Posters

P1

STRUCTURAL CHARACTERIZATION OF PHOSPHATIDYLINOSITOL 4-PHOSPHATE II

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Phosphoinositides are essential regulators of fundamental cellular processes. Phosphatidylinositol 4-kinases (PI4K) initiate the canonical phosphoinositide biosynthetic pathway by phosphorylating D-4 hydroxyl of the inositol head group. Two types of human PI4K, designated as type II and III, are distinguished. The biological role of PI4K type II is diverse. PI4K II generates PtdIns4P pools and thus modulates biological membrane composition, membrane trafficking, endocytosis or signal transduction. Moreover, PI4K II is involved in Wnt signaling that regulates embryonic and bone development, tumorigenesis, neurogenesis and lipid and glucose metabolism. PI4K II also represents the important regulatory molecule of lysosomal -glucocerebrosidase delivery. Depletion of PI4K II results in Gaucher disease, lysosomal storage disorder caused by a defect in the degradation of glucosylceramide.

Significant biological relevance of PI4K II , its insufficient structural characterization and the fact that PI4K II doesn't share sequence homology with any other crystallized kinase lead us to its structural characterization. In order to increase the solubility of PI4K II , the sequence of T4 lysozyme was fused within this protein. It´s quite common method used for membrane protein crystallization. PI4K II with T4 lysozyme was expressed in *Escherichia coli* and purified using affinity chromatography and size exclusion chromatography. Obtained protein with high purity was used for crystallization trials. PI4 II crystallized in orthorhomboic space group P 2_122_1 (a = 79.55 Å, b = 78.71 Å, c = 104.75 Å, = = = 90°). Native crystals diffracted to resolution 2.8 Å. The phase problem was solved by MR SAD using SeMet crystals and the T4 lysozyme as a search model.

P2

HYDRATION OF AMINO ACID RESIDUES IN PROTEINS: WHAT CAN WE LEARN FROM DATA MINING?

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In structural biology, water has been for a long time considered a passive medium. Nevertheless, its complexity and importance for biological interactions has gradually been recognized in recent years. It is now accepted that water is a key determinant of protein structure, dynamics and function, and that water-protein interaction governs various processes, including protein folding, enzymatic catalysis, and molecular recognition. Water does not simply fill up the available space around proteins, but occupies specific sites and forms localized clusters, determined by its hydrogen bonding capabilities. Distribution of water around proteins was analyzed in several early studies of protein crystal structure, which revealed its preference for Glu, Asp and Arg side-chains and main-chain carbonyl group [1]. However, due to limited number of high resolution structures, hydration patterns could be investigated for only a few amino acids, such as serine and threonine, in these early studies. We therefore decided to make use of the immense growth of the Protein Data Bank (PDB) [2] in the recent years and perform a detailed analysis of hydration patterns for all 20 standard amino acids using high-resolution protein crystal structures.

We used a set of 3845 PDB entries with resolution better than 1.8 Å, maximum R-factor value of 0.22 and mutual sequence identity of the chains of 50% or less. We checked the quality of all structures with Molprobity program and generated all the crystalograpic neighbours of the unit cell. The contacts of each amino acid residue with waters within 3.6 Å were then detected. Residue conformations were clustered separately in each class defined by residue type, secondary structure (alpha helix/beta sheet) and chi1 rotameric state (g+/g-/t) using the quality threshold algorithm. The clusters of residues with the associated water molecules were then subjected to the method of density representation [3] in order to identify the most preferred location of water molecules. Briefly, a fourier transform technique was used to calculate structure factors from atom positions, and to convert them to electron densities. Water peaks were then detected and water positions, occpancies and B-factor refined using standard crystallographic procedures.

The result of our study is a detailed atlas of protein hydration, containig the most populated positions of waters around each residue type in various backbone and side chain conformational states. The analysis of high resolution crystallographic structures of proteins from the PDB database thus revealed the spatial distribution of the water molecules in the first hydration layer of proteins and to some extent also their dynamic properties, which can be estimated from their *B*-factors, a measure of atom's vibrations. The hydrated amino acid rotamers obtainded from our study can be used in many areas of structural biology, from molecular replacement and crystalographic refinement, to the improvement of accuracy of ab initio protein structure prediction methods.

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P3

CALMODULIN AND S100A1 PROTEIN INTERACT WITH THE INTRACELLULAR TERMINI OF THE TRPM4 CHANNEL

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TRPM4 belongs to the large family TRP channels, a group of non-selective cation permeable channels. This channel participates in processes ongoing in neurons, cardiomyocytes, T-cells, etc.. It has been proven link between defects of TRPM4 receptor and progressive familial heart block type 1B. TRP channels consist of six transmembrane helices and a pore-forming loop between S5 and S6, with different lengths of intracellular amino and carboxy termini. [1, 2] It has been shown that TRPM4 activity could be modulated by intracellular calcium binding proteins calmodulin (CaM) and S100A1. [3]

In this study, one CaM/S100A1 binding site was localized in TRPM4 N-terminal region (NT) S_{583}-A_{668}. Another CaM/S100A1 binding site was determined in C–terminus domain (CT) V_{1050}-S_{1114}. The results from the previous experiments suggest that CaM and S100A1 could bind to the same or overlapping binding site. [3] Fusion proteins of the corresponding lengths were expressed in *E.coli* and purified by affinity and gel permeation chromatography. Sequences of the proteins were verified by Mass Spectrometry. To characterize CaM/S100A1 binding site on intracellular termini regions of the TRPM4 surface plasmon resonance measurements were used. Potential changes in secondary structure TRPM4/CaM complex were checked by the circular dichroism. De Novo molecular models of TRPM4 termini combined with ligand docking by CaM provides a structural insight into the TRPM4/CaM binding. Several positively charged residues were identified to be responsible for binding of CaM and S100A1 within the mentioned domains. The binding of both domains to CaM and S100A1 respectively is Ca^{2+} dependent.

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P4

SYNTHESIS AND SPECTROSCOPY OF DERIVATIVES OF THE FLUORESCENT PROTEIN CHROMOPHORE

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The fluorescent protein is a valuable tool in biological imaging techniques. We synthesized several derivatives of the green fluorescent protein chromophore, in order to understand spectroscopic properties of this interesting structural label. They were characterized by ^{1}H NMR, MCD and Raman spectroscopies. The spectral properties will be used to obtain information on the geometry and the electronic structure. Understanding the spectroscopic and chemical properties and the chemical modification of the chromophore can lead to better labels, e.g. which would be more stable or not easily eliminated by the organism.

P5

CRUCIAL ROLE OF SPOIIE DURING *BACILLUS SUBTILIS* CELL DIFFERENTIATION

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One of the characteristics of sporulation process in *B. subtilis* is asymmetric division of the cell, which leads to formation of two unequally sized compartments. Despite the fact that they contain the same genetic information, these compartments follow separate fates, due to dissimilar gene expression driven by compartment-specific sigma factors. While the smaller compartment, the prespore develops into heat-resistant spore, the bigger compartment, the mother cell nourishes the smaller prespore and finally lyses.

Proper positioning of the asymmetric septum is crucial for the process of sporulation. It is partially triggered by sporulation specific protein, SpoIIE, an exclusive component of the sporulation septum. SpoIIE closely cooperates with tubuline-like protein FtsZ. FtsZ forms higher organized structures, so called Z-rings, which constitute a scaffold for assembly of division machinery. During sporulation, FtsZ forms helical structures emanating from midcell Z ring towards cell poles. SpoIIE, which also forms ring-like structures, most likely stabilizes FtsZ helices at polar positions, thus polar Z-rings are formed [1].

SpoIIE is 827-residue protein, which is thought to consist of three domains. Its N-terminal domain, which contains ten transmembrane helices, anchors the protein into the sporulation septum [2, 3]. The central domain of SpoIIE is conserved only among SpoIIE orthologues. This domain is thought to be the regulation domain of the protein. Central domain is considered to be involved in dimerization of SpoIIE and it is also responsible for interaction of the protein with FtsZ [4]. SpoIIE C-terminal domain is closely related to PP2C domains of eukaryotic Ser/Thr phosphatases, which regulate the stress response [5]. This domain is responsible for activation of the first compartment-specific sigma factor, ^{F}, in the prespore. Activity of ^{F} is regulated by the means of interactions between an anti- sigma factor SpoIIAB and an anti-anti-sigma factor SpoIIAA and SpoIIE phosphatase, resident in the sporulation septum. In the predivisional cell and in the mother cell, ^{F} is held inactive, in the complex with two molecules of SpoIIAB. In the presence of dephosphorylated SpoIIAA, SpoIIAA -SpoIIAB complex is preferably formed thus ^{F} is released and active. Since SpoIIAA is phosphorylated by SpoIIAB kinase and released from this complex, SpoIIE phosphatase activity is critical to maintain the level of dephosphorylated SpoIIAA molecules in the prespore to keep ^{F} active, and able to promote prespore-specific gene expression [8,9]. The structures of the SpoIIA proteins, respectively their domains, have been determined, revealing the phosphorylated and dephosphorylated forms of SpoIIAA [14,15], as well as the interactions of SpoIIAB with ^{F} and SpoIIAA [9,16]. Previously, using a random truncation library approach [17], a set of soluble SpoIIE fragments was identified. Out of these fragments, SpoIIE fragment (590-827) encompassing the PP2C phoshatase domain was over-expressed, characterized, and its crystal structure was determined [18].

