



Posters

P1

PIP2 AND PIP3 INTERACT WITH N-TERMINUS REGION OF TRPM4 CHANNEL

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The transient receptor potential melastatin 4 (TRPM4) is a calcium-activated non-selective ion channel that plays a plethora of roles in cell sensors systems. Regulation of heart functions and control of various types of neuronal activities are among its functions relevant to human physiology and diseases. It is known that phospholipids, especially PIP2, play a unique role in the regulation of TRP channels however the molecular mechanism of this process is still unknown. We characterized the binding site of PIP2 and its homolog PIP3 in the E733-W772 region of the TRPM4

N-terminus via biophysical and molecular modeling methods. The specific positions R755 and R767 in this domain were identified as being important for interactions with PIP2/PIP3. Site-directed mutagenesis of arginine residues caused a partial loss of PIP2/PIP3 binding specificity. The binding of PIP3 to TRPM channels has never been described before. These findings provide new insight into the ligand binding domains of the TRPM4 channel.

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P2

AUTOMATIC WORKFLOW FOR THE CLASSIFICATION OF LOCAL DNA CONFORMATIONS

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A large number of crystal and NMR structures reveals the considerable structural polymorphism of DNA at the local level. DNA is highly variable with dinucleotide steps exhibiting substantial flexibility in a sequence-dependent manner. The existing classification of DNA dinucleotides [1] is based on the considerable amount of manual work, which is time consuming and error prone. To overcome this limitation, we developed an automatic workflow for the classification of DNA dinucleotide conformations [2]. Using the workflow, dinucleotides with unassigned conformation can be either classified into one of already known 24 classes or they can be flagged as unclassifiable. If they

exist, new classes in the set of unclassified dinucleotides are automatically identified by our nonhierarchical single-pass clustering algorithm. The project illustrates the utility of various machine learning approaches in the classification of local DNA conformations.

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P3

MODELING OF BLOCKING OF NMDA RECEPTOR CHANNEL BY ENDOGENOUS NEUROSTEROIDS

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N-methyl-D-aspartate (NMDA) receptors (NMDARs) are a major class of excitatory neurotransmitter receptors in the central nervous system. They form glutamate-gated ion channels that are highly permeable to calcium and mediate activity-dependent synaptic plasticity. NMDAR dysfunction is implicated in multiple brain disorders, including stroke, various forms of neurodegeneration, chronic pain and schizophrenia. NMDARs are activated by a agonists glutamate and glycine, and their activity is modulated by allosteric modulators including endogenous neurosteroids pregnenolone sulfate and 20-oxo-5 β -pregnan-3 α -yl sulfate and their synthetic analogues.

We have used electrophysiological and molecular biology techniques in combination with molecular modeling to analyze molecular mechanisms of steroid action at

NMDARs. In agreement with our theoretical results, the results of our experiments suggest that neurosteroids bind in the extracellular vestibule of the NMDAR channel to prevent the permeation of small mono and divalent ions. In addition, the model of the NMDAR channel opening suggests an explanation for the different contribution of the GluN1 and GluN2B NMDAR subunits to the inhibition by the steroid.

Vojtech Vyklicky, Barbora Krausova, Jiri Cerny, Ales Balik, Martin Zapotocky, Marian Novotny, Katarina Lichnerova, Tereza Smejkalova, Martina Kaniakova, Miloslav Korinek, Milos Petrovic, Petr Kacer, Martin Horak, Hana Chodounska, and Ladislav Vyklicky; Scientific Reports, *accepted*.

P4

STRUCTURAL CHARACTERIZATION OF PHOSPHATIDYLINOSITOL 4-KINASE III β IN COMPLEX WITH ATP AND INHIBITOR

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Phosphatidylinositol 4-kinases (PI4Ks) are crucial enzymes for the maintenance of lipid homeostasis and for the control of intracellular trafficking. Their product – phosphatidylinositol 4-phosphate (PI4P) - is the main lipid marker of the Golgi and the trans-Golgi network and plays a role at the plasma membrane as well. It is also a precursor for higher phosphoinositols. Many, if not all single stranded plus RNA viruses including dangerous human pathogens, replicate in membranous organelles highly enriched in PI4P. Indeed, type III (a and β) PI4Ks were shown to be essential host factors for the replication of single stranded plus RNA viruses. Numerous viruses from Picornaviridae, Coronaviridae and Flaviviridae families depend on the enzymatic activity of PI4K III β . As such,

PI4K III β is a potential therapeutic target in the development of broad-spectrum virostatics. To understand the mechanism of PI4K III β inhibition at the atomic level we have solved crystal structures of PI4K III β in complex with an archetypal inhibitor and with ATP. These structures revealed that the inhibitors occupy the binding site for the adenine ring of the ATP molecule.

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P5

REPETITIVE EXTRAGENIC PALINDROMES FROM *HAEMOPHILUS PARASUIS* AND THEIR ASSOCIATED TRANSPOSASE RAYT

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Repetitive Extragenic Palindrome (REP) elements represent relatively well characterized types of noncoding repetitive DNA in bacteria. The REPs play a variety of roles in the cell and can be cleaved and transferred to the target site by the associated REP-associated tyrosine transposase (RAYT, Nunvar *et al.* 2010). The ability of RAYT to catalyze cleavage and recombination of REP sequences was experimentally confirmed for *E. coli* (Messing *et al.* 2012) and newly determined for *Haemophilus parasuis*. Recognition between single-stranded REP DNA and RAYT is necessary for nucleoprotein complex formation and subsequent DNA strand cleavage and transfer (Messing *et al.* 2012).

In this work, we studied solution conformations of several REP-related oligonucleotides from *H. parasuis* by circular dichroism spectroscopy. Our results indicate that REP oligonucleotides from *H. parasuis* form predominantly a monomolecular hairpin conformation in solution which may be important for RAYT recognition. Interactions between the fluorescently labeled oligonucleotide

with sequence of *H. parasuis* REP and the associated RAYT were determined by microscale thermophoresis technique. Data show that the RAYT protein interacts with REP DNA from *H. parasuis* with high affinity, dissociation constant of binding RAYT to REP DNA was determined as 5 ± 0.8 nM.

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P6

THE SEMIEMPIRICAL QUANTUM MECHANICAL SCORING FUNCTION FOR *IN-SILICO* DRUG DESIGN

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This poster introduces the quantum mechanics (QM)-based computer-aided drug design, especially using semiempirical QM (SQM) methods. Computer-aided drug design aims to reduce the cost of the drug development and also to bring deeper insight into the inhibitor binding to its target. Binding free energy (G_b°) between protein (P) and a ligand (L), which is related to the dissociation constant (K_i) of the P-L complex, is expected to be proportional to the ligand potency.

Free energy estimators are mostly referred to as scoring functions in the drug-design community. The score stands for the binding free energy or for some generalized quantity describing the ligand potency. Previously, we designed a scoring function based on the semiempirical quantum mechanical (SQM) PM6-DH2X method and applied it to several types of P-L complexes, namely the HIV-1 protease (PR) [1], cyclin-dependent kinase 2 (CDK2) [2], casein kinase 2 (CK2) [3], adenosine kinase [4], aldose reductase [5], serine racemase [6] binding to series of inhibitors. The score consist of the interaction energy, the desolvation free

energy, the change of the conformational 'free' energies of the protein and ligand upon binding and the entropy change. The most accurate up-to-date methods are used for the respective terms thus offering a balanced and reliable scoring function. Let us emphasize that each of the terms has a clear physical meaning and that these terms are not adjusted/weighted by any means (fitting parameters) to the experimental data. Construction of the scoring function from the physically meaningful terms is a significant feature since it allows us to gain a deeper insight into the nature of the P-L binding.

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P7

PDBCOP: PDB COMPARISON PROGRAM**Karla Fejfarová^{1*}, Jan Dohnálek^{1,2}, Jindřich Hašek²**¹*Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovského nám. 2/1888, 162 06 Prague 6, Czech Republic*²*Institute of Biotechnology AS CR, v.v.i., Vídeňská 1083, 142 20 Prague 4, Czech Republic
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Crystallographic structure determination has become a technique that can be readily applied to many research problems in biotechnology, biomedicine, and drug design. These applications should be based on only the reliable data, however, some of the models in the Protein Data Bank still contain errors of various kinds [1]. It should be emphasized that one cannot blindly accept the results of automated crystal structure solution and refinement procedures and that it is essential to carefully assess the model quality.

The most common validation tools, MolProbity [2], and the wwPDB validation server [3], check mainly the geometric quality of the model. In the final stages of the crystal structure refinement, it is also important to keep track of those atoms, for which parameters change significantly during the refinement cycles. These atoms, usually belonging to solvent molecules, may be the source of possible problems. With the size of a typical macromolecular structure, it is almost impossible to check all the atoms manually.

PDBCOP is a command line-based tool that assembles specific details about X-ray structure model from PDB file.

If called in mode with two input files, it compares two PDB files and prints a list of atoms, for which position or ADP changed more than a certain threshold. In mode with one input file, *PDBCOP* can list e.g. non-standard residues in the PDB file, all atoms with occupancy different from 0 and 1, and residues with alternate conformations. The Linux version of *PDBCOP* is available at <http://crysta.fzu.cz/pdbcop/>.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant No. EE2.3.30.0029), and by BIOCEV CZ.1.05/1.1.00/02.0109 from the European Regional Development Fund.

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P8

**RECEPTORS EXPRESSED ON THE NATURAL KILLER CELLS;
REVIEW OF THE MOLECULAR STRUCTURE DATA****Hašek Jindřich¹, Skálová Tereza¹, Kolenko Petr², Dušková Jarmila¹, Koval Tomáš²,
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Natural killer cells indispensable for innate immunity are in the center of very complex machinery of interactions between cell receptors and ligands ensuring signal transduction, recognition, etc. They have also complex mechanisms for activating the adaptive immune system. The resulting effect depends on numbers of cellular receptors expressed on the NK-cells, on the numbers of receptors on the surface of other the immune and the tissue cells in neighborhood, on a type of their clustering and on concentration of small and high molecular weight ligands secreted into the surrounding intercellular space. Thus, the immune response is always a complex interplay of many events. Intensity of response usually depends on clustering of receptors, on attachment of ligands on the intracellular side of receptors, and on number and strength of interac-

tions with extracellular ligands. Moreover, cells perceive more signal pathways, some acting in synergy, some against each other. The Table presented on poster may help to get a brief glimpse on complexity of the problem.

