cleavage is rate-determining for substrates with electron-withdrawing groups. The reaction mechanism of Kynase is thus very similar to that proposed by Braunstein in 1949.

SW02.W10–7
Methionine gamma-lyase as a target in pathogens

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Pyridoxal 5′-phosphate-dependent methionine γ-lyase (EC 4.4.1.11) catalyzes γ-elimination reaction of methionine and its analogs and β-elimination reaction of S-substituted cysteines. The enzyme presents in some bacteria and primitive eukaryotes. It might be a new target in pathogens as mammalian cells do not contain methionine γ-lyase. We performed the search of sequences ascribed to methionine γ-lyase in genomes of pathogenic bacteria. Sequences from genuses of *Brucella*, *Porphyromonas* and *Clostridium* were selected and analyzed. Genes coding methionine γ-lyase in genomes of *C. sporogenes*, *C. tetani* and *P. gingivalis* were cloned, expressed in *E. coli* cells and substrate specificity of the enzymes was confirmed. As starting molecule for a design of potential inhibitors of the enzyme we studied reaction of the *Citrobacter freundii* enzyme with non-protein amino acid (2R)-2-amino-3-[(S)-prop-2-enylsylfinyl]propanoic acid (alliin). It was demonstrated that methionine γ-lyase catalyzes the β-elimination reaction of aliin with formation of allyl 2-propenethiosulfinate (allicin), which is the best known active compound of garlic. The reaction entailes the inactivation of the enzyme in both γ- and β-elimination reactions and the lost of its three SH-groups. Crystal structures of inactivated wild type *C. freundii* enzyme and its mutant form with the replacement of active site cysteine 115 were solved (at 1.85 Å, 1.45 Å resolution) and analyzed. It was determined that allicin forms mixed disulfides with SH-groups of methionine γ-lyase. Structural consequences of Cys115 and Cys245 modifications for the catalysis of the γ- and β-elimination reactions will be discussed.

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SW02.W10–8
The intrigues and intricacies of quino-cofactor biosynthesis

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The enzymatic quino-cofactor family arises via post-translational modifications of aromatic amino acid side chains (tyrosine or tryptophan). These side chain modifications can occur within the active site of the cognate enzyme or via the complex processing of a ribosomally encoded peptide. This presentation will summarize recent structural, genetic and biochemical probes of the generation of the peptide-derived cofactor, pyrroloquinoline quinone (PQQ). Emphasis will be on five essential gene products, their interactions with one another and the necessity of combining anaerobic and aerobic reactions within a single biosynthetic pathway. (Supported by the National Institutes of Health, GM025765).

SW02.W10–9
Plant formate dehydrogenase: structure – function studies

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NAD^+-dependent formate dehydrogenase (EC 1.2.1.2, FDH) plays important role in bacteria, yeasts, fungi and plants. In plants FDH is situated in mitochondria in contrast to cytoplasm localization in microorganisms. Plant FDH is the universal protein of stress. Under different stress conditions content of the enzyme sharply increased and could achieve up to 9% of total mitochondria proteins. Bioinformatic analysis showed that many plants have a few genes of FDH.

In this report we will present data about study of two FDH – from *Arabidopsis thaliana* (AraFDH) and isoenzyme 2 from soya *Glycine max* (SoyFDH). cDNA of AraFDH and SoyFDH were optimized and cloned in *E. coli* cells. Both enzymes were expressed as soluble and active proteins with yield up to 1 g of target protein per litre of cultivation medium. Kinetic properties and stability of AraFDH and SoyFDH were studied at different conditions. It was found that palnt FDHs show very high resistance to inactivation by hydrogen peroxide which is presented in mitochondria at high concentration under stress conditions. Crystallization of the enzymes were carried out on earth and in space. Crystal structures of apo- and holo-forms were solved with resolution till 1.4 angstrem. Rational design of the SoyFDH resulted in new mutants with higher specific activity, better Michaelis constants and great improvement of thermal stability. Common and characteristic features of properties and structures of microbial and plant FDHs are discussed.

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SW02.W10–10
Management of properties and stability of recombinant formate dehydrogenase from soya Glycine max by single-point mutation

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NAD^+-dependent formate dehydrogenase (EC 1.2.1.2, FDH) is an enzyme of high scientific and practical interest. FDH was found in bacteria, yeasts, fungi, moss, lichen and higher plants.

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Plant FDHs show lower values of Michaelis constants both for formate and NAD^+ compared to ones from microorganisms. So, such FDHs can be successfully used for cofactor regeneration in fine organic synthesis especially for preparation of chiral compounds. Besides, in plants FDH plays a very important role in stress conditions. Its content dramatically increases during such stress conditions as drought, lack of oxygen, thermal discontinuity, pathogen infections, etc. Therefore, FDHs from plants are worth studying. The object of research in present work was formate dehydrogenase from soya *Glycine max*. The enzyme has the lowest values of Michaelis constants among even plant FDHs known at the moment.