Multidomain composition of the protein allows its multiple roles during the process of sporulation. The key regulator SpoIIE has at least three major roles in the process of sporulation. The first role of SpoIIE is to stabilize FtsZ spiral intermediates at polar positions and co-localize with FtsZ in the forming sporulation septum. The second role of SpoIIE lies in its contribution to prespore-specific ^{F} activation, which is an event linked to the completion of sporulation septum. Consequent compartment specific gene expression is arranged by the activity of cell-type specific factors, which sequentially activate each other in a cascade-like manner [6]. Dynamic localization studies us-

ing SpoIIE-GFP fusions have shown that after completion of sporulation septum, SpoIIE is released from the septum and transiently localizes to all membranes in the forespore compartment. However, after engulfment is initiated, SpoIIE relocalizes to the septal site of the membrane, indicating possible role of SpoIIE in the later stages of sporulation. It was also shown that SpoIIE relocalization to the engulfing septum is dependent on SpoIIQ, the protein which is a part of the channel, connecting prespore and mother cell compartments. Moreover, immunoprecipitation experiments brought the evidence that SpoIIE and SpoIIQ reside in a membrane complex together [7].

Although three roles of SpoIIE are described, not all mechanisms of its actions are fully understood. Moreover, SpoIIE assignment in the stage of engulfment is very little understood. To answer these issues, we gather further information by screening interactions between SpoIIE and proteins taking part in cell division and engulfment by bacterial two hybrid system.

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P6

MOLECULAR DYNAMICS CALCULATIONS OF CY3 AND CY5 CYANINE DYES TERMINALLY ATTACHED TO DNA

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Cyanine Cy3 and Cy5 are fluorescent dyes with many applications in nonlinear optics, laser physics and particularly in biomedical imaging and single-molecule spectroscopy.

In this contribution, the structure and dynamics of cyanine Cy3 and Cy5 dyes terminally attached to the 5'-end of the CCACTAGTGG oligonucleotide are studied by molecular dynamics (MD), metadynamics and DFT calculations. Stacking interactions between the distal indole ring and guanine base are characteristic for the structures of the most probable conformers and these are in excellent agreement with the experimental structures for both dyes.[1] Essential dynamics showed that there are two main modes which describe the relative motion of the cyanine dyes with respect the last GC base pair (Figure 1). Unlike for the Cy3 dye, the Cy5-DNA complex shows similar flexibility but due to the longer pentamethine chain the binding motifs with proximal indole ring are not accessible at 298 K.

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Figure 1: Alignment of the structures with respect to the last GC base pair (colored by the atom names). Only atoms of the indole rings and polymethine linker are shown for all the structures except A1 for which the side chains and linker atoms are represented by lines.

A) Rotational- translational motion of the Cy3 dye with respect to the GC base pair in the direction A1 (blue) › A2 (red) › A3 (tan) › A4 (green). The change of stacking interactions during the motion: in A1 the distal indole ring is stacked with G, in A2 the Cy3 dye is almost de-stacked, in A3 and A4 the proximal indole ring is stacked with C and G, respectively.

B) Two translational motions of the Cy3 dye with respect to the GC base pair in the directions: (1) A1 (blue) › B1 (pink) › B2 (cyan) with partial de-stacking, and (2) A1 (blue) › C (violet) in which G is replaced by C in the stacking interactions of the distal indole ring of Cy3.

P7

PURIFICATION AND INITIAL IMAGING OF THE TYPE I DNA RESTRICTION-MODIFICATION ENZYME ECOR124I BY ELECTRON MICROSCOPY

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Restriction enzymes protect prokaryotic cells from viral infection by recognition and degradation of invading DNA. These enzymes are typically coupled with specific modification enzymes that mark and protect cellular DNA from degradation by methylation. The type I DNA restriction-modification enzymes unite both functions in a large multisubunit complex [1, 2]. The prototype enzyme EcoR124I is composed of one HsdS subunit that binds to a specific sequence of DNA, two HsdM subunits that are responsible for methylation of the cellular DNA and two HsdR subunits that are required for restriction of the nonmethylated (invading) DNA. Basic quaternary structure of the whole R_2M_2S complex has been shown by negative-stain electron microscopy at low resolution [3]. Although high-resolution structures of the individual subunits have been determined by crystallography or molecular modeling, the current EM structure is insufficient for accurate fitting of the known atomic models and to uncover the mechanisms of EcoR124I assembly and function.

We used affinity chromatography and gel filtration to purify the R_2M_2S complex. Densitometry quantitative analysis of SDS-PAGE gels of purified complex indicated average stoichiometry of 1.6(R):2.0(M):1.1(S) suggesting that only about a half of the assembled complexes contained both HsdR subunits, likely because of significantly different binding affinity of the two HsdR subunits to the M_2S subcomplex (K_{D1} 0.6 nM vs. K_{D2} 190 nM).

The purified EcoR124I complex was negatively-stained in 2% uranyl acetate and imaged electron microscopy. Images of the negatively stained complex showed projections consistent with the previously determined structure of the EcoR124I complex in the open conformation [3]. The purified complex was also plunge-frozen in liquid ethane and imaged by cryo-electron microscopy. Micrographs were collected at 2-4 μm defocus using a FEI Tecnai F20 microscope. The micrographs show distinct particles of the presumed EcoR124I complex (~120 Å diameter), despite the relatively small size of the complete R_2M_2S complex (~450 kDa). Further, methods of single particle analysis [5] will be used to reconstruct the EcoR124I complex to high resolution that would then allow reliable fitting of the atomic models of the individual subunits.

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P8

CRYSTALLIZATION AND STRUCTURAL CHARACTERIZATION OF LINB86 HALOALKANE DEHALOGENASE MUTANT

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LinB is a microbial enzyme of the haloalkane dehalogenase family that catalyze the cleavage of the carbon-halogen bond in halogenated aliphatic pollutants, resulting in the formation of a corresponding alcohol, a halide ion and a proton. Haloalkane dehalogenase LinB isolated from a bacterium *Sphingobium japonicum* UT26 has relatively broad substrate specificity and can be potentially used for biosensing and biodegradation of environmental pollutants. Different variants of haloalkane dehalogenase LinB were constructed with a goal to study the effect of mutations on enzyme functions. In LinB86 (W140A + F143L + L177W + I211L) variant mutations lead to blocking of the main tunnel and opening of alternative way for connection the deeply buried active site with the surrounding solvent.

Crystals of LinB86 were obtained Index (Hampton Research, USA) and Morpheus (Molecular Dimensions Ltd., UK) screens. Full data set was collected on the beamline ID-29 of ESRF (Grenoble, France) at 2.43 Å resolution. Crystals belong to H-centered trigonal *H*32 space group. Matthews coefficient of V_M = 2.55 Å^3 Da^{-1} suggests that the crystals contain two molecules per asymmetric unit, which corresponds to a solvent content of 51.79%. Crystal structure of LinB86 was solved by molecular replacement using the coordinates from LinB32 (L177W) haloalkane dehalogenase mutant. The structure refinement is currently in progress.

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P9

CRYSTAL STRUCTURE OF PHOSPHOTRANSMITTER AHP2 AND MODELING OF ITS INTERACTION WITH SENSOR HISTIDINE KINASE CKI1 - TOWARDS ELUCIDATING MOLECULAR RECOGNITION IN THE MULTISTEP PHOSPHORELAY SIGNALING IN PLANTS

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In multistep phosphorelay (MSP) pathway, the His-containing phosphotransmitters (HPt) encoded by *AHP* genes in Arabidopsis act as signaling intermediates integrating signaling inputs from diverse sensor histidine kinases. In order to identify the molecular determinants of the previously reported specificity of the interaction between receiver domain of sensor histidine kinase CKI1 ($CKI1_{RD}$) and AHPs at the atomic resolution, we determined the structure of AHP2 via experimental phasing after lutetium soaking of AHP2 crystals at 2.53 Å resolution. The key residues responsible for the AHP2-$CKI1_{RD}$ interaction revealing strong protein-protein binding were identified via molecular dynamic simulations for 100 ns. The AHP2-$CKI1_{RD}$ interaction was confirmed via NMR measurements and resulting chemical shifts partially overlap with the model. Comparison of the AHP2-$CKI1_{RD}$ model with recently published structure of AHP1-$AHK5_{RD}$ suggests that the interacting surfaces differ significantly between both complexes, particularly in the amino acid residues mediating hydrophilic interactions. Thus, in spite the vast majority of interacting residues in the AHP proteins is recruited from highly conserved residues, small structural differences of both AHPs and AHK_{RD}s seem to be sufficient for determination of specific molecular recognition as could be seen by our bioinformatical and structural comparisons.