Main reason for joining the protein crystallography data with data provided by other biophysical methods is to get the 3D view of the intermolecular interfaces, which seems to be very useful for a rational design of medical treatment. Advantages of crystallography of protein complexes are namely in (a) visualization of intermolecular interaction modes (it allows controlled intervention into the immunological processes and thus a rational modulation of functions), and (b) in the fact that the inspected proteins are under similar stress as in the tissue (protein concentrations



in “crystals” are similar to those in the tissues). Protein crystallography helps in:

- elucidating the detailed mechanism of adhesion and signal transmission between cells,
- reliable confirmation of reasons why molecular partners responsible for immunological processes bind,
- visualization of the interface provides a basis for a rational variation of the process under study.

Regrettably, the 3D structure data and snapshots of the complexes with their interaction partners are very sparse by now. Thus, the review of NK-cell receptor interactions is intended namely for rational planning of the future experiments research in the field. Protein crystallography is a relatively slow tool, and thus a rational selection of a research targets is very important.

The summary of the NK-cell surface receptors shows a large pool of the NK-cells receptors and their interactions with hundreds of their ligands. The Table summarizes the available biochemical data on the biomolecular interaction partners [9, 11, 12 etc.] and mixes them with the structure data obtained by X-ray crystallography [10].

The NK-cell receptors are sorted according to their CD names, and their biochemical properties and interacting partners were compiled primarily from several recent reviews. The information on experimentally determined structures of receptors or ligands shown in red is extracted from the Protein structure data bank (PDB) [10] in December 2014. Some related proteins of interest without CD names are at the end of the Table.

Columns are divided into three groups. The columns A-H concerning the NK-cell receptor, J-R concerning the supposed external ligands, and S-U devoted explicitly to function and morphology of the ligand-receptor complex under interest.

The Table is hopefully self-explaining. E.g. the column H (“Expression confirmed in”) contains the observed appearance of the receptor on different cell types. As an example, the m-CD30 is expressed only if NK-cells are activated and its interaction with CD156 may lead to proliferation of lymphocytes and apoptosis, by also to tolerance under specific conditions. It is evident that the tabulation requires extensive use of abbreviations. They are in consistency with those used in literature when it was possible. The detailed list of the abbreviations used in Table is alphabetically sorted by the end.

The related proteins from different species are resolved by a prefix (e.g. h-human, m-mouse) and are sorted on rows nearby (columns A,J). The table lists namely human and mouse receptors. Information on others species are here accidental and very sparse. The rows with human receptors have white background. The non-human receptors are marked by grey background.

Interacting partners. The cellular receptor can have a number of interacting partners (listed in column G). Some of them have their principle role in natural processes in organisms, some have no evident function in organisms, some can have adverse function and other can be prepared on purpose artificially as a reaction of organisms to antigen (antibodies). Due to the high throughput methods for testing pairs of protein binding partners, the number of known interacting partners of receptors is continuously growing.

Thus, in spite of a large number of the listed ligands, the Table is far to completeness. The information on ligands in this Table is based on few recent reviews and the data extracted from the PDB. No global search of data scattered in individual journals, neither systematic search of ligands deposited in the PDB [10] were done. The columns “Molecular morphology” are planned to explain the domain structure, complexation with intra and extracellular ligands and the observed multimerization of receptors, or ligands, or complexes.

The structures solved in our laboratory are highlighted by rose background. They include: (a) the structures of NK-cell receptors (e.g. NKRP1A, NKRP1F, CD69) [4,5,6], (b) the structures of the protein molecules interacting with NK cell receptors (e.g. Clr-g, LLT1) [1, 2, 3], (c) studies of Fc fragment of IgG’s interactions with cellular receptors [7, 8], self-aggregation and formation of immuno-complexes via Fc fragment interactions [7], (d) the role of glycosylation on protein stability [8]. The structures of NK-cell related molecules determined worldwide are denoted yellow-green background.

The presented table of the structure data on NK-cell receptors and their ligands compiles a large volume of data from several available recent reviews. The data are accepted as they stated in the literature and no guaranty on correctness and accuracy is possible. The Table should be taken as a guide for the first orientation only. All facts should be verified elsewhere. The Table is updated occasionally by a staff involved in some special areas only and thus, it doesn’t cover the field completely. Therefore, it is open to changes suggested by external specialists. A current version of Table is available on the request from the first author (hasekjh@seznam.cz).

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P9

IDENTIFICATION OF THE CATALYTIC RESIDUES OF GLYCOSIDASES FROM *PAENIBACILLUS THIAMINOLYTICUS* AS A WAY TO ENGINEERING OF NEW GLYCOSYNTHASES

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Glycosyl hydrolases -D-galactosidase and -L-fucosidase cloned from *Paenibacillus thiaminolyticus*, are interesting not only because of their hydrolase activity but mainly, for their transglycosylation activity, which is important for synthesis of interesting glycosylated molecules. At the beginning of this work, we had no detailed information about the structure of these enzymes, because none of these enzymes had been crystalized yet. In this project both enzymes were studied using the combination of theoretical and experimental methods. In the theoretical part, the structures of enzymes were predicted by homology modeling and further studies of these structures proposed the possible catalytic residues in both enzymes. The experimental part is focused to confirmation of the predictions of catalytic amino acid residues from the theoretical part. This poster presents the final results, where the mutated enzymes -D-galactosidase_mut233, -L-fucosidase_mut186 and -L-fucosidase_mut239 were produced and purified. Furthermore -D-galactosidase_mut157 was purified in earlier experiments. All mutants were tested on se-

lected substrates. The results of these activity tests are helpful for better understanding the catalytic machinery of studied enzymes and lead us to possibility of transforming them into glycosynthases i.e. modified glycosyl hydrolases that lack their natural hydrolytic activity because of mutation of nucleophilic amino acid residue, but maintain their transglycosylation activity.

The project was supported by COST actions Multi-GlycoNano (CM1102, LD13024). Participation at the conference is supported by specific university research (MSMT No 20/2015, No MSMT 21/2015).

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P10

POSSIBILITIES FOR BIOMACROMOLECULAR CRYSTALLIZATION AT BIC CORE FACILITY AT CEITEC, BRNO

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Protein crystallization followed by X-ray diffraction is well established method for determination of the protein structure. It is historically the first technique developed and lead to determination of more than 93 000 deposited structures of proteins and their complexes up to date [1]. It is currently the only method that enables atomic resolution for structures of virtually unlimited size including proteins, nucleic acids and their various complexes.

We offer a wide range of crystallization techniques in the Biomolecular Interaction and Crystallization (BIC) Core Facility at CEITEC MU in Brno. The standard 96-well crystallization screening plates are set up by Mosquito (TTP Labtech) and Phoenix (Rigaku) robotic systems allowing for high throughput, precision and accuracy. The optimization of crystallization conditions is further simplified by Dragonfly (TTP Labtech) pipetting robot. The automation speeds up the whole process allowing to set up 1000 – 2000 different conditions with 100 ul of concentrated sample in one hour. The storage and crystal tracking

is ensured by Minstrel HT (Rigaku) equipped with UV detection and advanced software analysis.

Beside standard sitting/hanging drop crystallization screening, we are using specialized techniques of crystallization under oil or in capillaries. TG40 machine (Centeo) serves for temperature optimization screening. We also offer a wide range of additives and nucleants as well as heavy atom compounds for protein derivatization. Recently we broaden the spectrum of available methods by membrane proteins crystallization in cubic and sponge phase thanks to Mosquito LCP machine (TTP Labtech). Biophysical properties of the samples can also be determined using analytical ultracentrifugation, dynamic light scattering and CD spectroscopy available to users.

All the possibilities are available not only to research groups in CEITEC, but thanks to Open access grant also to any non-commercial users worldwide for free (in 2015).

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P11

NMR RELAXATION STUDIES OF RECEIVER DOMAIN OF CYTOKININ RECEPTOR CKI1_{RD} MUTANTS 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 pathway has a great influence on many aspects of growth and development of plants. This signaling system is based on phosphate transfer between the cytoplasmic 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.

Aspartic acid 137, present in the active site of CKI1_{RD}, plays an important role in the signaling pathway. This Asp 137 residue binds phosphate and transfers it to next protein involved in the signaling pathway. Two CKI1_{RD} proteins with mutations in their active sites (CKI1_{RD}-D137A and CKI1_{RD}-D137E) were expressed in *E. coli*, labeled by stable isotope ¹⁵N, and studied by NMR relaxation experiments. The results were used to characterize internal motions of the mutants.

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P12

SPECTRAL AND ELECTROCHEMICAL ANALYSIS OF miR-34a-5p

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MicroRNAs (miRNAs) are small (22 nt), single-stranded, non-coding RNAs known as regulators of gene expression at the mRNA level. Their impact on the final translation product is significant, therefore it is suitable to study them as biomarkers of various diseases namely diabetes, cardiovascular, neurodegenerative, viral diseases and cancer [1]. Our attention was aimed at the research of miR-34a-5p related to head and neck cancer (HNC) and also prostate cancer (PCa) where we obtained significant differences in miR-34a-5p expression status between health controls and patients with PCa. This miRNA, belonging to the miR-34 family is directly linked to p53 and Wnt pathways [2]. The tight connection between loss of tumor suppressor function and activation of oncogenic signaling has been proven for this miRNA. Beside the classical molecular-biological methods studying miRNAs, such as PCR, Northern Blot-

ting (NB), microarrays technologies, new biophysical approaches were applied [3]. The results from the PCR analysis were complemented with UV absorption spectra, CD spectra and linear sweep voltammetry in connection with adsorptive transfer stripping (AdTS) technique [4]. Both data of miR-34a-5p were completed by results of DNA(U), having the same oligonucleotide sequence as miR-34a-5p. The comparison of miRNA with DNA bearing uracil instead of thymine (DNA(U)) showed significant differences in structure-function relation. The stabilities of RNA and DNA structures were studied using CD and UV-absorption spectroscopy and expressed as melting points (32 °C for RNA and 46.5 °C for DNA(U)). The effect of substitution of ribose for deoxyribose was shown and structural diversity was confirmed also by electrochemical methods.

