have preserved affinity to the hIFNγ receptor, but to be deprived in their capability to trigger the intracellular signal transduction.

To this end a library of mutants was created and two potential hIFNγ antagonists were selected for further investigations: a single point mutant K88Q (Q substitution for K in position 88) and a double mutant with additional substitution in the N-terminus. Both mutants and the wild type hIFNγ were expressed in *E. coli* employing the established by us methodology for large scale production of aggregation-prone proteins in soluble native form. The purified mutants were screened for interferon activity (antiproliferative assay), binding affinity (isothermal titration calorimetry) and ability to compete with the wild type for the hIFNγ receptor (competition assay on WISH cells).

The selected mutants demonstrated 100 (single mutant) and 1000 (double mutant) times lower antiproliferative activity than the wild type. Measuring the binding thermodynamic parameters, we proved that the receptor binding affinity of both mutants was preserved, which is an indication for their potential to compete with the wild type hIFNγ for its receptor. Finally, the biological assay performed on WISH cells showed a distinct dose-dependent competition between the wild type hIFNγ and the mutants.

Based on the results presented in this study we conclude that the two hIFNγ mutants are potential candidates for autoimmune therapy based on selective suppression of the endogenous hIFNγ activity.

P-02.02.2-004
MANF may attenuate hypoxia/reperfusion-induced renal cell injury

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER (endoplasmic reticulum) stress-inducible protein and widely expressed in mammalian tissues. It has been identified as a secretory protein that protects cells against ER stress-induced damage. ER-stress is one of the main mechanisms that play a role in ischemia/reperfusion (I/R)- induced renal injury. Recent studies demonstrated that MANF can protect cardiac myocytes and cortical neurons against I/R-induced injury. Moreover, it has been suggested that it has a restorative effect in ischemic injury. Nevertheless, the function of MANF in I/R-induced renal injury is still not known.

In the present study, we investigated the function of MANF by manipulation its expression level in ischemic acute renal failure model established in proximal tubular kidney cells (HK-2 cells).

For this purpose, the cells were transfected with either MANF siRNA or MANF encoding plasmids for silencing or over-expression of MANF, respectively. Then, the cells were exposed to hypoxia-reperfusion (H/R) induction for indicated times. Evaluations of cell viability were determined with WST-1 reagent. The changes in protein levels of H/R-induced stress markers were analyzed byimmunoblotting.

The results showed that the overexpression of MANF has provided a significant resistance to H/R-induced cell death, whereas silencing of MANF has rendered the cells more susceptible to death. It was also determined that the pretreatment of cells with MANF conditioned medium caused a decrease in cell death.

Additionally, oxidative/nitrosative stress (OS/NS) and ER stress levels were decreased with over-expression of MANF and increased by silencing of MANF in HK-2 cells.

Taken together, our study suggests that MANF may have a protective role against H/R-induced renal cell injury, possibly through the reducing effects on OS/NS and ER stress.

P-02.02.2-005
His-flag tag as a fusion partner in insect expression system – gain or loss?

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Human interferon gamma (hIFNγ) is a glycoprotein playing major role in the regulation of innate and adaptive immunity. Glycosylation is not essential for hIFNγ activity but is important for its stability, half-life and protease resistance in blood. The commonly used hIFNγ in therapy and research is produced in *E. coli* and therefore is not glycosylated. Bearing in mind the above mentioned shortcomings of the non-glycosylated hIFNγ we expressed it in mammalian cells and transgenic mice, however very low yields were achieved. To obtain glycosylated hIFNγ, here we employed a secretory expression of N-terminal HIS-FLAG fusion protein in baculovirus-infected insect High Five® cells. This small hydrophilic tag is designed to not affect the proper folding of the target protein and to facilitate the detection and purification procedures. In parallel the same fusion was expressed in *E. coli* cells. The fusion proteins were purified to high degree of purity by affinity and size-exclusion chromatography. Bioassay carried out on WISH cells showed that the antiproliferative activity of both fusion proteins was 500 times lower than that of the native hIFNγ. This result shows that, in contrast to the generally hold view, the N-terminal HIS-FLAG tag interferes with the biological activity of hIFNγ despite of the protein glycosylation. In order to restore the biological activity we attempted to remove the HIS-FLAG tag enzymatically. Surprisingly, we found that the fusion protein obtained from insect cells was resistant to enterokinase, independently of the enzyme source and experimental conditions, whereas the protein isolated from *E. coli* was susceptible and the tag-free protein showed fully restored biological activity. We are prone to explain the enterokinase resistance of the fusion protein from insect cells with either the specific conformation of the glycosylated protein or with the interaction of the carbohydrate residues with the enzymatic activity of the enterokinase.

P-02.02.2-006
Development of fluorescence assay for high-throughput screening system based on flow cytometry for directed evolution of cellobiose dehydrogenase

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Cellobiose dehydrogenase (CDH) is an enzyme produced by *Phanerochaete Chrysosporium* and it has been already successfully cloned in other organisms. One of the most important roles of CDH is removing products of cellulose degradation. CDH is very important for biofuel and biosensor industry. For improvements of enzyme properties we have used directed evolution. The most important step is to develop screening system that reflects properties of interest. Screening in microtiter plates (MTP) is expensive, time-consuming and has low throughput with a small number of variants detected (10^3–10^4 in months).