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P10

RNA STRUCTURE PREDICTION BY KNOWLEDGE-BASED STATISTICAL POTENTIALS AND FRAGMENT ASSEMBLY

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New RNA structure prediction tools are needed to fast obtaining detailed structural information of new non-coding RNA sequences. Here we propose to use knowledge-based statistical potentials and a fragment-based modeling approach as input to predict RNA structure from sequence. We have downloaded a dataset composed of all x-ray determined RNA 3D structures from the Protein Data Bank (PDB). From the initial 1940 files, 3082 different RNA structures were selected after filtering small sequences (<20 nucleotides) and structures without base-pairing. The CD-HIT program was used on those sequences in order to derive the sequence families, which were 304 after filtering. With this dataset we have calculated several general statistics as percentage of canonical and non-canonical base pairs, stacking, sequence length and RMSD resolution. The SARA method as was used for generating an all-against-all alignment of the best representative of each RNA family from our dataset. Those alignments are being used as an input into the RNADOM program in order to derive a set of conserved RNA fragments. At the same time a set of structural properties was determined in order to describe the RNA structure, so the most informative ones will be checked for conservation against the fragment dataset, obtaining a series of knowledge-based statistical potentials from this dataset.

P11

INFLUENCE OF THE SUBSTITUTION EFFECTS IN THE PYRIDINE RING ON THE REACTIVITY OF THE TRANS-$[Pt(NH_3)_2(PYR)CL]^+$ COMPLEX

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Platinum anticancer complexes are administered in its inactive form and the hydrolysis step is needed for their activation. The activated drug reacts rapidly with DNA or proteins and the hydrolysis step is the rate determining step of the whole process. Thus, the modification of the speed of hydrolysis is one of the ways of a new drug development. In the present work we have studied the kinetics of the aquation reaction on the trans-$[Pt(NH_3)_2(pyr\text{-}X)Cl]^+$ complex (X = OH^-, Cl^-, NO_2^-, NH_2, SH, CH_3). All possible positions *ortho*-, *meta*- and *para*- of the substituent X in the pyridine ring were considered and reaction energy profiles, atomic charges, electron densities at bond critical points, ligand binding energies were calculated. All the structures along the reaction pathways were fully optimized using B3LYP/MWB-60(f)/6-31+G* method. Single point energies and molecular properties were evaluated by B3LYP/MWB-60(2fg)/6-311++G(2df,2pd) method.

The substituent ligand influences electron density on the pyridine ring and thus the electron donating ability of the heterocyclic nitrogen. The substituent ligand on the pyridine ring can be ordered according to their ability to promote the aquation reaction. The largest selectivity offers the *ortho*- position which offers almost 400 times difference in the rate of the reaction between the fastest *o*-NH_2 and the slowest *o*-Cl^- ligand: $NH_2 > OH^- > SH^- > CH_3 > NO_2^- > H > NO_2^- \sim Cl$.

This work is supported by the Czech Science Foundation (grant No. 208/12/0622).

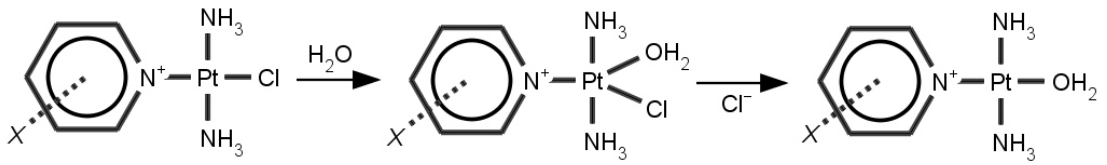

Figure 1. Reaction mechanism of the aquation reactions.

P12

CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF NEPENTHESIN-1

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Carnivorous pitcher plants of the genus Nepenthes secrete their own aspartic proteases, nepenthesins, to digest prey. Nepenthesins differ significantly in sequence from other plant aspartic proteases. This difference, which brings more cysteine residues into the structure of nepenthesins, in conjunction with putative N-glycosylation, can contribute to uniquely high temperature and pH stabilities of these proteases [1, 2].

In continuation of our previous study of the expression and biochemical and enzymatic characterization of a recombinant form of nepenthesin-1 (rNep-1) from Nepenthes gracilis [3], we report its crystallization and preliminary X-ray analysis. Crystals of rNep-1 in complex with the pepstatin A inhibitor have been grown using the hanging-drop vapour-diffusion technique. Diffraction data were collected to 2.9 Å resolution using synchrotron radiation at Bessy II of HZB, Berlin. The crystals belong to space group $P2_1$, with unit-cell parameters a = 54.4 Å, b = 86.6 Å, c = 95.8 Å, = 106°. The self-rotation function combined with solvent-content calculations and Matthews coefficient suggest presence of two molecules of rNep-1 in the asymmetric unit.

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P13

AMINOALDEHYDE DEHYDROGENASE ISOENZYMES FROM *PISUM SATIVUM* OXIDIZE *N*-ACYLATED AMINOALDEHYDES

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Plant aminoaldehyde dehydrogenases (AMADHs, EC 1.2.1.19) convert -aminoaldehydes to -amino acids. They play an important role in the catabolism of polyamines and production of various osmoprotectants. Plant AMADHs are usually homodimers with a subunit molecular mass of 50-70 kDa and show a typical structural fold of aldehyde dehydrogenases. Their substrate specificity is broad as they oxidize natural -aminoaldehydes originating from polyamines, -trimethylaminoaldehydes, aliphatic aldehydes as well as benzaldehyde or synthetic nitrogen-containing heterocyclic aldehydes. In this work, we tested *N*-acylated derivates of 3-aminopropionaldehyde (APAL) and 4-aminobutyraldehyde (ABAL) as substrates of two AMADH isozymes 1 and 2 from pea (*Pisum sativum*). All *N*-acyl- -aminoaldehydes were prepared by *N*-acylation of APAL or ABAL using chlorides of carboxylic acids and a heterogeneous catalysis with KF-Celite. We measured catalytic activity of both isozymes in the oxidation of sixteen different acylated aminoaldehydes comprising either a linear or branched acyl chain. All tested compounds were substrates of the two AMADHs. APAL derivatives were found better substrates than the corre-

sponding ABAL derivatives because of the higher relative reaction rate, lower K_m value and higher V/K_m ratio. Differences between substrates with a different length or branching of the acyl chain were not as significant as those between APAL and ABAL derivatives containing the same acyl chain. The conversion of the substrates was verified by LC-MS analysis of reaction mixtures. Substrate binding was evaluated also by molecular docking. In conclusion, the presence of a C2-C5 acyl chain bound to the amino group of the -aminoaldehydes APAL and ABAL does not prevent from the enzymatic conversion by plant AMADHs.

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P14

STRUCTURE AND FUNCTION OF THE C-TERMINAL HELICAL DOMAIN OF THE MOTOR SUBUNIT HSDR FROM THE TYPE I RESTRICTION-MODIFICATION SYSTEM ECOR124I

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The published structure of the HsdR subunit of EcoR124I [1] suggested the motor subunit to be a planar array of four functionally integrated domains, with the fourth, C-terminal domain being all helical and implied to play a role in complex assembly and/or DNA binding. However, the last 150 amino acids if this domain are unresolved in the crystal structure. A single point mutation lead to a new crystal structure that allowed to trace the backbone of the unresolved C-terminal residues, and homology and energetic modeling was applied to generate an all-atom 3-D model of wild-type HsdR and complemented by in vivo and in vitro studies to establish the function of the helical domain. In vitro DNA cleavage assays, gel mobility shift assays and in vivo restriction tests were performed on WT and mutant HsdR with selectively deleted parts of the helical domain. Our results strongly support the suggested role in subunit interaction and demonstrate the importance of the C-terminus in complex assembly.