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P13

ACTIVATION ROUTE OF THE *SCHISTOSOMA MANSONI* CATHEPSIN B1 DRUG TARGET

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The digestive protease cathepsin B1 (SmCB1) of the human blood fluke *Schistosoma mansoni* is a potential drug target for the treatment of schistosomiasis, a parasitic disease that afflicts over 200 million people worldwide. SmCB1 is biosynthesized as an inactive zymogen in which the pro-peptide operates as an intra-molecular inhibitor by blocking the active site. We have investigated the activation processing through which the pro-peptide is proteolytically removed and the regulatory role of sulfated polysaccharides (SPs) in this process. Crystal structures of three molecular forms of SmCB1 along the activation path-

way were determined, namely the zymogen, an activation intermediate with partially cleaved pro-peptide, and the mature enzyme. We have demonstrated that SPs are essential for the autocatalytic activation of SmCB1 as they interact with a specific heparin-binding domain in the pro-peptide inducing its complete processing. An alternative activation route for SmCB1 is mediated by *S. mansoni* legumain and is down-regulated by SPs, indicating that SPs act as a molecular switch between both activation mechanisms.

P14

PIP2 INTERACT WITH CYTOSOLIC N-TERMINAL REGION OF THE MELASTATIN CHANNEL TRPM1

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Transient receptor potential melastatin 1 (TRPM1) channel belongs to superfamily of ion channels that are mostly permeable to mono- and divalent cations and respond to various physiological stimuli like chemosensation, thermosensation and mechanosensation. TRP channels have

six transmembrane domains with a pore region between the fifth and the sixth segments. Cytosolic N-/C-tails are responsible for regulation of TRPs, which carry binding sites for signal molecules [1, 2]. 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 and loss of TRPM1 correlates with increased aggressiveness in melanoma [3, 4].

In this study using bioinformatic approach we identified phosphatidylinositol 4,5-bisphosphate (PIP2) binding site in region A451-N566 within the TRPM1 N-terminus (NT). Fusion protein of TRPM1-NT was expressed in bacteria *E. coli* Rosetta cells and purified in two-step purification protocol using affinity and HPLC gel chromatography. Alanine substitution mutagenesis screening revealed the crucial amino acids for these interactions. The equilibrium dissociation constants were estimated using surface plasmon resonance measurement. The CD spectra were used to characterize the interactions as well.

We identified the PIP2-binding site and found mutations that decreased the affinity of the TRPM1-NT/PIP2 interaction. Our results suggest that the sequence of

TRPM1-NT contains several basic amino acids which interact with anionic phospholipids. Moreover we have provided the structural insight to the TRPM1-NT/PIP2 interaction using computer ligand docking.

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P15

STRUCTURAL CHARACTERIZATION OF PHOSPHOPROTEIN PHOSDUCIN AND ITS INTERACTION WITH 14-3-3

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Phosducin (Pdc), a highly conserved phosphoprotein involved in the regulation of retinal phototransduction cascade, transcriptional control, and the modulation of blood pressure, is controlled in the phosphorylation-dependent manner including the binding to the 14-3-3 protein. However, the molecular mechanism of this regulation is largely unknown [1-3]. Here, the solution structure of Pdc and its interaction with the 14-3-3 protein were investigated using small angle X-ray scattering, circular dichroism, quenching of tryptophan fluorescence, analytical ultracentrifugation, hydrogen-deuterium exchange coupled to mass spectrometry and nuclear magnetic resonance. We show that the 14-3-3 protein interacts with and sterically occludes both the N- and C-terminal G_t binding interfaces of phosphorylated Pdc, thus providing a mechanistic explanation for the 14-3-3-dependent inhibition of Pdc function. The 14-3-3 protein dimer interacts with Pdc using surfaces both inside and outside its central channel. The N-terminal domain of Pdc, where both phosphorylation sites and the 14-3-3 binding motifs are located, is intrinsically disordered protein which remains likely highly flexible when bound to 14-3-3 indicating the fuzzy-like character of this complex. In addition, it has been speculated that the 14-3-3

protein binding decreases the rate of Pdc dephosphorylation after a light stimulus by virtue of its interaction with phosphorylated Ser54 and Ser73, thus lengthening the time that Pdc remains phosphorylated after a light exposure. Pdc is dephosphorylated *in vivo* by protein phosphatases 1 (PP1) and 2A (PP2A). Indeed, our dephosphorylation experiments with PP1 revealed that the 14-3-3 protein does slow down the dephosphorylation of doubly phosphorylated Pdc *in vitro*.

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P16

STRUCTURE-FUNCTION STUDY ON PLANT ALDEHYDE DEHYDROGENASES FROM FAMILY 2 AND FAMILY 7

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Aldehyde dehydrogenases (ALDHs) represent a super-family of NAD(P)⁺-dependent enzymes that catalyze oxidation of aldehydes to carboxylic acids. So far, 13 ALDH families have been described in plants. ALDHs play a crucial role in detoxifying aldehydes produced by various metabolic pathways and during adaptation upon various stress conditions. Plant ALDH2 family (EC 1.2.1.-) comprises mitochondrial and cytosolic isoforms and share ~ 60 % amino-acid identity with human ALDH2 (hALDH2). hALDH2 plays a role in ethanol metabolism by catalyzing the oxidation of ethanol-derived acetaldehyde to acetate. The first maize (*Zea mays*) ADLH2 gene was identified as a male fertility restorer RF2A followed by RF2B. Both genes code for homotetrameric mitochondrial ALDHs with an acetaldehyde activity. In contrast, the role and function of cytosolic ALDH2 members in maize is not known. ALDH7 (EC 1.2.1.31) is also known as 1-piperidine-6-carboxylate (P6C) dehydrogenase or antiquitin. Well-studied human ALDH7 (hALDH7) shares ~ 60 % sequence identity with plant orthologues. The enzyme primarily involved in the metabolism of lysine catalyzes the conversion

of α -amino adipic semialdehyde (AASAL) to α -amino adipate. Only little is known about substrate preferences of plant isoforms. Here we present a detailed biochemical characterization of plant ALDH2 and ALDH7 families by analyzing maize and pea ALDH7 (ZmALDH7 and PsALDH7) and four maize cytosolic ALDH2 isoforms RF2C, RF2D, RF2E and RF2F. Kinetic analysis demonstrates that AASAL is the preferred substrate for plant ALDH7. Aromatic aldehydes including benzaldehyde, anisaldehyde, cinnamaldehyde, coniferaldehyde and sinapaldehyde are the best substrates for cytosolic ALDH2. All ALDH2 isoforms display activity with 3-methyl-2-butenal (isopentenal), which is formed by the oxidation of isoprenoid cytokinins by cytokinin oxidase/dehydrogenase. To better understand substrate specificity, we solved the crystal structures of ZmALDH7, RF2C and RF2F with NAD⁺ at 2.95, 2.25 and 2.40 Å resolution, respectively and compared them with the human enzymes.

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P17

STRUCTURAL STUDY OF THE YEAST ENZYME NEUTRAL TREHALASE AND ITS COMPLEX WITH THE 14-3-3 PROTEIN

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The yeast enzyme neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae* hydrolyses the non-reducing disaccharide trehalose which serves as an energy source and a universal stress protectant in many different organisms. Enzymatic activity of Nth1 is enhanced by 14-3-3 protein binding in a phosphorylation-dependent manner. Nth1 activity is also regulated by Ca²⁺ binding to the EF-hand-like motif containing domain of Nth1 [1].

The native TBE PAGE and analytical ultracentrifugation show that Nth1 forms very stable complexes with yeast 14-3-3 proteins [1]. To study the structure of Nth1 alone and its complex with the 14-3-3 protein we used cir-

cular dichroism, H/D exchange coupled to mass spectrometry, chemical cross-linking [2] and small angle X-ray scattering (SAXS) [3]. At the same time protein crystallography of Nth1 alone and its complex with 14-3-3 protein is performed.

The low resolution structure of Nth1:14-3-3 protein complex revealed that 14-3-3 protein binding induces a rearrangement of the whole Nth1 molecule and that the region containing the EF-hand-like motif forms a separate domain which interacts with both 14-3-3 protein and catalytic domain of Nth1. We proved that integrity of the EF-hand-like motif is crucial for the 14-3-3 protein medi-



ated activation of Nth1 and for the Ca^{2+} binding. Our data suggest that the EF-hand-like motif functions as the intermediary through which 14-3-3 protein modulates the function of the catalytic domain of Nth1. These structural changes probably enable the substrate entry into the enzyme active site [3].

Our study of 14-3-3 protein complex with the fully active enzyme Nth1 offers a unique structural view of Nth1 activation enabling us also to better understand the role of the 14-3-3 proteins in regulation of other enzymes.

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P18

SITE-DIRECTED MUTAGENESIS OF TWO NUCLEOSIDE N-RIBOHYDROLASES FROM MAIZE

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Nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-) are glycosidases that catalyze the excision of the N-glycosidic bond in nucleosides to allow recycling of the nitrogenous bases and ribose. The enzyme comprises four aspartate residues located at the N terminus, which are involved in catalysis and coordination of a calcium ion at the active site. The binding of ribose moiety is highly conserved and NRHs impose a strict specificity for the ribose moiety. In contrast, the residues interacting with nucleobase highly vary. We identified two NRH subclasses in the plant kingdom; one preferentially targets the purine ribosides inosine and xanthosine while the other is more active towards uridine and xanthosine. Both subclasses can hydrolyze plant hormones - cytokinin ribosides. In this work, we combined a site-directed mutagenesis approach with kinetic and structural analyses to study nucleoside binding sites in two NRHs from maize (*Zea mays*), namely ZmNRH2b (GenBank accession number JQ594984) and ZmNRH3 (HQ825162). The first one is uridine specific

while the latter one is purine specific nucleosidase. The crystal structures of both ZmNRHs were solved at 1.75 and 2.51 Å resolution, respectively, and confirm that enzymes from both subclasses are dimers. Five ZmNRH2b variants and seven ZmNRH3 variants were prepared and changes in their kinetics were studied in detail. Replacement of two active-site histidine residues by alanine leads to two-fold lower specific activities. Replacement of a lysine residue, which protrudes into the active site from the second subunit and interacts with nucleobase, reduces the specific activity to 30% but increases hydrolysis of cytokinin ribosides compared to wild-type. Finally, replacement of active-site tyrosine and tryptophane residues alters substrate specificity for major substrates – xanthosine and uridine.

