column. The protein profile of all fractions was analyzed by one-dimensional gel electrophoresis technique. SDS-PAGE zymogram experiments using collagen and gelatin as substrates showed the presence of proteolytic enzymes in different protein fractions. Coagulation tests including aPTT, PT, and TT were performed as well. It was shown that the components of fraction #6 prolonged aPTT clotting time in 2.7 times, compared with the control. Chromogenic substrates such as $S_{2366}$, $S_{2251}$, $S_{2238}$ and $S_{2222}$ were used to evaluate the specific proteolytic activity of the protein fractions and to study the ability of the different fractions to activate the corresponding plasma proenzymes. It was revealed that fractions #3, #4 and #5 with different intensity cleaved all studied chromogenic substrates and fraction #9 activated prothrombin, factor X and protein C in plasma. Platelet aggregation assay showed


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that the components of fractions #3 and #4 induced aggregation in the rabbit platelet rich plasma and caused the same effect as $5 \times 10^{-6}$ M ADP. Molecular characterization of specific target proteins is in course. Acknowledgments: This research was supported by the Ministry of Education and Science of Ukraine [No. 0116U002527, 2016–2018].

## P-39-006
## Analysis of proteins and peptides of milk exosomes

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Exosomes are nanovesicles with a diameter of 40–100 nm. Recently the presence of proteins, peptides, DNA, mRNA and, especially, miRNA in their composition has been shown. Exosomes from the milk of animals and humans have been studied to a lesser extent then exosomes from culture media and plasma. It depends on the complexities with the isolation of exosomes due to the presence of a large number of impurity proteins that make it difficult to obtain pure preparations by standard methods. We have developed a modified protocol for exosome isolation from complex biological fluids such as milk. The obtained preparations were analyzed by electron microscopy, where the presence of exosomes was shown by immunocytochemical analysis. Besides, the method of affine chromatography with antibodies against exosome transmembrane proteins CD 9, CD 63 and CD 81 was used for additional purification of exosome preparations. The utilizing of flow cytometry showed the presence of these markers in exosome fractions after chromatography. Electrophoretic analysis and MALDI mass spectrometry were conducted to analyze major exosomal proteins. Also, for the first time, the presence of small proteins and peptides in the composition of milk exosomes was shown. For the analysis of these peptides, the destruction of exosomes and the separation of peptides by reversed-phase chromatography with subsequent mass spectrometric analysis were carried out. Thus, the study of protein and nucleic acid composition of milk exosomes gives us new information about the overall composition of exosomes and their potential significance in the physiology of the mammary glands and body as a whole, as well as their role in the development of the digestive and immune systems of newborns. Furthermore, extracellular milk vesicles are a new field of research, focusing on the possibility of their use in medicine as diagnostic and therapeutic tools. The study was supported with RSF project 18-74-10055.

## P-39-007
## Time-dependent analysis of *Paenarthrobacter nicotinovorans* pAO1 nicotine-related proteome

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*Paenarthrobacter nicotinovorans* is a soil Gram-positive Nicotine-Degrading Microorganism (NDM) that has found applications in the eco-friendly conversion of nicotine and nicotine containing waste to pyridine and pyrrolidine related green-chemicals. The nicotine catabolic genes on the 165 kb pAO1 megaplasmid have been sequenced, but little is known on how the cells cope with the accumulation and toxicity of the resulting nicotine metabolic by-products. In order to address this issue at the protein level, we performed a time-based proteomics study using reversed phase nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS). *P. nicotinovorans* was grown with and without nicotine and the cells harvested at 3 different time intervals: 7, 10 and 24 h post inoculation. The cells were lysed, cell free extracts were prepared and separated on 9–16% SDS-PAGE maxi gradient gels. The proteins were reduced, alkylated and in-gel digested using trypsin. The resulting peptide mixture was analyzed on a NanoAcquity UPLC (Waters, Milford, MA, USA) coupled to a Q-TOF Xevo G2 MS (Waters). Data analysis was performed using ProteinLynx Global Server v.2.4 (Waters, Milford, MA, USA), Mascot v.2.5.1 (Matrix Science, London, UK) and Scaffold (v.4.8.2, Proteome Software Inc., Portland, OR, USA). This approach allowed us to identify a total of 915 proteins grouped into 584 non-redundant clusters with an FDR of 0.3%. Currently, data analysis is being performed to link the observed differences in protein abundance to the accumulation of known nicotine intermediates. The mass spectrometry proteomics data have been deposited to PRIDE with the dataset identifier PXD012577 and can be made available upon request. This data provides insights into bacterial cells adaptation to the nicotine metabolic intermediates that are known to be toxic and to accumulate in the growth medium.

## P-39-008
## Human placenta exosomes: isolation, proteins and nucleic acids

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The placenta-derived exosomes are important in intercellular communication and immune function. Exosome bioactivity relies on different types of molecules since exosomes contain proteins, lipids and miRNA. In this work the protocol for exosomes isolation from the human placenta was developed. The exosomes were isolated from placentas of 20 healthy women by the combination of differential centrifugation, ultrafiltration and gel filtration. Further purification of the preparations by affinity chromatography on Sepharose bearing immobilized antibodies against exosome surface protein CD81 led to an isolation of highly purified exosomes (40–100 nm). The transmission electron microscopy (TEM) revealed the presence of spherical vesicles, with a typical cup-shape and diameters ranging from 40 to 100 nm. TEM with anti-CD63 and anti-CD81 immune labeling demonstrated the presence of exosomes in 40–100 nm membrane particles. Flow cytometry using antibodies against CD81, CD9 and CD63 also confirmed the presence of exosomes in preparation after gel filtration and affinity chromatography. Analysis of protein composition in highly purified placenta exosomes showed the presence of a small number of proteins. This indicates that some placenta proteins, which were previously described as exosomal, are not part of the exosomes, but co-isolate with them. Also the highly purified exosomes, we for the first time found that in addition to the large proteins (>10 kDa), exosomes having an affinity to CD81 contain more than 27 different peptides and small proteins of 2–12 kDa. For the analysis of nucleic acids, we obtained RNA samples of placenta exosome, carried out the analysis of distribution size in the Bioanalyzer and RT-PCR. This finding can be useful for revealing biological functions of pure placental exosomes. The study was supported partially under Russian State funded budget project (VI.62.1.5, 0309-2019-0003).


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## P-39-009
## Effect of $Zn^{2+}$ and toxic $Cr^{6+}$ ions on the proteome of *Arthrobacter globiformis* 151B

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We investigated, the gram-positive aerobic bacteria *Arthrobacter globiformis* 151 B, isolated from most contaminated site of Georgia (Kazreti). Mentioned bacteria is characterized with high bioremediation capability towards Cr(VI) and other metals ions. It exhibits resistance against high concentrations of Cr(VI), Zn (II) and other metallic ions. This bacterial species could effectively reduce highly toxic and carcinogenic Cr(VI) into Cr(III), which is less toxic. We have studied bacterial tolerance toward Cr(VI) and Zn(II) and accumulation of same metals on protein level by proteomic approach. Bacterial samples were prepared at 3 following conditions: 1. with Cr (VI); 2. with Zn(II); 3. without Cr(VI) and Zn(II). Bacterial cells were harvested in a time dependent fashion (36, 60 and 120 h after the starting of cultivation) and changes in proteome expression was analyzed using two-dimensional gel electrophoresis and liquid chromatography and mass spectrometry (LC-MS/MS) coupled with bioinformatics to identify proteins. Significant changes in protein expression included both up- and downregulation of different groups of proteins. Most remarkable changes were associated with metal-binding proteins and proteins involved in active transport. Parallel experiments with Atomic Absorption Spectroscopy revealed that reduced chromium appears mostly soluble and mainly associated with organics: especially with bacterial proteins. Zinc(II) ions enhances bacterial ability for Chromium accumulation. Zinc(II) also increases reduced Chromium-protein connections and their content increases after time (for 120 h). Our results signify that A. *globiformis* 151 B is naturally equipped at the proteomic level correspondingly with the relevant genes, to survive extreme toxic conditions, thus has great potential for bioremediation. Acknowledgements This work was supported by Grant #FR/218 018/16 from Shota Rustaveli National Science Foundation (SRNSF).

## P-39-010
## The surface plasmon resonance study of enzymatic biotinylated MDM2 various species

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The murine double minute 2 (MDM2) protein is at the center of an intense clinical valuation as molecular target for the management of cancer. The strategy of this course is to find inhibitor of the MDM2/p53 interaction and restore or increase p53 expression. The p53 protein is a key controller of the cell cycle regulation and plays a direct role on cancer development. The developed inhibitors must be characterized with strong affinity to MDM2 and specifically fit to the functional pocked of MDM2. Surface plasmon resonance (SPR) is a powerful technique used in high screening speed of drug discovery area. However, popular kinetics approaches are based on MDM2 immobilization to the solid surface and could disturb the functional center of MDM2/inhibitor interaction. It is not rare that immobilization of MDM2 is performed via amine or carboxylic groups that are around the functional center. The aim of this study was to perform safe and site specific immobilization with the enzymatic biotinylation of the MDM2 protein. The biotinylation process is via Avi-tag fused peptide and is conducted at posttranslational modification level. The efficiency of biotinylated and purified protein is around 60% and it was tested for various MDM2 species: human, mouse, rat, dog. The greatest effort was to performed by SPR technique the activity of the biotinylated proteins. The summarized results are in form of full kinetics rates and dissociation constants obtained for the MDM2 different species in complex with several inhibitors which are in development in clinical trials. We believe that presented studies will support the search of the leader of the MDM2/p53 complex inhibitor and thus speed up finding an active anticancer drug.

## P-39-011
## Design of peptides for spatial recognition of myeloperoxidase and myoglobin in express-diagnostics using biosensor format

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The aim this work is the search of oligopeptides complementary to target proteins – biomarkers of various cardiovascular conditions and development of basic architecture of microfluidic multiparametric biosensors. For this purpose, the visualization and analysis of protein structures (subunits in complex proteins or protein-substrate complexes) was carried out using developed Protein 3D software in the conjugated ionic-hydrogen bonds systems (CIHBS) rendering. Using this approach the peptide sequences for determination of myeloperoxidase (MP) and its subunits and myoglobin (MG) in express-clinical and pre-clinical diagnostics of cardiovascular diseases. Unlike most known cardiovascular biomarkers produced in cardiomyocytes that level correlates with myocardial damage, the level of MP produced by neutrophiles is a very useful parameter of clinical condition under cardiovascular surgery. MP is a complex 4-subunit haem-containing protein. The complex of MP and ceruloplasmin enables to design the complementary peptide by CIHBS-analysis. On the other hand, the analysis of intermolecular bonds of MP subunits enabled a peptide for determination of MP light chain to be accomplished. Another important cardiovascular marker is MG that is also a haem-containing protein, produced by myocard and characterized by the most expeditious appearance in blood. For these two proteins a number of sequences of oligopeptides has been proposed and synthesized. The synthesis of oligopeptides was carried out by the solid phase method using an Applied Biosystems 430A synthesizer: by the method in situ using N$\alpha$-Boc-protected amino acid derivatives. All peptides had a molecular weight and amino acid composition, coinciding with the calculated ones. The proposed peptides are tested using the Experion electrophoresis station and Pro260 chips. The results of specific binding of peptides and target proteins in electrophoresis demonstrated the perspective of using Protein 3D software.

## P-39-012
## Exosomes in lung cancer diagnosis

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Recently, we identified 4 membrane proteins which showed lung cancer specificity. In this study, we tried to determine whether


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the cancer specific membrane proteins can be detected on exosomes in the blood of cancer patients or not. A mouse xenograft model of human lung cancer carcinoma was constructed by injecting lung cancer cells subcutaneously into nude mice. The ELISA condition was optimized using blood samples of xenograft mice. The Protein G was coated on ELISA plate to ensure the antigen binding domain of the CD63 antibody is orientated away from the plate. The lung cancer specific expressed membrane proteins were detected by sandwich Exosome ELISA method in plasma samples of xenograft mice. There was a significant correlation between the size of the xenografted tumor and the amount of protein detected in the exosomes. In this study, we succeeded to detect lung cancer –specific membrane proteins in plasma exosomes. This success shows the possibility of novel lung cancer diagnostic methods in the future.

## P-39-013
## Characterization of heterogeneous bacterial periplasmic phospholipid binding protein samples under native and denaturating conditions

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Non-covalent protein interactions play a vital role in biology because proteins recognize all their binding partners (other proteins, lipids, carbohydrates, nucleic acids and small molecules) through noncovalent electrostatic, van der Waals, hydrophobic and hydrogen bonding interactions. Mass spectrometry performed under so-called "native conditions" (Native MS), which are conditions that preserve non-covalent interactions, can be used to determine the mass of biomolecules that associate noncovalently forming complexoforms (biomolecule complexes). Native MS also enables the characterization of other complexoform properties such as folding, assembly state, overall structure and post-translational modifications. Therefore, Native MS is a powerful tool for studying such interactions and determining the mass of biomolecules with high accuracy and sensitivity, providing insight into the composition, stoichiometry, network of interaction and overall shape of protein assemblies. On the other hand, intact mass analysis of complexoforms under denaturing conditions can be performed to obtain excellent resolution and mass accuracy of the partners that built complexoforms, on their purity, on their homogeneity and on their primary sequences. Here we report the application of native and denaturing MS to in-depth characterization of a bacterial phospholipid binding protein involved in the transfer of phospholipid across the periplasmic space in Gram-negative bacteria. This study showed that this protein may transport different phospholipid classes.

## P-39-014
## Curcumin as a regulator of protein expression in the liver of obese diabetic db/db mice

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Diabetes mellitus type 2 (DM2) is a metabolic disease characterized by deficient synthesis and/or insulin secretion, secondary to insulin resistance. In the liver, elevated concentrations of glucose and insulin modify gene expression. Curcumin is a powerful antioxidant and antidiabetic agent that regulates the gene expression of different signaling pathways through various transcription factors. The aim of this study is to analyze the effect of curcumin on the liver protein profile of obese diabetic *db*/*db* mice. Four groups of five mice, two healthy groups (WT) and two diabetic groups (*db*/*db*) were formed. Two groups were given curcumin (WT+C and *db*/*db*+C) at 0.75% w/w in diet. Liver proteins were separated by 2D electrophoresis. Differential protein expression analysis was performed on ImageMaster 2D Platinum software, proteins were identified by MALDI-TOF and subjected to enrichment analysis using STRING and DAVID databases. We found 36 proteins with differential expression due to diabetes and curcumin, which participate in metabolic pathways related to the metabolism of amino acids, carbohydrates and lipids; of these proteins found, seven prevented the expression change when the diabetic mice received curcumin. Proteins participate in carbohydrate metabolism (Dhtkd1, Idh3a, Mup2, Mup6), are associated with body composition and insulin resistance (Abhd14b, Ppp1r14d) or participates actively in the inflammatory process (Fgg). These proteins have been previously related to DM2. Interestingly, seven of these proteins were reversed in their expression by the effect of curcumin. Therefore, this study helps to elucidate the molecular mechanisms for which curcumin has beneficial effects in the treatment of DM2. This project was supported by CONACYT (IO10 / 532/2014) to JRE. Gerardo Silva Gaona was a fellowship from CONACYT (404080 and 291061).

## P-39-015
## Proteomic analysis of *Staphylococcus aureus* with a deletion of mazEF toxin-antitoxin system resulting in impaired ability to form a biofilm

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Toxin antitoxin (TA) system are small genetic elements, widespread in bacteria, known to play a role in stress response and biofilm formation. *Staphylococcus aureus* is an opportunistic pathogen of human and animals. Ability to form a biofilm is considered as an important virulence factor and the cause of chronic and relapsing infections. *S. aureus* carries a few TA systems, one of them is chromosomally encoded MazEF, linked to susceptibility to antibiotics


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and virulence. Here we explored an impact of MazEF system on biofilm formation and the respective changes in the proteome. Influence of *mazEF* deletion on biofilm was determined in assays with crystal violet and green fluorescent protein. For proteomics, intracellular proteins from biofilm and planktonic cells, as well as those released to growth medium were isolated and separated using two dimensional difference gel electrophoresis. MazEF deletion mutant exhibits significantly lower ability to form biofilm and this effect is reversible by supplementation. The number of differentiated proteins in delta *mazEF* was the highest in secretome (58), next in the intracellular proteome of planktonic and biofilm forming cells (32 and 24, respectively). Among proteins underexpressed in secretome of deletion mutant are alpha-hemolysin and proteases, known as virulence factors. In delta *mazEF* biofilm cells enzymes involved in glycolysis, teichoic acid synthesis and iron transport are downregulated, whereas those involved in amino acids metabolism are overexpressed. In planktonic cells the mutation have an influence on glycolysis and fatty acid metabolism. The lower ability to form biofilm and decreased expression of virulence factors in delta *mazEF* mutant suggest that this TA system has an impact on *S. aureus* virulence and the ability to modulate gene expression, which finds its reflection in the proteome. This study was supported by the National Science Centre (NCN, Poland), decision no. DEC-2014/13/B/NZ1/00043 (to BW).

## P-39-016
## Cytokine patterns in chronic kidney disease-mineral and bone disorders

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Mounting evidence revealed that progression of chronic kidney disease (CKD) is associated with serious complications, including mineral bone disorders and cardiovascular disease. Persistent, low-grade inflammation represents a hallmark of CKD, even from early stages. Proteomic biomarkers could have significant roles in early diagnosis and prognosis of CKD. In this context, the present study aims to focus on circulating pattern expression of mineral bone disorders biomarkers and mediators of inflammation in order to design a panel of biomarkers potentially useful in CKD progression. Multianalyte assays were performed on 86 CKD patients (stages 2–4) and 20 controls for semiquantitative determination of cytokines, chemokines, soluble proteins, using Proteome Profiler Human XL Cytokine Array Kit (R&D Systems). Dkk-1 and calcitriol levels were also assessed using Quantikine ELISA Human Dkk-1, and EIAab General Calcitriol ELISA kit. Mineral bone disorders biomarkers (osteoprotegerin, osteopontin, osteocalcin) were overexpressed in CKD group, and were correlated with the increased levels of pro-inflammatory cytokines (IL-6, TNF-α). Calcitriol level was decreased in CKD patients, and was correlated with Dkk-1 expression. A crosstalk between biomarkers level and CKD stages was also revealed. Circulating biomarkers assessment using advanced proteomic approaches will allow developing cytokine patterns for CKD progression. Further analysis on protein expression and signaling networks may lead to plainly establish the connection between mediators of inflammation, mineral bone disorders and kidney failure progression. Supported by Ministry of Research and Innovation in Romania, under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018 and grant COP A 1.2.3, ID: P_40_197/2016.