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P15

ASSEMBLY AND MOLECULAR ARCHITECTURE OF THE TSC1-TSC2 COMPLEX

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The TSC1-TSC2 complex plays an important role in the mechanistic target of rapamycin (mTOR) signalling pathway that integrates signals from extracellular growth factors, nutrients, energy deficit or inflammation and downstream controls cell metabolism and growth [1]. TSC1 is a ~130 kDa hydrophilic protein that shows no sequence homology to other known vertebrate proteins and likely guides and stabilizes the proper assembly of the TSC1-TSC2 complex [3]. TSC2 is a ~200 kDa protein that contains a conserved 163 amino acid region close to the C-terminus that is homologous to GTPase activating proteins (GAPs). The TSC1-TSC2 complex possesses RHEB GAP activity [4], and conversion of active GTP-bound RHEB into the inactive GDP-bound form by the complex downregulates the activity of mTOR complex 1 (TORC1) [1]. Pathogenic mutations in either *TSC1*) or *TSC2* lead to tuberous sclerosis complex (TSC), an autosomal dominant disorder characterized by neurological symptoms, skin and renal abnormalities [2]. Recently, it has been shown that TSC1 and TSC2 assemble into high molecular weight complexes (>1 MDa) [5,6]. However, the molecular architecture of this complex is unknown. We co-expressed epitope-tagged TSC1 and TSC2 in HEK 293T cells and imaged the affinity purified TSC1-TSC2 complexes by nega-

tive stain electron microscopy. Micrographs showed small rings of the complex that had a disk-like appearance. Initial alignments and classification of ~1000 particles revealed two kinds of rings: a smaller ring with ~70-Å diameter that seems to be composed of 5 subunits and a larger ~90-Å ring structure that may correspond to a heteroctamer of four TSC1 and four TSC2 subunits, according to the estimated average molecular mass of the complex. Ongoing image analysis aims to identify the different subunits in the two respective complexes and provide the structural basis for their function.

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P16

PHOTOSYSTEM II PSBO PROTEIN FROM HIGHER PLANTS

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Photosystem II (PSII) is a huge complex of proteins in thylakoid membranes of algae, higher plants, and cyanobacteria that conducts light-driven water oxidation and produces molecular oxygen, electrons and protons. The splitting of water and release of oxygen appear in the catalytic centre of PSII – the oxygen-evolving centre (OEC) that contains manganese-calcium cluster (4:1 Mn:Ca) situated near to the luminal surface of the transmembrane domain and hemmed by intrinsic and extrinsic components in thylakoid membranes. PsbO (33kDa), PsbP (23kDa), PsbQ (17 kDa), PsbR (10 kDa) are extrinsic proteins attached to the luminal side of PSII in higher plants, which keep stability of water oxidation site and right ionic environment during oxidation of water. The aim of our project is to receive recombinant PsbO proteins from *Spinacia oleracea* and *Pisum sativum*. The isolation of mRNA from leaves and convertion to cDNA were our first attempts to obtain the *psbO* gene. The conditions of polymerase chain reaction (PCR) were adjusted and DNA fragments encoding the *psbO* gene were acquired. Vector pBluescript II SK(+) was utilized as a cloning vector and pET-28b(+) vector was employed for overexpression of recombinant PsbO/HisPsbO proteins. Purification, crystallization and structure designation of the recombinant proteins PsbO/HisPsbO of the oxygen-evolving complex from *Pisum sativum* and *Spinacia oleracea* are under construction.

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P17

MOVING HILLS - NEW PARALLELIZABLE METADYNAMIC METHOD WITH FIXED AMOUNT OF BIAS POTENTIAL

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In the last seven years, there has been significant slowdown in the increase of computational power of single processor core. This limitation in molecular simulations was overcome by parallelization. But parallelization of the molecular dynamics and so the metadynamics, which we are focus on, is also limited, because of an increased time spent for interprocess communication. One way to overcome the problem is to reduce amount of data, which need to be transferred between processes. Our contribution to this problematics is Moving hills method. This method use multiple parallel simulation, same as Multiple walkers, but only one bias potential is assign to each parallel and position of the potential is updated according immediate state of the parallel. So the bias hill travels on the energy landscape as the current conformation is evolving. Shape of bias potential summed from all parallels converges as the system reaching an equilibration. The method was tested on small molecule and results were compared with other metadynamic methods. No significant differences in obtained free energy landscape were found.

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P18

INTERACTIONS BETWEEN RECEIVER DOMAIN OF CYTOKININ RECEPTOR CKI1$_{RD}$ AND AHP PROTEIN FROM *ARABIDOPSIS THALIANA*

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In our project, we studied the signaling pathway, called multistep phosphorelay signaling system in the plant *Arabidopsis thaliana*. The multistep phosphorelay signaling system has a great influence on many aspects of growth and development of plants. This signaling system is based on phosphate transfer between the cytoplasmatic membrane and nucleus. In the plant *Arabidopsis thaliana*, histidine kinase is phosphorylated upon signal recognition, and forwards the phosphate group through histidine phosphotransfer proteins to a response regulator protein located in nucleus, where the response take place. The input signal can be light, osmotic changes or hormones. The interactions between the two proteins were studied. The first protein was CKI1$_{RD}$ (Cytokinin independent 1 - receiver domain) and the second was protein from the AHP (*Arabidopsis* histidine phosphotransfer protein) family, both involved in the multistep phosphorelay. The interactions between these two proteins were studied by nuclear magnetic resonance. The proteins were expressed in *E.coli* and the protein CKI1$_{RD}$ was labeled by stable isotope ^{15}N.

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P19

CRYSTALLIZATION OF GLYCERALDEHYDE DEHYDROGENASE FROM *THERMOPLASMA ACIDOPHILUM*

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Due to the depletion of natural fossil resources, the development of environmentally gentle fuel and chemicals production processes is of key importance. One of such biotechnological approaches is a cell-free process called Synthetic Cascade Biomanufacturing.

The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (TaAlDH) is a part of cell-free system for production of isobutanol and ethanol from glucose. It participates in oxidation of D-glyceraldehyde to D-glycerate in this synthetic pathway. Wild type of TaAlDH has high substrate selectivity and product tolerance but leaves place for optimization. A mutant of TaAlDH (F34M+Y399C+S405N), which has a 55.7-fold increased activity of the wild type enzyme, enhanced NAD^+ acceptance and a slight solvent tolerance improvement, was obtained by a directed evolution approach.

For further optimization of TaAlDH functioning within the isobutanol and ethanol synthetic pathway structural information about the enzyme variant and its complex with the substrate is required. For this purpose crystallization of the enzyme by itself and in complex with cofactor or substrate, what will be followed by X-ray diffraction analysis, is used. Screening of crystallization conditions was performed by means of different crystallization screens using Gryphon crystallization robot (Art Robbins Instruments, USA).

Some hits were found in several conditions of Morpheus screen (Molecular Dimensions Ltd., UK). Results are well reproducible; crystals grow quite big (0.1 x 0.4 mm) but plane and multilayer. Preliminary diffraction analysis revealed bad quality of crystals, which diffracted only to 18-20 Å, so further optimization is necessary. For optimization diverse techniques were consequentially applied: variation of protein and precipitant concentrations, different pH of buffer, additive screen, addition of NAD^+ and glyceraldehyde and microseeding using Seed Bead (Hampton Research, USA). Certain improvement of crystals was observed in microseeding experiments.

P20

CHARACTERIZATION OF THE CALMODULIN AND S100A1 BINDING DOMAINS ON THE N-TERMINUS OF TRPM1 RECEPTOR

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Transient receptor potential melastatin 1 (TRPM1) belongs to the TRP family of non-selective cation permeable channels. TRPM1 is expressed in human melanocytes and bipolar cells in retina and participates in processes connected to vision. Mutations of TRPM1 gene are associated with congenital stationary night blindness in humans. [1, 2]

It is assumed that TRPM1 channel has six transmembrane domains with a pore domain between the fifth and the sixth segments. Intracellularly located N- and C-tails are responsible for regulation of TRP channels, which carry binding sites for signal molecules like calmodulin (CaM) or S100A1. [3]

In this study two independent CaM /S100A1 binding sites on the intracellular N-terminus of rat TRPM1 were characterized. Using bioinformatic approach we identified Ca^{2+}-dependent CaM /S100A1 binding sites in regions L242-E344 and A451-N566. Several basic and hydrophobic amino acid residues responsible for binding in these regions of TRPM1 to CaM and S100A1 were determined.

Fusion proteins were expressed in *E.coli* and purified according to the two-step purification protocol. Amino acid sequence was checked by mass spectroscopy. Fluorescence anisotropy, surface plasmon resonance and measurement of CD spectra were used to characterize the interactions between the binding sites of TRPM1 N-terminus and CaM /S100A1. Interactions were modeled using molecular molecular modeling. Experimental data also indicate that CaM and S100A1 bind to the same or overlapping binding sites.