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P19

SEDIMENTATION ANALYSIS OF THE INTERACTION BETWEEN ASK1 AND ITS BINDING PARTNERS

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Apoptosis signal-regulating kinase 1 (ASK1), a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, 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. ASK1 plays a key role in the

pathogenesis of multiple diseases including cancer, neurodegeneration, cardiovascular diseases and diabetes, thus being a promising therapeutic target against these pathologies. The enzymatic activity of ASK1 is tightly regulated by phosphorylation, oligomerization and protein-protein interactions. The formation of high molecular complexes, ASK1 signalosomes, was observed as an essential element for oxidative stress-induced cell death.

Thioredoxin (TRX) is an oxidoreductase which was found to bind the N-terminal domain of ASK1 in its inactive state and it prevents its activation. If exposed to reactive oxygen species (ROS), TRX dissociates from ASK1 through unknown mechanism. This leads to the subsequent binding of TRAF2/6 and full activation. The 14-3-3 protein was identified as one of the most important physiological regulators of ASK1.

It binds to the phosphorylated Ser967 at the C-terminus of the kinase domain ASK1 and maintains its inactive

state, thus preventing the signaling initiation. It has been previously shown that ASK1 is activated after dephosphorylation of Ser967 and dissociation of 14-3-3 in the presence of ROS but mechanism of inhibition is also unclear possibly similar to other 14-3-3 regulated complexes.

Here we present analysis of interactions of TRX or 14-3-3 protein with corresponding binding domains of ASK1 using AUC. We revealed possible binding interface between TRX and ASK1 and importance of different cystein residues in the binding mechanism [1]. We also characterized the complex with 14-3-3 revealing its weak and transient nature.

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P20

CRYSTALLOGRAPHIC AND CATALYTIC STUDY OF α -L-FUCOSIDASE FROM *PAENIBACILLUS THIAMINOLYTICUS*

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Fucosylated oligosaccharides have an important role in many biological processes such as fertilization, cell adhesion, cell proliferation and processes of inflammation. They are also responsible for existence of blood group antigens. They can also be used in pharmaceutical and food industry. Previous research indicates that α -L-fucosidase from *Paenibacillus thiaminolyticus* has great potential for artificial fucosylation which could be used in synthesizing of fucosylated oligosaccharides. Recombinant form of α -L-fucosidase was produced, purified and characterized.

Preliminary test of substrate specificity was conducted using selected substrates and ability to catalyze transglycosylation reactions was tested. Recombinant α -L-fucosidase was used for crystallization experiments. Conditions for crystal growth were found.

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P21

PROTEOLYTIC SYSTEM OF BLOOD-FEEDING TICKS: AN UPDATE ON PROTEIN STRUCTURES

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Ticks are blood-feeding ectoparasites that serve as vectors for transmission of diseases such as Lyme borreliosis and tick-borne encephalitis. There are two proteolytic systems in ticks that are essential for the interactions with the host and the transmission of the pathogens: (1) Protease inhibi-

tors from the tick saliva help to disarm the host haemostatic system and alter the inflammatory and host immune responses. (2) Proteases in the tick gut are responsible for digestion of host blood proteins, the ultimate source of nutrients for their growth, development and reproduction.



Proteins from both proteolytic systems are increasingly recognized as potential antigens for the development of “anti-tick” vaccines that protect against the ticks and reduce the risk of pathogen transmission. We present structural and functional analysis of the following members of these proteolytic systems: OmC2, a salivary protease inhibitor from the cystatin superfamily, is an immunomodulatory protein that suppresses the host’s adaptive immune response. The crystal structure of OmC2 (2.5 Å) was used to explain its inhibitory specificity against mammalian cysteine cathepsins from several types of immune cells. IRS-2, a salivary protease inhibitor from serpin super-

family, functions as a unique inhibitor of host acute inflammation and platelet aggregation. The inhibition mechanism of IRS-2 was described using the crystal structure (1.8 Å) of the protease-cleaved form of IRS-2. IrCD, a gut-associated cathepsin D protease, is critically involved in the initial step of blood protein digestion. Mechanisms of IrCD regulation were studied based on four crystal structures (1.5-2.3 Å) of the zymogen precursor, mature enzyme, and its complexes with active-site and exosite inhibitors.

P22

RETROVIRAL MATRIX PROTEINS INTERACTION WITH MEMBRANES

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Retroviruses are important mammalian pathogens. Despite an intensive research, some parts of the retroviral lifecycle remain unclear, for example interaction of the immature viral particles with plasma membrane of infected cells. Immature viral particles are formed by the main retroviral structural polyprotein Gag. N-terminal part of this polyprotein, i. e. matrix protein, is responsible for the interaction of the particles with plasma membrane and viral budding. In our work we compared matrix proteins from

Mason-Pfizer monkey virus (M-PMV), Human immunodeficiency virus (HIV-1) and Mouse mammary tumor virus (MMTV). We studied the interaction of the matrix proteins with artificial liposomes consisted of commercially available phospholipids as well as liposomes formed by phospholipids isolated from cell lines which contained also retroviral envelope proteins. We managed to find differences among individual retroviruses which will be presented and discussed.

P23

STUDIES OF LANTHANIDE COMPLEXES BY A COMBINATION OF SPECTROSCOPIC METHODS

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Chirality is important property of biomolecules, but its detection is often difficult. Therefore we investigated a series of lanthanide tris-(-diketonates) that are very effective for chirality sensing and amplification in biomolecular substrates. They are electrically neutral and can associate with various small organic molecules, such as chiral alcohols, amino alcohols or amino acids. In organic solvents they often produce a strong chiral signal.

Interaction of the Eu(FOD) complex with (*R*)- and (*S*)-enantiomer of 1-phenylethanol in n-hexane was studied using IR spectroscopy, Raman scattering, Raman optical activity (ROA), UV-Vis spectroscopy and ultraviolet circular dichroism (UVCD). Only ROA and UVCD spectroscopies

proved to be sensitive enough to the complexation of the studied chemical species.

The UVCD proved especially useful to determine the metal/ligand ratio in the complex. Although lanthanide tris-(-diketonates) are silent in UVCD spectra, symmetric UVCD signals induced around 300 nm were observed for Eu(FOD) upon complexation with (*R*)- and (*S*)- 1-phenylethanol.

In the future, structural variations of lanthanide tris-(-diketonate) complexes providing enhanced ROA signal are planned; also theoretical modeling may be useful to refine the chirality recognition in biological substrates.

P24

MONITORING OF PHOSPHORYLATION PROGRESS OF TYROSINE HYDROXYLASE AND INTERACTION WITH 14-3-3 ELUCIDATED BY NMR

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Human tyrosine hydroxylase 1 (hTH1) activity is regulated by phosphorylation of its N-terminus and by an interaction with the modulator 14-3-3 proteins. In order to monitor the structural changes within the regulatory domain of hTH1 (RD-hTH1, region of first 169 residues) caused by phosphorylation of S19 and S40 we have assigned NMR spectra by two different approaches. The non-uniform sampling approach (NUS) based on sparse multidimensional Fourier transform allows efficient acquisition of high dimensional NMR spectra. Increased dimensionality (5D) provided significant speed up of backbone and side-chain assignment of the unstructured RD-hTH1 region (about first 70 residues). The rest (structured parts) of RD-hTH1 was assigned by conventional set of 3D NMR experiments.

The quantification of quite complex binding mechanism between the phosphorylated hTH1 and 14-3-3zeta required novel approach. We used ^{31}P NMR to investigate interactions between 14-3-3 and minimalistic system comprising first 50 residues of hTH1 (hTH1_50), containing both phosphorylation sites of our interest (S19, S40). Dissociation constants between 14-3-3 and singly phosphorylated hTH1_50 (pS19 or pS40) were determined. Analysis of the NMR titration data revealed that a 14-3-3 dimer and the S19_S40-doubly phosphorylated hTH1_50 interact in multiple ways, with three major complexes formed. As the proof of principle of this methodology was successfully shown for hTH1_50 we continue in applying it for revealing of binding scenario between 14-3-3 and phosphorylated regulatory domain of hTH1 protein.

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P25

ROLE OF CH/ INTERACTIONS IN STABILIZING THE PROTEIN-CARBOHYDRATE COMPLEX

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Although CH/ interactions are relatively weak, it's assumed that they are very important for carbohydrate molecular recognition by proteins. The aim of our work was to study the influence of CH/ interactions on the stability of selected protein-carbohydrate complex. Study of this problem was carried out using 100 ns molecular dynamics simulations, for which complex of hevein domain (HEV32) and (GlcNAc)₃ carbohydrate was chosen. This complex was first studied under natural conditions in an aqueous medium. Then the hypothetical conditions with attenuation

of selected non-covalent interactions was carried out. Separate weakening of either CH/ interactions or hydrogen bonds was not enough sufficient to destabilize the complex. Only simultaneous weakening of CH/ interactions and hydrogen bonds together led to significant destabilization of complexes. It shows that CH/ and hydrogen bonds are working in concert to stabilize carbohydrate-protein complexes.



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LINK BETWEEN CYTOSKELETON AND SPORE FORMATION IN *BACILLUS SUBTILIS*

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Upon starvation, the rod shaped Gram-positive bacterium *Bacillus subtilis* can enter into differentiation process termed sporulation. Sporulation commences with an asymmetric cell division 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 forespore develops into heat-resistant spore, the bigger compartment, the mother cell nourishes the smaller forepore and finally lyses.

The earliest visible event in asymmetric cell division is the formation of a Z-ring by FtsZ, a tubulin like protein, and E-ring by SpoIIE at the future sporulation septum site. SpoIIE is a crucial component of the sporulation septum. SpoIIE remains at the polar septum until septum formation is complete and is later redistributed throughout the forespore membrane to perform its role in activation of the first compartment-specific sigma factor, σ^F . The possible third role of SpoIIE comes from the discovery that SpoIIE is subsequently recaptured at the forespore face of the polar septum where it may participate in peptidoglycan remodelling [1].

The rod shape of *B. subtilis* cells is maintained through its whole life cycle. As in most other bacteria, the cell wall is the key determinant of its cell shape. The coordinated action of two mechanisms of cell wall synthesis, one connected with cell elongation and the other with cell division seems to be responsible for rod shape maintenance [2]. The cell elongation is driven by the actin-like homologues

MreB, MreBH and Mbl. Recently, a well conserved membrane protein RodZ was discovered to be an important cell shape determinant in many bacteria [3].