The aim of this work was the development of screening system for mutant libraries of CDH expressed on surface of yeast cells based on fluorescent enzymatic assay and flow cytometry. The screening method should be capable of screening cellobiose dehydrogenase variants mutated for higher activity and higher thermostability by error prone PCR. The fluorescent assay was

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evaluated in MTP and compared with DCIP assay. For further work the fluorescent assay will be tested using yeast cells with expressed active CDH on yeast surface using fluorescent activated cell sorter detection system.

P-02.02.2-008
Purification, characterization and gene cloning of beta-galactosidase from *Arthrobacter sulfonivorans*

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Beta-galactosidase (EC 3.2.1.23) also known as lactase is the enzyme that typically catalyzes hydrolysis of beta-1,4-D-galactosidic linkages in beta-D-galactosides, including disaccharide lactose, with glucose and galactose as end reaction products. This enzyme is able to catalyze synthesis of oligosaccharides, in particular galactooligosaccharides via galactosyl transfer reaction.

Arthrobacter sulfonivorans beta-galactosidase of unique for prokaryotes extracellular localization may find application in food industry for manufacturing lactose-free dairy products and in pharmacology as bioactive principle of medicines prescribed for patients suffering from lactase deficiency.

The study was aimed at cloning of the gene encoding *A. sulfonivorans* beta-galactosidase, purification and characterization of the enzyme.

A novel extracellular beta-galactosidase from *A. sulfonivorans* was recovered with an overall 207-fold purification, a 7.7% yield and specific activity 16 300 $U \cdot mg^{-1}$ protein. The subunit molecular mass of the enzyme determined by SDS-PAGE analysis equalled 125 kDa. It was found that the enzyme displays pI 5.35, prefers ortho-nitrophenyl-beta-galactoside as substrate (Km 27 mM) and shows maximum activity at 40°C and at pH 7.5–9.5.

The beta-galactosidase gene was isolated from the genomic DNA library of *A. sulfonivorans*, sequenced, cloned and deposited in the GenBank database under accession number KM277894.1. It was established that the gene carries an open reading frame consisting of 3132 bp (1043 amino acids) and encodes beta-galactosidase referred to Glycosyl Hydrolase Family 2 (CAZy database).

P-02.02.2-009
Different splice-forms of TDRD7 protein mutated in cataract's and glaucoma's interacts with S6K1/2

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

The aim of our study was to confirm S6K-TDRD7 interaplay and study its role in cells.

Bioinformatical analysis of TDRD7 sequence revealed the presence of potential phosphorylation sites of S6K2. Using *in vitro* kinase assay, we have demonstrated that recombinant S6K2 phosphorylate 3 from 5 fragments of TDRD7. Formation of S6K2-TDRD7 complexes *in vivo* was further confirmed by co-immunoprecipitation using anti-S6K2 and anti-TDRD7 antibodies generated previously in rat brain lysates. This interaction was further confirmed by confocal microscopy, Oleksandr had shown that TDRD7 co-localize with S6K2 in HEPG2 cells, predominantly in perinuclear region, enreached for one of the TDRD7 isoforms identified previously.

Moreover, we have detected that C-terminal synthetic peptides of S6K2 with methylated Arg interfere with TDRD7 from HEPG2 lysates. The physiological characteristics of S6K2-TDRD7 interaction and the role of this complex formation in neuropathology's development need further investigation.

P-02.02.2-010
Very stable high molecular mass multi-protein complex from human placenta

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Many biological function of placenta are performed not just a set of individual proteins, but also different oligomeric structures and complexes. Herewith, activities of complexes may considerably differ from activities of individual proteins. Therefore, identification and characterization of placental multi-protein complexes is an important step to understanding the placenta function.

The aim of the present work was to investigate a composition and biological functions of the very stable high molecular mass multi-protein complexes (SPC) from placenta of healthy mother.

We isolated SPCs (~1000 kDa) from the soluble fraction of three human placentas. Light scattering measurements and gel filtration showed that the SPC is stable in presence salts, acetonitrile and Triton X-100 in high concentrations, but efficiently dissociates in the presence of 8 M urea and 50 mM EDTA. Such a stable complex is unlikely to be a random associate of different proteins. It was shown the SPC includes a number of proteins with molecular weights of 2 to 180 kDa. Several protein components of the SPC were identified, including serum albumin, transferrin, IgGs, annexin A5 and other proteins. Serum albumin, transferrin and protein with molecular weight 14,1 kDa are the main proteins of the complex. It was shown high the SPCs from three placentas possesses DNSase and catalase activities. An addition, investigation of cytotoxic effect on human cancerous cell lines has shown that the SPCs reveal high cytotoxicity.

P-02.02.2-011
Antibody-cytochrome b5 fusion protein, characterization and applications for antibody development process

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Antibodies have recently become an essential tool being a part of immunodiagnostics, therapeutics and as a valuable instrument in life science research. An enormous number of options utilizing a various tags were used to create a universal antigen-binding domain, which can be easily detectable, highly soluble and might be produced in high yields with low costs, but no multipurpose solution exists yet.

We addressed the question whether a single tag could be found for enhancing solubility of recombinant Fab antibody fragment and providing its detection and accurate quantification by rather simple method.