## P-39-017
## The investigation of hypochlorite-induced oxidative modification of fibrinogen by high-resolution mass spectrometry

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Fibrinogen molecules that are the highest vulnerable target for reactive oxygen species among other of blood plasma proteins are constantly exposed to the attack of free radicals. Post-translational oxidative modifications of fibrinogen cause disorders in the functional properties of the protein and, as a consequence, lead to fibrin assembly with an abnormal architecture and a reduced strength and elasticity. The alternative assembly of the three-dimensional structure of fibrin network in the case of fibrinogen oxidation may be due to the oxidative modifications of specific methionine residues belonging to Aα-, Bβ-, and γ-polypeptide chains. When studying the oxidative modification of fibrinogen by mass spectrometry, we analyzed the control fibrinogen samples, which were not subjected to induced oxidation, and the oxidized samples that were treated with hypochlorite (50 μmol/mg protein). By applying the mass-spectrometry method, a wealth of amino acid residues localized within all three polypeptide chains and main structural elements of fibrinogen was revealed to be involved in oxidation. The mass-spectrometry data indicated that: region E was the least susceptible to oxidation compared to other structural elements of the protein. The structures of both the hole 'a' and the knob 'A' remained non-oxidized, the chemical structure of knob 'B'; was completely retained, in the hole 'b'; structure the only Met367 was modified. None of the thrombin-binding residues was subjected to a chemical alteration that the thrombin-binding properties of fibrinogen are retained during oxidation, which ensures one of the key stages of fibrin formation. The study was performed with the budgetary support of State assignment (subject no. 0084-2014-0001) and was supported by the RFBR, research project No. 18-04-01313_a. Mass spectrometry data were obtained with the support of the Russian Science Foundation No. 16-14-00181.

## P-39-018
## Label-free proteomic analysis reveals changes in blood coagulation and red-ox homeostasis in the heart of cystathionine β-synthase deficient mice

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Homocysteine (Hcy) arises from the metabolism of the essential dietary protein amino acid methionine (Met). Cystathionine β-synthase (CBS) catalyzes the first step of the transsulfuration


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pathway that affords cysteine. Human CBS deficiency causes severe hyperhomocysteinemia (HHcy) and diverse clinical manifestations, including oxidative stress and pathologies in the cardiovascular system. However, mechanisms underlying these are not fully understood. We hypothesize that CBS deficiency induces changes in gene expression that impair heart homeostasis and can lead to changes in blood coagulation and thrombotic complications. To identify the genes involved and gain an insight into thrombotic functions of Cbs we analyzed the heart proteome of Cbs−/− (n = 17) and Cbs+/+ mice (n = 16). Using label-free quantitative mass spectrometry approach mice heart proteomes were analyzed. Proteins with a min. of 2 identified peptides and $P$ values <0.05 were considered as differentiating. Bioinformatic analyses were carried out using DAVID resources. We identified 40 heart proteins whose expression was significantly altered as a result of the Cbs gene inactivation. 12 were up-regulated and 28 down-regulated. The most striking features were upregulation of haptoglobin (Hp) and down-regulation of acetyl-CoA acetyltransferase (Acat1), in the Cbs−/− mice. The GO analysis revealed that the affected proteins participate in cell redox homeostasis (Prdx3,Gpx3,Park7) and regulation of blood coagulation (Apoe,Kng1). The most overrepresented KEGG pathway is the complement and coagulation cascades (Seprina1a,Serpina1c,Serpina1d,Fga,Kng1). These findings suggest that Cbs deficiency has an effect on heart proteome and induces changes associated with diverse cellular processes, including blood coagulation and platelet activation. Deregulation of genes involved red-ox reactions, suggests that Cbs deficiency increases oxidative stress. Supported by NCN grants: 2014/15/N/NZ5/01647, 2015/17/D/NZ5/03444.

## Education, training, and career planning in molecular life sciences

### P-40-001
### Progress with co-learning

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After evolving the training arrangements for the biochemistry introductory course, applying the principle of constructive alignment, we found real effects on the satisfaction and the success rate of students. We also noticed a saturation phenomenon, that is the success rate did not evolve anymore. Assuming that at least part of the public was still not engaged in a learning process, we decided to introduce specific methods of cooperation between students, which promote co-learning. These activities can be synchronous or asynchronous, in either face-to-face or remote mode. An example, in synchronous mode, is the organization of tutorials in teams of 4 students (2 pairs). This forces students to cooperate to find solutions and promotes co-learning. Each team deals with a different exercise. Once the exercise is resolved, the pairs exchange between teams and explain their respective exercises. Rotation between teams continues until all pairs have known of all the results. The exchanges are facilitated thanks the "active" rooms, equipped with mobile boards and tables. In addition we organize many remote asynchronous activities, through the digital working environment on the university website. Forums are organized to allow students to ask questions about lectures and tutorials, and other students are encouraged to answer. Chats are also scheduled before the exams, in remote synchronous mode, allowing students to ask and answer questions! The trust feeling established with the students, the kindness and the consideration contribute to the motivation of the students, one of the levers of the success. Making them active and making them feel that they are masters of their destiny is another. The impact is very encouraging for the teaching staff, the success rate has been steadily increasing over the past 5 years and now stands at more than 70% of students engaged in a learning process. Our observations during their implementation show significant gains for student learning.

### P-40-002
### Improving quantitative literacy in incoming biomedical science students.

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Quantitative literacy can be defined as the ability to interpret and communicate numbers and mathematical information throughout everyday life. We have observed that a significant proportion of tertiary students studying biochemistry, molecular biology and/or biomedical science, even by the end of their degrees lack basic quantitative literacy to the extent that it will be detrimental to future employment and further study. Considering that biology is rapidly becoming a more quantitative and predictive science, the extent of this detriment is likely to increase. By analysing student quantitative literacy in detail, using specific diagnostic tests and conducting detailed error analysis on assessment tasks, we have been able to categorise the main sources of student error and conceptual misunderstanding. We have also observed students have difficulty moving from abstract to applied mathematical problems demonstrating that this is strongly linked to reading comprehension. We have used this analysis to develop a comprehensive intervention strategy in the form of a core quantitative literacy subject introduced into the first year of our biomedical science course. To promote student engagement, the subject ostensibly addresses quantitative literacy through the prism of basic biology and biomedicine, emphasizing practical applications. As mathematics is often a source of anxiety in adult learners we have adopted a blended mode of delivery where students are supported to work at their own pace and use portfolios as reflective learning tools. Analysis of follow-up diagnostics and summative assessment indicated that students who have undertaken the subject significantly improved their quantitative literacy skills.

### P-40-003
### Creating an archive of audio files in order to improve students' overall academic activity

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Complexity and volume of medical content make medical studies difficult. This regards mostly biomedical subjects at the beginning of the educational program. These fields of science are constantly updated with new research results. Combination of different forms of teaching facilitates the achievement of learning outcomes. The aim of the survey was the evaluation of the impact of audio files on the acquisition of knowledge of medical students. 89 participants were first-year students of the Medical Faculty. 58 students out of 89 were having average academic performance. The study was conducted in 2018. After selection, the participants signed informed consent. Two questionnaires were developed: for the assessment of students' needs and students' satisfaction. We prepared 3 different types of audio files: (i) short summary – 10 min; (ii) detailed explanation – 25 min; (iii) additional scientific data – 15 min. Preliminary findings: 59 ± 26 participants supposed audio files to be helpful; 76 ± 5 participants prefer to have audio files in the library, among them 60 ± 18 participants requested audio files for the topics beyond the syllabus. 52 participants answered that audio lectures are most effective for revision in the limited time.


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The preferable time was 25 min (44 participants). 60 participants assumed that audio files had a positive impact on their academic performance. 30 students indicated 10 min as the best duration for the audio lectures, while the number of the students preferring 25 min files were decreased from 44 to 21. 62 participants liked files with scientific information, while 16 were not interested in it. Finally, 84 students indicated that they wanted audio lectures in other subjects. According to our results, most of the students found audio lectures useful in improving academic performance. We suppose that audio files are accessible for delivering scientific information to the students. *The authors marked with an asterisk equally contributed to the work.

## General topics – various

### P-41-001
### The novel PII-like protein PotN from *Lactobacillus brevis* subsp. *gravesensis* interacts with the ABC-transporter ATPase PotA

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PII proteins play a key role as sensors for energy state and availability of a carbon/nitrogen source for the cell. The competitive binding of ATP or ADP allows PII proteins to modulate various cellular functions depending on the current state of cell energy and plastic homeostasis. Among bacteria of the genus *Lactobacillus*, only 4 species have genes encoding PII-like proteins in the genome. Here we report the first biochemical characterization of a novel PII-like protein termed PotN from *Lactobacillus brevis* subsp. *gravesensis*. In contrast to the classical PII encoding monocistronic genes *glnB* or *glnK*, which are typically organized in an operon with ammonium permease gene *amtB*, PotN is encoded in an operon together with the *potABCD* genes, encoding the ABC transporter for spermidine/putrescine. Based on the location of proteins in one operon, the interaction of PotN with these proteins and participation in the control of polyamine transport can be assumed. By using bacterial two-hybrid system we have shown that PotN interacts with PotA. *In vitro* the soluble C-terminal domain of PotA was able to bind with PotN. The efficiency of this interaction can be modulated with ATP and ADP reflecting the possible mechanism controlling the activity of these proteins depending on the energy availability for the cell. This work was supported by the Ministry of Science and Higher Education of Russian Federation (Project No. 6.13498.2019/13.2).

### P-41-002
### Production and solubilisation of synthetic peroxidase gene BbePOX1 from *Branchiostoma belcheri* in *Escherichia coli*

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Peroxidases are catalytic enzymes that reduce hydrogen peroxide to oxygen and water and also oxidize a various substrates. Due to this biochemical function, they play an important role in many biological processes such as defense mechanisms, immune response and pathogenicity. They are widely used in various branches of biotechnological industry, but the problem of using this enzymes is, that it is usually difficult to obtain an enough yield of enzyme or for their high-cost production. Thyroid peroxidase from *Branchiostoma belcheri* belongs to the group of heme peroxidases, as well as other enzymes in this group, contains a porphyrin core and uses a heme *b* or posttranslationally modified heme as a redox cofactor for the hydrogen peroxide catalysis mediated by single- or double-electron oxidation of many molecules, including aromatic molecules. The aim of our research is to produce thyroid peroxidase from *B. belcheri*, for the structural studies and also because of its possible use in biotransformations. The BbePOX1 gene sequence was prepared synthetically, codon-optimized for expression in *Escherichia coli* and cloned into the pET-21a vector. The aims of the project are to select the optimal conditions for the soluble production of thyroid peroxidase. To achieve the highest yield of protein with the highest degree of solubility and enzyme activity we have focused on the optimal temperature of production, selection of suitable host strain, selection of cultivate media, fermentation conditions and also effective purification conditions procedures. After isolation of a sufficient amount of active recombinant thyroid peroxidase, we will be able to study its structure and also it could find use in many processes and biotransformation reactions, such as the eugenol bioconversion to vanillin, where the heme peroxidase is needed to efficiently cleave the double bonds in the reaction.

### P-41-003
### Identification of novel mitotic substrates of the PP4 Ser/Thr phosphatase

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Reversible protein phosphorylation driven by protein kinases and protein phosphatases, is involved in the regulation of various cellular events, including DNA replication and repair, differentiation, cell proliferation and death. While the role of protein kinases in the regulation of these processes is relatively well known, it is still not fully understood how their opposing enzymes, the protein phosphatases act. Our goal is to identify and characterize novel mitotic substrates and regulators of the poorly studied Protein Phosphatase 4 (PP4) and explore how dephosphorylation of its substrates contributes to cell division regulation in fruit flies. PP4 is an evolutionarily conserved heterotrimeric PP2A-type Ser/Thr phosphatase with an unexplored role in mitosis. Applying biochemical and proteomic approaches, we successfully identified more than 60 putative targets of PP4 that are all involved in cell cycle regulation and showed that at least 12 of them could make a direct physical interaction with the enzyme. We also found, that besides the well-characterized substrate-binding EVH1 domain of Falafel (the regulatory 3 subunit of the *Drosophila* PP4), its SMK1 domain, whose function has been unknown, could also be involved in the substrate binding. We therefore started to narrow down the binding surfaces between SMK1 and its putative interactors to uncover the mechanism this domain applies in substrate recognition. This would allow us to better understand how PP4 is involved in mitotic regulation. This work was supported by grants from The National Research, Development and Innovation Office (OTKA-PD115404), Ministry for National Economy of Hungary (GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00032)


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and Hungarian Academy of Sciences (Bolyai Fellowship (bo_329_15) and Lendület Program Grant (LP2017-7/2017)).

## P-41-004
## Exploring the mechanism of action of EcAMP1R2, a designed antimicrobial peptide

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The current drought in the pipeline of antibiotic drugs and the spread of antimicrobial resistance are a major threat to public health. Antimicrobial peptides (AMPs) are membrane-active peptides that have drawn the attention of the scientific community due to their potential as alternative antibiotics. We have studied the membrane activity of *Ec*AMP1R2, a designed AMP highly specific towards *Escherichia coli*. Concentrations above the minimum inhibitory concentration of *Ec*AMP1R2 cause hyperpolarization in *E. coli*, ruling out the paradigmatic 'pore formation mechanism of action' for this peptide. Studies with large unilamellar vesicles that mimic the lipid composition of the membranes of *E. coli* show that *Ec*AMP1R2 promotes fusion of vesicles containing cardiolipin (CL), a phospholipid that modulates protein complexes of energy transducing membranes. We hypothesize that the observed hyperpolarization might be caused by some sort of malfunctioning of proteins involved in the dissipation of the electrochemical potential. Coarse-grained molecular dynamic simulations suggest that indeed, *Ec*AMP1R2 hampers the availability of CL molecules to interact with the ATP synthase, an interaction extensively reported to be crucial.

## P-41-005
## Novel fibrinogenolytic enzyme form cultural liquid of *Pleurotus ostreatus*

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Use of proteases allows to obtain partly hydrolyzed forms of macromolecules with unique properties. The importance of proteases for the study of fibrinogen structure and functions bid scientists to look for new sources of highly specific proteases. Thus the propose of present work was to study the content of cultural liquid of Pleurotus ostreatus looking for the fibrinogen-specific proteases. *P. ostreatus* was cultured during 14 days at 27 °C. Culture media was collected and protein fraction was salted out by NaCl that was later dialyzed. Resulting solution was lyophilized. Powder was resolved in 0.05 M Tris HCl buffer pH 7.4 with 0.13 M NaCl prior the experiments. Products of hydrolysis were characterized by SDS-PAGE under reducing conditions. Molecular weight of proteins was determined using densitometry software Totallab TL100. MALDI-TOF analysis of enzyme was performed using a Voyager-DE. Enzyme electrophoresis allowed detecting three bands. Densitometry allowed us to detect minor proteolytic enzymes with molecular weights 60 and 80 kDa and major enzyme with molecular weight 40 kDa. MALDI-TOF analysis of enzyme allowed to detect the main peak occurring at mass/charge ratio of 42413. Analysis of the products of fibrinogen hydrolysis by proteases using SDS-PAGE demonstrated the exceptional cleavage of Aa-chain of fibrinogen. Moreover, only full-length fraction of fibrinogen Aa-chains (Aa1-610) were hydrolyzed. Minor polypeptide fraction of A$\alpha$-chains initially digested by plasmin (Aa1-583) remained intact. It can be an evidence that protease from the cultural liquid of *P. ostreatus* cleaves AaK583-M584 peptide bond of fibrinogen as plasmin does, however (in contract to plasmin) does not cleave the rest of a molecule. Application of this enzyme can allow us to obtain high-molecular weight forms of fibrinogen molecule with cleaved Aa584-610 peptides that can provide new information concerning the role of this peptide in functions of fibrinogen. *The authors marked with an asterisk equally contributed to the work.

## P-41-006
## Dynamics of synthetic processing bodies

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In addition to vesicle-like compartments, cells can coordinate reactions in space and time by forming membraneless organelles (MLOs). The reversible assembly and disassembly of these dynamic compartments is regulated by the liquid-liquid phase separation (LLPS) of nucleic acids and proteins, mainly RNA-binding proteins containing low complexity domains enriched in specific amino acids. Here, we reconstitute synthetic MLOs and investigate the fundamental biochemical processes underlying their dynamics focusing on the LLPS of DEAD-box ATPase Dhh1, associated with processing body formation in yeast. We identify ATP and RNA as key molecular triggers that regulate protein-protein interactions and modulate the phase diagram. The binding between Dhh1 and ATP nucleates the formation of the protein-rich condensates, whereas the binding of RNA promotes droplet growth and tunes the viscosity and surface tension of the viscoelastic droplets by establishing multivalent interactions. By cyclically adding and removing ATP we reproduce the reversible assembly and disassembly of the protein-rich condensates, mimicking molecular mechanisms that cells have likely developed to regulate the dynamics of processing bodies. We recapitulate this process on a droplet microfluidic platform, which allows to overcome the limitations of traditional methods and investigate the dynamics of the phase transition on biologically relevant timescales and in cell-like volumes (pL) not accessible by bulk techniques. The phase transition is induced on-chip by rapidly mixing a homogeneous protein solution with molecular triggers followed by compartmentalization into a water-in-oil emulsion, representing cell-like confinements. The quantification of the kinetics of droplet growth inside these compartments contains important information on the mechanisms underlying LLPS. Specifically, with our approach we are able to show that droplet growth is dominated by droplet coalescence rather than by Ostwald ripening.