In our previous work we have characterized RodZ protein from *B. subtilis* and have demonstrated that RodZ is likely an essential protein and an important part of cell shape determining network in *B. subtilis* [4]. Here we examine the role of RodZ in sporulation and show that RodZ localizes to the polar septum during sporulation and co-localizes with sporulation specific SpoIIE protein. We also discovered that RodZ directly interacts with SpoIIE. We propose that RodZ is not only important cell shape determinant during vegetative growth, but is also required for asymmetric cell division which is a prerequisite of resistant spore development.

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P27

IN SILICO STRUCTURAL AND THERMODYNAMIC STUDIES OF THE 14-3-3-COMPLEXES

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14-3-3 proteins are important regulatory proteins found in large abundance within the brain. Mammals have seven known isoforms of 14-3-3, and the protein family is vital for the survival of the organism. 14-3-3 proteins form functional dimers, and bind to hundreds of phosphorylated proteins. Associated with multiple diseases [1] such as insulin sensitivity, the Creutzfeldt-Jakob disease and small-cell lung cancer, understanding the principles driving the interactions of 14-3-3 proteins is an important goal that can advance medical research and molecular biology. We study both the intra-molecular interactions of 14-3-3-

dimers, and the interactions with some of their well known protein partners using computational methods and experimental approaches such as molecular dynamics, advanced free-energy calculations and NMR spectroscopy [2].

The interactions between the 14-3-3- protein and selected phosphopeptides were studied by Hamiltonian Replica Exchange Molecular Dynamics (H-REMD), and potential-of-mean-force (PMF) methods allowing the calculation of binding affinities. Combined with a novel reaction coordinate approach (distancefield) that was recently

proposed [3], these methods allow for the extensive sampling of the binding/unbinding pathways. The calculated binding affinities are compared with the available experimental data.

Comparison of the behavior of selected 14-3-3- variants in both the monomeric and dimeric states was performed by set of conventional Molecular Dynamics (MD) simulations. The impact of selected mutations at the N-terminal interface on the dimer stability was investigated. Structural changes induced by the C-terminal tail and phosphopeptide binding in different quaternary structure arrangements of the protein were analysed in detail.

The combination of these methods, provide deeper insight into the molecular processes affecting the behavior of 14-3-3 proteins, and allow for a more accurate prediction of binding affinities towards biologically relevant interaction partners.

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P28

BIOPHYSICAL AND STRUCTURAL STUDIES OF A NEW TYPE OF TRANSPOSASES – RAYTs

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Genes of REP-associated tyrosine transposases (RAYTs) have been found in many bacterial species. Members of the RAYT enzyme transposase – recognize, bind, cleave, and reinsert specific single stranded DNA elements, so called repetitive extragenic palindromes, REPs. The nuclease activity of RAYTs has been proven only for the *Escherichia coli* RAYT [1], and for *Haemophilus parasuis* in our laboratory, and the mechanism of the complex process of transposition is understood very poorly, and up to now, only crystal structure of the complex between *E. coli* RAYT transposase and its REP DNA has been determined (PDB code 4er8, [2]). The novelty and challenging nature of the RAYT/REP complex inspired our study of this system. Bioinformatic analysis of genomic databases revealed several hundred of RAYT-associated REP sequences, we have characterized some of their biophysical properties, and discovered surprising conformational diversity of these palindromic DNA elements that reaches far beyond the expected hairpin stem-loop architecture. We further have identified RAYT genes in several bacteria and selected eight most promising candidates according to the

values of sequence-calculated hydrophilicities. These eight RAYT genes have been cloned and expressed in *E. coli*. Production of most of these enzymes is challenging at the expression step, as their nuclease activity is deleterious for cells, they also have very low solubility, and extremely limited stability, they precipitate or aggregate shortly after purification. Despite these obstacles, we have succeeded in stabilizing the *H. parasuis* RAYT, been able to measure its various properties as thermostability, and CD spectrum, and also determine the binding affinity to the recognition REP oligonucleotide (dissociation equilibrium constant determined by the thermophoresis is about 6 nM). Crystallization trials and cleavage experiments with *H. parasuis* RAYT are under way.

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P29

SPECTRA MODELLING COMBINING MOLECULAR DYNAMICS AND QUANTUM MECHANICS

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Vibrational spectroscopic methods are powerful tools for studies of biomolecular structure. Such ability of the Raman scattering or infrared absorption is greatly enhanced by interpretation and simulation of the spectra by theoretical calculations. However, quantum mechanical (QM) spectra modelling of big systems, such as large biomolecules, are highly computationally demanding and from certain size practically impossible. Therefore there is a need for simpler, but accurate approaches.

We use the autocorrelation function of the polarizability and dipole moment obtained from dynamical trajectories to calculate Raman scattering and absorption intensity. For example, total polarizability of a single box is generated from molecular ones calculated at the QM level. This approach thus allows calculating spectra of much larger systems compared to usual QM approaches.

To estimate the accuracy, as an initial model a water box was used, different box sizes and approaches to pro-

cess dynamical trajectories were compared. Molecular dynamics with the Amber99 force field was compared to Amoeba09 force fields and ab-initio MD using the PBE functional. The simulated spectra were dependent on the method, but in general features agreed with the experiment. As a further application, Raman spectral dependence on pH for water solutions was examined both experimentally and theoretically. In the future, we plan to model spectra of proteins and include molecular chirality.

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P30

STRUCTURAL CHARACTERIZATION OF INTERACTIONS BETWEEN HUMAN GLUTAMATE CARBOXYPEPTIDASE II AND HYDROXAMATE-BASED INHIBITORS

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Small molecule ligands targeting human glutamate carboxypeptidase II (GCPII) are used in diverse diagnostic and therapeutic applications ranging from prostate cancer imaging to the therapy of neurological disorders. Such inhibitors typically consist of a glutamate moiety linked to a zinc-binding group to ensure high specificity and affinity for GCPII, respectively. At present, there are no structural data describing interactions between GCPII and inhibitors harboring a hydroxamate function, the prominent zinc-

binding function used in the field. Here we report X-ray structures of several complexes between GCPII and hydroxamate-based inhibitors. Our structures reveal unexpected positioning of hydroxamates in the internal GCPII pocket that differs markedly from binding modes of matching prototypical GCPII inhibitors featuring different zinc-binding groups. The data can be exploited for the structure-assisted design of novel GCPII-specific inhibitors.

P31

STRUCTURAL AND FUNCTIONAL STUDIES OF HUMAN HISTONE DEACETYLASE 6**Pavlíček J., Škultétyová L., Trapl D. and Bařinka C.***Institute of Biotechnology AS CR, v. v. i., Vıdeňská 1083, 142 20 Prague 4, Czech Republic*

Acetylation and deacetylation of lysine represent an important regulatory step influencing functions of many proteins. However, specific processes regulated by protein acetylation are still poorly understood. Many important effects of histone deacetylases and their inhibitors have been described on biological level only, without deeper understanding of their molecular mechanisms.

Human histone deacetylase 6 (HDAC6) is a major cytoplasmic deacetylase. This enzyme is structurally unique because of the presence of two catalytic domains in single polypeptide chain. It has an ability to deacetylate a broad spectrum of substrates, among them for example alpha-tubulin. Besides other roles, HDAC6 is involved in process of degradation of misfolded proteins through the formation of the aggresome. Thus, its inhibition has important clinical effects in various neurodegenerative diseases.

However, no detailed molecular structure of HDAC6 is available yet.

Here we report expression, purification and characterization of various HDAC6 constructs and mutants thereof. Recombinant proteins were successfully produced in several expression systems, including yeast *K. lactis* and mammalian HEK293 cells, and purified constructs were biochemically characterized. To identify novel substrates we assayed HDAC6 against a microarray featuring ~ 7 000 acetylated peptides derived from human acetylome. The analysis of site-directed mutants revealed the importance of numerous residues of the predicted substrate-binding pocket for the enzyme activity and stressed the significance of the C-terminal deacetylation domain. Screening of crystallization conditions yielded microcrystals that are at the moment being improved to obtain diffraction quality samples suitable for structure solving.

P32

INHIBITORY PROTEIN VARIANTS DERIVED FROM AN ALBUMIN-BINDING DOMAIN SCAFFOLD TARGETING HUMAN IL-23 CYTOKINE**L. Vaňková¹, M. Kuchař¹, R. Osička², H. Petroková¹, J. Černý¹, P. Šebo^{1,2} and P. Malý¹**

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This work was aimed to generate a collection of recombinant binders of human interleukin-23 (IL-23), which is a key element of pro-inflammatory IL-23-mediated signaling. IL-23, a heterodimeric cytokine of covalently bound p19 and p40 subunits, plays a pivotal role in the development of chronic autoimmune diseases, such as psoriasis, inflammatory bowel disease and multiple sclerosis. Binding of the IL-23 to its receptor (IL-23R) leads to triggering of the Jak/Stat signaling cascade that results in the secretion of inflammatory modulators such as IL-17A, IL-17F, IL-22 that thereby play a major role in downstream pro-inflammatory processes.

A high-complex combinatorial library derived from a three-helix bundle scaffold of the albumin-binding domain (ABD) of streptococcal protein G and ribosome display were used to select for high-affinity binders of human p19

protein, the alpha-subunit of the IL-23 cytokine. A collection of p19-binding proteins (called ILP binders) was used to identify a group of binding proteins that inhibited binding of p19 (IL-23) to its cognate receptor.

The binding of ILP binders to the p19/IL-23 is demonstrated using ELISA and the prediction of blocking function for several selected ILP variants is documented using docking of p19 to IL-23R homology model based on known IL-6/IL-6 receptor crystal structure. Inhibitory function of these variants is further confirmed using cell-surface competition binding assay. Our data document that the three-helix bundle scaffold of ABD is useful for generation of novel epitope-mapping tools important for development of novel IL-23-based next-generation therapeutics.



