Posters

P1

INTERACTION OF ZINC-DEPENDENT NUCLEASE FROM S1-P1 FAMILY WITH RNA

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Members of the S1-P1 nuclease family are zinc dependent phosphoesterases found in plants, fungi, protozoa and some bacteria. These nucleases have many important roles in organisms, such as specific apoptotic functions, stress response to viroid pathogenesis [1], scavenging for nutrients, or symbiont-host interactions [2]. They are often utilized in biochemistry and biotechnology and have potential medical applications [3]. S1 nuclease from *Aspergillus oryzae* is a single–strand specific nuclease with 3'-mononucleotidase activity and 5'-mononucleotides, mononucleosides as well as phosphate ions are its products and at the same time competitive inhibitors.

From previous studies non-specificity of S1 nuclease to nucleobases and its ability to remodel its active site is known [4]. The aim of this work is to analyze the differences in the binding of DNA and RNA in the S1 nuclease active site. Our study is based on two new structures at high resolution of S1 nuclease in complex with uridine and cytidin-5'-monophosphate (products of RNA cleavage) and their comparison with the already known structures of complexes of S1 nuclease with DNA products. Structural data are complemented with biophysical techniques in order to explain observed differences in the activity of S1 nuclease against DNA and RNA.

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P2

STRUCTURAL STUDIES OF THE 14-3-3 PROTEIN AND NEUTRAL TREHALASE (Nth1) COMPLEX

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14-3-3 proteins are a family of highly conserved molecules which were found in all eukaryotes. They interact with and regulate the function of hundreds of different proteins by recognizing specific phosphoserine- or phosphothreonine-containing motifs. Thus 14-3-3 are involved in many important physiological processes such as: regulation of cell cycle, signal transduction, metabolism, gene transcription or apoptosis. We focused on understanding of the 14-3-3 protein function in the regulation of the neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae*. This enzyme hydrolyses disaccharide trehalose and helps yeasts to survive different stress conditions. Nth1 can be phosphorylated by PKA and/or CDK1. Its activity is enhanced by the yeast 14-3-3 protein (Bmh1) binding [1, 2] and/or by Ca^{2+} binding within the EF-hand-like motif containing domain [3].

For revealing of the mechanism of the 14-3-3- and Ca-dependent activation of Nth1, solving the structure of Nth1 and its complex with 14-3-3 protein we used site-directed mutagenesis, enzyme activity measurements, MST, H/D exchange coupled to MS, SAXS and protein crystallography.

Our crystal structure of full-length Nth1 in complex with 14-3-3 protein provides a detailed mechanistic insight

into the role of 14-3-3 proteins in activating Nth1 [4]. We proved that 14-3-3 protein binding induces a rearrangement of the whole Nth1 molecule and enables the proper three-dimensional configuration of the pNth1 catalytic and calcium-binding domains relative to each other. The complex formation stabilizes the intrinsically disordered N-terminal part of Nth1 and moreover 14-3-3 protein directly interacts also with the separate Ca-binding domain of Nth1. Thus the EF-hand-like motif can function as an intermediary through which the 14-3-3 protein modulates the structure and function of the catalytic domain of Nth1. This process stabilizes the flexible part of Nth1 active site, so called "lid" loop, which completes the active site of pNth1 by providing side-chains important for catalysis and is crucial for the Nth1 activation. Our crystal structure of fully active Nth1 bound to yeast 14-3-3 protein Bmh1 provides the first high-resolution view of the neutral trehalase from eukaryotic organism as well as highlights the ability of 14-3-3 proteins to modulate the tertiary structure of a multi-domain binding partner.

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P3

STRUCTURAL ANALYSIS OF RESURRECTED ANCESTRAL HALOALKANE DEHALOGENASES

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Ancestral sequence reconstruction is a powerful approach allowing the resurrection of ancient enzymes based on sequences predicted by a phylogenetic analysis [1]. In this project, we predicted five ancestral enzymes (AncHLD1-5) of haloalkane dehalogenase belonging to subfamily II and representing the ancestors of the thoroughly characterized dehalogenases DbjA [2], DbeA [3], DhaA [4], DmxA[5] and DmmA[6]. Resurrected enzymes were overexpressed in *Escherichia coli*, purified to homogeneity, concentrated to 10 mg/ml and used for crystallization experiments. All crystallization trials were performed by using the sitting-drop vapor-diffusion method at 23 °C. The crystals of AncHLD2, AncHLD3 and AncHLD5 grown during the initial screening, were used for X-ray diffraction data collection and a complete data sets were collected to a resolution of 1.7, 1.3 and 1.8 Å resolution, respectively. Obtained microcrystals of AncHLD1 and AncHLD4 are currently optimized by variation of enzyme concentration, pH and precipitant concentration. The structure of AncHLD2, AncHLD3 and AncHLD5 was solved by molecular replacement using the structure of DbjA (PDB ID 3AFI) [7] as a search model. Similarly to descendant haloalkane dehalogenase enzymes, ancestral enzymes consists of two domains, the conserved main domain formed by eight-stranded -sheet with 2 lying in an antiparallel orientation with respect to the direction of the -sheet surrounded by 6 -helices. The second variable cap domain consists of five -helices. During refinement of the crystal structure of AncHLD2, two chloride anions were detected in the vicinity of the enzyme active site. The first chloride anion occupied the product-binding site and interacts with two conserved halide-stabilizing residues. The second chloride anion was located about 10 Å from the product-binding site and is buried deep in the protein core. The second halide-binding site has previously been observed in the structure of DbeA, the closely related descendant counterpart of AncHLD2. Comparison of structures of ancestral enzymes with structures of descendants accompanied by dynamic simulations will provide detailed insight into their catalytic properties.

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P4

GRAND THEFT AMONG BACTERIA AND PHAGES: THE STRUCTURE OF THE GENE TRANSFER AGENT

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Gene transfer agents (GTA), the virus-like particles of certain alpha-proteo-bacteria, represent the most sophisticated system of horizontal gene transfer in prokaryotes discovered to date. Here, we report the structure of *Rhodobacter capsulatus* GTA.

A novel purification method of GTA was developed, combining anion-exchange chromatography on methacrylate monolith columns with ultracentrifugation in sucrose gradient. This method resulted in > 95 % pure sample of full and empty GTA particles with ratio = 2:3. Cryo-EM studies revealed functionally important differences between GTA and bacteriophages in the capsid geometry. There are two types of GTA particles. The minor one (1 % of all particles) possess icosahedral head, with the triangulation number T = 3. The more prevalent one (99 % of all particles) possess oblate head (icosahedron shortened along fivefold axis), with the triangulation number for the center body Q = 3, resulting in the compression value in respect to spherical capsid of dQ = -3. To our knowledge this is the first structure with negative dQ value ever observed.

The capsid is composed out of major capsid protein with HK97 fold, decorated with head spikes on the capsid pentons. The portal and the tail proteins are homologous to those of phages from the family *Siphoviridae*. The tail of GTA contains 6 hexamer rings, with an estimated rise of 38.1 Å, the relative twist of the disks is yet undetermined. The portal, neck, and tail of the full GTA particle contain internal central density of unknown origin, which is not observable in the empty particle. Similar structure is not present in phages and may play a role in transducing of a signal to initiate genome-release. This differs from the conformational changes of baseplate, tail, and neck that trigger the genome release of phages.

The baseplate of GTA possess C3 symmetry and has been reconstructed to the resolution of 6.1 C. It is the only part of GTA structural locus with higher than 50% sequence similarity to known phages. Gp12 forms hexamer disc at the tip of the tail, gp13 forms central hub, and gp15 forms receptor binding trimers connected to the hub. The tail fiber is encoded in genome locus distinct from that, which encodes the rest of the baseplate, revealing complex evolution of the baseplate assembly.

Our results show more distant evolutionary relationship between GTAs and phages then previously thought, and reveal a strategy that enabled bacteria to utilize its parasite for own purposes.

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P5

STRUCTURAL STUDY OF STAPHYLOCOCCAL PHAGE phi812K1/420 PROTEINS BmpA, BmpB and BmpC

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Staphylococcus aureus causes a range of illnesses from minor skin infections to life-threatening pneumonia, meningitis, and sepsis. Furthermore, many *S. aureus* strains, particularly those isolated from hospitals, carry genes for resistance to antibiotics. Annual medical expenses caused by *S. aureus* infections in the EU and US were estimated to be more than €380 million [1] and $2 billion, respectively. In 2017, the World Health Organization listed *S. aureus* among the twelve most threatening antibiotic-resistant pathogens for which new treatments are urgently needed. Phage therapy is an alternative to antibiotics for treatment of staphylococcal infections. Phage phi812K1/420 infects 95% of Methicilin-sensitive *S. aureus* strains and at least 75 % of Methiciline-resistant strains [2]. However, before it can be applied as therapeutics, it is important to know the detailed structure of the virus particle. Here we show the current state of structural research of phage phi812K1/420 proteins BmpA, B, and C. BmpA and BmpB are being optimized for expression. BmpC was succesfully crystallyzed and diffraction data were recently collected. All proteins are part of the base plate, which is responsible for the attachment of the phage to host cell. The structure determina-

tion of the base plate proteins can reveal important informations about the infection process. We anticipate our study to be another step in introduction of staphylococcal phages as therapeutic agents.