## P-41-008
## Macromolecules involved in otoliths biomineralization

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Biomineralization is a process of mineral formation under biological control. Biominerals are a combination of a crystal phase


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deposited on a matrix composed of macromolecules, such as proteins, saccharides or lipids. The presence of the organic matrix influences the properties and functions of biominerals. In a group of macromolecules which control biomineralization, proteins are especially important. Biomineral-associated proteins affect the polymorph, size and shape of mineral phase. Otoliths, the ear stones of teleost fish, are one of the biominerals whose formation is highly controlled by proteins. Major role of otoliths is sound transduction and sensation of linear acceleration. Similar structures, called otoconia, are located in the mammalian inner ear. Our studies are focused on characterisation of organic composition of common carp otoliths. The project consists of a few sections. The first part was focused on otolith extraction and purification, the second – on organic matrix extraction, with a special attention to proteins. A protocol for protein extraction included decalcification in EDTA and protein precipitation was developed. The obtained macromolecules were then examined by chromatographic and electrophoretic techniques. Proteins were analysed with LC-MS/MS. Proteomic studies indicated the presence of several proteins associated with the otolith matrix such as otolith matrix protein, otolin-1 or otogelin. In order to characterize individual macromolecules, attempts were made to separate proteins. *In vitro* biomineralization tests analysed the effect of purified macromolecules on calcium carbonate crystals. We believe that our results have great importance for understanding the mechanism of otolith biomineralization. Additionally, since fish are the model organisms for otoconia studies, gathered details could be useful in search of resolving balance disorders problems like BPPV.

## P-41-009
## PDIA3/ERp57, a multifunctional protein

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PDIA3 is a member of the protein disulfide isomerase family mainly found in the endoplasmic reticulum where it modulates the folding of newly synthesized glycoproteins. PDIA3 is also involved in several human diseases due to its multi-subcellular localization, its overexpression in cancer and above all flexible binding sites. Taking into account the ability of PDIA3 to interact with a series of small ligands, e.g. flavonoids, the thermodynamic parameters, binding constants and the effects on enzymatic activity of the interaction between PDIA3 and a wide range of flavonoids were characterized. Two flavonoids, silibinin and punicalagin, showed the ability to bind and modify protein properties in the micromolar range of concentration. In order to understand if the silibinin and punicalagin binding effects are only PDIA3-specific, the aim of this study is to perform a comparative study between PDIA1 and PDIA3. Flavonoids' binding and the effects on both proteins were evaluated by quenching analysis of the protein intrinsic fluorescence, differential scanning calorimetry and isothermal titration calorimetry assays, while disulfide reductase activity was assayed with a fluorescent substrate. The punicalagin and silibinin exhibit different behaviours on both PDIAs suggesting that the interactions between flavonoids and PDIAs involve different binding sites. The ability of the silibinin and punicalagin to modulate PDIAs could be used in the treatment of diseases in which the PDIAs are overexpressed, such as in different types of cancer, in platelet aggregation where PDIA3 plays an important role and as adjuvant in chemotherapy promoting endoplasmic reticulum stress and apoptosis.

## P-41-010
## Mouse IgG3 as a highly protective antibody that inspires development of new biotherapeutics

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Mouse antibody of class IgG3 provides efficient protection against several lethal pathogens in animal models. This subclass has a few properties that are unique among IgGs: it is able to oligomerize, binds multivalent antigens with high affinity and agglutinates red blood cells. IgG3 activates complement and immune effector cells, although IgG3-specific receptor is unknown. The exceptional features of IgG3 correlate with the protection after passive immunization using this isotype. While many reports confirmed the IgG3 properties, their molecular mechanisms are unknown. We performed functional analyses of IgG1/IgG3 hybrid antibodies with swapped constant domains and we discovered that the second constant domain of heavy chain (CH2) determines antibody oligomerization and increases functional affinity to multivalent antigen. Moreover, IgG3-dependent hemagglutination was enhanced by CH2. We were also trying to identify IgG3-specific receptor on mouse immune cells. Our findings confirmed existence of high-affinity IgG3-receptor that is likely a complex of several membrane proteins. The discovery that IgG3 unique properties are determined by the CH2 domain gives hope of generation of IgG3-inspired humanized antibodies that might provide protection against existing or emerging life-threatening bacteria.

## P-41-011
## Enhancing the catalytic power of serine hydroxymethyltransferase to produce commercially valuable compounds

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Serine hydroxymethyltransferase (SHMT) is a pyridoxal-5′-phosphate (PLP)-dependent enzyme that, under biological conditions, requires tetrahydrofolate (THF) cofactor to catalyze the reversible conversion of L-serine to glycine and THF to 5,10-methylene THF. Under specific conditions, SHMT can also catalyze the reversible retro-aldol cleavage of 3-hydroxy-amino acids in a THF-independent mechanism. Taking advantage of these characteristics, SHMT is being explored by scientists and engineers as a powerful biocatalyst for the stereoselective synthesis of a broad variety of α,α-dialkyl-α-amino acids in an isomerically pure manner, which are of great value for the pharmaceutical industries. In order to gather a better knowledge about the stereoselective mechanism catalyzed by SHMT and gather a better knowledge on how it can be improved, computational methods were used to gather an atomistic portrait and an energetic profile of the reaction. To this end, the wild-type and mutated forms of SHMT were subjected to molecular dynamic simulations, and the mechanism studied using the ONIOM QM/MM methodology based on the DLPNO-CCSD(T)/CBS//B3LYP/6-31G(d,p): AMBER scheme. The VMD, molUP, AMBER, and Gaussian09 software were used to perform the calculations and analyze the results. The computational results are in fully agreement with the available experimental results and provide for the first time, the


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differences on the stereoselective catalytic mechanism of SHMT, that result from two mutations of Glu51. The QM/MM also provides important clues about the catalytic process and now can be used as a blueprint to test other mutations and in the development of more efficient bioengineered enzymes in the production of α,α-dialkyl-α-amino acids. This will be very important to enhance the synthesis of commercially valuable compounds, namely the precursors in the synthesis of drugs, such as, as myriocin and lactacystin.

## P-41-012
## *Candida utilis* expression system for the production of human enterokinase light chain (hEKL)

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Proteases are a highlighted group of enzymes that correspond to approximately 60% of the total worldwide enzyme sales, representing one of the most important enzymes due to their applications in detergent, food, pharmaceutical, chemical, leather, paper and pulp, and silk industries. That is the reason why it is necessary to look for possibilities of alternative expression systems which might be expression system of *Candida utilis*. Food yeast *C. utilis* has appeared as an excellent host for recombinant protein production and sufficient intracellular production of recombinant proteins have been already achieved. In the current research, we focused on increasing production of enterokinase by expression in yeast *C. utilis* which has been approved as a GRAS microorganism (Generally Recognized as Safe) and preferred property of use is that its secretome does not contain proteolytic enzymes that are redundant in the medium for more demanding purification and possible degradation of the product. In order to control the expression system, it is possible to use constitutive glyceraldehyde-3-phosphate dehydrogenase- GAP promoter, because it does not inhibit growth and is not toxic to cells. Enterokinase is a serine protease that has the ability to cleave exactly at the N-terminus of the substrate in the (Asp)4Lys sequence and it does not leave amino acid residues on N-terminus, also it is an ideal tool for cleaving recombinantly produced fusion proteins. The aim of our work is to optimize the enterokinase sequence by codon usage and consequently prepare the pGAP-EKop construct by inserting the gene that codes enterokinase without recognition sequences for Kex2 and Ste13 proteases.

## P-41-013
## Identification and optimization of expression conditions of the recombinant alcohol dehydrogenase with the application in biotransformation processes

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Biotransformation refers to a process for chemical modification of compounds from which other chemical compounds are formed in the presence of a substance, called biocatalyst. In practice, these processes can be used to produce expensive products in various industries, e.g. biotechnology, pharmaceutical, food or cosmetic industries. A significant biocatalyst can also be an enzyme called alcohol dehydrogenase (ADH), which under certain conditions is capable of interconversion between aldehydes or ketones and their corresponding alcohols. This enzyme is an oxidoreductase that is often used in processes of biotransformation in food or cosmetic industry. However, to be capable of designing and preparing an efficient regenerative biotransformation system by which it would be possible to provide a sufficient quantity of alcohol at commercial scale, an ADH with highest temperature tolerance is needed. The ADH produced by an aerobic, Gram-positive bacteria Rhodococcus ruber, was found out to be a good candidate to test this hypothesis. In our experiment, we prepared a recombinant E. coli strain with R. ruber ADH gene and set basic conditions to ensure successful expression of RrADH, e.g. the temperature of cultivation, the selection of a suitable E. coli strain and the determination of the proportion of the soluble fraction, since only the soluble enzyme is active. We have succeeded in expressing the recombinant RrADH protein in E. coli but, despite attempting to increase the proportion of the soluble fraction in several ways, we have failed to achieve a higher proportion of the soluble component of this enzyme. Therefore, we are currently testing an effect of adjuvants to avoid a creation of inclusion bodies which cause this insolubility. We also investigated the biotransformation properties of ADH from Saccharomyces cerevisiae, so the final biotransformation results from the yeast and bacteria ADHs can be compared and evaluated.

## P-41-014
## Rabphilin3A interacts with SNAP25 in PC12 cells

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The liberation of neurotransmitters to the synaptic space is a deeply complex process that needs an accurate control of the vesicles recruitment. Numerous proteins are gathered to the presynaptic space and many of them share a common structural motif: the C2 domain, which is regulated by its ability to bind $Ca^{2+}$, phospholipids and other proteins, providing this domain with the capability to fine-tune the broad range of vesicle release modes. Rabphilin3A (Rph3A) is a membrane trafficking protein involved in the $Ca^{2+}$-dependent regulation of secretory vesicle exocytosis in neurons and neuroendocrine cells. In this work, we have used in situ protein ligation assay (PLA) to characterize the molecular determinants driving the Rph3A-SNAP25 interaction in PC12 cells. We observed that these interactions occur both in the cytosol and at the plasma membrane. These signals correspond to populations of transport and synaptic vesicles that might contain Rph3A-SNAP25 and vesicles docking respectively. Furthermore, staining HA-Rph3A and myc-SNAP25 by immunofluorescence demonstrated that both proteins localize as expected. Site-directed mutagenesis of important aminoacidic residues located at the C2B bottom α-helix as well as two regions of the SNAP25-N helix showed that these two motifs are important for the Rabphilin3A-SNAP25 interaction at the plasma membrane and contribute to explain some of the numerous steps and interaction events which take place at the presynaptic neuron.


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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## P-41-015
## Production, purification and description of thermostable catalase-peroxidase and its mutated form in *E. coli*

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Catalases are widely used in various industrial applications (food industry, pharmaceuticals, biopolymerization, biosensoring, medical technologies, textile industry, etc.). In our work we have purified and described catalase AfKatG from thermophilic archeon produced in *E. coli* using different conditions to homogeneous protein quality and activity. We worked with four different growth media (two complex batch, autoinduced and chemically defined fed-batch). We used hemin as an additive in all media as AfKatG is a heme-enzyme and we proved complex media to be better for production of this enzyme. Another strategy was using different production strains where we got highest soluble amount from E. coli strain MC4100. To produce also soluble mutated type AfKatG (single mutations to suppress catalase and improve peroxidase activity) we had to use BL21 and lower temperature (20 °C). We used a simple three steps purification – heat treatment, IMAC nickel column and dialysis against water. We determined pH and temperature optima for both catalase and peroxidase activity and used calorimetric studies of the thermal denaturation to study the melting point of non-mutated AfKatG. We studied the secondary structure of the enzyme by using circular dichroism in far to middle ultraviolet spectrum and we determined its tertiary structure by HPLC. We studied activity of mutated AfKatG and as predicted it showed no catalase activity. However, there was no difference in peroxidase activity compared to unmutated AfKatG. In further studies we will focus on creating new mutations in AfKatG to enhance peroxidase activity in order to be able to use it in biotransformation reactions. The produced unmutated enzyme can be used directly in need of a thermostable catalase.

## P-41-016
## Identification and characterisation of the major yolk granule protein isoforms in the high and low density yolk granule subpopulations present in the sea urchin egg

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The yolk granule is a ubiquitous organelle found in the eggs and embryos of many organisms. The classic view of the yolk granule as a store of nutrients for the developing embryo has been supplanted by data suggesting a more dynamic role for this organelle. In the work reported here, we have fractionated the yolk granule population in sea urchin eggs into two subpopulations distinguishable by their buoyant densities in sucrose gradients. The subpopulations were shown to possess differing gelatin-cleavage activities. Interestingly, different isoforms of the major yolk granule protein were found to be present in the subpopulations. We have previously shown that the egg-localised, major yolk granule protein underwent calcium-dependent changes in their secondary and tertiary structures. These changes correlated with the major yolk granule protein binding to liposomes and inducing liposome aggregation. In the work reported here, the major yolk granule protein isoforms could be distinguished by their buoyant densities, as well as differing susceptibilities to digestion by chymotrypsin and interactions with the non-ionic detergent TX-100. The presence of two isoforms of the major yolk granule protein will be discussed in the context of our understanding of the functional capabilities and increasingly complex roles played by this protein.

## P-41-017
## Partial loss of pancreas endocrine and exocrine cells of human ARX-null mutation: consideration of pancreas differentiation

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*Aristaless*-related homeobox gene (*ARX*) mutation leads to several neurological disorders including X-linked lissencephaly with abnormal genitalia (XLAG), West syndrome and Partington syndrome, with XLAG being the most severe form. Although some of the brain pathology of XLAG has already been described, the crucial extra-brain symptoms are severe growth retardation, transient hyperglycemia and intractable diarrhea. Since ARX expresses in the islets of Langerhans during the embryonic stage, these visceral phenotypes may be related to a loss of ARX function, which develops endocrine cells in the pancreas. We investigated the abnormal pancreatic development of XLAG patients with *ARX*-null mutation. We performed immunohistochemistry of XLAG pancreases, using the antibodies against glucagon, insulin, somatostatin, pancreatic polypeptide, ghrelin, Brn4, Nkx2.2, Mash1, amylase and pancreatic lipase. The glucagon- and pancreatic polypeptide-producing cells were found to be completely deficient in the islets of Langerhans. We also discovered marked interstitial fibrosis, small exocrine cells with loss of amylase-producing cells and an enlargement of the central lumen of the glandular acini. These pathological findings indicate that ARX contributes not only to endocrine development, but also to exocrine development of the human pancreas, and its deficiency may lead to the severe phenotypes of XLAG patients.

## P-41-018
## Plasma radiation source for biochemical research

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Plasma Activated Water (PAW) is applied in practice of biochemical research and in industry for sterilization of food. To obtain it, cold plasma generators are used. At a high specific power of plasma source the active species with a high probability are terminated in interactions between themselves. This leads to a decrease in the effectiveness of the method. We have created a spark discharge plasma radiation generator in air, which can be used to produce Radiation Activated Water (RAW). Water is treated by spark discharge plasma radiation; the discharge area is 3 cm from the RAW surface. Discharge power 0.4 J/s, pulse repetition rate 50 Hz. The features of reaction kinetics in water under the action of plasma radiation were investigated. The main mechanism is indirect effect of radiation. Active species first generated in water under plasma radiation, and then they interact with chemicals dissolved in water. That is, does not difference to treat water with dissolved chemicals directly by radiation, or to introduce the chemicals into the water after treatment with radiation. Unlike the RAW, in PAW the main processes occur at the time of plasma contact with water. The primary active species formed directly under the action of radiation are hydroperoxide radicals, hydrogen peroxide, nitrogen oxides. Secondary active


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species in RAW are hydrogen peroxide, nitrous acid and complex decaying into peroxynitrite and peroxynitrous acid during not less than 4 days. The reaction with active species in RAW lasts up to 4 days. Active species have both oxidizing and reducing properties, exhibit a strong antimicrobial effect. There is a delay in the action of radiation, since a significant concentration of active species is achieved in water not less than 1 min after switching on the radiation source. RAW characteristics are: pH ~ 3, ORP = 790 mV (SHE), conductivity ~ 1 mS/cm. The specific energy cost of producing RAW is about 10 times less than PAW. *The authors marked with an asterisk equally contributed to the work.

## P-41-019
## O-GlcNAcylation as marker of antioxidant effects of vitamin E-stabilized ultra high molecular weight polyethylene in human osteoblast

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High Crosslink process was introduced in the development of joint prosthetic devices, in order to decrease the wear rate of ultrahigh molecular weight polyethylene (UHMWPE) but it also triggers the formation of free radicals and oxidative stress (OS), which affects the physiological bone remodeling, leading to osteolysis. Vitamin E stabilization of UHMWPE was proposed to provide oxidation resistance without affecting mechanical properties and fatigue strength. The aim of this study is to evaluate the antioxidant effect of vitamin E added to UHMWPE on OS induced osteolysis, focusing in particular on the evaluation (by western blot analysis) of protein O-GlcNAcylation, OGA and OGT levels considered markers of cellular response to OS. O-GlcNAcylation levels increased in presence of vitamin E blended UHMWPE, in particular with not crosslinked vitamin E stabilized UHMWPE ($P < 0.005$) while, conversely, they fall in absence of vitamin E. Significant increase ($P < 0.01$) of OGT protein was found in presence of not crosslinked Vitamin E blended UHMWPE, whereas a significant increase ($P < 0.05$) of OGA enzyme was observed in Vitamin E absence. The OGT/OGA expression ratio show a behavior consistent with the observed O-GlcNAcylation levels. Our results suggest that the Vitamin E stabilization of UHMWPE: (i) seems to improve the ability of osteoblast to respond to oxidative stress, inducing cellular mechanism of defense, such as dynamic O-GlcNAcylation in order to promote cell survival; (ii) could contribute to reduce oxidation- induced osteolysis and the consequent loosening of the prosthetic device, therefore improving the longevity of total joint replacements.