P33

STRUCTURAL ANALYSIS OF THE ASK1-CD:14-3-3 COMPLEX BY SMALL ANGLE X-RAY SCATTERING

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Apoptosis signal-regulating kinase 1 (ASK1) is a Ser/Thr protein kinase that plays an essential role in stress and immune responses. Its increased activity has been linked to the development of several diseases such as cancer, cardiovascular and neurodegenerative diseases. The whole enzyme consists of almost 1400 amino acids. Catalytically active is only a kinase domain located approximately in the middle of the molecule and this is the only domain that has been crystallized so far [1]. 14-3-3 form a family of evolutionary conserved regulatory proteins that participate in a variety of intracellular processes through binding interactions with hundreds of diverse cellular proteins. In an inactive state ASK1 kinase is bound to the 14-3-3 protein, which is a negative regulator of ASK1 [2]. However, the mechanism whereby the 14-3-3 protein inhibits the kinase activity of ASK1 remains unknown.

To elucidate the mechanism of this inhibition, the catalytic domain of ASK1 (ASK1-CD) and the C-terminally truncated form of the 14-3-3 protein (14-3-3 C) were purified

and a structural characterization of the ASK1-CD:14-3-3 C complex was performed using analytical ultracentrifugation and small-angle X-ray scattering. The analytical ultracentrifugation measurements revealed that ASK1-CD interacts with the 14-3-3 C protein in 2:2 stoichiometry with K_D in micromolar range. Small angle X-ray scattering showed that molecular envelope of the complex is highly asymmetric suggesting that the 14-3-3 C dimer binds only one chain of ASK1 dimer.

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P34

STRUCTURE-FUNCTIONAL STUDIES OF HALOALKANE DEHALOGENASES

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Haloalkane dehalogenases (EC 3.8.1.5) are bacterial enzymes cleaving a carbon-halogen bond by a hydrolytic mechanism in a broad range of halogenated aliphatic compounds [1]. The enzymes can be potentially applied in bioremediation, biosensing, biosynthesis, cellular imaging and protein immobilization [2]. Structurally haloalkane dehalogenases belong to the α -hydrolase superfamily with two domain organization: an α -hydrolase core domain and α -helical cap domain, which lies on the top of the core domain. Active site residues are located in a hydrophobic cavity at the interface between the two domains and are connected to the protein surface by several tunnels.

Nowadays more than 20 proteins and their mutant variants from haloalkane dehalogenases family are systematically studied. The main target is focused on research of proteins such as DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DbeA of *Bradyrhizobium elkanii* USDA94, LinB of *Sphingobium japonicum* UT26 or novel haloalkane dehalogenases DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marynobacter sp.* ELB 17, etc.

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P35

ENGINEERED ANTICALINS TARGETING HUMAN PROSTATE-SPECIFIC MEMBRANE ANTIGEN

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Modern diagnostic and imaging approaches aimed at various tumors require protein target allowing a drug to distinguish between carcinoma and healthy cells. In the case of prostate cancer (PCa), which is by far the most prevalent tumor in men, prostate-specific membrane antigen (PSMA) serves as such a target. Beside the PCa it is also expressed on the neovasculature of most solid tumors. Anticalins are a prominent members of a rapidly growing family of non-immunoglobulin protein scaffolds. Exploiting combinatorial libraries the Anticalins can be engineered to specifically bind virtually any target.

In presented work we have used DNA library based on human lipocalin gene sequence to select Anticalins specific for GCPII. Phage display together with ELISA screening were used to select first generation of GCPII binding Anticalins. The most promising variants were further sub-

jected to affinity maturation, where new DNA library was obtained by random mutagenesis of selected clones and another selection procedure via phage and bacterial surface display was applied then. The binding properties were analysed by ELISA and SPR measurements which revealed improvement from nanomolar (about 10 nM dissociation constant) in the first generation clones) to subnanomolar ($K_D \sim 500$ pM) affinities for GCPII in the third generation clones. Subsequent immunofluorescence microscopy using cell lines expressing PSMA on the surface showed specific binding of the best clones to PSMA positive cells. This observation was then confirmed by flow cytometry on live cells. Thus we have proved the usability of selected Anticalin clones for selective labelling cells expressing PSMA on the surface and their potential for *in vivo* bioimaging applications.

P36

STRUCTURAL CHARACTERIZATION OF NAD(P)H:QUINONE OXIDOREDUCTASE WrbA FROM *E. COLI* IN COMPLEX WITH BENZOQUINONE

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The protein WrbA from *E. coli* belongs to the family of flavodoxine-like proteins and participates in protection of bacterial cell from oxidative stress. It acts as a homotetramer with one molecule of FMN specifically bound per protein monomer as a co-factor. WrbA catalyses transfer of two electrons from NADH to electron acceptor using a ping-pong kinetic mechanism, where oxidized quinone binds after NAD moves out from the active site. Similar kinetic mechanism, presence of analogous co-factor and common structural features demonstrate the close relationship of WrbA with eukaryotic dimeric FAD-dependent oxidoreductases.

In order to understand the structural basics of benzoquinone binding and probable mechanism of quinone reduction we have determined the crystal structure of WrbA in complex with FMN and benzoquinone at 1.33 Å resolu-

tion. WrbA adopts a β -twisted open-sheet fold typical for flavodoxines. Three monomers contribute to each of four identical active sites. The molecule of benzoquinone is stacked in the active site between aromatic ring of FMN and Trp97, which is the ideal position for electron transfer tested by QM/MM calculations of charge transfer rates. Comparison of the structure of WrbA-FMN benzoquinone complex with the structure of dimeric mammalian oxidoreductase in complex with duroquinone (pdb id 1qrd) reveals similar monomeric fold and the position of the quinone ring. The active site of WrbA is fully occupied by benzoquinone and cannot accommodate the second substrate NADH. This finding is consistent with ping-pong kinetic mechanism proposed for WrbA.

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P37

APPLICATION OF MULTISPECTRAL CAMERAS FOR ESTIMATING NITROGEN AND GRAIN YIELD IN PADDY FIELDS

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Nitrogen is an important variable for paddy farming management. Today several methods are available for monitoring the nitrogen content of rice during its various growth stages. However, monitoring still requires a quick, simple, accurate and inexpensive technique that needs to be developed. In this study, Tetracam Agriculture Digital Camera, as a re-engineered multispectral camera, was used to acquire high spatial and temporal resolution images to determine the status of nitrogen and predict the grain yield of rice (*Oryza sativa* L.). Twelve pots of rice were subjected to four different N treatments (0, 125, 175 and 250 kg ha). Three replicates were arranged in a randomized complete block design to determine the status of N and predict rice

yield. The images were captured at different growth stages (i.e., tillering, panicle initiation, booting and heading stage) of rice in each pot. Nitrogen and grain yield were significantly correlated with Normalized Difference Vegetation Index (NDVI) ($R = 0.78$) and Green Normalized Difference Vegetation Index (GNDVI) ($R = 0.88$), especially at the panicle initiation and booting stages, respectively. The study demonstrated the suitability of using the Tetracam images as a sensor for estimating chlorophyll content and N. Moreover, the findings showed that the images revealed their potential use in forecasting grain yield at different growth stages of rice.

P38

INTEGRATION OF SPIDER WORKFLOWS INTO SCIPION FOR USER-FRIENDLY ANALYSIS OF ELECTRON MICROSCOPY DATA

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In three-dimensional electron microscopy, there are 7 to 10 general-purpose image-processing software packages, and a few dozen other packages which perform specific functions or upon specific types of samples. It can be useful to mix and match the best features of the different software packages, but to do so, data historically often had to be converted from one format to another, which presented a barrier with regard to interoperability.

Scipion is a workflow integration system, among the functions of which is to manage image-processing projects, consolidate access to different software suites, trace the inputs and outputs of each step, monitor processes, etc. SPIDER is one of the oldest image-processing suites currently in use, available since 1978, with over 600 functions and its own internal scripting language. The goal of the project we are presenting is to provide a framework to integrate the functionalities of SPIDER, at the procedural level, into the Scipion environment. Some advantages of

Scipion to manage SPIDER workflows are as follows. First, display of outputs can be customized according to the data type, e.g., an image, a set of images, a 2D plot, a volume, etc. Second, inputs and outputs are classified into data types, and subsequent procedures will list, as possible options for an input, existing files of the appropriate data type. Third, some parameters can be tested interactively before execution by using “wizards” in real time, rather than by trial & error.

As a case study, the SPIDER workflow for 2D multivariate data analysis was integrated into Scipion. Of particular importance is the method to parse SPIDER procedures from Scipion to retrieve the necessary information about the script parameters. The next tasks will be integration of SPIDER’s random conical tilt and projection-matching workflows, two of the workhorses of SPIDER usage.

P39

MECHANISM OF STAPHYLOCOCCUS AUREUS CELL WALL PENETRATION BY PHAGE PHI812/K1

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S. aureus phage phi812/K1 is polyvalent T4-like bacteriophage with contractile tail. This phage is approved to use as an antimicrobial agent in Slovakia and the Czech Republic. Attachment of the phage to the host cell surface, tail contraction and penetration of cell wall are essential steps for successful infection. However, detailed mechanism of cell wall penetration is unknown. Our results show 3D reconstruction of phage tail tube penetrating the cell wall. The mechanism of genome delivery has been studied by

Cryo-electron tomography. During attachment and contraction, many structural changes happen. Phage baseplate bind to receptors on cell surface, tail sheet rearrange and contract tail. Non-contracted tail tube is ejected and pierces outer cell membrane and cell wall. Then, virus genome is released and replication cycle starts. Determination of mechanism of cell wall penetration and genome delivery is necessary step to understanding of whole replication cycle of virus.

P40

CRYOEM RECONSTRUCTION OF SMALL NUCLEOPROTEIN COMPLEXES: APPLICATION TO THE ECOR124I RESTRICTION-MODIFICATION ENZYME

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Cryo-electron microscopy has undergone significant advances in hardware development that push limits of 3-D structure determination of large macromolecular complexes of proteins and nucleic acids. New generation of electron microscopes (such as FEI Titan Krios) with parallel sample illumination, stable electro-magnetic lenses and new specimen compustage provided a platform for obtaining data extending to near-atomic resolution. Additionally, advent of direct detectors with 4-times better sensitivity and capability to record several images per second revolutionized the field of cryo-electron microscopy of biological specimen.