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P6

CRYSTALLIZATION STUDIES OF NOVEL HALOALKANE DEHALOGENASE DgaA FROM *GLACIEOCOLA AGARYLITICA* NO2

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Haloalkane dehalogenases (HLDs) are microbial enzymes that have attracted significant interest because of their ability to catalyze the irreversible hydrolysis of a wide range of halogenated compounds. These enzymes can be used as potential applicants in industrial catalysis, in the bioremediation and the biosensing of environmental pollutants. Novel haloalkane dehalogenase DgaA (EC 3.8.1.5, HLDs) belonging to the superfamily of a hydrolases, was isolated from a psychrophilic and moderately halophilic organism, *Glaciecola agarilytica* NO2, that was found in marine sediment collected from the East Sea, Korea. The purified protein dialyzed against 50mM Tris HCl buffer (pH 7.5) overnight and stored at 193 K was used for crystallization experiments in the concentration of 13.6 mg ml^{-1}. Screening for crystallization conditions has been performed by Oryx crystallization robot (Douglas Instruments, Ltd., UK) using sitting drop vapour diffusion method. Commercial crystallization screen Index HR2-144 (Hampton Research, USA) was used for screening of DgaA crystallization conditions. Futher optimization to find successful crystallization conditions will be starting point for further research focused on structure determination and and description of protein function.

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P7

PHAGE ADHESION TO *S. AUREUS*: STRUCTURE-FUNCTIONAL STUDIES OF RECEPTOR BINDING PROTEIN 1

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Infection caused by antibiotic-resistant *S. aureus* strains are difficult to treat and can induce life-threatening symptoms. Because of the slow development of new antibiotics, alternative treatments are being looked for. One of the promising approaches is *phage therapy* employing bacterial viruses (phages) [1, 2]. Phage phi812K1/420 from the family *Myoviridae* infects and kills *S. aureus* cells [3]. The phage can infect 95 % of *S. aureus* strains, including those resistant to antibiotics. Such a wide host range makes the phage phi812K1/420 a promising agent for phage therapy.

Receptor Binding Protein 1 (RBP1) is located in the baseplate of the phage phi812K1/420 [4]. At the onset of infection, RBP1 binds to specific receptor at the surface of *S. aureus* and enables attachment of the phage to the bacterial surface. The goal of this project is to determine the RBP1 structure using X-ray crystallography and cryo-electron microscopy. Key interactions of RBP1 with *S. aureus* cell wall receptors will be studied with biochemical and biophysical assays, such as glycan array or difference scanning fluorimetry. RBP1 characterization will help to com-

plete our understanding of the phage infection process and allow design of phi812 variants targeted against specific *S. aureus* strains.

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P8

STRUCTURAL ALPHABETS FOR CONFORMATIONAL ANALYSIS OF NUCLEIC ACIDS

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We present a universal nucleic acids structural alphabet suitable for conformational analysis of DNA as well as RNA structures. The underlying local conformational classes assigned at a level of dinucleotide "step" are characterized by seven torsion angles of the sugar-phosphate backbone and two torsion angles around two glycosidic bonds, supplemented by empirically determined parameters. Our previous definition of 44 DNA conformational classes [1,2] was extended to incorporate also RNA conformers.

The conformational classes are built on a subset of RNA conformers defined previously [3] and classes newly defined by means of hierarchical clustering of available structural data. The necessary structural information was extracted from a sequentially non-redundant set of high resolution crystal structures containing about 60 and 60 thousands steps for DNA and RNA, respectively. We have obtained more than 70 conformational classes covering the combined structural variability of DNA and RNA.

We have found that many of the previously defined DNA conformational classes are shared among nucleic acids and are suitable for RNA description. They differ in relative occurrence, whereas in DNA B-forms prevail and A-forms are less common, A-forms are the most prevalent in RNA. The most significant feature of new conformational classes unique for RNA is the high occurrence of non-stacked steps. In spite of the apparent structural flexibility of RNA, the conformations rarely found in DNA comprise only about one third of all characterized classes.

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P9

STRUCTURE OF PROTEIN FROM NECK OF BACTERIOPHAGE INFECTING *STAPHYLOCOCCUS AUREUS*

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Bacteriophage phi812 is a lytic phage from the family *Myoviridae*. Phi812 infects most staphylococcal strains including those resistant to antibiotics [1]. The structure of this bacteriophage was previously determined by cryo electron microscopy [2] but functions of individual proteins remain unclear. Here we present our work towards resolving the structure of protein gp99 that forms the neck region of phi812. The protein may play a role in a regulation of the genome release from the virion during infection. Gp99 was cloned and expressed in *E. coli* and purified by affinity and size exclusion chromatography. A suitable crystallization condition was found and X-ray diffraction data with a resolution of 2.3 Å were collected. To solve the phase problem, a variety of methods had been tried, including molecular

replacement, heavy atom soaking and co-crystallization, and seleno-methionine incorporation. The structure of gp99 will help explain the mechanism of bacterial infection by bacteriophage. As there is a rising number of antibiotic resistant bacterial strains causing severe illnesses, phage therapy has a high potential. For this purpose, detailed knowledge of bacteria-phage interactions is vital.

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P10

VIRION STRUCTURE AND INHIBITION OF GENOME RELEASE OF HUMANECHOVIRUS 18

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Echovirus 18 (E18) is one of the most common enteroviruses, which cause aseptic meningitis, leukoencephalitis, and acute exanthema. No treatments against E18 are available and its structure is unknown. Here we present a cryo-EM study of E18 genome release and its inhibition by a capsid-binding compound. The structure of E18 virion was determined to the resolution of 3.2 Å. We show that binding of an inhibitor WIN51711 replaces a natural pocket factor within capsid protein VP1, but its binding does not cause any changes in the overall capsid structure relative to the native virus. However, the compound effectively decreases infectivity of the virus, probably by blocking of the genome release. Structure of so called "A" particle of E18 shows changes in its capsid that enable subsequent genome release. Similar to other enteroviruses the E18 A-particle contains pores along twofold symmetry axes.

P11

FUNCTIONAL CHARACTERISATION OF NOVEL CYSTEINE PROTEASE INHIBITOR FROM *FASCIOLA HEPATICA*

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Fasciolosis caused by the liver fluke *Fasciola hepatica* is a worldwide spread parasitic disease of domestic ruminants responsible for considerable economic losses in the cattle industry. In addition, fasciolosis is now recognized as an emerging human disease. This work is focused on FhCY2, a member of cystatin superfamily encoded by *F. hepatica.* FhCY2 is expressed from metacercarial to adult stages in several tissues and also found in *F. hepatica* excretory-secretory products (ESP). Sequence alignment analysis and homology modelling revealed that FhCY2 belongs to the type 1 cystatin (stefin) family, but surprisingly it has also typical features of mammalian members of the type 2 cystatin family, including two disulfide bridges and a signal sequence. Recombinant FhCY2 showed a broad inhibitory specificity towards various mammalian cysteine cathepsins and ESP proteases, including cathepsin L1 (FhCL1), the most abundant ESP protease. FhCL1 was produced by recombinant expression as a stable active-site mutant and used for the preparation of FhCL1-FhCY2 complex for crystallographic analysis. We propose that FhCY2 acts as a physiological regulator of endogenous parasite proteases as well as modulator of the host proteolytic system, and represents a potential vaccination target.

P12

STRUCTURES OF THREE INFECTION CYCLE INTERMEDIATES OF COXSACKIEVIRUS A6

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Coxsackievirus A6 (CVA6) has recently overtaken CVA16 and Enterovirus 71 as the primary causative agent of epidemics of hand, foot and mouth disease (HFMD) that usually affects children but occasionally spreads also to adolescents and adults. Millions of self-limiting HFMD cases and several hundred of deaths are reported each year, predominantly in the Asian-Pacific region. However, no vaccine against CVA6 is available, and vaccine development against CVA16 and EV71 has proven challenging and even trivalent vaccine candidates fail to cross-protect. Recently published structural characterizations of CVA6 [1,2] lack structural data of the native antigenic, mature virion. Unlike other picornaviruses, CVA6 virus particle preparations yield visibly only expanded and empty capsid conformations [1, and our observations]. By using a very large cryo-electron microscopy (cryo-EM) dataset, we were able to detect a small subset of mature virions during the 3D classification steps of single particle reconstruction. Here, we present high-resolution cryo-EM structures of CVA6 infection cycle intermediates, including mature virion, expanded and empty capsids to 3.4, 2.8 and 3.2 Å resolution (at FSC = 0.143), respectively. Structure comparisons, conformational changes and their implications will be discussed.