## P-41-020
## Inhibition of adenosine deaminase activity by novel synthesized piperazine class compounds

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Adenosine deaminase (ADA) is an important enzyme in purine metabolism. The increase of the ADA activity is documented in such pathologies as diabetes, tuberculosis, cancer, rheumatoid arthritis, etc. The substrate of ADA, the adenosine, suppresses the inflammation and plays the role in the protection against injuries. The increasing of ADA activity in the extracellular medium or pathological effusions results in decrease of adenosine concentration and aggravation of inflammation. Therefore, the inhibition of ADA is considered as beneficial tool for regulation and remission of inflammation. This work describes the *in vitro* inhibition of purified from bovine lung ADA by novel synthesized tertiary amino alcohols substituted by piperazine ring. The screening of 15 compounds was carried out. Among them, the compounds, containing in the piperazine ring the phenyl and heliotropine substitutes were more effective. The $IC_{50}$ values for 6 potent compounds in inhibition of ADA were between 3.5–15.5 μg/mL. The average value of $IC_{50}$, for the compounds, in their turn, containing in the heliotropine ring different substituent groups was of 20 μg/mL. The inhibition of ADA by effective one – GGN 322*HCl the $IC_{50}$ value was equal to 15.2 μg/mL. The inhibition was of competitive nature with Ki = 2.5 μM. For the compounds containing as a substitute phenyl group in the piperazine ring, the average $IC_{50}$ value was of 10 μg/mL. The most significant results among them was registered for the PO191*2HCl, with benzhydryl (diphenylmethyl) as a substitute group. The $IC_{50}$ value for it in inhibition of ADA was a rather low 3.5 μg/mL (6.5 μM). The Ki for this compound was 1.5 μM and the nature of inhibition was competitive. The constant of bimolecular interaction of PO191*2HCl with the tryptophan residues in ADA ($K_{SV}$) was evaluated from the fluorescence quenching, using Stern–Volmer equation as of $0.145 \pm 0.027$.

## P-41-021
## Interaction of PCID2 and NudC proteins of *Drosophila melanogaster in vitro*

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There are many proteins and protein complexes involved in the mRNA transport from the nucleus to the cytoplasm in Drosophila melanogaster cells. PCID2 protein as one of the participants in this process binds mRNA in the nucleus and enters the cytoplasm, changing partners in the transport complex. In our laboratory we purified the PCID2 complex from cytoplasm and detected its interaction with the NudC (nuclear distribution protein). We investigated interaction between PCID2 and NudC proteins. First of all we generated antibodies to NudC and confirmed PCID2 and NudC proteins interaction by immunoprecipitation. We continued by testing whether PCID2 protein has a separate domain for interaction with NudC protein. We divided PCID2 into the following domains: N-terminal, C-terminal (WD-domain) and the "middle" domain (MID) located between them. Moreover, MID- and WD-domains together constitute a functional PCI-domain. We also divided NudC protein into N- and C-terminal domains in the same way. We expressed the 6xHis-tagged proteins related to said domains and full-size PCID2 and


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NudC proteins. Using the Pull Down method we verified interactions between full-size NudC protein and PCID2 protein domains, and similarly, between full-size PCID2 protein and NudC protein domains. In conclusion we showed that PCID2 C-terminal domain interacts with full-size NudC protein (both NudC domains), full-size NudC protein is able to dimerization. This work was supported by a grant from the Russian Foundation for Basic Research (project #18-34-00151).

## P-41-022
## Internalization of enzyme L-lysine α-oxidase in isolated segment of rat small intestine

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L-lysine α-oxidase (LO) enzyme belongs to a family of L-amino acid oxidases and demonstrates significant antitumor effect by parenteral administration. LO is considered to be a promising anticancer agent for clinical trials, particularly for the treatment of patients with colorectal cancer. LO was purified to homogeneity from fungal strain–*Trichoderma* cf. *aureoviride* Rifai VKM F-4268D. Application of fungal protein as an intravenous drug is rather complicated and very rare. Previously published data on the internalization of intact proteins in small intestine inspired us to investigate the possibility of oral LO administration. It was determined that LO incubation in the presence of proteolytic enzymes does not lead to the loss of its enzymatic activity. Experiments were carried out on a model of isolated inverted segments of rat small intestine. A conjugate of LO with Acridinium (LO-Acridinium) was used to quantify the LO internalization. Isolated segments of the small intestine (n = 4) of regular inbred rats were inverted and immersed into the incubation medium with LO-Acridinium. After 30 min samples were taken from the incubation medium and from segments of the intestine. Relative luminescence level was determined in samples by the standard flash chemiluminescence method in relative light units. Using experimental data, it was calculated that 0.1% of the LO-Acridinium was absorbed per 1 cm of intestinal segment. Since the average length of rat small intestine is about 110 cm, the total amount of enzyme absorbed was 11%. Based on the optimal therapeutic parenteral dose of LO for mice (400 U/kg), the calculated dose for oral administration should be approximately 4000 U/kg. The results suggest that the Acridinium-LO can be absorbed in the rat small intestine for a physiologically adequate time. Experimental data were used to predict the therapeutic dose for oral administration. The publication was prepared with the support of the "RUDN University Program 5-100".

## P-41-023
## Cell death and its molecular signals of HeLa cells induced by body fluid of *Turritopsis* spp.

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*Turritopsis* spp. (Hydorozoa, Anthomedusae) drastically metamorphoses from fertilized ovum to planula, polyp and medusa during their growth process. In addition, they possess a unique life cycle which is "the rejuvenation" from medusa to polyp. During these metamorphoses, the cell differentiation and cell death are conjectured to be occurred actively, and the molecular signals to lead these activities are thought to be expressed remarkably in these cells. The signals may have potentials to apply for regeneration medicine and anti- aging. If human cancer cells are induced metamorphosis by these signals, the application for the cancer treatment is also promising. In this study, the morphological change of HeLa cells by body fluid of two species of *Turritopsis* spp. (*Turritopsis* sp. and *Turritopsis rubra*). *Turritopsis* sp. and *Turritopsis rubra* are known their higher and lower rates of "the rejuvenation" than other *Turritopsis* species, respectively. First, HeLa cells, human malignant epithelial cells, were cultured in DMEM added each body fluid extracted from the mature medusae of *Turritopsis* sp. and *Turritopsis rubra*. As the result, in both kind of body fluid, HeLa cells shrunk, unstuck from the culture dish and formed membrane blebs. DAPI stain was showed that these morphological change of HeLa cells were cell death. Based on the result, *Turritopsis* sp. and *Turritopsis rubra* was suggested to possess the signals to lead HeLa cells death. Moreover, HeLa cells were cultured on the PVDF membranes which were absorbed proteins in each body fluid. These cells were unstuck from the membranes after shrinking. This result indicates that the signals induced cell death are proteins. The proteins detection and their functional mechanism will be discussed which are related to the signals induced HeLa cell death in the poster session.

## P-41-024
## Changes in rumen microbiota of cattle with the simultaneous introduction of iron and copper nanoparticles and quorum sensing suppressants

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One of the promising areas is the development of new solutions to manage the sense of quorum (QS) in bacteria. The aim of the present work was to study the effect of iron nanoparticles (NPs) (80 nm in size with a specific surface of 15 $m^2/g$) and copper nanoparticles (Cu NPs) (97 nm in size with a specific surface of 24 $m^2/g$) together with anti-quorum substances (AQ) on rumen microbiocenosis of cattle. Water extract of Quercus cortex was used as AQ substances in the ratio of 1:10 at a dose of 0.64 mL/kg body weight. The composition of extract was determined on gas chromatograph (Shimadzu, Japan). The study was carried out on 13-month bulls of the Red Steppe breed. Animals were divided into 3 groups (n = 5): group I received Quercus cortex extract and Fe NPs (1.6 mg/kg body weight); group II – extract


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of Quercus cortex and Cu NPs (0.6 mg/kg of animal weight); group III – control – without additives. The taxonomic composition of rumen was determined by NGS sequencing using MiSeq instrument (Illumina, United States). It was found that the use of AQ substances and Fe NPs in the diet (group I) was accompanied by an increase in the number of *Bacteroidetes* by 17.5%, *Proteobacteria* by 22.1% and *Candidatus Saccharibacteria* (by 50.0%) compared to control. Analysis of species diversity showed a decrease in the number of *Firmicutes* by 13.9%, *Streptococcaceae* by 8.8%, compared with the control, and an increase in *Butyrivibrio* and *Pseudobutyrivibrio* to 1.8 and 2.0% of the total. Using the AQ substance and Cu NPs (Group II), an increase in the number of representatives of *Bacteroidetes* (by 14.5%), *Proteobacteria* (by 36.8%) and a decrease in *Firmicutes* (by 13.1%) was registered. The components contained in Quercus cortex extract control the sense of quorum (QS) in bacteria, causing a suppression of their sensitivity. The study was performed in the framework of the project #0761-2019-0005.

## P-41-025
## Rumen microbiome of cattle after introduction of ultrafine particles in feed

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The organism of animal represents a whole world with many ecological niches for microorganisms. Changes in rumen microbiocenosis has a direct impact on the productivity of animals. The aim of the present work was to study the effect of ultrafine particles (UFPs) of $SiO_2$ and FeCo alloy on rumen microbiocenosis of cattle. Bulls of the Red Steppe breed 13 months of age were studied. Animals were divided into three groups (n = 5). Animals of group I were injected with UFPs of $SiO_2$ (13 mg/kg) and were fed with the basic diet, group II with UFPs of FeCo alloy (5 mg/kg), and group III without additives. Advanced Powder Technologies LLC, Tomsk, manufactured UFP of FeCo, $SiO_2$ of spherical shape. UFPs of $SiO_2$ were 40.9 nm, UFPs of FeCo – 62.5 nm. The taxonomic composition of ruminal composition was determined by NGS sequencing method on MiSeq instrument (Illumina, United States). 16S libraries were prepared according to the Illumina workflow with primers. The libraries were sequenced using the MiSeq v3 reagent kit with 2 × 300 base pairs. UFPs have been found to affect the rumen microbiome of animals. The microbiocenosis of rumen of animals in the control group is 100% represented by bacteria. Introduction of $SiO_2$ UFPs (group I) to the diet reduced the number of the *Bacteroidetes* by 50%, *Firmicutes* by 69.2% compared with the control and *Proteobacteria* (less than 2% of the total). The use of UFPs of FeCo alloy (group II) in the diet increased the number of *Firmicutes* taxon by 6.9%, but reduced the number of bacteria belonging to the *Bacteroidetes* phyla by 12.4% compared with control and *Proteobacteria* (less than 2% of the total). The use of UFPs in the composition of diets led to a change in the number of individual groups of bacteria in rumen microbiocenosis of cattle, it promoted the change in the digestibility of the nutrients of feed. The study was performed in the framework of the project #0761-2019-0005.

## P-41-026
## Copper ultrafine particles induce caspase-3 expression

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Nowadays, new medicines based on ultrafine particles (UFPs) emerge along with traditional medicines containing microelements. At the same time, various pathological conditions still can develop. The aim of the research is to assess the expression of Caspase-3 in hepatocytes as an indicator of programmed cell death against the background of the introduction of Cu UFPs. Studies were performed on Wistar male rats. They were enterally (with water) fed with aqueous suspension of Cu UFPs (55.0 ± 15.0 nm) once daily within 1 week at a dose of 2.0 mg/kg animal weight. Sampling was performed once every day. Immunohistochemical studies were performed on paraffin sections of liver using monoclonal antibodies (Caspase-3) and Bio Genex Super Sensitive Detection System, USA. Immunosensitive cells were counted per 1000 among hepatocytes and expressed in . Experimental studies on animals were carried out in accordance with the instructions recommended by the Russian Regulations, 1987, and "The Guide for the Animals" (National Academy Press, Washington, D.C. 1996). Statistical data were processed using Statistica 10.0 software (StatSoft, Inc., USA) and MS Excel 2000 software. Mean (M) and standard error of the mean (±SEM) are presented. The significance of differences was assessed by Student's *t*-test, the differences $P \leq 0.05$ were considered statistically significant. Expression of Caspase-3 enhanced by 40% ($P \leq 0.05$) after triple administration. The established property of Cu NPs to increase the readiness for apoptosis can be used in chemotherapy of malignant tumors, where apoptosis of cells containing mutations occurs. However, further study is needed. The study was performed in the framework of the project #0761-2019-0005.

## P-41-027
## Influence of wolfram nanoparticles on the stability of the emotional state and behavior of rats in the "Open field test"

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The effect of wolfram nanoparticles on the nervous system (motor performance, emotional poise) using the "Open field test" was assessed in our scientific research. The changes of the level of vertical and horizontal motor activity (VMA, HMA), emotional stress and fear, and the level of curiosity were estimated. The study was conducted in male Wistar rats. (n = 60) weighing 110–180 g. The experimental groups were injected once intraperitoneally with nanoparticles of wolfram in dose 1.5 and 15 mg/kg, respectively. The control group was injected once intravenously with 0.9% sodium chloride solution. There was the increase in the level of all tested parameters on the 1st day: HMA by 10%, VMA by 33%, grooming behavior by 67% ($P < 0.05$), defecation by 12% comparing to the control group. There was the increase in all indicators apart from the investigatory reaction on the 7th day: HMA by 17% ($P < 0.05$), VMA by 22%, grooming behavior by 46% ($P < 0.05$), defecation by 6%. There was the increase in HMA by 22% ($P < 0.001$), VMA by 24% ($P < 0.01$), grooming by 75% ($P < 0.001$) and defecation by 33% ($P < 0.05$) on the


FEBS Open Bio **9** (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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14th day in the second experimental group. It should be emphasized, the activity of second group animals increased from 1 to 14 days of observation. There was the tendency to increase indications of investigatory reaction, motor performance and emotional poise when injected with nanoparticles of wolfram in dose 15 mg/kg. The highest level of activity was recorded in third group on the 14th day of the experiment, there was a statistically reliable increased HMA by 31% ($P < 0.001$), VMA by 15%, grooming by 79% ($P < 0.001$) relative to the control. Similarly, the indicators of activity were changed relative to the control on 1st and 7th days. The results of our studies have shown nanoparticles of wolfram effect on the nervous system, increasing motor activity and emotional stress of animals. This research was conducted within the scientific project (Project No. 0526-2019-0001). *The authors marked with an asterisk equally contributed to the work.

## P-41-028
## Mapping of MPP1 palmitoylation sites using acyl-PEG-exchange (APE) method

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Protein S-palmitoylation is a reversible post-translational modification that alters the localization, stability, and function of numerous proteins in the cell and is currently intensively explored. MPP1 (Membrane Palmitoylated Protein 1)/p55 belongs to the MAGUK family of scaffolding proteins, and was recently found to be crucial molecule that regulates lateral membrane heterogeneity in erythroid cells. MPP1 contains four cysteine residues in position 94, 179, 242 and 454 that can be potentially acylated, however it is uncertain which one is naturally modified. As palmitoylation of MPP1 seems to be crucial for its function, the aim of this study was to identify the number of MPP1 palmitoylation sites by using Acyl-PEG-Exchange (APE) method. This novel method allows fast and sensitive determination of the number of endogenous protein S-acylation sites in whole cell lysates and also in purified protein. Briefly, free SH groups are blocked, and thioester linkage between palmitate and cysteine are cleaved. Newly released cysteines then bind a PEG mass-tag of defined mass providing therefore an increase in the protein molecular mass and changes in the electrophoretic mobility that can be easily visualized by western blotting. Supported by the National Science Centre, Poland, 2016/21/B/NZ1/02821.

## P-41-029
## Computational study of diarylethene-based photocontrolled peptide inhibitors for the human p53/MDM2 complex

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It is well known that Mdm2 is an important regulator of the p53 tumor suppressor. The level changes of the MDM2 can have an impact on control of cell cycle and contribute to oncogenesis. That is why, MDM2 is promising target for cancer treatment. In the present work we tried to develop and validate peptidomimetics of the interaction between p53 and MDM2. The pDI (Ac-LTFEHYWAQLTS-NH2) peptide was used as a template. This peptide was modified by adding of photocontrollable part in two positions (Ac-LTF-X-EYWAQL-X-S-NH2). As a result, cyclic peptide which could exist in two interconvertible photoforms (next called: open/close) was obtained. For stability estimation of complexes "MDM2-open" and "MDM2-close" MD simulation was used. According to MD simulation, the mobility of MDM2 in complex with open (range: 0.07–0.19 nm) and close (range: 0.05–0.18 nm) is stable and almost identical. Nevertheless, in both forms (MDM2 – open/close) were detected serious local geometric differences. There is only a steric interaction between photocontrollable part (close) and MDM2 (located just near Phe 54 and Gln 59). In turn in "MDM2 – open" photocontrollable part of peptide is shifted by 0.45 nm and tightly stuck between Met 62, Phe 55 and Gln 59. In both cases hydrophobic parts of Leu 26 and Ile 99 making strong steric interaction, the average distance between them are 0.56 (open) or 0.55 (close) nm. In this manner, a significant part of binding interface is shielded. However, the H-bond with His 96 is unstable in "MDM2 – close" complex. That definitely has a negative effect on binding between MDM2 and closed form of peptide. And finally as expected, the open form of peptide (Kd = 15 nM) was more active compared to the closed one (Kd = 237 nm) during biological testing.

## P-41-030
## Interaction of coagulation factor XIII zymogen to subpopulations of activated platelets

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Coagulation factor XIII (fXIII) is a pro-enzyme circulating in blood at 14–28 μg/mL (~ 88 nM). Its active form, the transglutaminase fXIIIa, is a critical regulator of fibrinolysis. The objective of the present study was to quantitative investigating fXIII zymogen binding to the PS-positive and PS-negative subpopulations of activated platelets. These subpopulations were recently shown to be heterogeneously distributed in arterial thrombi and this also raises a question about distribution of factor XIII. Platelets were isolated from whole blood by washing and gel-filtration. Gel- filtered platelets were stimulated with thrombin (100 nM), ADP (100 μM) or CRP (20 μg/mL) Flow cytometry and confocal microscopy were used to investigate interaction of FITC-labeled FIII with the membranes of activated platelets. The present study was shown fXIII binds at ~ 700–800 molecules per a PS-positive platelets (produced with thrombin or CRP) when 100 nM fXIII is added, with minor binding to PS-negative ones (when produced with thrombin or CRP), ADP-activated or resting platelets. FXIII binding is calcium-independent, linear and non- saturable within a wide range of concentrations for all cases. FXIII interacts with platelets not directly, but through fibrin(ogen) that is in turn bound to integrin αIIbβ3 or retained on platelet surface by crosslinking and polymerization. Exogenous fXIII (as well as platelet-derived fXIIIa and tissue transglutaminase) is predominantly located in the procoagulant platelet "caps" and co-localized with fibrinogen. Strong platelet stimulation, fibrinogen, and αIIbβ3 play essential roles in fXIII binding,


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without any of them fXIII does not binding to platelets. The preferential binding in the cap-like structures might be important for increasing local fXIII concentration in platelet thrombi. This study was supported by the Russian Foundation for Basic Research grant 19-04-00615. *The authors marked with an asterisk equally contributed to the work.