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P21

STRUCTURAL STUDIES ON PDC/14-3-3 PROTEIN COMPLEX

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Phosducin (Pdc), a highly conserved 30 kDa phosphoprotein, regulates visual signal transduction by interacting with the beta and gamma subunits of the retinal G-protein. Pdc was also suggested to be involved in transcriptional control, the regulation of transmission at the photoreceptor-to-ON-bipolar cell synapse, and the regulation of the sympathetic activity and blood pressure. The function of Pdc is regulated by phosphorylation at Ser54 and Ser73 in a process that involves the binding of phosphorylated Pdc to the regulatory 14-3-3 protein. The 14-3-3 proteins are highly conserved dimeric molecules that regulate the function of other proteins through a number of different mechanisms. The exact role of the 14-3-3 protein in regulating Pdc function is still unclear, but it is entirely possible that 14-3-3 either sterically occludes and/or affects the structure of Pdc. Both 14-3-3 binding motifs are located within the N-terminal domain of Pdc, which participates in the binding to the beta and gamma subunits of the retinal G-protein as well as contains the SUMOylation site Lys-33.

Our previous study revealed that phosphorylated Pdc and the 14-3-3 protein form a stable complex with 1:2 molar stoichiometry. Complex formation with 14-3-3 affects the structure and reduces the flexibility of both the N- and C-terminal domains of dpPdc, suggesting that dpPdc undergoes a conformational change when binding to 14-3-3. To further investigate this interaction and mainly the 14-3-3 protein-mediated conformational changes of Pdc,

we performed structural studies using Bio-NMR, SAXS and tryptophan fluorescence whose results are presented here.

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P22

STRUCTURE-FUNCTION STUDY ON ENZYMES LINKED TO CYTOKININ METABOLISM - ALDEHYDE DEHYDROGENASES AND NUCLEOSIDE N-RIBOHYDROLASES

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Plant hormones cytokinins are irreversibly inactivated by cytokinin oxidase/dehydrogenase (CKO). Adenosine/adenine moiety and corresponding aldehyde are produced in this metabolic pathway. We focused on two enzyme groups possibly involved in metabolism of these products. They include aldehyde dehydrogenases (ALDHs) oxidizing aldehydes to corresponding acids and nucleoside N-ribohydrolases (NRHs) catalyzing conversion of adenosine to adenine. While nucleoside N-ribohydrolases (E.C. 3.2.2.-) belong to the family of hydrolases catalyzing the cleavage of the N-glycosidic bond in purine and pyrimidine nucleosides, aldehyde dehydrogenases (ALDHs) are $NAD(P)^+$-dependent oxidoreductases (EC 1.2.1.-). ALDHs are considered as general detoxifying enzymes, which eliminate biogenic and xenobiotic aldehydes to the corresponding carboxylic acids. Up to date, 13 ALDH families have been described in plants but only a small number of the enzymes have been functionally characterized despite the existence of a large number of coding genes. We analyzed ALDH2 and ALDH7 families from maize (*Zea mays*). The ALDH2 family expanded significantly during evolution of terrestrial plants and the number of family members varies substantially between species. Maize ALDH2 (ZmALDH2) includes six genes coding for mitochondrial and cytosolic enzymes while ALDH7 family includes only one gene. Some ALDH2 family members were originally identified as genes restoring fertility in plants (called RF2), which have the ability to suppress the male-sterile phenotype and restore the production of pollen to plants carrying the deleterious mitochondrial genome. Two genes *RF2C* and *RF2F* coding for two ZmALDH2 enzymes and the gene coding for ZmALDH7 were cloned and expressed in *E. coli*. The obtained recombinant proteins were thoroughly characterized and their identity was verified by MALDI-TOF peptide mass fingerprinting. All studied ALDHs utilized NAD^+ but not $NADP^+$ as a coenzyme. They were crystallized and their crystal structures were solved. They represent the first structures of plant ALDH2 and ALDH7 family members. Gene expression analysis using TaqMan probes revealed RF2C as a predominant *ALDH2* gene expressed in all plant tissues while other *ALDH2* genes are less abundant and specific to some developmental stages.

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P23

STRUCTURAL AND CATALYTIC PROPERTIES OF -L-FUCOSIDASE FROM *PAENIBACILLUS THIAMINOLYTICUS*

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-L-Fucosidase is a glycosidase with ability to catalyze removal of -L-fucose residues from the non-reducing terminus of glycoconjugates. This enzyme is also able to catalyze transglycosylation reactions. Fucosylated oligosaccharides have an important role in many biological processes, they can also be used in pharmaceutical and food industry. An application of enzymes could facilitate the process of synthesis of these compounds. Previous studies have shown that -L-fucosidase from *Paenibacillus thiaminolyticus* may have great potential for synthesis of glycosylated molecules. Recombinant form of this enzyme was prepared. The protein was purified and characterized. Several sugar acceptors were tested in transglycosylation reactions. 3D-model of -L-fucosidase will be prepared. Crystallization experiments will be carried out provided that a sufficient quantity and appropriate purity of this enzyme is obtained.

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P24

STUDIES OF SELF-ASSEMBLY PROPERTIES OF *BACILLUS* SPORE COAT PROTEINS

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When challenged by starvation, *Bacillus subtilis* produce a dormant cell type, called a spore. Spores are distinctive by a unique capability to withstand extreme environmental conditions. Fundamental importance to spore resistance but also for its germination is the coat. The coat is proteinaceous multilayered shell that provides mechanical integrity and excludes large toxic molecules while allowing small nutrient molecules to access germination receptors beneath the coat. Spore coat is formed by over 70 proteins, ranging in size from about 6 to 70 kDa. The process of the coat assembly which is rather poorly understood, represents a central objective of this research. We have focused on a small group of proteins, called morphogenetic which act in controlling the deposition of the various coat components around the forming spore, namely SpoIVA, SpoVID, SafA and CotE. We are also interested in proteins localized directly on the spore surface - in the outermost part of the coat called crust CotY, CotZ, CotV and CotW. In our previous work we examined the protein-protein interactions between these proteins using genetic, biochemical and biophysical methods (Krajcikova et al., 2009, Mullerova et al. 2009, Qiao et al., 2012, Qiao et al., 2013). Besides confirming the previously revealed interaction between SpoVID and SafA (Costa et al. 2006) we showed that there are also direct contacts between the other key morphogenetic proteins SpoVID-SpoIVA, SpoVID-CotE and SpoVID-SpoIVA. Our data imply that due to the physical association the crucial morphogenetic proteins can form a basic skeleton where other coat proteins would be attached. We have also revealed that the crust proteins CotY, CotZ and CotW, CotV form a protein complex and most of the proteins have a high tendency to make the homo-oligomers.

Consequently we investigated the self-assembly properties of spore coat proteins by electron microscopy. Currently, there are no structural information about the individual coat components. In order to gain insights into the structure of the spore coat of *B. subtilis* we have prepared a set of recombinant coat proteins including CotY, CotE, CotZ, CotV, CotW, SafA and SpoVID and analyzed these proteins both as single species and in combination. A number of self-assembled structures including two-dimensional crystals and helical fibers were discovered thus clearly indicating that coat proteins have an intrinsic ten-

dency for self-organization into higher order assemblies. We conclude that these assemblies could play a role in coat formation.

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P25

INTERACTION OF M-PMV MATRIX PROTEIN MUTANTS WITH MEMBRANES

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Mason-Pfizer monkey virus (M-PMV) is a betaretrovirus which is used for studies of the late phase of the retroviral life cycle. Matrix protein is the N-terminal part of the main structural polyprotein Gag of the virus, which is important for the transport of immature viral particles to the plasma membrane and interaction with it. Previously we described structure determination of two double mutants of the matrix protein, i.e., T41I/T78I and Y28F/Y67F that are deficient in budding. In this work we studied the interaction of the wild-type matrix protein and the double mutants with main cellular phospholipids. For NMR measurements water-soluble phospholipids with fatty acid residues with 8 carbons were used instead of insoluble phospholipids with natural fatty acids. Series of NMR experiments were measured in which we observed chemical shift changes in 2D ^{1}H-^{15}N HSQC spectra to define interacting residues of the proteins. For the determination of the equilibrium dissociation constants K_D of individual interacting pairs we used chemical shift changes of ^{31}P spectra.

P26

DETECTION OF MOLECULAR CHIRALITY IN LANTHANIDE COMPLEXES BY THE RAMAN OPTICAL ACTIVITY

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Since conventional structural analysis offers rather limited means for the chirality detection, a series of lanthanide tris-(-diketonates) was developed as effective receptors for a better chirality sensing in biomolecular substrates. These lanthanide complexes containing -diketonate ligands are electrically neutral; they can further coordinate with various small organic molecules such as chiral alcohols, amino alcohols or amino acids in organic solvents and produce a strong chiral signal.