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P13

CONFORMATIONAL BIASES OF Tau PROTEIN'S MICROTUBULE BINDING REPEAT REGIONS

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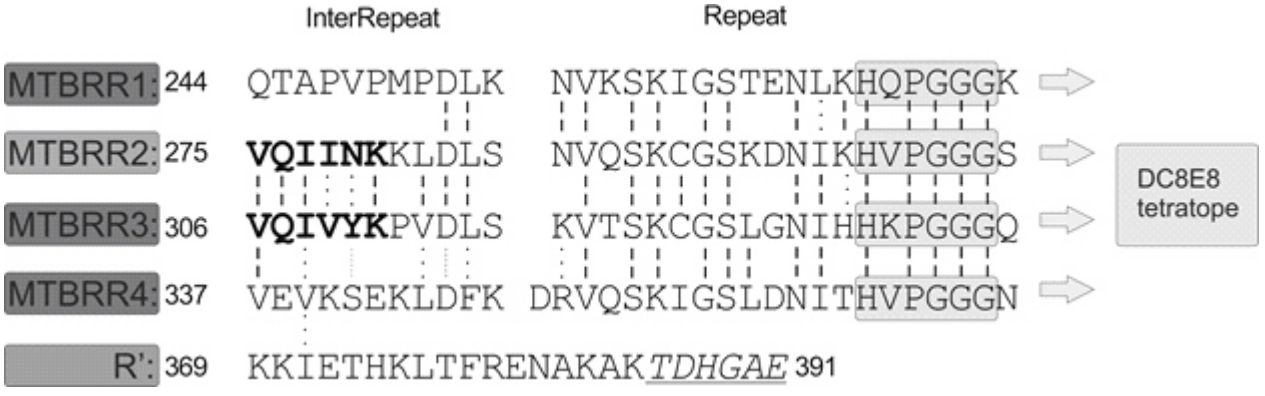
Accumulation of intrinsically disordered protein tau in the form of insoluble aggregates is a common feature of neurodegenerative tauopathies. Monoclonal antibody DC8E8 is able to inhibit tau-tau interaction and therefore it holds promise for the immunotherapy of Alzheimer's disease. The active vaccine based on the DC8E8 epitope peptide has successfully passed the phase 1 clinical trial [1]. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeats (MTBRs) of tau. However, the affinity of DC8E8 for its MTBR epitopes differs and descends as follows: MTBR2 > MTBR1 > MTBR3 > MTBR4. These differences in the antibody affinity for highly homologous epitopes can be attributed to different conformational biases of epitope peptides for the bound conformation. The crystal structure was solved so far only for the complex of MTBR2 peptide with DC8E8 Fab. We have performed 300 ns long molecular dynamics simulations of 18 amino acids containing peptides from all four MTBRs in NAMD program with CHARMM36m force field suitable for simula-



P15

FACILITIES AT THE CENTRE OF MOLECULAR STRUCTURE OF BIOCEV

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The Centre of Molecular Structure (CMS) consists of three core facilities: i) Biophysical Measurements; ii) Crystallization and X-ray Diffraction; iii) Structural Mass spectrometry. The CMS thus provides complementary tools for the studies of the biophysical properties, three-dimensional structure and structure:function relationships of biological macromolecules.

The Biophysical Techniques facility offers circular dichroism spectroscopy (Chirascan Plus CD spectrometer), spectrophotometry (Specord 50 Plus UV/Vis spectrophotometer), differential scanning fluorescence (Prometheus NT.48), dynamic light scattering (Zetasizer Nano ZS90) and differential scanning calorimetry (Microcal VP-DSC). These devices are used for the determination of size, structure and stability of biomolecules, and for the study of conformational changes and thermodynamics of temperature transitions. Isothermal titration calorimetry (Microcal iTC200), microscale thermophoresis (Monolith NT.115 and NT.LabelFree) and surface plasmon resonance (ProteOn XPR36) allow to characterize protein-protein interactions and protein-ligand interactions (small molecule, DNA, RNA, peptides, sugars, lipids…) that can be measured under close to native conditions.

The Crystallisation and Diffraction facility includes an ArtRobbins Gryphon dropsetter, which allows to set-up nanodrop crystallisation plates, and a Formulatrix RI1000 hotel for the storage of crystallization plates and for the automated, remote-access monitoring of crystallization droplets using visible, polarized and UV light. A glovebox, a cold room and a warm room (all equipped with stereomicroscopes) allow crystallisation and crystal manipulation in different environments (including in oxygen-free conditions). The Bruker D8 Venture diffractometer equipped with a liquid metal jet X-ray source (plus its ISX stage for in situ diffraction) is the core equipment for diffraction data acquisition.

The Structural Mass Spectrometry facility offers access to an ultra-high resolution 15T-Solarix XR FT-ICR (Bruker Daltonics), and to a MALDI-TOF Autoflex Speed (Bruker Daltonics) mass spectrometers. This instrumentation allows determination of precise mass of biological macromolecules, characterization of posttranslational modifications, peptide mass fingerprinting, detection of small molecule/metabolites and monitoring of protein structural changes/protein-protein interactions under physiological conditions by hydrogen-deuterium exchange and chemical cross-linking. An Agilent Technologies 1200 HPLC system coupled to the 15T-SolariX XR mass spectrometer is used for the separation of complex peptide mixtures, proteins and metabolites.

Together with the core facilities of CEITEC, the CMS is part of Czech Infrastructure for Integrative Structural Biology (CIISB), national affiliated centre of Instruct-ERIC (European Integrated Structural Biology Infrastructure).

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P16

STRUCTURE-FUNCTION ANALYSIS OF DhmeA, A HALOALKANE DEHALOGENASE OF SUBFAMILY III FROM *HALOFERAX MEDITERRANEI*

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Haloalkane dehalogenases (HLDs), which catalyse the cleavage of the carbon-halogen bond of organohalogen compounds, are recognized as the key tools in many industrial and biotechnological processes [1]. The HLDs are composed of two domains a main domain containing catalytic residues and a cap domain. According to phylogenetic and structural analyses, HLDs can be divided into three subfamilies - HLD-I, HLD-II and HLD-III, which differ in the cap domain architecture and the composition of their catalytic pentads [2]. Unlike the HLD-I and HLD-II members, which structures have been studied by X-ray crystallography, the structural information on HLD-III enzymes is missing. A major problem with structural characterization of HLD-III members is the fact that these enzymes form polydisperse high-molecular weight oligomeric states [3, 4], which hampers their structural

analysis. Here, we report an optimized method for recombinant production and purification of DhmeA from the halophilic archeon *Haloferax mediterranei*. The strategy employs a robust recombinant expression in *Escherichia coli* together with protein purification through a poly-histidine affinity tag and size-exclusion chromatography. Subsequent biophysical characterization by differential scanning fluorimetry and dynamic light scattering revealed that the newly developed purification method significantly improved monodispersity of DhmeA. Our findings pave a way for probing DhmeA structure by high-resolution techniques such as X-ray crystallography and single-particle cryo-electron microscopy analysis. Cryo-electron microscopy data was collected and initial 3D model of DhmeA was inferred. Future work will focus on 3D structure reconstruction, model building and experimental validation.

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P17

STRUCTURAL STUDIES OF THE *MYOVIRIDAE* BACTERIOPHAGE 812 PORTAL COMPLEX

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The Twort-like bacteriophage 812 from the *Myoviridae* family is a promising therapeutic alternative for treatment of antibiotics-resistant bacterial infections. We previously reported high-resolution cryo-electron microscopy reconstructions of the isometric head and the double-layered tail of this phage [1]. The portal domain is located in the neck region at the interface between the head and the tail and serves as a channel for DNA translocation. *In situ* cryo-EM reconstruction of the portal complex from phages after genome release, obtained at 3.8 Å resolution, reveals that the portal adopts a dodecameric ring-shaped conformation with an outer diameter of 13 nm. A prominent crown composed of axially stacked C-terminal helices forms a funnel-like structure opening towards the head interior. In contrast, a preliminary 6.5 Å cryo-EM electron density map of a recombinant portal complex shows that the latter assembles predominantly into 13-meric structures with a diameter of 15 nm and presents conformational differences in the crown and the stem regions. These differences likely stem from mis-folding in solution in absence of phage chaperones, but they could also correspond to structural rearrangements experienced by the portal complex during different stages of phage maturation.

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P18

EXPRESSION, PURIFICATION AND STRUCTURAL ANALYSIS OF RECOMBINANT CHRONIC BEE PARALYSIS VIRUS RNA-DEPENDENT-RNA-POLYMERASE

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Colony collapse disorder is a phenomenon, which causes rapid decline in bee colonies. It is a global problem for bees and beekeepers. Although the origin and mechanism of this process is still not know, one of the agents, which cause the collapses are virus infections. One of these viruses is chronic bee paralysis virus (CBPV), a positive single strained RNA virus. We aim to determine the structure of CBPV RNA-dependent-RNA-polymerase. We used *E.* coli bacterial expression system, as well as, baculovirus expression system in *SF9* cell line, which is more phylogenetically related to bees, the natural hosts of CBPV. We found significant differences in solubility of CBPV RNA polymerase produced by these expression systems. Partial solubility of recombinant CBPV RNA polymerse produced by *SF9* cell line enabled further experiments leading to the preparation of pure protein, its crystallization, and subsequent structure determination. Knowledge of the structure of CBPV RNA polymerase may allow development of anti-viral compounds.

P19

STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF AICHI VIRUS RNA DEPENDENT RNA POLYMERASE

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Aichi virus is a single stranded plus RNA (+ RNA) virus which causes gastroenteritis in humans. Aichi virus is also used as a model organism for studying related more virulent viruses like SARS, HCV, West Nile virus, Yellow fever virus etc. Protein $3D^{pol}$, RNA dependent RNA polymerase, is a key enzyme for the life cycle of a + RNA viruses. The $3D^{pol}$ enzymes catalyze formation of phosphodiester bond between RNA nucleotides. The picornaviral RNA replication is protein $3D^{pol}$ activity dependent. The RNA replication is curtail for both viral genome multiplication and viral protein translation.

+ RNA viruses hijack phosphatidylinositol 4-kinases (PI4Ks) to generate membranes highly enriched in phosphatidylinositol 4-phosphate (PI4P). These viral induced membrane structures serves as structural scaffold for RNA replication and also to protect from innate intracellular immunity. We studied interaction of $3D^{pol}$ with PI4P lipid using recombinant $3D^{pol}$ from Aichi virus 1 (AiV), Poliovirus 1 (PV), Coxsackievirus b3 (CV) and Enterovirus 71 (EV). The polymerase activities of $3D^{pol}$ enzymes were compared by PETE fluorescence assay [1]$_{pol}$

We also determined the crystal structure of Aichi 3D at 3.8 Å resolution. The structure revealed an overall conserved fold. However, the Aichi $3D^{pol}$ has a different N-terminal structure compared to other related picornaviruses (PV, CV, EV), where the N-terminal residue is buried in a pocket at the base of fingers domain. Our structure reveals that Aichi $3D^{pol}$ can not stabilize itself by a slip of its first redue in the hydrophobic core of the protein.