## P-41-031
## Effect of biogenic and pyrogenic $SiO_2$ NPs on the viability and the level of reactive oxygen species

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Silica ($SiO_2$) in plant tissues is believed to be a component for enhancing mechanical properties and as a physical barrier. In recent years chemistry based on green approach as well as research on new bio-replacements for materials produced by traditional industry seem to have been an effective strategy to prepare and study various form of nanoparticles. In this paper we compared the biological effects of both silica nanoparticles extracted from *Urtica dioica* L. and pyrogenic commercially available silica nanoparticles. TEM and SEM analysis confirmed size of tested silica between 8 and 20 nanometers as well as their amorphous structure. We report that biogenic silica (bio$SiO_2$) in terms of chemical composition has similar composition to synthetically produced silica (pyr$SiO_2$). The compounds' cytotoxic activity and intracellular reactive oxygen species (ROS) generation were determined in relation to cells. The cytotoxic effects of the NPs were determined after exposure to different concentrations (0–200 μg/mL) at 24, 48 and 72 h. The cell viability was measured using spectrophotometric (MTT) and fluorimetric (Hoechst 33258) assays. The impact of the nanoparticles on the level of intracellular reactive oxygen (ROS) spices was assessed using $H_2$DCF-DA probe. The results demonstrated that $SiO_2$ NPs extracted from stinging nettle presents higher toxicity than pyrogenic NPs in immortalized human microvascular endothelial cells (HMEC-1). The study also confirmed that bio$SiO_2$ has higher influence on ROS production than pyr$SiO_2$. The level of ROS is time and concentration dependent. It was proved that biogenic nanoparticles are safe for the endothelial cells in appropriate concentration. What is more, they show both good biocompatibility and a high impact for future studies.

## P-41-032
## High-throughput SELEX analysis of YY1 and YY2 interaction with DNA

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Human transcription factor Yin Yang 2 (YY2) is a recently discovered paralog of YY1, with pronounced sequence similarity and partially similar function. YY1 is composed of N-terminal disordered regulatory domain and C-terminal DNA-binding domain; a similar bipartite structure is presumed for YY2. The DNA-binding domains of YY1 and YY2 were shown to recognize the same consensus sequence in a standard SELEX assay. However, YY2 does not bind all the YY1 sites *in vivo* and some of the commonly recognized promoters are oppositely regulated by YY1 and YY2. We employed high-throughput SELEX-seq analysis to determine the DNA sequence recognized by YY1 and YY2, both full-length and truncated to DNA-binding domains. The recognized oligos were selected with the use of EMSA and subject to Next Generation Sequencing. Such methodology yielded several hundred thousand readouts for each sample, which enabled us to obtain novel and reliable data. We found that YY1 and YY2 prefer different sequences flanking the core motif on the 3′ side. We also proved that sole DNA-binding domains of YY1 and YY2 recognize the same sequence as do respective full-length proteins. However, sequences with tandem repeats of the consensus motif were significantly more enriched by the full length YY1 and YY2 then by their DNA-binding domains alone. This suggests that YY1's and YY2's N-terminal regulatory domains influence their interaction with DNA.

## P-41-033
## Comparative evaluation of collagens isolated from hydrobionts and mammals

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Collagen is an important structural polypeptide. In nature, there are five different types of these proteins. Typically, the type of collagen is determined by the type of tissue from which isolated. Data on the structure, composition and properties of collagen hydrobionts is much less compared with the results of the study of this proteins for mammals. The aim of the research was to first compare the molecular masses of collagens isolated from hydrobionts (Hypophthalmichthys molitrix and Todarodes pacificus) with the molecular weight of collagen fractions isolated from mammals (*Bos taurus*). Collagen extraction was performed according to a standard procedure. After homogenization of the skin in 1 M citric acid solution, the resulting suspension was centrifuged at 4500 rpm, the supernatant was collected, and electrophoresis was performed on 8% PAAG. As a result, a significant difference was found in the molecular weight of protein fragments for collagen of hydrobionts and mammals. For the obtained fractions of collagen conducted a study of the sensitivity to collagenase. The data obtained allow us to conclude that there are significant differences in the structure of collagen hydrobionts and mammals. The splitting of proteins by the action of collagenase indicates that the isolated proteins can be attributed to the family of collagen proteins. Further research is related to the establishment of the secondary structure of the proteins and the determination of their amino acid sequence.

## P-41-034
## Deciphering the role of the *Pseudomonas aeruginosa* PA4400 Nudix protein in various metabolic pathways

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*Pseudomonas aeruginosa* is an ubiquitous bacterium found in different environments and in association with various organisms. In humans, it causes a broad spectrum of opportunistic infections that are difficult to eradicate. Thus, there is an urgent need to search for novel virulence – associated factors, which might serve as targets for antibacterial therapies. Nudix pyrophosphatases are widely distributed among all classes of organisms. These enzymes catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives, compounds either toxic or demanding tight control during cell cycle. Nudixes have also been shown to contribute to virulence of some pathogenic bacteria species. The PA4400 protein is a functional homologue of the first characterized Nudix family member – the *Escherichia coli* MutT protein which has been shown to play an important antimutator role. Inactivation of the *P. aeruginosa*


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*PA4400* gene caused an increase in spontaneous mutation frequency. In addition to this observation we have found that deletion of *PA4400* affects pyocyanin production and severely impairs swarming motility and sensitivity to piperacillin. Mutated strain exhibits weakened growth in M9 minimal medium. The C-terminal part of PA4400 is 40% identical to a consensus sequence of thiamine monophosphate synthases, suggesting that this protein is bifunctional and apart from its antimutator functions also plays a role in thiamin biosynthesis. Indeed, when the M9 medium was supplemented with thiamine no differences in bacterial growth between the mutant and the parental strain was observed. The ongoing experiments will explain the mechanism of action of PA4400 in various metabolic pathways. This work was supported by grant no: UMO-2014/15/B/NZ6/02562 from the National Science Center.

## P-41-035
## Application of nucleoside phosphorylases for the preparation of biologically active nucleosides: search of optimal substrates

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Enzymatic reactions are widely used for the preparation of biologically active nucleosides. Nucleoside phosphorylases (purine nucleoside phosphorylase EC 2.4.2.1, uridine phosphorylase EC 2.4.2.3, thymidine phosphorylase EC 2.4.2.4) effectively catalyze a transfer of the carbohydrate moiety from one heterocyclic base to yield a new nucleoside the corresponding heterocyclic bases and monosaccharide 1-phosphates. An enzymatic transglycosylation is the two coupled consecutive equilibrium reactions of phosphorolysis of nucleosides (Nuc). Phosphorolysis step: Nuc1 + Pi ↔ Sug-P + Base1; Synthesis step: Base2 + Sug-P ↔ Pi + Nuc2. We have compared the different substrates in these reaction and found that the highest yield of product may be achieved when 7-methyl-(2-deoxy)guanosine (7MeGuo or 7MedGuo) or α-D-(2-deoxy)ribose-1-phosphate (Rib-1-P or dRib-1-P) are used as substrates in this reaction. We have developed useful protocol for the preparation of these derivatives. In conclusion, we have developed optimal strategy for the preparation of pyrimidine and purine ribo- or 2-deoxyribonucleosides using nucleoside phosphorylases. This work was supported by Russian Science Foundation (project No. 16-14-00178).

## P-41-036
## Detergent-free solubilization of a double-Cys mutant of the HEAG2 channel from the membranes of eukaryotic cells

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Detergent-free solubilization of membrane proteins is a promising method for the isolation of membrane proteins, while preserving a native lipid environment. One of the recently developed methods uses a copolymer of styrene and maleic acid (SMA) for this purpose. Particles containing a small quantity of membrane lipids and an SMA copolymer are called SMALP (styrene maleic acid lipid particles). In this work, we used SMA to solubilize the voltage-dependent potassium channel HEAG2 from whole eukaryotic cells and their isolated membrane preps. The studied channel bore an affine 1D4 tag at its C-terminus and two cysteine mutations introduced at positions D339 and M474 that may be used to stabilize the channel in a closed state, similar to HERG. The recombinant protein was expressed in an eukaryotic COS-1 cell line. The HEAG2-D339C-M474C channels solubilized in SMALPs were purified using affinity chromatography, as described in. To determine the hydrodynamic diameter of the particles, we used dynamic light scattering (DLS). Using single particle electron microscopy, we confirmed the formation of nanoparticles with a diameter of ~ 15 nm. Based on the obtained images, the preliminary electron density of the SMALP-HEAG2-D339C-M474C-1D4 complex was reconstructed, comparable to that of the wild type HEAG2 channel. Further work is in the process of preparing the complexes for cryo-EM analysis. This work was partially supported by grants from the Russian Science Foundation for young scientists (18-74-00087) and RFBR (18-504-12045). Electron microscopy was performed at the User facility Center of the Biological Faculty of Moscow State University "EM for life sciences".

## P-41-037
## *Chaetopterus* luciferase: partial purification of the protein and cofactors required for bioluminescence

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Decades of research of *Chaetopterus variopedatus*, a bioluminescent marine polychaete, yielded conflicting data regarding the number and function of the reaction components involved in *Chaetopterus* light emission. Early studies of the worm's 12th segment by O. Shimomura revealed the presence of photoprotein requiring 5 cofactors (oxygen, $H_2O_2$, $Fe^{2+}$, and two unknown low-molecular-weight components) to produce visible light. The function of $Fe^{2+}$ and $H_2O_2$ was later contested in the experiments with *Chaetopterus* secreted luminous mucus, which showed that these cofactors either have no stimulatory effect or even inhibit the mucus luminescence. Further research has suggested the involvement of riboflavin or its derivatives in *Chaetopterus* light emission reaction, but didn't confirm the presence of other low-molecular-weight components in this bioluminescence system. In the present study we designed a new method for the separation and partial purification of the protein (30 kDa) and low-molecular-weight components of the *Chaetopterus variopedatus* bioluminescent system. None of the separated compounds displayed independent light emission, but an intense *in vitro* luminescence was triggered upon the addition of $Fe^{2+}$ to the reaction mixture containing the purified protein and the separated low-molecular-weight component. Moreover, the luminescence intensity showed a linear dependence on the amount of the low-molecular-weight compound. This fact leads to an important insight into *Chaetopterus* bioluminescence system, suggesting that it belongs to the luciferin-luciferase type, rather than a photoprotein, that it was previously considered to be. Further isolation and structural characterization of the *Chaetopterus* bioluminescence system components will hopefully shed light on the true nature of this reaction. This work was supported by the Russian Science Foundation grant 18-74-10102.


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## P-41-038
## The role of cAMP-dependent RpfA protein synthesis in the reactivation of dormant mycobacteria

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Earlier, the role of protein RpfA, was established when the dormant mycobacteria (DM) become active. We found that the active synthesis of Rpf proteins occurs at late stages of reactivation. It cannot be ruled out that under these conditions there is a mechanism that turns on at an early stage and leads to the expression of Rpf proteins. The purpose of this work was to elucidate the molecular mechanisms leading to the synthesis of RpfA. Earlier we found that reactivation of mycobacteria is stimulated by exogenously added cAMP. In the *Mtb* genome, the cAMP-dependent transcription factor (cAMP – TF) encoded by the *Rv3676* gene is annotated. *Mtb* strain with overexpression of this gene was designed. This strain rapidly grew *in vitro* and *in vivo* under stress conditions. Bacteria of this strain retained the plasmid with the Rv3676 gene *in vivo* even in the absence of a selection agent, which indicated the advantage for survival upon increased expression of cAMP-TF. The obtained results demonstrated the importance of Rv3676 for maintaining the viability of *Mtb* cells under unfavorable *in vitro* conditions and their virulence *in vivo* and were similar to those obtained by overexpression of adenylate cyclase Rv2212. A new mechanism for reactivation of DM is proposed, in which adenylate cyclase is activated and intracellular level of cAMP increases, resulting in activation of cAMP-TF. This, in turn, triggers the expression of 150 genes, including *rpfA*. At the next stage of reactivation, RpfA protein is synthesized and PknB protein kinase is activated, through the products of its enzymatic activity (muropeptides), which triggers PknB autophosphorylation reaction, followed by activation of a number of processes, resulting in cell division stimulation. This work was financially supported by the Russian Science Foundation grants 16-15-00245 (*in vitro* experiments) and Ministry of Science and Higher Education of the Russian Federation (*in vivo* experiments).

## P-41-039
## Novel high-throughput label-free method for experimental protein interactomics

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A high-throughput label-free method is developed for simultaneous real-time measuring the kinetics of several protein-protein interactions (PPI) for tasks of dynamic interactomics. The method overcomes the issue of expensive consumables and low productivity of modern label-free instruments that are among the limiting factors of experimental interactomics, in which every research require characterization of thousands of pairwise PPI. For the first time, we employ glass microarrays to realize concurrent recording of a vast number of PPI. No additional mass spectrometry analysis is required as each microarray spot corresponds to a known protein target. The developed biosensing prototypes were tested for promising antibody selection for *in vitro* diagnostics of prostate cancer and for rapid simultaneous measuring of multiple molecular markers of tumors with a unique combination of optical instruments and magnetic nanoparticles. The developed method was also applied to investigation of kinetics of 2 types of magnetic particles (MP): (i) "hard" MP based on composite nanomaterials used as a mobile solid phase controlled by an external permanent magnets, and (ii) "soft" iron oxide MP used as nanolabels detected by nonlinear magnetization at combinatorial frequencies. The concept of simultaneous use of 2 different types of MP in two distinct modalities opens new opportunities for development of rapid highly sensitive analytical methods. These approaches may expand the arsenal of methods of functional proteomics and facilitate resolving of a wide range of problems in system biology, e.g., investigation of molecular mechanisms of functioning of living systems, functions of new proteins, identification of potential targets for development of new therapeutic preparations. Various aspects of this multidisciplinary research were partially supported by Russian Foundation for Basic Research (grants 16-33-60228, 18-33-20252 and 18-29-09169) and Skoltech Systems Biology Fellowship.

## P-41-040
## Solving the mystery – molecular identification of vertebrate 4-oxo-L-proline reductase

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In vertebrates, a 4-oxo-L-proline reductase (EC 1.1.1.104) catalyzes the reduction of 4-oxo-L-proline to 4-hydroxy-L-proline in the presence of NADH. The molecular identity of this enzyme has remained unknown since 1962, when its activity was described for the first time in rabbit kidney. Thus, the aim of the current investigation was to identify the gene encoding 4-oxo-L-proline reductase and verify the activity of the recombinant enzyme toward both 4-oxo-L-proline and its structural analogues. Following a three-step chromatographic purification procedure, the 4-oxo-L-proline reductase activity was purified approximately 280-fold from rat kidney. The yield of the purification reached 25% and the activity was eluted as a single peak throughout the whole process, indicating the presence of a single enzyme species. The resulting protein preparation was then analyzed by tandem mass spectrometry (nanoUPLC-Q-TOF) and 3-hydroxybutyrate dehydrogenase type 2 (BDH2) was found as the only possible candidate for the reductase. The recombinant BDH2 was produced in *E. coli*, purified to homogeneity and shown to catalyze the reduction of 4-oxo-L-proline *in vitro*. To conclude, our results indicate that BDH2 is indeed 4-oxo-L-proline reductase. As 4-oxo-L-proline is present in human naive $CD4^+$ T cells and HEK 293T cell line, and its physiological importance is still missing, our findings open novel opportunities to determine the potential source of this metabolite and to disclose the (patho)physiological significance of both 4-oxo-L-proline and the 4-oxo-L-proline reductase in humans. It is tempting to speculate that 4-oxo-L-proline may be a "dead end" metabolite resulting from an as yet unknown metabolic pathway and the 4-oxo-L-proline reductase may be a novel example of the metabolite proofreading enzyme. This investigation was supported by the DSM 501-D114-86-0117600-20 from the Polish Ministry of Science and Higher Education.


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## P-41-041
## Identification of Dps protein in culturing medium of *Escherichia coli*

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Proteins of the Dps family protect genomes from various stresses, usually using positively charged N-termini for structure-specific binding to DNA, and ferroxidase centers for removing toxic iron ions from the vicinity of the nucleoid. Being permanently bound to DNA, Dps molecules can also be found in complexes with structured RNAs. However, Dps from *E. coli* was recently detected in membrane structures, where the presence of nucleic acids is not *a priori* assumed, and the N-terminal signal peptide for the exported proteins have been found in the Dps2 protein of *D. radiodurans*. This implies a possibility of the Dps participation in some processes that go beyond the boundaries of the bacterial cell. Here we show that Dps of *E. coli* is present in the extracellular milieu, although it does not have a signal peptide for secretion. *E. coli* BL21*(DE3) cells and their transformants, carrying the pGEM_dps plasmid for overproduction of recombinant Dps, were used as the main objects in the study, while the dps-null mutant of *E. coli* MG1655 was chosen as the control strain. The cells were harvested at the stationary phase. The presence of Dps in cell lysates and culturing medium was simultaneously evaluated by electrophoretic fractionation followed by Western blot analysis with antibodies against Dps. No bands were detected in samples of the control strain, which excludes cross-reaction of antibodies with other ferritins of *E. coli*. Both lysates and *E. coli* BL21*(DE3) culturing medium contained Dps, the amount of which was much larger inside the bacterial cells. Overproduction of Dps changed this ratio, significantly increasing the portion of secreted protein. Although leakage from dead cells occurs in both cases, this difference assumes secretion of at least excessive Dps. If so, it might be involved in export of short RNAs outside the cell, but the mechanism of its own passage through the membrane remains unclear. The study was supported by RSF (grant No. 18-04-0348).