We used this new instrumentation to image the type I restriction-modification enzyme EcoR124I that is at the edge of standard cryoEM procedures and reconstructions: it is only ~500 kDa large, has low symmetry and most likely is flexible in its open conformation [1]. The data obtained from Titan Krios with a direct detector provided data that can be processed and lead to high-resolution 3D reconstruction. However, we had to develop a new approach for processing of the aquired data to extract the individual EcoR124I particles from imaged micrographs. We used

wavelet filter (i.e. dyadic and stationary wavelet transform with different thresholds) to enhance the contrast of the particles in the micrograph that facilitated identification of their positions and automated picking from thousands of collected micrographs. Next, we obtained the initial low-resolution model of the EcoR124I structure from cryo-electron tomography, a technique that allows 3-D reconstruction without any initial assumption about the 3-D structure. The average from ~300 subtomogram particles was used to automatically locate particles in the filtered micrographs, using the algorithm implemented in the image processing package Relion [2]. Our next efforts are focused on refining the 3D structure to subnanometer resolution using the original dataset obtained from Titan Krios.

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P41

THEORETICAL STUDY OF CARBONIC ANHYDRASE IX

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Carbonic anhydrases (CA) are a group of metalloenzymes that catalyze reversible hydration of carbon dioxide to bicarbonate. Isoforms of CA play a vital role in various processes including lipogenesis, glyconeogenesis, pH, and CO₂ homeostasis. In this work, an isoform IX was studied by the means of theoretical methods. This isoform is over-expressed in solid tumors as a response to hypoxia conditions caused by insufficient supply of oxygen due to a massive tumor growth. CA IX helps to keep intracellular pH to a physiological level, but at the same time, it significantly lowers extracellular pH, which results in a higher risk of tumor cell invasiveness. CA IX is therefore a negative marker for people suffering from a cancer [1].

CA IX consists of proteoglycan domain (PG), catalytic domain (CA), transmembrane helix and intracytoplasmatic portion. The catalytic domain has a globular shape and accommodates a conical cavity where the active site is located [2]. The PG domain is a unique structural element to the isoform IX. It is assumed that it is a key to the excellent catalytic properties of CA IX. Despite numerous experimental efforts, the structure and exact function of the PG domain is not known. We employed molecular dynamics

simulations to reveal its structure and function. Several constructs were simulated on μ s time scales including the PG domain alone and constructs including both the CA and PG domains. Results indicate that the PG domain is rather unstructured portion of CA IX.

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ALTRUISTIC METADYNAMICS

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Molecular dynamics simulation is still computationally too expensive to efficiently simulate slow molecular processes such as protein folding or protein-ligand binding. This significantly limits its application in protein design, drug discovery and related fields. Numerous enhanced sampling techniques have been developed to address this problem, including metadynamics [1]. This method uses a bias potential to "flood" free energy minima and to reduce free energy barriers. A parallel variant of metadynamics – multiple walker metadynamics [2] – was developed early after introduction of basic metadynamics algorithm. Multiple walker metadynamics is a parallel version of metadynamics simulation during which all parallels (walkers) share their bias potential. Systems represented by individual walkers are same, only the initial conditions of simulations are different. This allows for efficient simulation in a parallel computing environment.

In this project we introduce a modification of multiple walker metadynamics where individual walkers may repre-

sent different, yet similar, systems, for example different mutants of a protein or a protein with different ligands. This can in future become a basis for parallel screening of protein mutants or parallel screening of ligands. Preliminary data on GPCR-ligand docking will be presented.

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P43

A PLANT-INSECT INTERACTION IN 3D**J. Srp^{1,2}, P. Pachel^{1,3}, P. Řezáčová^{1,3}, J. Vondrášek¹, M. Nussbaumerová¹, M. Horn¹,
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LdCD is a digestive cathepsin D-type protease of the Colorado potato beetle, *Leptinotarsa decemlineata*. Using two approaches, protease activity measurements and MicroScale Thermophoresis binding analysis, we demonstrated that LdCD is inhibited by the interaction with PDI (Potato Cathepsin D Inhibitor), a Kunitz-type wound-inducible protein from potato leaves. This suggests that LdCD is a target for PDI acting as an antifeedant in plant defense against insect herbivory. Recombinant LdCD was produced in *Pichia pastoris* and its crystal structure was de-

terminated at 1.95 Å resolution. PDI was purified from potato and its crystal structure was determined at 2.1 Å resolution. We propose the interaction mechanism of LdCD with PDI based on a docking model. Crystallization of LdCD-PDI complex is currently in progress aimed to describe a new mechanism for natural inhibition of aspartic proteases.

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INFLUENCE OF PHOSPHATE ION BOUND TO ACTIVE CENTER OF TBN1**J. Stránský^{1,2}, T. Koval³, T. Podzimek⁴, P. Lipovová⁴, J. Matoušek⁵, A. Týcová^{5,6}, P. Kolenko³,
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Tomato multifunctional nuclease (TBN1; UniProt accession no. Q0KFB0), which belongs to the nuclease type I family, plays an important role in specific apoptotic functions, vascular system development, stress response, and plant tissue differentiation [1]. Furthermore, TBN1 exhibits anticarcinogenic properties [2]. The enzyme possesses endonuclease and exonuclease-like activity on single stranded and double stranded RNA and DNA and on structured RNA, with production of mono- and oligonucleotides from 3'-end of nucleic acids [3]. Based on the crystal structure of TBN1, the phospholipase activity of the enzyme was discovered [4]. TBN1 consists of 277 aminoacids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated).

Three crystal structures of TBN1 were solved in our group: one structure of wild type TBN1 and two structures of mutant N211D [4]. The common motif conserved among all known structures is formation of super-helices,

where protein molecules are related by 3_1 screw axis. The contacts are provided by the active site of one molecule and a surface loop (SDR-loop) of a neighboring molecule. However, packing of super-helices to final crystal differs across different crystallization conditions. The conserved formation of intermolecular contacts in crystals suggests the way of assembly of molecules into oligomers in solution, which were observed by dynamic light scattering. The interaction of the active site and the surface loop is best resolved in the currently reported structure, where the active centre at the zinc cluster is occupied by phosphate ion. It correlates with behavior of TBN1 in phosphate buffer. The phosphate ion binds differently than corresponding ions in known structures of AtBFN2 from *Arabidopsis thaliana* [5]. Also presence of phosphate in the zinc cluster leads to more open TBN1 active center. Properties of mutants, designed to modify dimerization and activity of TBN1, suggest that deliberate disruption of the loop-active site



contacts by mutations limits expression of the active enzyme. Therefore formation of TBN1 oligomers together with phosphate binding are hypothesized to have regulatory roles in apoptotic-like and senescence processes in plant cells.

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P45

HOMOLOGY MODELING OF GPCR: OXYTOCIN RECEPTOR

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G-protein-coupled receptors (GPCRs) [1] are a large family of seven-transmembrane domain receptors that have been increasingly studied in recent years and are of big importance for several fields of science and pharmaceutical industry. However, there are not many GPCRs with an experimentally determined structure at the atomic resolution. Oxytocin receptor is one of GPCRs which, by activating G_q protein - phospholipase C - inositol-1,4,5- triphosphate pathway, increases the cytoplasmic Ca²⁺ concentration. By binding to this receptor, oxytocin plays very important roles in sexual reproduction, labors, maternal bonding, and many emotional and social behaviors. Here, we used homology modelling [2, 3] to obtain a model of oxytocin receptor, based on the sequence similarity with a receptor which does have a determined 3D structure. The model that we obtained was simulated in several different environments including the membrane build of 1-palmitoyl 2-oleoyl phosphatidylethanolamine molecules. By exploring the free energy surface of oxytocin, we have found distinctive energy minima which, we assume, correspond to

different stable conformers of this hormone. One of the possible reasons for their existence could be found in the mechanism of oxytocins binding to its receptor. Therefore, the simulations included the oxytocin receptor - hormone interactions as well.

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P46

STRUCTURE OF BILIRUBIN OXIDASE FROM *MYROTHECIUM VERRUCARIA* WITH LIGAND IN ACTIVE SITE

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Bilirubin oxidase from plant pathogen *Myrothecium verrucaria* (BO, EC 1.3.3.5) is a blue monomeric multi-copper oxidoreductase (MCO) catalyzing oxidation of substrates consisting of tetrapyrrole (especially bilirubin to biliverdin), diphenols and aryl diamines. BO consists of 534 amino acid residues with a molecular mass of 60 kDa. Similarly to other MCOs the substrate binding site of BO is comprised of one Cu ion (T1) coordinated by one cysteine, two histidines and one methionine and behaves as an acceptor of an electron from substrate. Four electrons are transferred from T1 to the trinuclear copper cluster (TNC) coordinated by eight histidines, where molecular oxygen is reduced to water. BO is capable to oxidize a great variety of organic compounds with many applications in industry (e.g. pulp bleaching, delignification, drug detection and degradation of herbicides [1, 2, 3]). Two structures of BO have been already published in PDB (2XLL [3], 3ABG [4]), but none of them with a ligand in the substrate binding site. The substrate binding mechanism and the way of the electron transport from substrate to T1 site are still to be determined.

We solved two structures of BO using X-ray crystallography - native structure at 2.3 Å resolution and a structure with a small ligand in the substrate binding site at 2.6 Å resolution. The phase problem was solved by molecular replacement using a previously reported structure of BO (PDB code 2XLL [3]) as a model. Both structures are in the same space group F222, which is different in comparison to previously deposited structures (P1 in 2XLL [3], P6₃ in 3ABG [4]). Two molecules were localized in asymmetric unit. On the basis of our results we suggest two possible electron transfer routes from substrate to the T1 center.

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CRYSTALLOGRAPHIC CHARACTERIZATION OF THE OUTER MEMBRANE LIPOPROTEIN FrpD FROM *NEISSERIA MENINGITIDIS*

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FrpD is an Iron-regulated outer membrane lipoprotein, which is highly conserved in a set of strains representative of all serogroups of *Neisseria meningitidis*. The biological function of FrpD remains unknown but FrpD is likely to participate in the anchoring of the Type I-secreted FrpC protein to the bacterial cell surface. FrpC belongs to the Repeat in Toxins (RTX) protein family and binds FrpD with very high affinity ($K_d = 0.2$ nM) [1]. However, the mechanism of FrpD-FrpC interaction is unknown due to the ab-

sence of any structural information on these proteins. This project is aimed to determine the structure of the FrpD protein.