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P20

SmSP2: AN ANTI-HEMOSTATIC SERINE PROTEASE SECRETED BY THE BLOOD FLUKE PATHOGEN, *SCHISTOSOMA MANSONI*

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Schistosomiasis caused by parasitic blood flukes of the genus *Schistosoma* is the second most important parasitic infection after malaria with more than 240 million people infected. There is an urgent need to identify novel anti-schistosomal targets for therapeutic interventions. Our work is focused on *S. mansoni* serine protease 2 (SmSP2). It was localized in the tegument and esophageal glands, ovaries, testes and vitelaria of adult schistosomes by immunofluorescence microscopy and *in situ* RNA hybridization. Enzyme activity measurements and immunoblotting identified SmSP2 in the excretory/secretory products. Recombinant SmSP2 was produced in the *Pichia pastoris* expression system and its cleavage specificity was investigated using combinatorial substrate libraries and 3D model analysis. SmSP2 was found to activate plasmin, the key component of the fibrinolytic system, and releases vasoregulatory kinins from kininogen. Our results suggest that SmSP2 plays a role in host-parasite interactions and represents a potential target for novel drug or vaccine interventions.

P21

THE BENCHMARK OF ^{31}P NMR PARAMETERS IN PHOSPHATE: A CASE STUDY ON STRUCTURALLY CONSTRAINED AND FLEXIBLE PHOSPHATE

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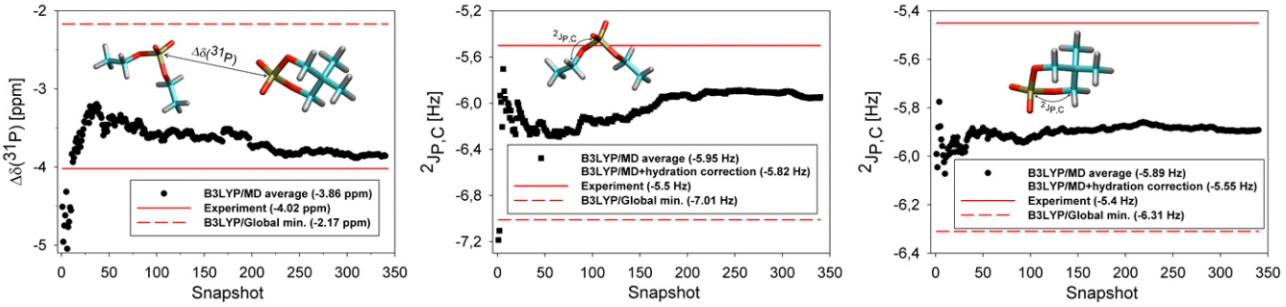
A benchmark for structural interpretation of the ^{31}P NMR shift and the $^{2}J_{P,C}$ NMR spin–spin coupling in the phosphate group was obtained by means of theoretical calculations and NMR measurements in diethylphosphate (DEP) and 5,5-dimethyl-2-hydroxy-1,3,2-dioxaphosphinane 2-oxide (cDEP). The NMR parameters were calculated employing the B3LYP, BP86, BPW91, M06-2X, PBE0, KT2, KT3, MP2, and HF methods, and the 6-31+G(d), Iglo-n (n = II, III), cc-pVnZ(n = D, T, Q, 5), aug-cc-pVnZ(n = D, T and Q), and pcS-n and pcJ-n (n = 1, 2, 3, 4) bases, including the solvent effects described with explicit water molecules and/or the implicit Polarizable Continuum Model (PCM). The effect of molecular dynamics (MD) on NMR parameters was MD-calculated using the GAFF force field inclusive of explicit hydration with TIP3P water molecules. Both the optimal geometries and the dynamic behaviors of the DEP and cDEP phosphates differed notably, which allowed a reliable theoretical benchmark of the ^{31}P NMR parameters for highly flexible and structurally constrained phosphate in a one-to-one relationship with the corresponding experiment. The calculated ^{31}P NMR shifts were referenced employing three different NMR reference schemes to highlight the effect of the ^{31}P NMR reference on the accuracy of the calculated ^{31}P NMR shift. The relative (^{31}P) NMR shift calculated employing the MD/B3LYP/Iglo-III/PCM method differed from the experiment by 0.16 ppm while the NMR shifts referenced to H_3PO_4 and/or PH_3 deviated from the experiment notably more, which illustrated the superior applicability of the relative NMR reference scheme. The $^{2}J_{P,C}$ coupling in DEP and cDEP calculated employing the MD/B3LYP/ Iglo-III(DSO,PSO,SD)/cc-PV5Z(FC)/PCM method inclusive of correction due to explicit hydration differed from the experiment by 0.32 Hz and 0.15 Hz, respectively. The NMR calculations demonstrated that reliable structural interpretation of the 31P NMR parameters in phosphate must involve both the structural and the dynamical components.



P22

TEST OF GENETIC CODE EVOLUTION HYPOTHESES: REVERSE EVOLUTION OF SPECIFIC TARGET PROTEINS BY mRNA-DISPLAY TECHNIQUE

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Although extant proteins consist of 20 different amino acids, it has been proposed that primordial proteins consisted of a smaller set of "early" amino acids and that additional "modern" amino acids have gradually been recruited into the genetic code [1-3]. This naturally leads to the questions: can structured and functional proteins be constructed using the "early" amino-acid alphabet? Can extant proteins be reverse-evolved while preserving their structure/function?

To test this, protein databases have been inspected to select model extant protein candidates with different structural folds. Our preliminary search contains proteins with both catalytic and binding/interaction functions.

The selected protein targets were "reverse-evolved" *in vitro* into variants where the "modern" amino acid were randomized by "early" ones. The libraries of randomized genes were incorporated into a genotype-phenotype linkage to be compatible with an appropriate library display (mRNA display [4]) and selection method. The selection of successful candidates was based on conservation of structure and/or function and the most "successful" variants will be characterized.

This research will inform us of the essentiality of "modern" amino acids for building protein structure/function and thus will provide a direct test of the hypotheses about early proteins. In addition, proteins constructed from a limited amino acid alphabet are of importance in protein engineering and synthetic biology. Finally, this area touches upon the very basic link of protein sequence-structure-function that lies at the core of many biotechnological and biomedicine problems and has express implications for construction of artificial biochemistries.

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P23

CRYSTAL STRUCTURE OF HONEYBEE HEXAMERIN 70B PROVIDES INSIGHT INTO REGULATION OF JUVENILE HORMONE LEVELS IN HAEMOLYMPH OF PUPAE

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The most abundant proteins in haemolymph of honey bee (*Apis mellifera*) larvae and pupae are hexamerins. These haemocyanin derived proteins are produced by a larval organ „fat body" and serve as a source of aminoacids for the development of pupae that do not consume food. Furthermore, there has been an evidence that hexamerins act as juvenile-hormone binding proteins but the mechanism remained unknown. Here we present a structure of native hexamerin 70b, isolated from bee pupae, determined to the resolution of 2.0 Å by X-ray crystallography. Hexamerin 70b forms hexamers with 32 symmetry. Each subunit contains an enclosed hydrophobic cavity occupied by one molecule of putative juvenile hormone. We suggest that the juvenile hormone can be released only upon proteolytic digestion of hexamerin. Therefore, the hexamerin consumption is linked to release of free juvenile hormone that may affect development of pupae. This regulation mechanism might be universally conserved among holometabolous insects.

P24

CRYSTALLIZATION STUDIES AND PRELIMINARY X-RAY CRYSTALLOGRAPHIC ANALYSIS OF A NEWLY PREPARED HISTIDINOL–PHOSPHATE PHOSPHATASE Tt82 FROM *THERMOCOCCUS ONNURINEUS*

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Dephosphorylation of histidinol–phosphate is the eight step in the histidine-biosynthetic pathway and is catalyzed by histidinol-phosphate phosphatase [1]. The main goal of this research was to optimize crystallization conditions and obtain suitable monocrystals of a newly prepared and until now uncharacterized protein Tt82 from *Thermococcus onnurineus*, which is considered to be a histidinol–phosphate phosphatase. Crystal screening was performed with crystallization kit Index-HR2-144 (Hampton Research, USA) using sitting drop vapor diffusion technique at 295 K. The most suitable crystals for diffraction experiment were grown from the precipitant solution containing 0.2 M magnesium chloride hexahydrate, 0.1 M Tris pH 8.5 and 25% w/v PEG 3350 (condition No. 85). Native data set was collected to the resolution of 1.6 Å at the BESSY-II synchrotron operated by Joint Berlin MX-Laboratory (Berlin-Adleshof, Germany). Although attempting to solve the crystal structure using molecular-replacement method with homologues (PDB codes 1qyi, 3pib, 3kbb, 2hdo, 3r3h, 2vvl, 3iru) as search models, all trials using MOLREP [2] and Phaser [3] resulted in failure. Therefore, heavy atom derivative crystals were grown from the precipitant solution No. 85 from Index-HR2-144 (Hampton Research, USA) adding 1 mM manganese (II) chloride and data set is about to be collected. Diffraction data will be used for further research, mainly for solving the structure by heavy atom derivatization method.

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P25

ABBREVIATION PARADISE: THE Taz2 (CBP) TAD (C/EBP) INTERACTION

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Cyclic-AMP-response element-binding protein (CREB)-binding protein, (CBP), and its paralog p300 are histone acetyl transferases playing a critical role in embryonic development, cell growth control, division as well as homeostasis. Via its Taz2 domain, they interact with multiple transcription factors to regulate gene expression. These transcription factors include CCAAT-enhancer-binding proteins, the C/EBP family. Through transactivation domain (TAD), C/EBPs recruit the co-activators (p300, CBP) that open up chromatin structure and mediate its phosphorylation through the recruitment of specific kinases. C/EBP binds to the closed chromatin and acts as a pioneering factor for initiating tissue-specific gene expression at several promoters. Here, we present the detailed structural characterization of the C/EBP interaction with CBP.