In the current study, interaction of the [Eu(FOD)$_3$] complex with (*R*)- and (*S*)- enantiomer of 1-PhenylEthanol in n-hexane was studied using Raman Optical Activity (ROA) and ultraviolet circular dichroism (UVCD) spectroscopies. Since resonance phenomenon in Raman scattering was observed in the studied systems due to the correspondence of Europium electronic transition energy to the laser excitation wavelength, about a 10^2-fold enhancement if compared to non-resonant vibrational ROA. This enabled shorter detection times as well as lower sample concentrations.

The UVCD was used to support the results obtained by ROA measurements and determine the metal/ligand ratio in the complexes. Although lanthanide tris- (-diketonates) are silent in the UVCD spectra, symmetric UVCD signals induced around 300 nm were observed for [Eu(FOD)$_3$] upon complexation with (*R*)- or (*S*)-1-PhenylEthanol. Further experiments with structural variations of lanthanide tris-(-diketonate) complexes and theoretical modeling can further refine the chirality sensing systems for chiral recognition in biological substrates.

P27

PROTEIN CRYSTALLIZATION PROJECTS AT THE FACULTY OF SCIENCE OF THE USB CB

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The protein crystallography is one of the methods used to study the protein structures and to describe their function and mechanism. The most important and necessary condition to used protein crystallography is obtaining of well-diffractable monocrystals. Different crystallization techniques such as standard, advanced and alternative methods are used to crystallize soluble and membrane proteins and protein complexes. Research projects are designed for graduate students and postgraduates who work on these projects on different levels. Finally developed methods and obtained crystallization and crystallographic data are compiled and results are published in scientific journals.

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P28

STRUCTURAL CHARACTERIZATION OF THE THIOREDOXIN-BINDING DOMAIN OF PROTEIN KINASE ASK1 AND ITS INTERACTION WITH THIOREDOXIN

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis in various cells and plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin (Trx) and the 14-3-3 protein that both function as inhibitors of ASK1 [1]. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear.

To better understand the role of Trx binding in the inhibition of ASK1, we prepared the isolated Trx-binding region of ASK1 (ASK1-TBD), performed its structural and biophysical characterization and studied its interaction with Trx under both reducing and oxidative conditions. Data obtained from analytical ultracentrifugation, time-resolved fluorescence anisotropy measurements, circular dichroism and small-angle X-ray scattering suggest that: (1) ASK1-TBD is a compact monomeric and rigid domain that under reducing conditions forms with Trx a stable and well defined complex with 1:1 molar stoichiometry; (2) the structural integrity of the catalytic Trp-Cys-Gly-Pro-Cys motif of Trx is essential for its binding to ASK1-TBD; (3) Trx interacts with the region of ASK1-TBD located in the vicinity of Cys250; and (4) Trx binding does not induce significant structural change of ASK1-TBD. In addition, it seems that the interaction between ASK1-TBD and Trx does not involve the disulfide bond formation, as has been suggested in the literature [2,3].

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P29

KLK2- AND KLK11-BINDING PROTEINS AS NOVEL CLASS OF RECOMBINANT CAPTURE LIGANDS USEFUL FOR IMPROVED PROSTATE CANCER DIAGNOSTICS

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Human kallikrein 2 (KLK2) and kallikrein 11 (KLK11), members of the kallikrein gene family of trypsin-like serine proteases, have recently been indicated as novel promising oncomarkers of the prostate cancer (PC), the second most common cause of male cancer-related deaths in the Western world. Currently, the only clinically validated diagnostic approach for the PC diagnosis relies on serum level detection of Prostate Specific Antigen (PSA), known also as KLK3, and the assessment of the ratio between total and free PSA. Yet clinically largely used, the antibody-based PSA assay fails in prediction of early stages of PC and does not precisely distinguish between malign form of the PC and benign prostate hyperplasia. This leads to the identification of group of false indicated PC patients that have to undergo cost-effective and risky prostate tissue biopsy. To overcome this problem together with limitations of PSA detection for the early detection of the PC, there is a need to develop multifactorial ELISA kits or novel biosensors, as more complex PC prognosticators, for more accurate prediction of cancer presence, stage and metastasis.

Here we present generation and characterization of novel binders targeting human KLK2 (called KLA binders) and KLK11 (HIP binders) that were selected by ribosome display from a high-complex combinatorial library, derived from an albumin-binding domain (ABD) scaffold. We demonstrate a preliminary assessment of binding properties of the most promising KLA and HIP variants and their thermal stability studies performed by Thermal shift assay (TSA).

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P30

THE ROLE OF THE NON-PHARMACOPHORE POCKET OF GLUTAMATE CARBOXYPEPTIDASE II IN THE DESIGN OF SMALL-MOLECULE INHIBITORS

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Inhibitors targeting human glutamate carboxypeptidase II (GCPII) are used in various diagnostic and therapeutic applications and typically consist of a C-terminal docking moiety and a functionalized distal moiety linked *via* a zinc-binding group. While details of interactions between GCPII the C-terminal part of an inhibitor are reasonably well established, there is a limited amount of structural information related to interactions between GCPII and distal functionalities. Here we present a comprehensive structural study aimed at dissecting the impact of physicochemical characteristics of distal functionalities on GCPII binding/affinity. To this end we determined crystal structures of eight complexes between GCPII and phosphoramidate-based inhibitors featuring an invariant P1' glutamate and a variable P1 distal functionality. While the positioning of the phosphoramidate-glutamate functionality in the GCPII binding site is identical for all compounds tested, there are profound differences in GCPII interactions with distal groups and those are reflected in varied affinities and inhibition modes. Our data unravel a complexity of different binding modes of inhibitors within the S1 site of GCPII and can be exploited for the design of novel GCPII-specific compounds.

P31

CRYSTAL STRUCTURES OF COMPLEXES OF GCPII WITH P1'-DIVERSIFIED UREA-BASED INHIBITORS

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Urea-based inhibitors of human glutamate carboxypeptidase II (GCPII) have advanced into clinical trials for imaging prostate cancer and its metastases. In parallel efforts, agents with increased lipophilicity are designed and evaluated for targeting GCPII residing in the neuronal compartment. Here we report the structural and computational characterization of six complexes between GCPII and P1'-diversified urea-based inhibitors that have the C-terminal glutamate replaced by a more hydrophobic moiety. Our results provide a detailed description of interactions governing the recognition of non-glutamate residues in the S1' pocket of the enzyme. We observed the flexibility of the S1' pocket, most notably that of Phe209, Leu428, and Lys699 side chains, as well as the structural rearrangement of the amino acid segment Leu259 – Gly263, that allows GCPII to accommodate non-glutamate moieties at the S1' site. The X-ray structures are complemented by the quantum mechanics calculations that provide a quantitative insight into the GCPII/inhibitor interactions. Presented data can be used for the rational design of novel glutamate-free GCPII inhibitors with improved physicochemical properties.

P32

ASSEMBLY AND MOLECULAR ARCHITECTURE OF PI4K II COMPLEXES

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The phosphatidylinositol 4-kinases (PI4K) catalyze the production of phosphatidyl inositol phosphates (PIPs) that regulate membrane associated signal transduction and protein trafficking in eukaryotic cells [1]. The PI4Ks are also hijacked by several RNA viruses to generate membranes enriched in phosphatidylinositide 4-phosphate lipids that are used as viral replication platforms [2]. Two types of PI4K's have been identified in mammalian cells: the type II kinases (and isoforms) that are stably associated with membranes and the type III kinases (and isoforms) that are soluble and remain in cytosol [3]. While type III kinases share homology to structurally well characterized PI3 kinases the structure of type II kinases is unknown.

We expressed and purified a recombinant PI4K II kinase and discovered that it can assemble into large multimeric complexes. Here, we present the initial structural analysis of these complexes that were imaged by negative stain and cryo electron microscopy. Electron micrographs showed heterogeneous particles of globular shape ranging from 110 to 310 Å in diameter. The particles were separated into three groups based on their overall diameter, aligned and classified in 2D using EMAN package. Most particles (~70%) appeared as small compact spheres of ~110 Å diameter. About 17% particles had an oval shape with dimensions ~250 x 200 Å. Interestingly, about 13% of the particles formed large rings with ~310 Å outer diameter, ~140 Å thickness and ~90 Å inner hole. While the small particles likely represent a tight assembly of several PI4KII molecules (54 kDa), the two larger particles exhibit similar architecture as some other membrane binding proteins – respectively, the intermediate particles appear as compact assemblies of the human caveolin-3 protein [4] and the large rings are not dissimilar to assembled ESCRT proteins at the necks of budding viruses from the cell membrane [5].