## P-41-042
## Heterologous expression of fungus *Neonothopanus nambi* hispidin-3- hydroxylase in yeast *Komagataella phaffii* (*Pichia Pastoris*)

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The fungus *Neonothopanus nambi* is the first eukaryotic bioluminescent organism with fully discovered enzymes, involved in luciferin biosynthesis. One of the crucial reactions in this pathway is luciferin formation by hydroxylation of hispidin catalyzed by hispidin-3-hydroxylase (*H3H*). Even though *H3H* gene shows sequence similarity with 3-hydroxybenzoate 6-monooxygenase genes, the mechanism of luciferin production remains poorly understood. Therefore the production of functional enzyme suitable for structural and kinetic investigations is an important task. We have found that *E. coli* expression platform wasn't able to produce H3H in a soluble and active form. So we chose *Pichia* like more preferable host. We have engineered *H3H* gene with hexahistidine extension sequence at 5′-end and cloned into pPICZa vector under AOX1 promoter control. Also construction contains a prepro-peptide sequence to target desire protein to secretory pathway. Expression vector has been linearized with *Mss*I and electroporated in to *Pichia pastoris* strain X33. Transformants carrying multiple copies of the H3H gene have been identified on selection plates containing of 3 mg/mL Zeocin. Single clones were inoculated 10 mL BMGY medium in 50-mL tubes and cultivated at 30 °C overnight. Then cells were collected and transferred into BMMY medium containing 1.5% (v/v) of methanol. Methanol was added a final concentration of 1% (v/v) every 24 h. *H3H* expression level was evaluated by immunodot-blotting with mouse Anti-His Tag HRP-conjugated monoclonal antibody. We have found that H3H didn't pass through cytoplasmic membrane and localized in membrane fraction. Moreover the maximum expression level of this enzyme has been reached at 22 °C during 36 h of induction phase. We hypothesize that membrane localization of H3H that shows cytoplasmic topology links to the membrane luciferase of *N. nambi*. This work was supported by Russian Foundation for Basic Research grant 18-34-20134.

## P-41-043
## Oxidized protein content corresponds with proteasome activity in the liver of rainbow trout treated by bacterial infection

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Protein oxidation results from cellular oxidative stress provoked by multiple stressors under fish cultivation, including infectious diseases, high rearing density, low water exchange, water temperature rise, hypoxia, handling, etc. Carbonylated protein content as well as the level of protein degradation relying on three main proteolytic systems, such as cathepsins, calcium-dependent proteases (calpains), and the ubiquitin-proteasome system, were assessed in the liver of rainbow trout, *Oncorhynchus mykiss*, one of the most widely cultured fish species worldwide, whose growth and survival always are threatened by a prevalence of infectious and non-infectious diseases. Protease activities are associated with normal fish growth and have seasonal variations. We revealed that protein oxidation level and proteolytic pathways, particularly proteasome activity, in reared rainbow trout liver changes in an orchestrated manner in physiologically growing fish, in fish treated by bacterial infection, and after antibacterial therapy. In infected fish, the disease state is accompanied by increase in both protein carbonyl concentration and proteasome activity. In recovering fish subjected to antibacterial therapy, the suppression in proteasome activity and lower level of protein carbonyls were detected. Our findings support the hypothesis that proteasome pathway is mainly responsible for degradation of oxidatively damaged proteins and considered as an indicator of fish health state. This work was supported by the Russian Science Foundation, project no. 17-74-20098.

## P-41-044
## Analysis of the impact of glucocorticoid receptor NR3C1 on RANKL gene (TNFSF11) expression

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Introduction: Glucocorticoids are steroid hormones with many different roles in the human body. These roles are the result of


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glucocorticoids interaction with the glucocorticoid receptor (GR) NR3C1. One of the diseases where this mechanism can be potentially implied is glucocorticoid-induced osteoporosis where active interaction of NR3C1 with receptor activator of nuclear factor κB ligand (RANKL) is expected. In this work, the impact of glucocorticoid receptor on RANKL gene expression was tested in human osteosarcoma (HOS) cell line. Materials and methods: Plasmid vectors that contain inserted gene NR3C1 (pCMV-NR3C1 and FLAG as a control) were used in this experimental work. After the transfection process, RNA isolation and reverse transcription were performed. The impact of different masses of NR3C1 on the RANKL gene expression was tested in the HOS cell line and estimated through quantitative polymerase chain reaction (Real Time-PCR). For this research, Roche LightCycler 480 was used. The analysis of relative changes in RANKL gene expression was performed using the Livak's method (2ΔΔCt). Results: Initially, the impact of 500 ng of glucocorticoid receptor NR3C1 on RANKL gene expression was tested and compared to control (FLAG) and untreated cells. Livak's method showed that there is an increase in gene expression in relation to untreated cells. Analysis of the influence of glucocorticoid receptor NR3C1 in concentrations of 250, 500, 1000 ng with respect to control showed the most significant increase in RANKL gene expression after the HOS cells were transfected with 500 ng of NR3C1. The relative expression in HOS cells with 1000 ng of GR NR3C1 was increased while reduced RANKL gene expression was observed with 250 ng of glucocorticoid receptor NR3C1. Conclusion: Our study confirms that glucocorticoid receptor NR3C1 is one of the regulatory molecules affecting the RANKL gene (TNFSF11) expression in human osteosarcoma cell line.

## P-41-045
## Insight into Se-nanoparticle cellular uptake and intracellular transport in cell culture

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Selenium is used as antioxidant and antitumor preparations in pharmaceutics. The dose and form of these elements are important factors that determine biological activity and toxicity; it proved that the reduction of the particle size increases efficiency. The aim of the work is to create stable selenium nanoparticles and to study their effect on eukaryotic cells. Selenium nanoparticles were synthesized in reaction of selenious acid (LenReactiv, Russia) and ascorbic acid. The size of nanoparticles were determined by the photon's cross-correlation method (Nanophox) and scanning electron microscope with X-ray diffraction Hitachi TM4000Plus. Eukaryotic cells – is fibroblasts – were isolated by enzymatic method. DMEM medium (PanEco, Russia) was used for culture cells, containing fetal bovine serum (FBS, 10%), and the penicillin−streptomycin solution (1%, 10,000 units of penicillin and streptomycin (10 mg in 0.9% NaCl)) in a humidified atmosphere of CO2 (5%) and air (95%) at 37 °C. Morphological evaluation was performed using Nikon Eclipse. The cellular uptake determine of transmission electron microscope JEM-2100. We obtained NP of selenium 50–100 nm. Selenium caused morphological changes in cell cultures: the cells changed their characteristic form, the cell proliferation slowed down, the number of cells decreased, it's indicated by low confluency, large conglomerates of particles with proteins were found. Next, using TEM, were studied fibroblast samples exposure selenium nanoparticles for 24 h. In cells cytoplasm there were vacuoles with a dark electrically impermeable content — selenium nanoparticles. We suppose that the cell phagocytes NP, then transports them along the cytoplasm. Vacuoles are evenly distributed in the cytoplasm of cells in the sample, whereas in the control group of fibroblasts there were no such inclusions. *The authors marked with an asterisk equally contributed to the work.

## P-41-046
## Protein interaction network of the mouse LOC66598 protein

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The mouse *3110001I22Rik* gene encoding the LOC66598 protein is located in the first intron of the *Bfar* (bifunctional apoptosis regulator) gene. Thus, recently it was renamed to BFAR isoform 3, although the encoded protein is completely distinct from other BFAR isoforms and is rather similar to Periphilin-1. Mice with the *3110001I22Rik* gene knockout did not show any significant phenotypic changes, and the function of this gene remains unknown. We found that the LOC66598 protein is expressed mainly in the nucleus and its overexpression can induce caspase-3-dependent apoptosis. To obtain a moderate level of LOC66598/EGFP fusion protein expression in NIH-3T3 cells, we utilized a lentiviral expression system induced by doxycycline. Looking for proteins interacting with LOC66598, we performed co-immunoprecipitation (GFP-trap, Chromotek) and subsequent analysis on the Orbitrap spectrometer. Initial analysis revealed interactions with 40S ribosomal proteins (S25, S27, S14, S19, S3), histones (H1.2, H1.4, H3.3C), proteins involved in splicing (SFPQ, SNRPA1, HNRNPA3, NONO, KHDRBS3) and in calcium signaling (HPCAL1, PTK2B). Interestingly, SFPQ and NONO proteins belong to the Drosophila behavior human splicing (DBHS) family of RNA-binding proteins and it was already reported that SFPQ-NONO heteromer may be involved in DNA unwinding and in DNA non-homologous end joining (NHEJ) as well may play a role in nuclear retention of defective RNAs. Our results suggest that LOC66598 could be an additional component of this complex. Observed LOC66598 interactions with histones could be mediated by its LGE domain since it is known that such a domain could be involved in the ubiquitination of histones. On the other hand, interactions with proteins involved in calcium signaling are consistent with the expression pattern of *3110001I22Rik* which is the highest in cells with high levels of calcium ions. This work was supported by the Polish National Science Centre (grant no 2014/13/B/NZ3/04650).

## P-41-047
## The stabilization of HIV-1 IN by Ku70 does not require a direct interaction between two proteins

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The integration of HIV DNA into host cell genome is an essential step in retroviral life cycle. The major player in this process is viral enzyme integrase (IN), and it requires binding of some cellular proteins for its functioning. Recently, some reports showed that Ku70, which is a part of the DNA-PK complex, binds HIV-1 IN and probably protects it from proteasomal degradation. The Ku70 protective effect may arise from a direct shielding of HIV-1 IN in its complex with Ku70. We have shown that HIV-1 IN bearing substitutions E212A/L213A (IN_mut) shows a weaker binding towards Ku70 *in vitro* and *in vivo*. To verify if IN binding to Ku70 indeed protects it from proteasomal degradation, we expressed wild type IN (IN_wt) or IN_mut tagged with HA epitope in 293T


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cells, analyzed protein expression and found no differences in the expression levels of IN with or without mutation. When Ku70 was transiently overexpressed in cells, the amounts of both IN_wt and IN_mut were elevated. Conversely, siRNA mediated knockdown or CRISPR/Cas9 based heterozygous knockout of Ku70 led to a significant decrease in the levels of both variants of IN. Also, we measured the dynamics of IN degradation in the presence of translation inhibitor cycloheximide. IN_wt degraded quickly with a half-life of 65 ± 10 min that is consisting with previous reports (A. Mousnier et al., 2007). The half-life of IN_mut was similar to that determined for IN_wt. Ku70 overexpression increased the half-life of both IN_wt and IN_mut to a similar extent (120 ± 15 min), whereas heterozygous knockout of Ku70 resulted in a 6-fold decrease in their expression. Interestingly, that the IN mRNA level remains constant in cells with different amounts of Ku70. Therefore, HIV-1 IN is indeed stabilized by Ku70 but this effect does not depend on a direct binding between these two proteins. This work was supported by RFBR grant 17-04-01178. *The authors marked with an asterisk equally contributed to the work.

## P-41-048
## Physicochemical characterisation of ligands of human protein kinase CK2– a tool to assess thermodynamic contribution of a halogen bond

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Halogenated ligands play a significant role in medicinal chemistry and drug design. In case of human protein kinase CK2, which regulates almost all cellular processes by phosphorylation of other proteins, the halogenated low-mass inhibitors are also considered to be the most selective ones. The first reported inhibitor of protein kinase CK2 was DRB – nucleoside analogue of ATP, and after the further modifications aimed at increase of binding affinity and selectivity, the 4,5,6,7-tetrabromo-1H- benzotriazole (TBBt) was synthesized. Since it has been suggested that inhibitory activity towards protein kinase CK2α is predominated by hydrophobic interactions, we used our former model of hydrophobic solvation. The values of excess volume, which represents the difference between the experimentally measured partial molar volume and the estimated *in silico* molecular volume, is attributed directly to the effect of reorganization of water molecules in the solvation shell and thus can be interpreted as a qualitative measure of solute hydrophobicity. We have characterized halogenated analogues of TBBt, purity of which was assessed by quantitative NMR method. The excess volume (β) of halogenated derivatives was found to be highly correlated with melting temperature of the protein-ligand complex, which however also increases with solubility of the solute, and $pK_a$ for dissociation of the triazole proton. We have confirmed that the partial molar volume varies in commonly used organic solvents that differed in hybridization of oxygen. The latter is believed to affect solvent halogen bond accepting propensities. Therefore, we confirmed that the excess volume (β) is sensitive for both specific and non-specific solvent-solute interactions, and may be thus used as a measure of solvophobic effect. This work was supported by Polish National Science Centre 2015/19/B/ST4/02156 grant. The equipment was sponsored in part by Centre for Preclinical Research and Technology (CePT).

## P-41-049
## Effect of the host molecule on the guest activity: binding of halogenated benzotriazoles to the catalytic subunit of human protein kinase CK2 in the presence of cyclodextrin

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Cyclodextrins (CD) are cyclic oligosaccharides that display a hydrophilic outer surface and a lipophilic central cavity. There are three natural CDs (α-CD, β-CD, γ-CD) that differ by the ring size and solubility. In aqueous solutions CDs are capable to form water-soluble inclusion complexes with various hydrophobic solutes, however at higher concentration they may also form self-assembled aggregates. Therefore, CD are widely used as carriers of drugs poorly soluble in aqueous solution. When improving the apparent solubility, inclusion complexes with cyclodextrins increase both bioavailability and stability of numerous low-mass drugs. Herein, we are studying a model protein-ligand system, using the catalytic subunit of human protein kinase CK2 (hCK2α) as the target biomolecule and a series of brominated benzotriazoles (analogues of tetrabromobenzotriazole, TBBt, the well known ATP-competitive hCK2α inhibitor). For a series of halogenated benzotriazoles we have measured the increase in their aqueous solubility caused by the presence of four different cyclodextrins (α-CD, β-CD γ-CD and 2HP-β-CD) using UV-Vis spectroscopy. Stability of inclusion complexes were also determined using $^1$H NMR and UV spectroscopy. Finally the interaction of γ-CD inclusion complexes with the target protein were assessed with Thermal Shift Assay (nanoDSF), and the binding affinities at normal conditions were additionally determined with Microscale Thermophoresis (MST). The results clearly show that the presence of cyclodextrin at a moderate concentration (10 fold excess) substantially improves solubility of halogenated benzotriazoles, however slight decrease of the binding affinity to the target protein was observed. This work was supported by the Polish National Science Centre 2015/19/B/ST4/02156. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT).

## P-41-050
## Does the ionic state of the solute affect organization of the solvation shell? Study of the pH-dependence of experimentally measured partial molar volume of 1*H*-benzotriazole

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Hydrophobic effect reflects the observed tendency of nonpolar substances to organize surrounding water molecules. Thermodynamic contribution of hydrophobic interactions to the binding affinity is of extremal importance in all kinds of drug design approach. There are several methods that help to determine experimentally solute hydrophobicity. Among them partition coefficients in water/octanol system or RT-HPLC derived retention times are commonly used as ADME parameters. However, both of them are determined from two-state equilibrium, so they must be regarded as relative rather than absolute ones. We have been testing applicability of experimentally determined partial molar volumes as a direct measure of hydrophobic interactions. Partial molar volumes can be determined accurately form density-molality relationship. Interestingly, the excess volume defined as the difference between the experimentally measured partial molar volume and *in silico* calculated molecular volume seem to


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be a reasonable estimator of hydrophobic contribution to protein-ligand binding affinity. According to this model, the density of solvent in the hydration shell depends on the structure and polarity of solute molecule. Herein, we demonstrate how the partial molar volume of 1*H*-benzotriazole depends on its ionic state. The densimetric measurements were performed at the temperature of 20–30 °C. We have screened pH range of 4–11, which enabled analysis of pure anionic and neutral states, but also their dynamic equilibrium. The obtained results indicate that we have developed the approach to demonstrate that ionic state of the molecule visibly affects the measured partial molar volume and thus affects the solvation shell. This work was supported by Polish National Science Centre grant 2017/25/B/ST4/01613. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT).

## P-41-051
## Heterogeneously halogenated benzotriazoles: promising inhibitors of protein kinase CK2

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Protein kinase CK2 is one of the key enzymes responsible for cell proliferation and survivability. Its overexpression is confirmed in many types of cancer cells. Therefore, CK2 become a very attractive target for drug design in anticancer therapy, since inhibition of this enzyme results in cell apoptosis. Many inhibitors that have been already designed are originated from halogenated benzotriazoles (Bt) or benzimidazoles. All these molecules act as CK2 competitive inhibitors blocking the ATP-binding site. One of identified compounds is tetrabromobenzotriazole – TBBt, which is moderately potent inhibitor of CK2 ($IC_{50}$ = 0.5 μM). We have been analysed position-dependent thermodynamic contribution of any halogen atom attached to the benzene ring of Bt to the inhibitory activity of variously halogenated benzotriazoles. For this purpose, we initially analyzed all nine possible substitution patterns on the benzene ring of Bt, showing that substitution of bromine in positions 5 and 6 of the benzene ring is crucial to maintain inhibitory properties of substituted Bt. Herein, we demonstrate the interplay within the mixed bromine/chlorine derivatives, in which chlorine was introduced as strong electron acceptor to enhance eventual halogen bonding involving bromine atoms. The binding affinities to the catalytic subunit of human protein kinase CK2 were tested for these Bt derivatives with the aid of Thermal Shift Assay. The method is based on ligand – induced variation in the protein thermal stability, commonly assessed by the middle-point temperature of protein denaturation (Tm). The obtained results correlate with those determined previously for a series of brominated benzotriazoles, pointing however the possible advantage of site-specific heterogeneous halogenation. The work was supported by Polish National Science Centre 2015/19/B/ST4/02156. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT).

## P-41-052
## Collagen peptides as a potential delivery tool to label cells

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Collagen is known as an extremely efficient metal binding molecule. The ions of $Fe^{3+}$ bind collagen resulting in strong condensing and stabilizing effect on collagen monolayers. Collagen is known to absorb silver oxide nanoparticles. But mechanisms of collagen sorption are not clear. In this work, we evaluated the possibility of sorption of silver nanoparticles by both collagen and collagen peptide molecules. For this purpose, a preparation of fish collagen obtained from the *Hypophthalmichthys molitrix* skin were used. Collagen peptides were obtained by treatment of collagen by collagenase. To saturate the preparations with silver, the samples were heated up to 40 °C and silver nanoparticles were added for 1 h. The unbound silver nanoparticles were washed away by PBS. The solutions of silver saturated collagen and collagen peptides were lyophilized and examined by energy dispersive X-ray spectroscopy (EDX). As expected, a large amount of carbon, nitrogen and oxygen was found in both collagen preparations. Interestingly, that the amount of silver was no more than 1.5% in the collagen untreated with collagenase. At the same time, the amount of silver in the collagen peptides preparation increased to 14–15%. We find this result promising as silver saturated collagen peptides bound to an antibody could be used as a delivery tool to specifically label cells, to monitor cell migration, for example. Moreover, fish collagen peptides as a delivery tool may be promising instrument to target cancer cells if saturated with appropriate silver concentration.