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P26

STRUCTURE AND REPLICATION OF *EMILIANIA HUXLEYI* VIRUS 86

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The *Emiliania huxleyi* virus 86 (EhV-86) is a Coccolithovirus from the family *Phycodnaviridae*. EhV-86 infects marine coccolithophore alga *Emiliania huxleyi*, which is found in almost all ocean ecosystems from the equator to sub-polar regions. *E. huxleyi* forms massive blooms, which can cover over 100,000 square kilometers and are visible from space. The blooms affect not only the properties of the surface ocean water, but also the global climate. These algae blooms collapse usually after 5–8 days because of infections by coccolithoviruses, most often by EhV-86 [1]. This virus is unique among other Phycodnaviruses, because it is enveloped by a lipid membrane. Thanks to the outer membrane EhV-86 exploits an animal-like membrane-fusion infection strategy and its progeny virions leave the algal host cell by budding [2].

We cultivate *Emiliania huxleyi* in large volumes and infect it with EhV-86. We purified the EhV-86 particles from the lysed alga culture and are working toward structure determination of EhV-86 by cryo-electron microscopy. To characterize the life-cycle of EhV-86 inside the alga cell, we are preparing thin lamellas (under 300 nm thick) of virus-infected cells using focused ion beam milling. These lamellas will be investigated by cryo-electron tomography.

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P27

NOVEL MACROCYCLIC INHIBITORS OF HUMAN CATHEPSIN D

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Human cathepsin D (HCD), a lysosomal aspartic peptidase, plays an important role in tumor progression and metastasis. It is an independent marker of poor prognosis in breast cancer. HCD is overexpressed and hyper-secreted in cancer cells and is involved in proteolysis of various intra- and extracellular factors and matrix remodeling. Our work is focused on the development of HCD inhibitors as novel tools for HCD regulation. We designed and synthesized a set of macrocyclic peptidomimetic inhibitors that were used for analysis of the macrocyclic scaffold and specificity of S1 and S2´ subsites. The high resolution crystal structures of three macrocyclic inhibitors in complex with HCD were determined and their binding mode was investigated using quantum chemical calculations. The work identified a novel inhibitor scaffold and interaction hot spots that can be exploited for the rational design of specific inhibitors of HCD and related aspartic peptidases as potential chemotherapeutics.

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P28

STRUCTURAL BASIS OF ASK1 INHIBITION BY DJ-1

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Human ASK1 (Apoptosis signal-regulating kinase 1) is a Ser/Thr kinase, belonging to subfamily of mitogen activated protein kinases (MAPKs). ASK1 is activated as a response to various stress stimuli, further transduces signal and activates response pathways playing role in apoptosis, differentiation, survival or immune response [1]. During normal conditions ASK1 is tightly regulated mostly by its direct physiological inhibitors 14-3-3 and Thioredoxin [2]. Recent studies, showed that a human protein/ nucleic acid

deglycase DJ-1 is another important negative regulator of ASK1.

DJ-1 is a versatile enzyme playing role as an oxidative stress sensor, redox-sensitive chaperone, protease and transcription factor, forming dimers when activated. All enzymatic activities of DJ-1 lead to protection against oxidative stress and cell death [3, 4]. There are two models describing role of DJ-1 as a direct negative regulator of ASK1, however the exact molecular mechanism is not known [5].

Both, ASK1 and DJ-1 play important role in tissue homeostasis and cellular stress response, thus deregulation of their activity in human results in various diseases and cancer [6-8]. Aim of this study is therefore: to describe regions of ASK1, DJ-1 and thioredoxin participating in the interaction and to provide a detailed molecular mechanism of ASK1 inhibition. Data obtained in this study can be further used to design drugs and therapeutic approaches to treat diseases caused by deregulated ASK1 activity.

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P29

VACCINIA VIRUS AS VECTOR FOR HUMAN RHINOVIRUS C3 EXPRESSION

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Human rhinoviruses (HRVs) belong to the family *Picornaviridae* of small, icosahedral, non-enveloped viruses containing 7.5 kb long positive-stranded RNA genomes. There are over 300 serologically distinct human rhinoviruses which are divided to A, B, and C types. Infected patients exhibit diseases ranging from mild upper respiratory tract common colds to severe lower respiratory tract bouts of bronchiolitis, wheeze, and asthma exacerbation. Viruses belonging to human rhinoviruses type C are more genetically diverse than viruses of A and B types and are more prone to stimulate asthma reactivity or more virulent. There are no specific treatments for HRV-C infections. Viruses of the C type use cadherin-related family member 3 protein as receptor for their cell entry. This receptor is not expressed in undifferentiated immortalized cells commonly used for propagation of viruses in tissue cultures, which complicates cultivation of HRV-Cs. In this work we plan to use recombinant vaccinia virus for delivery and expression of HRV-C3 in Hela cells. The recombinant vaccinia virus will contain HRV-C3 genome sequence and gpt/fluorescence selection marker integrated into vaccinia thymidine kinase locus. HRV-C3 will be expressed under the control of vaccinia early or early/late promoter combined with tetracycline-dependent operators. HRV-C3 is tagged with HA-tag or BoBS aptamer for further detection. Expressed and purified HRV-C3 will be used for structure determination by cryo-electron microscopy.

P30

EXPLORING DRUGGABLE HOT SPOTS IN *SCHISTOSOMA MANSONI* CATHEPSIN B1 FOR STRUCTURE-BASED DESIGN OF VINYL SULFONE INHIBITORS

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Schistosomiasis, caused by parasitic blood flukes of the genus *Schistosoma,* afflicts over 200 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a gut-associated peptidase that digests host blood proteins and is a drug target for vinyl sulfone inhibitors. We present a detailed inhibition profiling of SmCB1 with a set of vinyl sulfone peptidomimetic derivatives. They were screened against recombinant SmCB1 and against *S. mansoni* schistosomulas. This work provided two inhibitors of SmCB1 with the IC_{50} values in the sub-nanomolar range that are the most effective inhibitors of this enzyme reported to date. Their high resolution crystal structures in complex with SmCB1 were determined. Analysis of the inhibitor binding mode using quantum chemical calculations identified novel interaction hot spots that can be exploited for the rational design of anti-schistosomal chemotherapeutics.

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P31

HUMAN PROCASPASE-2 PHOSPHORYLATION AT BOTH S139 AND S164 IS REQUIRED FOR 14-3-3 BINDING

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Caspase-2 engages the mitochondria-dependent apoptotic pathway by inducing the release of cytochrome c. Previous studies in *Xenopus laevis* oocytes have identified that phosphorylation of procaspase-2 (proC2) at several residues under nutrient abundance conditions suppresses its activation and subsequently blocks the apoptosis through process involving the binding of the scaffolding protein 14-3-3 [1]. To elucidate the mechanism of the 14-3-3 protein-mediated inhibition of caspase-2 in human, we used various biochemical and biophysical approaches to identify phosphorylated motifs of human proC2 required for 14-3-3 binding. Using fluorescence polarization assay, native electrophoresis and analytical gel filtration we show that the 14-3-3:proC2 complex assembling is governed by both of these binding sites: S139 and S164 [2].

Characterization of proC2 in solution showed its monomeric status (methods of analytical ultracentrifugation and small-angle X-ray scattering). By continuing studies of the 14-3-3:proC2 complex we determined that the dissociation constant between 14-3-3:proC2 is in a nanomolar range and the stoichiometry of the interaction is 2:1. Data also indicate that other regions of proC2, in addition to phosphorylation motifs, may be involved in the interaction with 14-3-3. Limited proteolysis of proC2 alone and in the complex with 14-3-3 protein by trypsin and chymotrypsin found out the capability of 14-3-3 to protect the proC2 N-terminal part against proteolytic degradation. The circular dichroism spectra measurements detected significant changes in the tertiary structure of proC2 after 14-3-3:proC2 complex formation.

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P32

STRUCTURE-FUNCTIONAL STUDIES OF PROTEIN P4 FROM BACTERIOPHAGE 8

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The dsRNA bacteriophages of *Cystoviridae* family package their genome into empty capsid – procapsid, which protects the genome from degradation inside as well as outside host cell. The genome packaging is performed by a molecular motor - P4 proteins, which are components of procapsid. The P4s possess an NTPase activity that converts the chemical energy from ATP hydrolysis to a mechanical movement of packaging ssRNA precursors into a procapsid, where the replication and transcription of dsRNA occurs [1- 3]. The P4s are RNA helicases belonging to the Superfamily 4 of helicases with characteristic presence of conserved sequence motifs (H1, H1a, H2, H3 and H4) [1- 3]. The RNA helicases cause the distribution of RNA-protein complexes and carry out RNA unwinding [2]. The P4 assembles into hexameric ring (Fig.1), which has on the outer perimeter NTP-binding sites and the nucleic acid binding sites are located in the central channel. Each P4monomer can be divided into N-terminal, core NTPase domain with sequence motif and C-terminal domain, which is inserted into the central channel of hexamer and its conformational changes regulate ring stability and ATPase activity of P4s [1, 3-4]. Here we grow the monocrystals of the 8 P4 protein (Fig. 2) in the crystallization conditions of 100mM sodium acetate (pH 4,6) and 2,2 M ammonium sulphate. These conditions were suitable enough to be a good starting point for the next crystallization experiments with RNA assembled to P4.