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P33

CRYSTALLIZATION AND PRELIMINARY DIFFRACTION ANALYSIS OF DHA57, DHAA80 AND DHAA106 MUTANTS FROM *RHODOCOCCUS RHODOCHROUS* NCIMB 13064

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Haloalkane dehalogenases catalyze a reaction of great environmental and biotechnological significance: conversion of halogenated aliphatic hydrocarbons to their corresponding alcohols. Mutagenesis, focused on the entrance of tunnels in DhaA structure, produced protein variants with significantly improved activity towards 1,2,3-trichloropropane (TCP). The DhaA57, DhaA80 and DhaA106 proteins were constructed in order to determine the 3D structure of the mutants at atomic resolution to explore the importance of tunnel's mutation to the enzymatic activity to TCP degradation.

The DhaA mutant forms were crystallized using the sitting-drop vapor-diffusion method. Diffraction data for DhaA57 and DhaA80 proteins were collected at the MX14.2 beamline operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II electron storage ring (Berlin-Adlershof, Germany), data sets for DhaA106 were collected at the X-ray diffraction station at the X-ray diffraction station at the Institute of Molecular Genetics AS CR in Prague. Diffraction data sets for DhaA57, DhaA80 and DhaA106 proteins were gathered to the 1.20 Å, 1.45 Å and 1.69 Å resolutions, respectively.

The DhaA57 and DhaA80 crystal structures were solved characterized and deposited in RCSB protein data bank under 4F5Z pdb code for DhaA57 and 4F60 pdb code for DhaA80 protein. Model building and refinement of the DhaA106 protein is currently in progress.

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P34

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF *RALSTONIA SOLANACEARUM* LECTIN MUTANT

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Ralstonia solanacearum is a gram-negative plant pathogen with wide radius of hosts (potato, tomato, banana, …). Among other ways it uses carbohydrate-binding proteins as a strategy to invade a plant. Understanding of this interaction can help in prevention or treatment of infections caused by *R. solanacearum.*

Sugar binding protein RSL from *Ralstonia solanacearum* was previously characterized in our group [1]. In this project, we examined a two-point mutant RSL_S15 QH60Q produced in the *E.coli* host cells. FPLC on the mannose-agarose column was used to purify the protein, which was subsequently subjected to in-depth analysis. Isothermal titration calorimetry was used to assess thermodynamic parameters of protein binding to various ligands. Protein showed the highest affinity towards fucose-containing saccharides. Also, near-UV CD spectroscopy revealed changes of tryptophan peaks while being titrated with L-fucose. The protein was crystallized by a hanging drop technique and crystals were subsequently used to determine the structure using X-ray crystallography. Diffraction data were collected at high resolution of 1.0 Å and

structure was solved by molecular replacement using the native protein structure.

At the same time, other mutants of the lectin are studied in our group. This combined approach enables us to deeper understand the relationship between lectin sequence-structure and its binding properties including fine specificity towards various ligands.

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P35

STRUCTURAL ANALYSIS OF A NOVEL HALOALKANE DEHALOGENASE DBEA FROM *BRADYRHIZOBIUM ELKANII* USDA94

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A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkanii* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. The DbeA protein was crystallised using the sitting-drop vapour-diffusion method. The crystals of DbeA belong to the primitive orthorhombic space group $P2_12_12_1$. Crystal structure of a DbeA enzyme has been solved and refined to 2.2 Å resolution. The enzymatic molecular structure of DbeA was compared with those of known haloalkane dehalogenases already deposited in Brookhaven Protein Data Bank.

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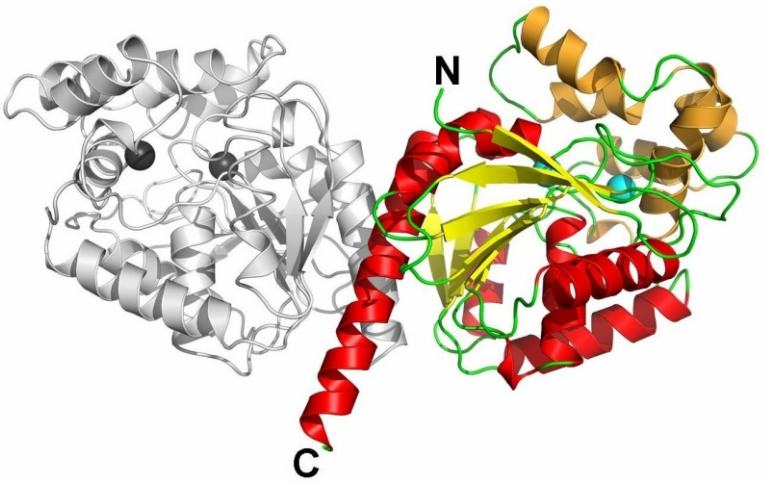

P36

MOLECULAR DYNAMICS COMPARISON OF *E. COLI* WRBA APOPROTEIN AND HOLOPROTEIN

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WrbA is a novel multimeric flavodoxin-like protein of unknown function. A recent high-resolution X-ray crystal structure of *E. coli* WrbA holoprotein revealed a methionine sulfoxide residue with full occupancy in the FMN-binding site, a finding that was confirmed by mass spectrometry. In an effort to evaluate whether methionine sulfoxide may have a role in WrbA function, the present analyses were undertaken using molecular dynamics simulations in combination with further mass spectrometry of the protein. Methionine sulfoxide formation upon reconstitution of purified apoWrbA with oxidized FMN is fast as judged by kinetic mass spectrometry, being complete in ~5 hours and resulting in complete conversion at the active-site methionine with partial conversion at second, heterogeneous sites. Analysis of methionine oxidation states during purification of holoWrbA from bacterial cells reveals that methionine is not oxidized prior to reconstitution, indicating that methionine sulfoxide is unlikely to be relevant to the function of WrbA *in vivo*. Although the simulation results, the first reported for WrbA, led to no hypotheses about the role of methionine sulfoxide that could be tested experimentally, they elucidate the origins of the two major differences between apo- and holoWrbA crystal structures, an alteration of inter-subunit distance and a rotational shift within the tetrameric assembly. Furthermore the MD results hint the possibility of communication between binding sites suggesting that FMN binding to WrbA might be cooperative. Indeed, a ready mechanism for allosteric signal transmission exists in the fact that each FMN binding site comprises residues from three of the four WrbA subunits. However there are no conclusive experimantal results so far.

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PUMP-PROBE MOLECULAR DYNAMICS (PPMD) SIMULATIONS IN GROMACS AND ITS TESTING ON THE PDZ DOMAIN

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Despite the fact that allostery plays a crucial role in the protein function, this complicated phenomenon is still not fully understood. Allostery involves gradual changes in the conformation propagating throughout the molecule on the timescales from picoseconds to microseconds. Such action is beyond the capabilities of classical MD. Pump-probe MD is based on the idea that artificial oscillating forces can be applied on selected part of the molecule (pump) and resultant signal transmits across the protein. The residues involved in the dissemination can be identified by employing the Fourier transform (probe). This method was incorporated into GROMACS programme package and different settings were tested on the PDZ domain. If the oscillation is applied on each residue in the separate simulations and all residues in each simulation are probed, it is possible to create the map of allostery, where every residue-residue interconnection should appear. Moreover these data can be statistically analyzed. One PPMD simulation is quite computationally undemanding, since we plan to establish the user-friendly scientific web-portal, where all programs and algorithms would be provided.

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P38

INTERDOMAIN COMMUNICATION IN THE ENDONUCLEASE/MOTOR SUBUNIT OF TYPE I RESTRICTION-MODIFICATION ENZYME ECOR124I

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Restriction-modification systems protect bacteria from foreign DNA. Type I restriction-modification enzymes are multifunctional heteromeric complexes with DNA cleavage and ATP-dependent DNA translocation activities located on endonuclease/motor subunit HsdR. The recent structure of the first intact motor subunit of the Type I restriction enzyme from plasmid EcoR124I suggested a mechanism by which stalled translocation triggers DNA cleavage via a lysine residue on the endonuclease domain that contacts ATP bound between the two helicase domains. In the present work molecular dynamics simulations are used to explore this proposal as the protein samples conformational space. MD suggests that the Lys-ATP contact alternates with a contact to a nearby loop housing the conserved QxxxY motif that had been implicated in DNA cleavage. This model is tested here by using in vivo and in vitro experiments. The results indicate how local interactions are transduced to domain motions within the endonuclease/motor subunit.