## P-41-053
## No compartment for proteins – an approach for isolating differentially located intermediate filaments

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Desmin is a muscle-specific intermediate filament (IF) protein located in cytoplasm. Lamin B on the other hand is a type V IF protein located under the nuclear envelope and is farnesylated to re-enforce the membrane connection. The isolation of IF proteins is challenging since they are elongated and highly polymerized structures. Isolation procedure for IFs frequently requires denaturing conditions. However, our aim was to isolate desmin and lamin B while preserving native protein structure for downstream assays. Zebrafish (*Danio rerio*) skeletal muscle tissue was used for protein isolation. The tissue was first mechanically disrupted via mortar and pestle followed by sonication. An isolation buffer containing both ionic and non-ionic detergents, and reducing and chelating agents, was used for chemical disruption. A dialysis step was added to procedure to remove reducing agent since the lysate was intended to use for co-immunoprecipitation. The problem regarding our co-immunoprecipitation assay was risen from the fact that the targeted proteins are located in the biochemically and biophysically diversified compartments generated by nuclear envelope structure. Combining the compartmentalization "problem" with the entangled organization of IFs, finding the proper isolation method was a real obstacle. By using this method, isolation of two differentially located IF proteins in a non-denaturing condition was successfully achieved. This study had been funded by the Scientific and Technological Research Council of Turkey, Project number 214S174 to P. Dincer.


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## P-41-054
## Mapping of recognition sites of monoclonal antibodies responsible for the inhibition of pneumolysin functional activity

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Pneumolysin (PLY) is the major protein virulence factor of *S. pneumoniae* that causes effects contributing to the pathogenesis of acute lung and cardiac injury. The virulence of PLY is mainly based on its ability to form pores in the cellular membrane causing cell lysis. Therefore, PLY-neutralising strategies are of high demand and antibodies could be useful tools for neutralisation of this toxin. This study was aimed at developing PLY-specific monoclonal antibodies (MAbs) and detection of their binding sites essential for PLY functional activity. Ten MAbs against PLY were developed and their ability to neutralize cytolytic activity of PLY was investigated *in vitro* using human erythrocytes. Three MAbs (clones 3A9, 3F3 and 6E5) were found to be neutralizing. To localize the binding sites of the neutralizing MAbs, eight overlapping PLY fragments were constructed and expressed in *E. coli*. The neutralizing MAbs were analysed for their reactivity with PLY fragments by Western blot. MAbs 3A9 and 6E5 recognized only one PLY fragment comprising aa 321–471 but did not react with any of the overlapping fragments spanning the sequence between aa 321–453. This suggested that the epitopes of these neutralizing MAbs are located in the PLY region of aa 454 and 471. MAb 3F3 did not react with any overlapping PLY fragments suggesting that it recognizes a conformation-dependent PLY epitope. According to the crystal structure of PLY (PDB, 5CR6), the sequence recognized by the neutralising MAbs 3A9 and 6E5 is located within the cholesterol-binding loop required for PLY interaction with membrane cholesterol. Our study suggests that antibodies directed to the cholesterol-binding loop could be employed as efficient PLY-neutralizing agents. This research is funded by the European Social Fund under the No 09.3.3-LMT-K-712 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure.

## P-41-055
## Biophysical characterization of the calcium/calmodulin-dependent protein kinase kinase 1 (CaMKK1):14-3-3 protein complex

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The calcium/calmodulin-dependent protein kinase kinase 1 (CaMKK1) is a serine/threonine protein kinase playing an important role in the cascade responsible for Ca2+/calmodulin signalling. This signalling cascade is involved in neural development, bone mass accrual, gluconeogenesis, protein translation, appetite control, axonal elongation and memory formation. CaMKK1 is activated by $Ca^{2+}$/CaM-binding in response to increased intracellular $Ca^{2+}$ levels and the active CaMKK1 is an upstream activator of CaMKI and CaMKIV kinases. The activity of CaMKK1 is inhibited by the cAMP-dependent protein kinase (PKA) in a process involving the binding of the scaffolding protein 14-3-3 whose role in the regulation of CaMKK1 is still unclear. CaMKK1 contains five PKA phosphorylation sites but only two of them were suggested to be the 14-3-3 protein binding motifs. The main aim of this study was the preparation and the biophysical characterization of the complex between phosphorylated CaMKK1 and 14-3-3g using analytical ultracentrifugation and small angle X-ray scattering (SAXS). Recombinant CaMKK1 (kinase dead mutant) containing only two PKA phosphorylation sites/14-3-3 binding motifs (Ser74 and Ser 475) was prepared using Escherichia Coli expression system and purified using nickel chelating chromatography and size exclusion chromatography. Phos-tag SDS-PAGE and mass spectrometry were used to verify the stoichiometric phosphorylation of prepared CaMKK1 by PKA. Analytical ultracentrifugation (sedimentation velocity) measurement revealed the concentration-dependent dimerization of CaMKK1 in solution as well as stable complex formation between doubly phosphorylated CaMKK1 and 14-3-3g protein. Data obtained from SAXS measurements were used for an ab initio reconstruction of the CaMKK1:14-3-3g complex. This work was supported by the Czech Science Foundation (project 19-00121S).

## P-41-056
## The hybrid molecule of adenylate cyclase toxin and α-hemolysin translocates its cell-invasive enzyme into cell cytosol and targets CD11a-positive cells

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The Bordetella pertussis adenylate cyclase toxin (CyaA) and Escherichia coli α-hemolysin (HlyA) are cytolytic leukotoxins of the Repeat in toxin (RTX) family. CyaA is capable to directly penetrate target cells across their cytoplasmic membrane without the need of endocytosis and delivers into the cell cytosol its adenylyl cyclase (AC) enzyme domain. The Hly portion of CyaA mediates toxin binding to the integrin CD11b/CD18 of myeloid phagocytic cells and translocates the AC domain into target cells. Chimeras of the cyaA and hlyA genes were constructed and the acylation status of the produced CyaA-HlyA chimeras were inspected by FT-ICR mass spectrometry. Membrane binding, cell invasive and hemolytic activities were further determined using sheep erythrocytes. We show that the AC domain of CyaA have to be fused to the adjacent AC-to-Hly-linker segment and the pore-forming domain of CyaA in order to be efficiently translocated across the cell membrane. The C-terminal acylated and RTX domains of CyaA then can be replaced with the corresponding domains of the HlyA. Further, the myristoyl-activated hybrid molecule targeted and penetrated cells expressing the integrin CD11a/CD18. This suggests that the key element of the cell specificity of the hybrid is located in the C-terminal portion derived from HlyA. *The authors marked with an asterisk equally contributed to the work.

## P-41-057
## S-substituted cysteine sulfoxides as promising antifungal prodrugs

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Bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, and the fungus *Candida albicans* are the


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most common sources of nosocomial infections. The current arsenal of antifungal drugs, such as azoles or amphotericin B, is limited and many of them show high toxicity for prolonged treatments, which demands the search for new antifungal compounds. Natural products continue to represent a rich source for the discovery or design of new compounds with non-canonical structural patterns. In our previous works we have demonstrated that C115H methionine g-lyase (EC 4.4.1.11, C115H MGL) effectively catalyzes the β-elimination reaction of S-alk(en)yl-L-cysteine sulfoxides with the formation of thiosulfinates, analogues of nature antibacterial agent of garlic, allicin. Thiosulfinates formed in binary systems C115H MGL + propiin, methiin, alliin, and ethiin have an antimycotic activity *in vitro* against *C. albicans* ATCC 24433 reference strain and *C. albicans* 1937, 1946, and 1947 clinical isolates from cystic fibrosis patients with minimum fungicidal concentrations of 1.65, 5.8, 1.67 and 2.6 mg/mL respectively. *In vivo* experiments revealed that the binary systems combined with amphotericin B have a synergistic or additive effect. Synergistic interactions were observed for the binary system C115H MGL+methiin combined with amphotericin B and caspofungin. This work was supported by the Russian Science Foundation (project No. 15-14-00009).

## P-41-058
## Biocompatibility assessment for organic semiconductor materials used in biosensor electronics

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Developing improved methods for prevention of diseases is of crucial importance nowadays. Non-invasive monitoring and diagnostic systems based on organic semiconductors have a huge potential in the context of their integration in wearable on-skin electronics, which has to be non-toxic and safe. However, there are just very few studies addressing biocompatibility of semiconductors used in organic field-effect transistors (OFETs). In the present work we addressed this issue and explored the most "classical" organic semiconductors such as fullerene $C_{60}$, naphtho [2,3-b]naphtho[2′,3′:4,5]thieno[2,3-d]thiophene (DNTT), anthra [2,3-b:6,7-b']dithiophene (ADT) and pentacene. We used human embryonic lung fibroblasts (HELF), obtained from the Research Centre for Medical Genetics collection, as the model living system. Cells were grown on substrates with pre-evaporated thin films of studied materials. The first group of cells was fixed after 30 h of incubation, the second – after 96 h, the last – after 14 days in standard conditions (37 °C, $CO_2$ 5%). For evaluation of the materials toxicity, a flow-cytometry (FC) and immunofluorescence microscopy were used with antibodies to H2A histone family member X (phosphorylated form) and 8-oxoguanine. To compare differences in cell metabolism between the control and experimental groups total RNA was removed, real-time polymerase-chain-reaction (PCR) was carried out with primers to the genes: *TBP, CCND1, NRF2, NOX4, BAX, BCL2, TNF, IL1, IL6, TLR9, NFKB1, BECN1*. FC analysis and microscopy showed no changes in DNA, but PCR demonstrated an increased level of apoptosis, autophagy signal in cells grown on pentacene. Increased level of *NFKB1* expression and decreased *CCND1* were detected in every group. There were no significant changes in other examined genes. Based on the obtained results for the specified cell-based system, we conclude that all investigated materials, except pentacene, have a potential to be biocompatible.

## P-41-059
## Creation of a new expression system for phenylacetone monooxygenase (PAMO) from *Thermobifida fusca* and fusion systems based on it

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Bayer-Villiger monooxygenases are capable of oxidizing aldehydes and ketones to esters. One of the members of this group of enzymes is phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*. It is a soluble NADPH-dependent enzyme consisting of one FAD-containing subunit. PAMO has broad substrate specificity. It has high temperature and chemical stability, which makes PAMO promising for use in real enzymatic processes. For the expression of PAMO in *E. coli* cells, a vector under the control of the araBp promoter is usually used. It requires expensive L-arabinose as an inducer. In this work, it was proposed to create an expression system for PAMO and fusion systems based on PAMO and PseFDH using the vector pET28a, since it uses IPTG or lactose as an inducer and the last one is much cheaper than L-arabinose. Due to high cost of NADPH, a regeneration system of the $NADP^+$ is required for the industrial use of PAMO. For this purpose, various dehydrogenases are widely used. Formate dehydrogenase (FDH) catalyzes the oxidation reaction of formate ion to carbon dioxide with the combined reduction of $NAD^+$ to NADH. An enzyme from *Pseudomonas* sp. 101 with changed coenzyme specificity from $NAD^+$ to $NADP^+$ was obtained in our laboratory. Preparation of fusion enzymes consisting of two enzymes in one polypeptide chain is promising approach to increase efficiency of process. The goal of this work was to obtain plasmids containing the genes of the three PAMO variants with different His-tag locations, and PAMO-based fusion systems using two different mutant $NADP^+$-specific PseFDHs. The PAMO gene was synthesized according to the sequence in GenBank AAZ55526.1. The expression of all the resulting constructs was carried out. Enzymes have been isolated and purified and their catalytic activity has been evaluated. The reported study was funded by RFBR and Moscow city Government according to the research project No. 19-34-70036.

## P-41-060
## Glycosomal protein import: a new target against trypanosomiasis

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Chagas disease, a parasitic condition caused by *Trypanosoma cruzi*, was originally a neglected tropical disease, but it has spread from endemic countries and currently starts to threaten human health and welfare worldwide. Only a limited number of


FEBS Open Bio 9 (Suppl. 1) (2019) 65–431 DOI: 10.1002/2211-5463.12675
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treatment options are available which additionally suffer from toxicity, limited efficacy and increasing resistance. Therefore, identification of new macromolecular drug targets and small-molecule modulators is of utmost importance. Glycolysis is the only source of energy in trypanosomatids and unlike in most other cells it is compartmentalized in unique organelles – the glycosomes. Recent studies demonstrated that blocking the glycosomal protein import selectively kills *Trypanosoma*. Peroxins are central components of the translocation machinery for glycosomal matrix enzymes. We used structure-based design methodology to obtain inhibitors of selected peroxins. Here we describe the screening, hit evaluation and optimization results which led to biologically active trypanocidal small molecules. X-ray structures are additionally provided which characterize the binding interactions at the molecular level to explain SAR (structure-activity relationship) profiles. Our results provide solid ground for further development of peroxin targeting compounds as drugs against trypanosomiasis.

## P-41-061

## Cloning, expression and purification of human acetylcholinesterase and butyrylcholinesterase

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Human acetylcholinesterase (*h*AChE, EC 3.1.1.7) and human butyrylcholinesterase (*h*BChE, EC 3.1.1.8) are targets of numerous natural and synthetic inhibitors, including therapeutics, wide range of toxic esters as organophosphorus pesticides, insecticides and nerve agents. Current work was focused on establishing the expression system for recombinant cholinesterases production and subsequent enzyme purification. The cell line Expi293 were transiently transfected by a pcDNA3.4 vector encoding the *h*AChE and *h*BChE proteins and incubated in serum-free medium for the next 120 h for recombinant protein expression. At the end of the expression, the cell culture supernatant was collected, and proteins were directly subjected to purification steps or stored at −80 °C. Both cholinesterases were purified using affinity chromatography – procainamide sepharose for hAChE and Huprezine resin for hBChE. The level of protein expression and efficacy of purification steps were inspected by Ellman's method. The protein purification procedure was analyzed using SDS-PAGE analysis followed by Western blotting using anti-His tag antibody. The kinetic parameters of recombinant enzymes were validated by values of $IC_{50}$ of standard inhibitor e.g. donepezil and ethopropazine, which were compared with published results. Acknowledgements: This work was supported by the Czech Science Foundation (GA CR): 18-01734S and by Ministry of Health of the Czech Republic (no. 16-34390A). *The authors marked with an asterisk equally contributed to the work.

## P-41-062

## Proteomic changes in *Bacillus subtilis* caused by overexpression of HtrA protease

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Heat-stress inducible protease HtrA from *Bacillus subtilis* performs a quality protein control by hydrolysis of denatured proteins, thereby, protecting cells from various stresses. The aim of the work was to establish the changes in protein profile of *B. subtilis* caused by the HtrA overexpression. The *htrA* gene from *B. subtilis* 168 was cloned into the shuttle-vector pDG148 downstream of an IPTG inducible $P_{spac}$ promoter by resulting pDG-HtrA plasmid which then was transformed into *B. subtilis* 168. *B. subtilis* 168 transformed with empty pDG148 plasmid served as a reference. The overexpression of HtrA led to higher tolerance to stresses and to enhanced biofilm formation. Indeed, *B. subtilis* pDG-HtrA was characterized with increased transcription from promoters of *eps* and *yqxM* genes involved in biofilm matrix formation. For deeper insight into cellular processes changing due to HtrA overproduction background, we analyzed the proteomic profile of biofilm cells of *B. subtilis* pDG-HtrA in compare with the reference. The biofilms of these strains were collected from the plates after 24 h of cultivation, the cells were disintegrated on a homogenizer and their proteomes were analyzed with two-dimensional electrophoresis. Interestingly, in *B. subtilis* pDG-HtrA the increased expression of ABC transporter OppA was observed. Since OppA is required for the uptake of quorum sensing peptides, competence development and initiation of sporulation, the increased uptake of regulatory peptides could explain the formation of rigid and dense biofilm by *B. subtilis* pDG-HtrA in response to HtrA overexpression. Thus, HtrA in *B. subtilis* seems to be a pleiotropic protein involved in biofilm formation, quorum sensing, and stress resistance. This research was supported by the Ministry of Education and Science of the Russian Federation (assignment # 6.13469.2019/13.2).

## P-41-063

## Preparation and characterization of D-amino acid oxidases from yeast *Ogateya parapolimorpha* DL1

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D-amino acid oxidase (EC 1.4.3.3, DAAO) is enzyme, which plays important role in living cells. DAAO catalyzes reaction of oxidation of D-amino acids to corresponding α-keto acids with production of ammonia and hydrogen peroxide. DAAO is widely used in fine organic synthesis, medical diagnostics and pharmaceutical industry. DAAO is also the enzyme of high fundamental interest. Usually, two types of DAAO are present in living organisms: DAAO with wide substrate specificity profile ("classical" DAAO) and the enzyme which is highly specific to D-Asp and D-Glu (also called as D-aspartate oxidase, EC 1.4.3.1, DASPO). Genome analysis of the yeast *Ogateya parapolimorpha* DL1 revealed presence of four genes which can encode probable D-amino acid oxidases, while in other methylotrophic yeasts, like *Candida boidinii*, only one gene of DAAO and only one gene of DASPO are present. So, we decided to clone genes, express in


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*E. coli* and characterize all four enzymes of interest. As result all tentative D-amino acid oxidases from the yeast *O. parapolimorpha* DL1 were prepared and studied. Catalytic parameters were determined with different D-amino acids. All enzymes have different substrate specificity profiles and one of them shows substrate specificity profiles similar to DAAO from yeast *Candida boidinii*. Effects of pH and temperature on the enzyme activity and stability were studied. Model structures of all enzymes were constructed, and putative catalytically important residues in the active sites of enzymes were proposed. This work were supported by Russian Foundation for Basic Research (grant 17-04-001487a).

## P-41-064
## Functional studies on human EXOG provide insights into its role in mitochondrial DNA repair

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Human EXOG (EXOG) is a mitochondrial inner membrane nuclease that plays an essential role in human mitochondrial DNA repair. Its 5′-exonuclease activity, as well as substrate specificity conferred by the C-terminal Wing domain allow for precise processing of DNA damage. Based on the amino acids sequence, EXOG belongs to ββα-Me nuclease family of non-specific nucleases and contains N-terminal transmembrane domain that promotes its exclusive mitochondrial localization and confines the enzyme to the inner membrane of mitochondria. It is not well understood how the lipid environment of the mitochondrial inner membrane affects EXOGs activity. Here, we examine the effect of different lipids on the activity of purified EXOG. We show that the stability and activity of EXOG is modulated by the constituents of the mitochondrial inner membrane.