The native and Se-Met substituted variants of recombinant, truncated version FrpD₄₃₋₂₇₁ protein were prepared and crystallized using the sitting-drop vapour-diffusion method. The crystals of native FrpD₄₃₋₂₇₁ protein belong to the hexagonal space group P6₄, while the crystals of Se-Met substituted FrpD₄₃₋₂₇₁ protein belong to the primi-



tive orthorhombic space group $P2_12_12_1$ [2]. The crystal structures of native and SeMet labelled FrpD proteins were solved to an atomic resolution of 2.3 and 1.40 Å, respectively. The refined crysCtalographic models comprise 224 of the 229 residues with the one N-terminal and four C-terminal residues being disordered. The two refined structures are largely identical, with the coordinate root-mean-square deviation (r.m.s.d.) of 0.56 Å of over 224 aligned residues. FrpD protein displays a compact slightly concave globular shaped structure. The FrpD fold is based on three central α -sheets, flanked by 3 short helices and one long C-terminal α -helix. Structure-based analysis was made using A

DALI (distance matrix alignment server) and *PDBeFold* server database search for similar three-dimensional structures using the refined model of FrpD as the target. Performed analysis indicated that FrpD fold did not contain the topology sufficiently similar to establish structural homology with other known proteins, indicating that FrpD corresponds to a novel fold.

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STRUCTURE-FUNCTIONAL ANALYSIS OF THE HALOALKANE DEHALOGENASE DmxA FROM *MARINOBACTER SP.* ELB17

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The selected enzyme DmxA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5; HLDs), catalyzing the hydrolytic conversion of halogenated aliphatic compounds to their corresponding alcohols, was isolated from *Marinobacter sp.* ELB 17 (DmxA). DmxA is one of the 10 members of α -hydrolases, which are already on the market for the practical use for such applications as biodegradation, biosensing, protein tagging for cell imaging and protein analysis, decontamination of warfare agents, production of optically active hydrocarbons and alcohols.

DmxA is an extremoenzyme, exhibiting height enantioselectivity, however reveals the highest activity at height temperatures (the maximal activity towards 1,3-diiodopropane was detected at 55 °C and pH 9.0), what highlights it among the other HLDs.

Diffraction crystals of DmxA were refined up to the resolutions 1.45 Å. Diffraction data for DmxA were collected using Pilatus 6M-F detector at the wavelengths of 0.972 Å on the beamline ID29, at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France).

Crystal of DmxA belonged to $P2_12_12_1$ space group, with the unit-cell parameters: $a = 43.371$, $b = 78.343$, $c = 150.51$ Å; $\alpha = \beta = \gamma = 90.0^\circ$ and contained 2 molecules in the asymmetric unit. The structure was solved by molecu-

lar replacement with *MOLREP* from the CCP4 software suite by using the coordinates of *Rhodococcus rhodochrous* (PDB entry 4E46; 48% sequence identity for 142 residues and 63% sequence similarity).

Structurally DmxA showed the typical composition of the molecule of the other members of the superfamily of α -hydrolases. The proteins have a globular shape and are composed of two domains: a highly conserved α -hydrolase main domain, which is the scaffold-like for the catalytic residues, and a smaller helical cap domain. The core domain is composed of eight α -strands, within antiparallel (β -sheet). The central β -sheet is flanked on both sides by α -helices: four are on one side and two are on the other side of the β -sheet. The second domain, the cap structure is located at the C-terminal end of the β -sheet and is composed of α -helices, covering the active site, which has revealed the catalytic pentad essential for their activity: Asp 105, His 273, Glu 129, Gln 40, Trp106. Presenting on the both in monomeric and dimeric form, a dimer enzyme of the α uniquely formed by the covalent disulfide through Cys 294, the homo-dimer is chosen as biological asymmetric unit.

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EXPRESSION OF TOXIC RECOMBINANT PROTEINS FOR STRUCTURAL STUDIES IN THE *E. COLI* LEMO21(DE3) EXPRESSION SYSTEM

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Recombinant DNA technology as well as heterologous expression and purification systems available today would allow us to obtain any desired protein in a sufficient amount and in an appropriate quality for detailed structure characterization. Nevertheless, production of some proteins remains still problematic. One of the major obstacles is the toxic effect of some proteins on the host expression system. A special approach is required to solve this problem and there are different possible ways how to overcome these expression difficulties in various expression systems.

The *E. coli* expression system is a well known and widely used system for heterologous protein production which has many advantages such as low cost, low time consumption, high yields of proteins, etc. On the other hand, problems in expression of many types of proteins are frequent. The first step is to define or estimate the character of the protein toxicity. There are several aspects which would have to be taken into account: whether the codon composition is compatible with the production system, a possible detrimental function of soluble protein over-expressed in host system or high levels of insoluble protein production leading to a significant metabolic drain for cells.

In our study we used the *E. coli* Lemo21(DE3) system to achieve and subsequently optimize expression of a toxic recombinant protein from *Legionella pneumophila*. A gene for protein designated Lpn3 has been identified in the genome of the *L. pneumophila* as 3' nucleotidase/nuclease and this protein is homologous to class I nucleases.

The gene for Lpn3 nuclease contains 882 bp and the whole protein consists of 285 amino acid residues with a

molecular mass of 32 kDa. Sequence analysis shows that the first 27 amino acids of the N-terminal end of the protein represent a signal peptide, which directs the protein out of the bacterial cells and it is cleaved off during the transport.

Several vectors from the Oxford Protein Production Facility UK (OPPF-UK) were tested for optimal expression of Lpn3 nuclease in the *E. coli* Lemo21(DE3) expression system. The whole Lpn3 gene was cloned to the pOPINE vector, while the nucleotide sequence without the N-terminal signal peptide was inserted into pOPINE, pOPINMaIE, pOPINDsbA, pOPINP, pOPINTolB, and pOPINS.

Successful Lpn3 expression in Lemo21 cells was achieved in the cases of pOPINMaIE and pOPINP constructs, which are primarily determined for periplasmic expression of recombinant proteins. A key factor for optimization of expression conditions was determination of suitable concentration of rhamnose in the media. Optimal concentration causes complete suppression of basal expression before IPTG induction. Other important parameters were temperature and time of growth before and after inductions. All these steps were necessary to effectively tune the expression level of this difficult recombinant protein.

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STRUCTURAL ANALYSIS OF FROG VIRUS 3 BY ELECTRON MICROSCOPY AND TOMOGRAPHY: COMPOSITION AND MORPHOLOGY OF ITS LARGE VIRION WITH INNER MEMBRANE

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Nucleocytoplasmic large dsDNA viruses (NCLDV) are among the largest and most complex viruses known, with the capsid size ranging from 200–800 nm in diameter [1]. Here, we focused on ranaviruses (~200 nm in diameter), global pathogens with increasing importance, which exist in two infectious forms: naked capsids and enveloped virions [2]. In order to determine the structure of the two respective forms of the virus, we purified and imaged virions of the specific type of ranavirus, frog virus 3 (FV3), by

cryo-electron microscopy and tomography. Electron micrographs and subtomogram average showed large capsids with three distinguishable layers enclosing the electron-dense core of packaged dsDNA. The inner shell presumably corresponds to an internal membrane, the intermediate layer to a proteinous capsid shell and the outer layer to an external lipid envelope. Overall, the FV3 capsid exhibits similar morphology and structural features as related viruses from the family *Iridoviridae*, PBCV-1 and



CIV [3]. Subtomogram averaging of individual extracted virions was done in *Bsoft* [4] using icosahedral symmetry. The average is consistent with the single particle reconstruction and further image analysis are undertaken to identify the putative special vertex for genome ejection, specific for the naked capsid form. Next steps are directed towards determining the structural and mechanistic features of the ranavirus replication cycle and structural basis for cell entry by the enveloped virions, determining the structure of the surface glycoproteins which mediate the entrance.

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PHASE TRANSITION APPROACH ON THE INTERPRETATION OF THE CHEMICAL OSCILLATION IN THE BELOUSOV-ZHABOTINSKY REACTION

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Actively studied over the last decades the Belousov-Zhabotinsky (BZ) reaction [1] is no lost interest in the scientific world nowadays. The BZ reaction was devised as a primitive model of citric acid cycle [2]. To the surprise of the authors, it brought about the phenomenon of chemical clock (in mixed systems) or spontaneous pattern formation (in still compartments). The distinct feature of the BZ reaction is that it possible to observe development of complex patterns in time and space by naked eye on a very convenient human time scale of dozens of seconds and space scale of several millimetres. The BZ reaction can generate up to several thousand oscillatory cycles in a closed sys-

tem, which permits studying chemical waves and patterns without constant replenishment of reactants. The sensitivity of the system to the external conditions changes make possible to investigate the change the pattern formation process in response on the modulating agent strongest factor (shape geometry in our case).

There are several hypothesis followed the target pattern propagation and wave evolution in the system [3,4], but most of them concentrate on the cascade of the chemical transformations [5,6] or process taking place in the reaction-diffusion system model [7]. Our investigation create new factors to forecast the chemical system behaviour un-



Figure 1. The wave evaluation course tracing in the tree colour channels demonstrate the reliable differences in the red colour channel. This fact allows us to suppose that along with changes in a chemical compound (have been traced by pixel intensity time oscillation in wave shape in green and blue colour channel) examined system is leading by some other accompanied process (phase transitions).

der changed environment conditions and their impact on the main structure formation of waves. The formation of structures presented as the combination of two processes which was detected by independent analysis of blue and red channel of the colour camera (Figure 1.). While blue and green colour channel follows the $\text{Fe}[(\text{phen})_3]^{2+}$ and $\text{Fe}[(\text{phen})_3]^{3+}$ - reaction catalyst and redox-indicator - concentration oscillation, oscillation in red colour channel was followed completely different behaving process. We suggest that the process observed in the red channel has the character of phase change and propose it to be connected with formation of Br_2 taking place during all reaction evaluation time. Our hypothesis have an agreement with other scientists work [8,9]. In this work are present the experimental results to prove that BZ system wave structure evolution response on changes in geometrical conditions are not only a reflection of chemical kinetics modification, but also directly dependent on the phase transition derived due to structural changes in Br-contained reaction compounds. We propose a model of the process which explains most of the distinct aspects of the structure formation based on deduced hypothesis.

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A course of applications of groups in crystallography for beginners and slightly advanced is planned.

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