The work is supported from European Regional Development Fund-Project "Mechanisms and dynamics of macromolecular complexes: from single molecules to cells" (No. CZ.02.1.01/0.0/0.0/15_003/0000441).

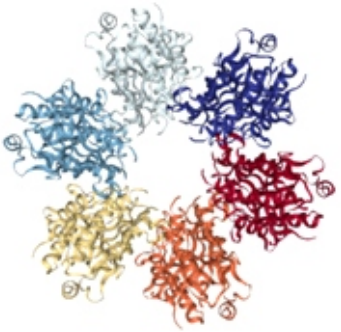

Fig. 1. The tertiary structure of P4 hexamer from phage 8. The monomers are distinguished by different colors [Data from PDB: http://www.rcsb.org/3d-view/4BLQ/1].

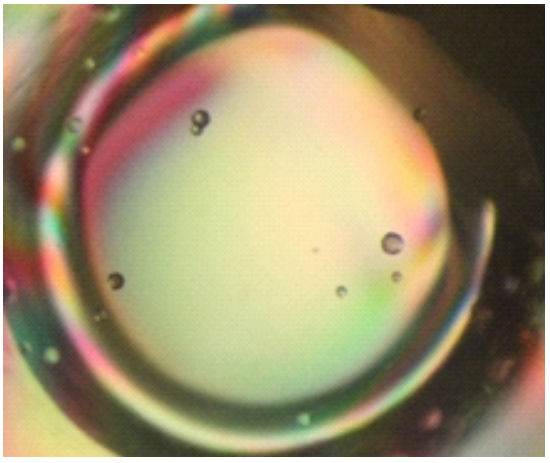

Fig. 2. Crystals of protein P4 [5,5mg/ml] from condition with 100 mM sodium acetate (pH 4,6) and 2,2 M ammonium sulphate

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P33

INTERFERONS TYPE II AND THEIR RECEPTORS R1 AND R2 IN FISH SPECIES: STRUCTURE, FUNCTION AND EVOLUTION

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Study of accessory cytokines and signaling pathways in fish provide important clues to understanding of evolution of the antiviral defense system in vertebrates. Interferon gamma (IFN-) is one of the key players in the regulation of immune system of vertebrates especially in response to viral infections. We studied phylogeny, biophysical and structural aspects of IFN- and its receptors in fish species to fill up the knowledge gap about its evolution and structural properties in fish species. In this study we determined the crystal structure of structure of IFN- from olive flounder (Paralichthys olivaceus, PoliIFN-) at 2.3 Å resolution. The overall fold of PoliIFN- is similar to the other known IFN- structures but significant differences were observed, namely the additional -helix G at the C-terminus and a different angle between -helices C and D. By comparing the known IFN- and structures of its two receptors R1 and R2 we suggest a putative structure of the binary and ternary complexes. Our computational analysis showed that three functionally tightly linked molecules, IFN- , IFN- R1, and IFN- R2, have probably underwent distinct evolutionary events. IFN- split into two distinct clusters of IFN- and IFN- related proteins. Genes for IFN- related proteins develop independently in different fish classes. Genes for IFN- receptor 1 also revealed the existence of two independently evolving receptor types. In contrast, there is no sign of existence of a second group of IFN- receptor 2.

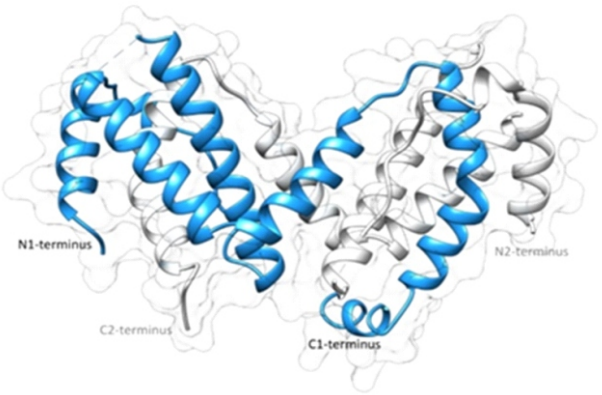

Figure Three-dimensional (3D) structure of the IFN- dimer from *Paralichthys olivaceus* oliIFN-). A crystal structure of PDB entry 6F1E. The monomer 1 is shown in blue, the monomer 2 in white, the solvent accessible surface is shown in white.

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P34

WEAK LIGAND BINDING: DATA PROCESSING AND ELECTRON DENSITY CALCULATION METHODS

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Analysis of interactions between macromolecules and small molecule ligands represents one of the leading courses to develop new drugs. A number of libraries containing from tens to thousands of small molecule fragments have been already used to screen potential binding sites [1]. These observations were later used for the design of a larger ligand composed of chemically linked fragments that would be of a biological importance. One of the problematic issues is weak (or partial) binding of a fragment that may lead to potential loss of information in calculated electron density.

We collected a medium resolution test dataset of an FAD-dependent enzyme that binds a ligand with partial occupancy. At first, we have analyzed the influence of diffraction limit estimation. After the careful diffraction data processing, we have tested several methods of electron density calculation (*e.g.* composite omit map, feature-enhanced map, polder map, *etc.*) [2-3]. Comparative analysis of the calculated electron densities was performed.

We show that weak reflections may significantly increase the quality of observed electron density for the ligand, and also an importance of various electron density calculation approaches in the validation of ligand binding. We have demonstrated that careful analysis of the data may lead from initial unobserved ligand binding to final determination and validation of partial occupancy ligand binding.

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P35

STUDY ON SEVERAL PLANT ALDEHYDE DEHYDROGENASES FROM MOSS *PHYSCOMITRELLA PATENS*

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Aldehyde dehydrogenases (ALDHs) represent a superfamily of $NAD(P)^+$-dependent enzymes that catalyze oxidation of aldehydes to carboxylic acids. Aldehydes are known for being highly reactive and toxic at higher concentrations and ALDHs play a crucial role in detoxifying aldehydes produced by various metabolic pathways and during various stress conditions such as salinity, heat, cold and drought. At least 13 ALDH distinct families are found in plants. ALDHs sharing more than 40% sequence identity belong to the same family. ALDHs share a common fold of three domains and carry a catalytic cysteine residue. While certain algae genomes contain up to nine *ALDH* genes, the genome of a moss *Physcomitrella patens* already comprises 21 genes sorted in many more families. Therefore, the moss *Physcomitrella* represents an important non-vascular plant model to study an evolution of *ALDH* genes.

Here, we focused on ALDH2, ALDH10 and ALDH12 family members from the moss and compare them with their orthologues in maize (*Zea mays*). Plant ALDH2 family comprises mitochondrial and cytosolic isoforms split into ALDH2B and ALDH2C subfamilies, respectively. While mitochondrial ALDH2 isoforms display acetaldehyde activity across the whole plant kingdom, the role of cytosolic isoforms is much more diverse. They can oxidize various aliphatic and aromatic aldehydes including benzaldehyde or coniferaldehyde from phenylpropanoid pathway and many others. The genome of *P. patens* contains two *ALDH2* genes coding for PpALDH2A (belonging to ALDH2C subfamily) and PpALDH2B from ALDH2B subfamily. ALDH10 family represents peroxisomal aminoaldehyde dehydrogenases also known as betaine aldehyde dehydrogenases and the family is represented only by a single *ALDH10* gene in moss. Finally, the ALDH12 family represents mitochondrial 1-pyrroline-5-carboxylat hydrogenases (P5CDHs). There is usually a single *ALDH12* gene in both lower and higher plants. P5C is an oxidation product of proline. Proline is known to accumulate in plants during various environmental stresses including drought, high salinity, high light and other oxidative and biotic stresses. We performed X-ray crystallographic study combined with site-directed mutagenesis and enzyme kinetics to analyze active sites and to identify key residues affecting the substrate or coenzyme preferences of above mentioned ALDH family members.

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P36

STRUCTURAL INSIGHTS INTO THE REGULATION OF CAMKK2 BY CALMODULIN AND 14-3-3 PROTEIN

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Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a member of the Ca^{2+}/calmodulin-dependent kinase (CaMK) which is indispensable for cell function, gene expression and apoptosis activation. Malfunction of this protein is often associated with neuropathology, genital carcinogenesis and obesity, all making it a promising therapeutic target. CaMKK2 is activated via binding Ca^{2+} sensor protein calmodulin (CaM) and inhibited by 14-3-3 protein in a phosphorylation-dependent manner. However, the precise molecular mechanisms by which these binding interactions affect CaMKK2 are still unclear.

To elucidate the structural basis of 14-3-3 and CaM binding on CaMKK2 we used analytical ultracentrifugation, small-angle X-ray scattering and methods coupled with mass spectrometry. Analytical ultracentrifugation showed that phosphorylated CaMKK2 and 14-3-3 protein form a stable complex with 1:2 molar stochiometry. Our data from SAXS suggest that the complex has an asymmetric shape and CaMKK2 is located outside the central channel of the 14-3-3 dimer. Moreover, 14-3-3 appears to directly interact with several regions of CaMKK2 outside the 14-3-3 binding motifs, including the kinase domain. SAXS-based modeling of CaMKK2:CaM complex revealed multiple conformations, where nearly half conformers adopts a compact structure. Simultaneously, hydrogen/deuterium exchange and chemical crosslinking coupled with mass spectrometry suggested that CaM also interacts with the kinase domain of CaMKK2. Direct interaction between CaM and the kinase domain has been previously described only for one related CaMK, Death-associated protein kinase. Altogether, it seems that catalytic domain plays an important role in both activatory and inhibitory interactions. However, these suggestions need to be supported by high-resolution structure of both complexes.