## P-41-065
## Biochemical and biophysical characterization of the metalloprotease Wss1 involved in DNA repair

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The metalloprotease Wss1 has been identified in the yeast *Saccharomyces cerevisiae* (*Sc*Wss1) as a crucial protein in DNA repair that prevents the adverse effects of DNA-protein cross-links (DPCs). Wss1 targets DPCs and cleaves the protein component of DPCs in a DNA-dependent manner. However, it remains unclear how DNA is required for Wss1 to cleave its substrates. In this study, we will carry out biochemical and biophysical studies to investigate the structure of Wss1 in the absence and the presence of DNA. We identified and cloned Wss1 homologs from several organisms to identify a homolog that is well expressed and soluble. The promising Wss1 homolog, which is highly solubly expressed in *E. coli*, was then subjected to biochemical and biophysical studies. Although the protein that we are focusing on is a predicted functional homolog of *Sc*Wss1, we have verified its functions both *in vivo* and *in vitro*, and confirmed that it has a similar in function to *Sc*Wss1. The Wss1 homolog reported here was also expressed and purified in large scale for protein crystallization. The crystallization screening was carried out in the absence and presence DNA using sitting-drop vapor diffusion technique. Crystals of the protein-DNA complex were observed. These crystals weakly diffracted X-ray to 4–5 A; they are currently being improved to obtain high-quality diffraction that is suitable for structure determination.

## P-41-066
## N-terminal processing of matriptase is differently regulated in epithelial cells

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Matriptase is a member of type II serine protease family. It plays important roles in barrier function of epithelial cells. However, the molecular regulation mechanism of matriptase activation is still unclear. It was considered that N-terminal processing of matriptase, which cleaves the peptide bond at G149, is required for its auto-activation. In this study, we constructed a matriptase wild-type expressing plasmid and a G149A mutant plasmid and transiently transfected to HEK293 and MCF-7 cells. Western blot results showed that matriptase activation was still found in G149A mutant-transfected HEK293 cells, but not in MCF-7 cells. To further study the effect of G149A mutant, cytoplasmic and out-membrane fractions were separated, an 85 kDa band, which was believed to be the C-terminal band after N-terminal cleavage at G149, was found both in wild-type and G149A mutant HEK293 cells, however, this band was not found in G149A mutant MCF-7. Interestingly, activated matriptase was not found in out-membrane of HEK293 cells, only the 130 kDa zymogen was found both in wild-type and G149A mutant HEK293 cells. However, compared with wild-type, G149A mutant produced less amount of matriptase in the out-membrane. In conclusion, the N-terminal processing at G149 of matriptase was differently regulated in different epithelial cells. At least in HEK293 cells, cleavage at G149 is not required for matriptase auto-activation.

## P-41-067
## New amphiphilic polymers designed for studies of membrane proteins by the method of surface plasmon resonance

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It is interesting membrane proteins, specifically lipid-protein, protein-protein and protein-ligand interactions. Physicochemical parameters of these interactions can be studied using surface plasmon resonance (SPR). The problem is that membrane proteins are insoluble in aqueous solutions. this can be solved by using detergents, bicelles, nanodiscs, amphiphilic peptides, organofluoric surfactants and amphiphilic polymers. The most interesting for us are amphiphilic polymers such as A8-35. We


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synthesized a new molecule consist of A8-35 and peptide which can bind to streptavidin. new polymer can solubilize membrane proteins. Three peptide ligands: WSHPQFGG, GGGGCWHPQAGC (with intermolecular disulfide bond) & CGGGWSHPQFEK were synthesized by the solid-phase method using Fmoc-protected amino acids. They have amino acid sequence HPQ which specifically binds to streptavidin. It was proved by competitive immunoassay. The ligand WSHPQFGG showed a weak affinity for streptavidin. The ligand GGGGCWHPQAGC & CGGGWSHPQFEK was covalently attached to the amphiphilic polymer A8-35. Modifications of amphipol A8-35 do not cause steric or other difficulties for the functioning of the membrane protein. Functionality of protein was proved by flash photolysis assay. This work was supported by the Ministry of Education and Science of the Russian Federation. (grant no. 6.3157.2017/PP).

## P-41-068
## Key nucleolar proteins in mouse spermatogenesis

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Spermatogenesis is a complex process during which cells undergo multiple changes including the transition from spermatogonia to spermatocytes, round spermatids and, finally, spermatozoa. At this time the reassembly of the nucleoli is also taking place. Number and size of nucleoli reduce and it undergoes gradual degradation in meiotic prophase I. During degradation part of the material migrates into cytoplasm and participates in formation of a specific to male germ cells structure – chromatoid body (CB), which is involved in acrosome formation and mitochondria migration. Almost complete absence of transcriptional activity of late stage spermatozoa's nuclei is a characteristic feature of spermatogenesis. At this point the chromatin is highly condensed due to substitution of histones with protamines. Multifunctional proteins fibrillarin, nucleolin, nucleophosmin and SURF6 take part in ribosome biogenesis, participating in rDNA transcription and rRNA processing. We have showed that all these proteins were present in multiple nucleoli of spermatogonia. During meiosis in spermatocytes we detect several chaotically located structures of irregular shape that contain all four proteins. At the same time we observe the formation of the CB and its marker protein DDX4 colocalizes with SURF6. In round spermatids only small dots containing fibrillarin remain present. Our attention was drawn to the unexpected presence of SURF6 in acrosomes and tails of spermatozoa. Following immunocytochemical and western-blot studies of mature epididymal spermatozoa confirmed our observations. Thus, we demonstrate that SURF6 leaves the nucleoli earlier that other nucleolar proteins and is situated in the CB. It is shown for the first time that this participating in ribosome biogenesis protein SURF6 remains in spermatogenic cells until the stage of mature spermatozoa, which are transcriptionally inactive. The study was supported by the Russian Foundation for Basic Research (grant 18-34-00767). *The authors marked with an asterisk equally contributed to the work.

## P-41-069
## Study of the NdCTR1 chelating properties and development of its purification method

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Copper (Cu) is an essential trace element which acts as a cofactor of cuproenzymes and also directly regulates cellular processes. Despite the need of copper for all living organisms free Cu ions are toxic as they provoke ROS formation. Free Cu ions play role in inherited Cu-related diseases as Wilson's disease, neurodegenerative disorders like Parkinson's or Alzheimer's diseases and tumor growth. It was shown that Cu chelation therapy is a promising approach but at the moment only small synthetic chelators with plenty of side effects are used and there's no safe substitution for them. In particular work we propose 55 a.r. copper transporter 1 (CTR1) extracellular N-terminal domain (NdCTR1), which has three Cu-binding motives, as a natural selective Cu chelator. NdCTR1 with GST affinity tag was cloned in *E. coli*. It increased bacterial resistivity to Cu, Ag ions and Ag nanoparticles ($Ag^+$ are similar to $Cu^+$) despite transformed cells accumulated more metal than the control. By chromatography and immunoprecipitation it was shown that it's NdCTR1 responsible for metal chelation. Resulting recombinant protein demonstrated high potency to aggregation due to GST dimeric nature and hydrophobic a.a. cluster on the NdCTR1 C-terminus as was shown by bioinformatics analysis. At the same time, it was found that NdCTR1 can be purified without any affinity tags on $Ni^{2+}$ column as NdCTR1 itself contains 10% His residues. For reasons unknown it was impossible to express sole NdCTR1 in bacterial system so GB1 solubility tag was fused to the truncated NdCTR1 without mentioned hydrophobic cluster. We purified highly soluble GB1-NdCTR1 on $Ni^{2+}$-charged column and removed GB1 by thrombin proteolysis. At the moment studies of purified NdCTR1 are undergoing. Usage of NdCTR1 as a safe and effective natural Cu chelator are discussed. The work was supported by RFBR grants 18-015-00481 and MK2718.2018.4.

## P-41-070
## The impact of zinc oxide and titanium dioxide nanoparticles and co-exposure NPs and UV radiation on human skin cells

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Due to rapid development of nanotechnology, which is one of the key technology of the 21st century, human exposure to nanomaterials is growing dramatically. Consequently, possible hazard for human health is being created, because the unique properties of nanoparticles (NPs), despite have an impact on their pharmacological activity and common uses, can also be responsible for their adverse and toxic effect. One of a major routes of exposure for NPs is skin. Currently, zinc oxide ZnONPs and titanium dioxide $TiO_2$NPs nanoparticles are the two most commonly components used in sunscreens due to their ability to block ultraviolet radiation, reduce the photoallergy, as well as their high photostability. For this reason, we have been focused on toxicological interaction NPs and the effects co-exposure of NPs and UV radiation on human skin cells including comprehensive characterization of NPs in biological system. The cell lines: human dermal fibroblasts (HDF), immortalized human keratinocytes from normal skin (HaCaT) and human melanocytes (HEMas) were used to *in vitro* assessment the toxicity of NPs. Inhibition of


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cell growth was investigated by mitochondrial metabolic potential. Cellular uptake and ultrastructural changes of NPs was examined by transmission electron microscopy (TEM). Cell death was determined by flow cytometry. Protein level was detected by using Western Blot. The obtained results have demonstrated that exposure to ZnONPs significantly decrease viability of skin cells and inhibit cell proliferation, which haven't been observed for $TiO_2$NPs. Moreover, only ZnONPs lead to transformation of melanocytes to melanoma, induce inflammatory and mixed types of cell death. What is important, co-exposure both of types of NPs and UV radiation enhance toxic effect on melanocyte cells, which indicates high risk associated with the application of NPs in sunscreens. This work was supported by grant 2016/21/N/NZ3/03276 from the Polish National Science Center (NCN).

## P-41-071
## High-throughput analysis of ubiquitination cascade by chloroplast E3 ligases in the reconstituted bacterial system

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Since the majority of the chloroplast proteome is nucleus-encoded, biogenesis and proper functioning of this organelle is highly dependent on its protein import capabilities. Translocon complexes localized at the outer envelope membrane (TOC) and the inner envelope membrane (TIC) transport pre-peptides across the respective membranes. A recently identified E3 RING-type ubiquitin ligase embedded in the OEM, called *suppressor of ppi1 locus1* (SP1) targets components of TOC and designates them for degradation by the ubiquitin-proteasome system (UPS). More recently, it has been shown that SP1 interacts with SP2 and CDC48, an Omp85-type β-barrel channel located in the OEM and a cytosolic AAA+ chaperone, respectively. Interestingly, SP1 is also present in the peroxisomal and mitochondrial outer membranes. The model plant *Arabidopsis thaliana* encodes two ubiquitin ligases homologous to SP1, the SP1-Like1 (SPL1) and the SP1-Like2 (SPL2) that share topological and significant sequence identity with SP1. Their subcellular localization is also alike, except that for SPL2 no peroxisomal association has been observed. Any further knowledge, especially about their ubiquitination substrates is lacking however. Our current aim is to elucidate the functions of SPL1 and SPL2. We employed several approaches based on co-immunoprecipitation (CoIP) and *in vitro* ubiquitination assays to identify SP1L substrates. We also adapted an *in vivo* ubiquitination system reconstituted in bacterial cells to identify E2-E3 pairs and putative substrates of these novel E3 ligases. We then further modified this system to determine the type of ubiquitin chain linkage that directs the modified proteins to different cellular fates. Obtained results provide an insight of the role fulfilled by SP1-Like ubiquitin ligases in a plant cell. This work was funded by National Science Centre, Poland (NCN) grant 2015/19/D/NZ1/02790 awarded to WB.

## P-41-072
## A polyamorous repressor: deciphering the evolutionary strategy used by the phage-inducible chromosomal islands to spread in nature

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*Staphylococcus aureus* pathogenicity islands (SaPIs) are a family of related 15–17 kb mobile genetic elements that carry and disseminate superantigen and other virulence genes. SaPIs reside passively in the bacterial chromosome, repressed by a master repressor called Stl, encoded by the own SaPI. The key feature of their mobility and spread is the induction by helper phages of their excision, replication, and efficient encapsidation into specific small-headed phage-like infectious particles. After infection or induction of a resident helper phage, SaPIs are de-repressed by the specific protein-protein interaction of phage proteins with Stl. SaPIs have developed a fascinating mechanism to ensure their promiscuous transfer by targeting with the Stl repressor structurally unrelated phage proteins performing the same conserved function. Combining structural biology approach and functional characterization *in-vivo* and *in-vitro* we decipher the molecular mechanism of this elegant strategy by which the SaPI hijacks the phage process to sense the starting of the lytic cycle. Our structural studies show that the Stl of the island SaPIbov1 combines a canonic HTH N-terminal domain to bind DNA, and sequentially acquires new domains which act as recognizing modules for the different phage proteins (anti-repressors). Our *in-vivo* and *in-vitro* data deciphers the molecular mechanism that underlies the interaction between the Stl repressor and different phage coded anti-repressors, showing how each Stl module mimics the substrate for each anti-repressor type. The interaction of Stl with different types of anti-repressor always disrupts the Stl dimer, implying the DNA dissociation and SaPI derepression. Our results establish the molecular mechanism of the interaction event that detonates the intra- and inter- generic transference of the clinically relevant SaPIs. *The authors marked with an asterisk equally contributed to the work.

## P-41-073
## hIL7-BAPmut fusion protein production and application for the specific scFv selection from combinatorial antibody library

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Human Interleukin-7 (hIL7) – cytokine which plays important role in the development and regulation of immune system (B and T cells proliferation). The results of modern studies testify about IL-7 antitumor effects in tumors such as glioma, melanoma, lymphoma, leukemia, prostate cancer, and glioblastoma. That why it's can be promising therapeutic candidate. Antibodies is one of the most widely used diagnostic tools for the biological molecules investigation. Single-chain antibodies (ScFv) compare to full-length antibodies have several advances: smaller sizes, animal-free antibody production, reproducibility, high-throughput, rapid and cost effective production, and can be genetically fused with marker protein. The aim of our investigation is the obtaining of bifunctional fusion protein hIL7-BAPmut for one-step selection


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of scFv, specific to hIL7, from combinatorial antibody libraries. The fusion protein, consisting of hIL7 for scFv binding and bacterial alkaline phosphatase with enhanced catalytic activity (BAPmut) for immune complex detection, was developed. The DNA sequences encoding human IL-7 and BAPmut were subcloned into pET24a(+) plasmid vector under control of T7 promoter. E. coli BL21(DE3) cells were transformed with pET24-IL7-BAPmut expression vector and protein synthesis was induced with auto-induction protocol. Target protein was accumulated in the form of bacterial inclusion bodies. IMAX was used for purification of solubilized proteins with subsequent optimization of renaturation method. Bifunctional activity of IL-7-BAPmut fusion protein after renaturation was confirmed in ELISA and Western blot. It was shown that application of hIL7-BAPmut allows at least three times shortening the time of the screening of immune combinatory libraries of variable genes of IgG and does not require using of specific primary and secondary antibodies.

## P-41-074
## Analysis of butyrylcholinesterase interactions with old inhibitors and new reactivators

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Inhibition of the enzyme butyrylcholinesterase (BChE) in human tissues by binding of compounds to its active site serine is important for the detoxification and scavenging of xenobiotics such as organophosphates (OP). Although BChE is generally considered as having no physiological function, a growing body of evidence indicates that BChE plays a central role in the development of the symptomatology of Alzheimer's disease and related dementias. The most likely function for BChE is as a backup for acetylcholinesterase (AChE) and protection of synaptic AChE from man-made and naturally occurring poisons. Newly considered strategies in medical protection against nerve agents focus on the use of exogenously administered BChE. The overall idea is to administer such an enzyme in combination with a specific oxime, to scavenge an OP before it can reach and inhibit native AChE, thus helping organism detoxification from the excess OP. Starting with a directed library of pyridinium aldoximes, we identified efficient reactivators of sarin, cyclosarin, VX, and tabun-BChE conjugates and kinetically characterized their interactions in detail. Moreover, for several oximes BChE reactivation potency was showed to be promising when compared to the standard oximes used in medical practice. However, an absence of universality of reactivators underlies comprehensiveness of the reactivation mechanism and importance of the stabilization of the oxime group in vicinity of the phosphorus conjugated at the catalytic serine. Its convenient position for the nucleophilic attack is the major criteria for efficient reactivation of OP-BChE conjugates. Notwithstanding, we identified several efficient reactivators of phosphorylated BChE that, due to a cumulative capacity to reactivate both AChE and BChE, possess the potential for bioscavenging of OP. Acknowledgments: This work was supported by the Croatian Science Foundation (IP-2018-01-7683).

## P-41-075
## Insights on the mechanism of action of class IIa bacteriocins

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Bacteriocins are ribosomally synthesized peptides with antimicrobial activity against pathogenic Gram positive bacteria. These peptides have high potency and low toxicity, and are usually specific for one or a few target microorganisms. Sakacin-A is a class IIa bacteriocin, produced by *Lactobacillus sakei*, with a specific anti- *Listeria* activity. For this reason, it has the potential to be employed to reduce the risk of *L. monocytogenes* poisoning of ready-to-eat food products. Class IIa bacteriocins exert their anti-*Listeria* activity by binding to a transmembrane receptor on the target cells, but the exact underlying mechanism has yet to be fully elucidated. The binding properties of the N- and the C-terminal domains of sakacin-A were tested against different microorganisms, by using confocal laser scanning microscopy and peptides conjugated with 5(6)-carboxyfluorescein, to investigate the molecular mechanism responsible for the specific recognition of *Listeria*. In addition, the full length peptide was inserted into liposomes of appropriate composition – where the C-terminal domain is hidden into the lipid bilayer, whereas the N-terminal half is exposed to the solvent – to perform pulldown experiments on bacterial lysates. Analysis of the liposome-bound proteins confirms a specific interaction between sakacin-A and transmembrane receptors on *Listeria* cells. Our data point to a general role of the N-terminal domain in the binding to different Gram positive bacteria, although this region is not "*per se*" sufficient for the specific recognition of *Listeria*. In conclusion, a better understanding of the mechanisms underlying the specificity and anti-microbial activity of sakacin-A will pave the way for a more efficient exploitation of class IIa bacteriocins as food preservative or as an alternative to non-protein antibiotics.


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