Rational design approach is successfully used in this laboratory to study structure-function relationship of different enzymes. Analysis of SoyFDH structure surface revealed the Phe residue in 290 position which is localized in coenzyme-binding domain of active site and its replacement may course changing in enzyme properties. Alignment of FDH amino acid sequences from different sources showed presence of residues Asn, Tyr, Asp, Ser. Computer modeling of influence of different amino acid changes of Phe290 was carried out and the most promising mutations were chosen for future work. Results of modeling shown that some of the Phe290 replacements may cause the formation of additional hydrogen bonds in the enzyme structure. Mutant SoyFDHs with changes of Phe 290 by Asn, Asp, Ser, Tyr, Gln, Glu, Thr and Ala were prepared and purified. Catalytic properties and stability of the mutant enzymes were studied. It was revealed, that practically all mutation influenced on Michaelis constant with formate, but K_M^{NAD+} remained practically unchanged. Thermal stability of mutant SoyFDHs were studied by analysis of inactivation kinetics as well as by differential scanning calorimetry. It was found that mutations of Phe290 resulted also in significant increase of enzyme stability. One of the best enzymes SoyFDH Phe290Asp was 44 times more stable and had Tm 7.8°C higher, then that for wt-SoyFDH.

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SW02.W10–11
Investigation the role of Met104 in catalytic activity and thermal stability of D-amino acid oxidase

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D-amino acid oxidase is a FAD-containing enzyme catalyzing D-amino acids oxidation to corresponding α-keto acids. DAAO plays important role in regulation of many processes in living cell (especially in mammals). The enzyme is also of high practical interest for pharmaceutical industry, fine organic synthesis and analytical biotechnology. It is used for biocatalytic conversion of cephalosporin *C* into 7-amino cephalosporanic acid (7-ACA) being the key precursor for synthesis of cephalosporanic antibiotics. D-amino acids oxidase from the yeast *Trigonopsis variabilis* (TvDAAO) is the most appropriate enzyme for application due to the best properties among all known DAAO's. Nevertheless wild type TvDAAO often possesses drawbacks for practical processes. Properties of enzyme can be improved by protein design technique.

Here we will present the results of protein engineering of TvDAAO. Computer analysis of TvDAAO structure and docking of substrates to active site revealed flexible amino acid residues, including Met104, located at the entrance and controlling access of substrates to the active site. To clear out the role of Met104 residue in catalysis and thermal stability eight substitutions to small and bulky amino acids were proposed based on computer simulation of potential mutants. Site-directed mutagenesis of Met104 was performed to introduce chosen substitutions. All mutants were obtained in soluble and active form. Study of properties showed that volume of residue in the 104th position has a crucial influence on thermal stability and catalytic activity. So introduction of bulky residues led to significant changes of thermal stability and affected on catalytic efficiency with the most of substrates. To investigate interaction between Met104 and spatially closed Phe54 several double mutants were obtained and characterized.

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SW02.W10–12
Glutathione S-transferase alpha from Esox lucius liver: purification and characterization

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Glutathione S-transferases (GST) are one of key enzymes involved in a xenobiotic transformation and protection cells against oxidative stress. GSTs are phase II biotransformation enzymes and are proposed as biomarkers of environmental pollution. In the large family of GST proteins, various isoforms differ significantly in their catalytic properties. In this study, one isoform of a cytosolic glutathione transferase was purified from the liver of freshwater fish pike (*Esox lucius*) by affinity chromatography. The obtained protein was identified by MALDI-TOF-MS as an alpha class GST, had an isoelectric point of about 6.4 and was composed of two subunits each with a molecular weight of 25235.36 Da. This GST showed significant activity towards mammalian alpha class GSTs substrate 1-chloro-2,4-dinitrobenzene and ethacrynic acid and towards 4-nitroquinoline 1-oxide found to be a preferred substrate of mammalian Mu- and Pi-class GSTs. Kinetic analysis with CDNB as the substrate revealed a K_m of 0.75 mM and V_{max} of 12.81 μM/min per mg of protein. It had maximum activity at a pH 9.0, while in various other organisms most GSTs pH optimum is within neutral values. It also shows a low thermal stability with 80% loss of its initial activity at 45°C for 20 min. A comparison with higher vertebrates and some fish species GSTs suggests that the purified protein in general is a typical member of the family of cytosol glutathione S-transferases.

SW02.W10–13
Engineering DNA methyltransferases for a novel cofactor

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Cofactors are metals, or organic compounds, which play fundamental roles in enzymes. Cofactor engineering has only been partially pursued and rarely have natural cofactors been substituted with synthetic organic molecules. Herein, we have designed a synthetic compound presenting in its structure few key modifications that, in turn, an engineered enzyme can exploit to achieve cofactor specificity, tight binding and orthogonal recognition of it in stead of the natural cofactor. The cofactor was designed

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