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P37

MOLECULAR MECHANISMS OF DNA INTERSTRAND CROSS-LINK FORMATION

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DNA interstrand cross-links (ICLs) are a common lesion generated in DNA spontaneously or induced by chemical agents *e.g.* chemotherapeutics such as Cisplatin, nitrogen mustard, psolaren. In ICLs two opposite strands of double helix are covalently linked blocking DNA replication and transcription [1]. Presence of ICLs in genomic DNA contributes to aging, neurodegeneration, and cancer. Apurinic/apyrimidinc (Ap) sites are form of DNA damage occurring frequently and also spontaneously in genomic DNA. Two equilibrating forms of Ap site are cyclic hemiacetal and ring-opened aldehyde. These unstable moieties can easily undergo amino-catalyzed or spontaneous strand cleavage generating an electrophilic 2,3-didehydro-2,3-dideoxyribose sugar remnant. A ring-opened aldehyde form of Ap sites can react with nucleophiles, for example adenine residues on the opposing DNA strand formin ICL with relatively high yields (20%). This can generally at sequences 5‘-ApT/5‘-AA and under physiologicaly relevant conditions [2, 3]. Therefore it is feasible that Ap-ICLs are generated within the genetic material of living cells. Recently, it has been shown that N-glycosidic bond forming the ICL is cleaved by Endonuclease VIII-like 3 (NEIL3), leading to unhooked crosslink in S phase. A critical step is programmed collapse of replication forks stalled by the ICL. Replication fork converge on the Ap-ICL, promoting NEIL3 dependent glycosylase cleavage of the N-glycosidic bond and following the nucleotide insertion across from the unhooked lesion, leading to strand extension beyond the unhooked lesion [4].

The focus of this work is the preparation of Ap-ICL, its characterization and mechanisms of its formation. In order to understand the molecular mechanisms of the repair we employed crystallization of purified Ap-ICL with and without DNA repair protein. Additionally, we have set up

mechanistic experiments *in vitro* to reveal and calculate the probability of Ap-ICl formation and its stability *in vivo*. We characterize how environmental conditions affect these processes. Our work suggest that Ap-ICL is relatively a stable lesion in comparison with Ap site itself that is easily fragmented. Our experiments extended the understanding of ICL formation, repair and rough estimates of occurrence of this lesion *in vivo*.

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P38

STRUCTURAL STUDIES OF 14-3-3 COMPLEXES WITH PEPTIDES CONTAINING 14-3-3 BINDING MOTIFS OF PROTEIN KINASE CAMKK2

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Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a member of the Ca^{2+}/calmodulin-dependent kinase (CaMK) family involved in adiposity regulation, glucose homeostasis and cancer, and is a potential target for therapeutic intervention. This upstream activator of CaMKI, CaMKIV and AMP-activated protein kinase is negatively regulated by phosphorylation, which triggers an association with the scaffolding protein 14-3-3. Mechanistically, 14-3-3 proteins constrain the conformation, occlude sequence-specific and structural features, promote protein-protein interactions, or prevent the aggregation of their binding partners. Studies have shown that CaMKKs bind to various 14-3-3 protein isoforms and that the conserved motif containing phosphorylated Ser^{74} in CaMKK1 (corresponding to Ser^{100} in CaMKK2) functions as the primary 14-3-3 binding site. Furthermore, a second 14-3-3 binding motif containing phosphorylated Ser^{475} in CaMKK1 (Ser^{511} in CaMKK2) has also been suggested. Although the putative C-terminal 14-3-3 binding motif (sequence RSLpSer^{511}AP) is a canonical "mode I" 14-3-3 binding site (RXX(pS/pT)XP, wherein pS/pT is phosphoserine or phosphothreonine and X is any residue), the N-terminal motif (sequence RKLpS^{100}LQE) contains a Gln residue at the position +2 relative to the phosphorylated residue $pSer^{100}$. Bioinformatics survey of 14-3-3 binding sites revealed that Gln is seldom found at +2 because the Pro residue and, to a lesser extent, Ser, Gly and Asp also, are frequently found at this position.

To elucidate the structural basis of interactions between 14-3-3 proteins and the 14-3-3 binding motifs of CaMKK2, we solved the crystal structures of phosphopeptides pepS100 (sequence RKLpSLQER) and pepS511 (sequence RSLpSAPGN) bound to 14-3-3 and 14-3-3 , respectively. The 14-3-3 isoforms were selected based on quality of the resulting crystals. The crystal structures were solved by molecular replacement using the structures of 14-3-3 (PDB ID: 4FJ3) and 14-3-3 (PDB ID: 2B05) as search models, and refined at a resolution of 2.68 and 2.84 Å, respectively. The final electron densities allowed us to build seven residues (KLpSLQER) of pepS100 and six residues (RSLpSAP) of pepS511. The crystal structures showed that both phosphopeptides interact with the amphipathic groove of 14-3-3 similarly to other 14-3-3 complexes. Nevertheless, in the case of the N-terminal motif, the interaction between the side-chain of Gln at the position +2 relative to $pSer^{100}$ and the phosphate group appears to change the direction of the polypeptide chain. Interestingly, the superimposition of this structure with that of the ternary complex between the phosphopeptide derived from the C-terminus of plant plasma membrane H^+-ATPase, plant 14-3-3C, and fusicoccin showed that the fusicoccin binding cavity stays empty due to the abrupt change in the direction of the C-terminal part of pepS100. Therefore, this protein-protein interaction might be stabilized by small-molecule compounds, as previously reported for other 14-3-3 complexes, which is a potential strategy to inhibit the CaMKK activity.

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P39

ELECTRONIC SCULPTING OF AT2R LIGANDS BY WELL-TEMPERED AND FLYING GAUSSIAN METADYNAMICS

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One of the important regulators of aldosterone secretion and effector controlling blood pressure is angiotensin II. It acts through two main receptors: AT1R (the angiotensin II type-1 receptor) and AT2R. Second one, AT2R, is a promising target for anticancer drugs. Magnani and co-workers [1] have demonstrated that selectivity towards AT2R (and not to AT1R) can be enhanced in peptides with large population of cis peptide bond preceding proline (Pro7 in angiotensin II). Accordingly, the replacement of His6 by aromatic residues with different propensities can form CH/ interactions with Pro7. In order to design new angiotensin derived AT2R ligands we have carried out molecular simulations of model peptides Ace-Xxx-Pro-Nme, with tyrosine, phenylalanine, 4-nitrophenylalanine and O-phosphotyrosine as Xxx. Cis/trans isomerisation takes place in tens to hundreds millisecond time scales, which is hardly accessible in classical unbiased simulations. Therefore, we used metadynamics [2] to predict equilibrium populations of cis peptide bond. Populations of cis peptide bond determined by 400 ns well-tempered metadynamics simulations were predicted as: 17 ± 3 % (exp. 40 %) for tyrosine, 35 ± 6 % (exp. 25 %) for O-phosphotyrosine, 20 ± 4 % (exp. 20 %) for phenylalanine and 14 ± 4 % (exp. 5 %) for 4-nitrophenylalanine, respectively (mean ± s.d.) as Xxx. The accuracy of these results are currently being improved by combination with Flying Gaussian metadynamics, the new method developed by our group.

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P40

CONSTRUCTION OF VECTORS ENABLING EUKARYOTIC EXPRESSION OF ANTIBODY FABS AIMED AT CRYSTALLOGRAPHY OF TAU FILAMENT CORE

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In Alzheimer disease and other tauopathies, tau protein is the constituent of neurofibrillary tangles [1]. Ultrastructurally, tau inclusions are made of paired helical filaments (PHFs) and straight filaments [2]. Protein tau emerges as a promising target for developing disease-modifying drugs [3]. Precise atomic-level structural information of the tau fibrils has been recently elucidated using cryo electron microscopy [4], but there are remaining unresolved questions. Structural study of complexes between tau protein fragments derived from the PHF core [5] and monoclonal antibodies specific for a conformation of tau in PHF core may help to elucidate missing parts of assembled tau in tauopathies.

Tau protein alone is not forming crystals, but using binding partners, e.g., monoclonal antibodies, it may be possible to stabilize distinct (*in situ*) fold of recombinant tau and likely crystallize the complex [6]. Fabs of monoclonal antibodies can thus serve as surrogate tau protein binding partners to aid tau crystallization. Fabs are traditionally prepared by papain digestion of intact antibody; however, these Fabs may be heterogeneous and may contain papain, which can cleave sensitive tau molecule during co-crystallization. Alternatively, recombinantly-expressed Fabs can be used. Recombinant Fabs are homogeneous and pure and therefore more suitable for crystallization. Expression of such recombinant antibodies requires the knowledge of the antibody sequence and a suitable cloning vector.

Aim of this work was to create a new expression vector yielding a high expression of recombinant Fab in CHO cells. The vector should have a suitable antibiotic resistance and contain restriction sites for facilitating cloning. We constructed vector pHu_7 derived from pCMV-BD vector (Stratagene), expressing recombinant proteins under the CMV promoter with a human immunoglobulin kappa light chain signal sequence allowing for secretion of the protein. Subsequently, we have inserted light and heavy chain of DC8E8, MN423 and DC25 antibody Fab [6,7]. We have verified the expression of the antibodies from the constructed vectors by a small-scale pilot experiment in CHO cells.

Summarizing, we successfully constructed expression vectors carrying the light and heavy chains of several monoclonal antibody Fabs. In the next steps we will purify Fab fragments on a large scale, characterize their quality for crystallographic experiments and co-crystallize them with selected variants of tau protein.