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STRUCTURES OF RECEPTORS AND LIGANDS OF MAMMALIAN NK CELLS

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Natural killer cells (NK cells) are large granular lymphocytes, a sort of lymphocytes discovered in the early 1970s. They comprise ca. 10 % of lymphocytes. They are able to kill virally infected and tumor cells. Unlike T-cells, the activity of NK cells is innate, they do not need to have previous experience with a tumor – they are natural killers.

We solved an X-ray structure of an extracellular part of mouse Clr-g [1], a ligand for NK receptor NKR-P1F. The diffraction data of a rod-like crystal were measured at synchrotron BessyII of HZB in Berlin and processed up to 1.95 Å. The protein forms dimer similar to that of CD69.

The dimer differs slightly from CD69 dimer in mutual orientation of both monomers. This effect was observed also for other similar protein dimers and seems to be an effect of flexibility of the dimers and not a characteristic of the dimerization mode of the protein.

A model of a complex with its binding partner, NKR-P1F, was designed based on electrostatic complementarity of both molecules. In this model, the complex is not formed in "face to face" mode, as is usually expected; rather the dimer of NKR-P1F embraces one of the monomers of Clr-g.

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P40

ENHANCEMENT OF LINEAR VS. NON-LINEAR MOTIONS IN PROTEIN FOLDING

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Time scales of molecular simulations are limited by available computational resources. To overcome this obstacle, many enhanced sampling methods have been developed. Metadynamics method disfavors previously visited states of the studied system to improve sampling in directions of selected degrees of freedom. Enhancement of sampling of linear collective motions of a molecule has been tested in the framework of metadynamics. However, atomic motions are not linear. In this project we test enhancement of non-linear collective motions in the simulation of folding of tryptophan cage mini-protein. Several folding-unfolding transitions were observed in 200 ns simulations, despite the fact that significantly longer time scales are necessary to fold this mini-protein in conventional simulations.

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CRITICAL PARAMETERS FOR S-SAD PHASING – REAL CASE EXPERIENCE

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As X-ray diffraction technologies develop, *de novo* phasing of protein structures by single-wavelength anomalous dispersion by sulphur (S-SAD) is simplified and more common. However, anomalous differences in the sulphur atomic factors are in the order of errors of measurement, which makes careful intensity reading and data processing crucial. The new structure of a small 12 kDa protein with 4 sulphur atoms per molecule was phased with anomalous data up to 2.3 Ĺ but the data did not enable a straightforward structure solution. Data processing was performed using XDS [1] and scaling using XSCALE. The sulphur substructure was determined by SHELXD [2] and phases were obtained by density modification and autotracing in SHELXE [2]. Both programmes strongly depend on input parameters and default values did not lead to the correct phases. Therefore a systematic search of optimal values of several parameters was used to find a solution. This method helped to confirm sulphur substructure and to differentiate the handedness of the solutions. Moreover, a script for comfortable conversion of SHELX outputs to MTZ format was developed, using programmes included in the CCP4 package [3]. The previously unsolvable protein structure was successfully resolved with the described procedure.

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EXPLORING FREE ENERGY SURFACE OF OXYTOCIN BY USING WELL-TEMPERED METADYNAMICS

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Well-tempered metadynamics (WTmetaD) is one of the enhanced sampling methods for free energy calculations. [1] In this work it was used to explore the free energy surface of neurohypophysial hormone oxytocin, which led to defining different stable conformations of this hormone. The difficulty of choosing the right collective variables (CVs) in the metadynamics as biased simulations was avoided by using dimensionality reduction techinuque - Principal Component Analysis, from the trajectories obtained using unbiased simulations (Molecular dynamics and Replica Exchange Molecular Dynamics). [2] The WTmetaD simulations were done in both implicit (30ns long simulation) and explicit (20ns long simulation) solvent in order to observe their possible impact on the conformation of the molecule. After exploring the free energy surface of oxytocin three distinctive energy minima were found and so we assume that it has three stable conformers.

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CRYSTALLIZATION OF FRPC PROTEIN FROM *NEISSERIA MENINGITIDIS*

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Neisseria meningitidis is a Gram-negative bacterium that colonizes the nasopharynx of approximately 10% of population without causing any symptoms. However, the bacteria can penetrate across the nasopharyngeal mucosa, gain access to the bloodstream and pass the blood-brain barrier, inducing devastating invasive meningococcal diseases such as septicemia and/or meningitis.

Under conditions of limited iron availability, *N. meningitides* was shown to produce RTX1 (repeat in toxin) family proteins secreted through the type I pathway, the so-called FrpC-like proteins. These are characterized by the presence of a variable number of carboxyl-proximal glycine and aspartaterich repetitions of a nonapeptide RTX consensus motif (L/I/F)*X*GG*X*G(D/N)D*X*. But the biological activity of meningococcal FrpC-like proteins remains unknown.

It was reported that the FrpC protein undergoes a unique calcium-dependent autocatalytic processing at an Asp-Pro peptide bond that is accompanied by formation of high molecular weight oligomeric species of FrpC that contain subunits covalently cross-linked through a new type of isopeptide bond. The results strongly suggest that FrpC is also processed and cross-linked when meningococci grow in the calcium-rich environments of the mucosal secretions of nasopharynx and/or in the plasma and liquor during invasive infections.

One of the directions to investigate the function of FrpC protein is to study its structure. And the "clip-and-link" self-processing activity is one of the crucial aspects in purification step.

Initial crystallization trials of FrpC protein were performed using the sitting-drop vapour-diffusion at both 4 ^{o}C and 18 ^{o}C. The commercial screeening kits Morpheus, Structure, Pact Premier, MIDAS, PGA Screen and JCSG+ (Molecular Dimensions, Suffolk, England), PEG Suite (Qiagen sciences, Maryland, USA), Index Screen (Hampton Research, Aliso Viejo, USA), JCscreen Plus (Jena Bioscience, Jena, Germany) were tested to determine initial crystallization conditions using Gryphon high throughput crystallization robot (Art Robbins Instruments, USA).

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DEVELOPMENT OF LACTIC ACID BACTERIUM CAPABLE OF BINDING SHIGA TOXIN ON THE SURFACE

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Lactic acid bacteria (LAB) have long history of safe usage in the dairy industry. Because of their important role in human intestine and potential health-promoting effects, LAB are considered as promising vectors for the delivery of different recombinant proteins to the human intestine. Infections with shiga toxin-producing bacteria, like enterohaemorragic *Escherichia coli* and *Shigella dysenteriae*, represent serious medical problem. Recombinant lactic acid bacteria could be engineered to bind shiga toxin on their surface by displaying binding proteins against B subunit of shiga toxin (StxB) on the surface of LAB. Small engineered binding proteins, like designed ankyrin repeat proteins (DARPins), derivatives of B domain of staphylococcal protein A (affibodies) and derivatives of albumin-binding domain (ABD) of streptococcal protein G, were reported as promising alternatives to antibodies for many applications.

In the first part of the present study, we tested the efficiency of the display of binding proteins on the surface of model LAB *Lactococcus lactis*. We have prepared and evaluated all three types of binding proteins (DARPins, B domain, ABD) on the surface of *L. lactis*.

In the second part we focused on the expression of recombinant StxB and further development of binding proteins against StxB using ABD library (1). We have prepared functional recombinant Stx1B and Stx2B as targets for selection of binding proteins. After five cycles of ribosome display and screening of positive clones with ELISA, we selected four potential Stx1B binders. Our future work will focus on further characterization of selected binders and their functional display on the surface of LAB.

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NMR STUDY OF HALOALKANE DEHALOGENASE DHAA: RESONANCE ASSIGNMENT AND RELAXATION

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Nuclear magnetic resonance (NMR) is a method well suited for studies of biomacromolecules at the atomic level. Wild type haloalkane dehalogenase DhaA, obtained from the bacterium *Rhodococcus rhodochrous*, is a 33.5 kDa enzyme capable of hydrolytic biodegradation of aliphatic alogen-hydrocarbons yielding a halide, a proton and a primary alcohol. The goal of the project was to describe the molecular motions of DhaA. As the first step, it is necessary to assign the resonance frequencies. The sample for assignment was uniformly triply labeled with ^{2}H, ^{13}C and ^{15}N isotopes and for relaxation study was doubly labeled with ^{2}H and ^{15}N isotopes. The experiments utilizing transverse relaxation-optimized spectroscopy (TROSY) were performed. The assignment of 222 residues was achieved, which represents approximately 90 % of the observed backbone amide peaks in the ^{1}H-^{15}N TROSY spectrum. TROSY-based R_1, R_2 and ^{15}N-{^{1}H} steady-state NOE experiments were carried on the 950 MHz US^2 Bruker spectrometer and they were used to provide information on molecular motions on the fast time scale.