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P40

CRYO-ELECTRON MICROSCOPY OF MAMMALIAN CELLS

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Until recently, it was not possible, due to technical and methodological limitations, to study the structures of macromolecular complexes in living cells. Cryo-EM studies have been performed on isolated macromolecular complexes and organelles. In contrast cells had to be fixed, stained by heavy metals, and sectioned for imaging in an electron microscope. However, current advances in focused ion beam milling (FIBM) and cryo-electron tomography (cryo-ET) permit imaging of macromolecular complexes in near native state in sections of vitrified cells.

Here we present optimization of FIBM protocol to prepare lamellas from mammalian tissue culture cell lines for cryo-ET. Cells grown on golden electron microscopy holey carbon coated grids with Quantifoil® were blotted and vitrified in liquid ethane using Vitrobot Mark 4 (Thermo Fisher Scientific) to prevent formation of crystalline ice. Subsequently, lamellas were milled using focused beam of gallium ions on dual beam scanning electron microscope Versa 3D (Thermo Fisher Scientific). We strive to prepare lamellas with maximal thickness of 250 nm so that they are suitable for imaging in transmission electron microscope operated at 300 kV. Grids with milled lamellas are then transferred, in cryogenic conditions, to electron microscope Titan Krios (Thermo Fisher Scientific) for cryo-ET of selected objects of interest inside the cell.

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P42

STUDY OF DYNAMICS OF INTRINSICALLY DISORDERED PROTEIN MAP2C USING NMR RELAXATION

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Intrinsically disordered proteins (IDPs) are macromolecules lacking unique 3D structures. In past two decades, it was revealed that a significant part of eukaryotic genome consists of IDPs. In spite of the absence of defined structures, IDPs are necessary for proper function of cells. [1]

One of the largest IDPs studied with atomic reoslution is Microtubule Associated Protein 2c (MAP2c), that belongs to a MAP subfamily expressed in neurons during neuronal differentiation in the developing nervous system and can be found mainly in their dendrites [2]. MAP2c regulates dynamics and structure of microtubules in a phosphorylation-dependent manner, which is essential for the correct function of cytoskeleton of neural cells and its dysfunction may be one of the reasons of the development of neurodegenerative diseases [3].

To understand the functions of MAP2c, knowledge of its conformational properties is required. NMR relaxation a powerful method well suited to provide information about dynamics of IDPs such as MAP2c. The relaxation study of MAP2c revealed presence of partially ordered regions that correlate well with known or proposed binding sites of the protein.

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INTERACTION OF MOMLV AND MMTV MATRIX PROTEINS WITH MEMBRANEOUS PHOSPHOLIPIDS

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The retroviruses are enveloped viruses, therefore during the late phase of their life cycle they need to recruit viral membranous envelope originating from the cytoplasmic membrane from the host cell. The interaction of viral immature particle with the membrane is facilitated by structural protein matrix protein (MA). Like all structural proteins, MA in immature viral particle is a part of Gag polyprotein and it is cleaved by viral protease after virus particle leaves host cell. MAs of most retroviruses are myristoylated and the myristoyl is necessary for the interaction with the membrane, since it serves as an anchor of whole Gag in phospholipid bilayer. The molecule responsible for the binding of MA to the proper membrane is phosphatidylinositol-4,5-bisphosphate (PIP), a phospholipid found exclusively in cytoplasmic membrane. It has been proven for HIV-1, that PIP significantly improves binding of retroviral MAs on the plasma membrane[1,2].

There are two main types of retroviral virus particle assembly. The B/D type retroviruses assemble the immature virus particle in the cytoplasm and it is then transported towards the membrane. The C-type Gag proteins are transported directly towards the plasma membrane and the formation of immature virus particle occurs on the membrane along with the budding. This type of retrovirus must also affect the interaction of gag with the membrane, since in C-type only single molecules interact with the membrane, while in D-type it is whole immature virus particle. In this work, we used MA of Moloney Murine Leukemia Virus (MoMLV) as a C-type retrovirus model and Mouse Mammary Tumor Virus (MMTV) MA as a model of B/D type retrovirus. We measured the binding of these MA proteins with liposomes of various phospholipid compositions and compared the strength of the interaction between these two proteins and also to compared it with other retroviral MAs (mainly with HIV-1 as a C-type and Mason-Pfizer Monkey Virus as a B/D-type).

The proteins were produced in *E. coli* cells. The myristoylation was facilitated by co-expression of MAs with yeast N-myristoyl transferase. The purified proteins were then used for interaction with artificial liposomes composed of different lipids and different amounts of PIP. The unbound protein was subsequently removed from the liposomes by ultracentrifugation in sucrose gradient and analyzed by SDS-PAGE.

This work was supported by Czech Science Foundation grant GA17-24281S.

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P44

ENGINEERED FRAGMENTS OF ANTI-PSMA ANTIBODIES AND THEIR USE IN IMMUNOTHERAPY OF PROSTATE CANCER

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Prostate-specific membrane antigen (PSMA) is an excellent biomarker for prostate cancer (PCa) imaging and therapy. In our ongoing efforts targeting PSMA, we have developed a new monoclonal antibody 5D3 with sub-nanomolar affinity for human PSMA. Next, we used protein engineering to construct single-chain variable fragment derivatives of 5D3 (5D3-scFv), optimized expression and purification protocols and verified their functionality in complementary *in vitro* assays. We are further extending our experimental approaches by exploiting the 5D3-scFv in immunological platforms including (i) the design of bispecific fusions that will engage effector immune cells (macrophages and T-cells); and (ii) construction of chimeric antigen receptors (CARs) to transduce T-cells. Both of these platforms will be then tested *in vitro* and *in vivo* for immunotherapy of PSMA-expressing tumor cells.

P45

STRUCTURAL AND ELASTIC PROPERTIES OF DNA MUTATION MOTIFS

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Mutations in human genes can be responsible for inherited genetic disorders and cancer. It has been shown that certain DNA sequences are more prone to mutate [1]. These sites are termed hotspots and exhibit a higher mutation frequency than expected by chance. In contrast, DNA sequences with lower mutation frequencies than expected by chance are termed coldspots. Mutation hotspots are usually derived from a mutation spectrum, which however often reflects particular population where an effect of a common ancestor plays a role. To detect coldspots and hotspots unaffected by population bias we analysed presence of germline mutations obtained from HGMD database in the 5-nucleotide segments which repeatedly occurring in genes associated with common inherited disorders [2], particularly in PAH, LDLR, CFTR, F8, and F9 genes.

Using molecular dynamics simulations and free energy calculations we have analyzed global bending properties of selected coldspots and hotspots with G/T, G/A and A/C mismatch base pairs. Coldspots were observed to be inherently more flexible than hotspots. In addition, we observed that mismatch G/T pair opens more easily towards minor groove than a canonical G = C base pair in the bent DNA conformation. This property might be critical for effective mismatch repair as aberrant DNA recognized by MutS protein is noticeably bent and mismatch base pair is disrupted and shifted towards minor groove where it is recognized by conserved amino acids from MutS .

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P46

DINUCLEOTIDE HYDRATION SITES

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Understanding of the biological function of DNA requires to understand its molecular structure. It is generally known that DNA is polymer made up of nucleotides that contain a phosphate group, a sugar group and a nitrogen base. The recurrent pattern of the sugar group and the phosphate group constitute the DNA backbone. DNA complexes are typically comprised of two or more strands. Functional forms of DNA are stabilized by base pairing, base stacking, and interactions with solvent. Influence of water molecules on the structure and function of DNA is an object of numerous researches [1, 2].

Our work is focused on the analysis of the relation between hydration sites and dinucleotide conformations. We used the previously published method [1] to identify the positions and intensities of the hydration sites of the defined dinucleotides conformational classes called ***NtC*** (Nucleotide Classes) [3]. The bioinformatic analysis is based on the crystal structures with a resolution better than 2.6 Å with the most frequent resolution being near 2.2 Å. The dinucleotides were classified into the ***NtC*** using the protocol [3] and further analysed separately for each of the 16 possible dinucleotide sequences. For the calculation of the average water positions, so called hydration sites [1], we took into account only the dinucleotides for which our database of available dinucleotide structures contains with more than 500 waters. The distributions of water molecules were expressed as pseudo-electron-density maps, and hydration sites were determined as maxima in the density map [1, 6]. The results will be presented in the Atlas of biomolecular hydration, Watlas, based on the recently published atlas of amino acid hydration [4, 5]. Here, we present results for selected ***NtC*** classes with the highest occupancy of water molecules.

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P47

THE THEORETICAL STUDY OF CHARGE TRANSFER EFFICIENCY THROUGH DAMAGED DNA DUPLEXES

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We theoretical studied the charge transfer efficiency for donor-acceptor pairs in DNA duplexes containing damaged nucleosides. The donor was 2-aminopurine (Ap) while the acceptor was guanine (G), 8-oxoguanine (OxoG) or 2,6-diamino-4-oxo-5-formamidopyrimidine (FapyG). The OxoG and FapyG are mutations of normal guanine that occur owing to interaction of DNA with free radicals. The human 8-oxoguanine DNA glycosylase 1 (hOGG1) repair enzyme that performs excision of damaged bases, however, the exact excision mechanism is currently unknown.

Recently, we proposed new catalytic scheme for hOGG1 enzyme [1, 2]. In the current study we wanted to find out whether the base excision by hOGG1 could be in principle monitored in real time employing fluorescence spectroscopy. In our previous studies on charge transfer the calculated donor – acceptor coupling integrals described successfully modulation of charge transfer efficiency that was measured in DNA molecules [2, 3]. In particular, the coupling integrals described quenching of fluorescence radiation from 2-aminopurine by guanine in relation with the hole transfer from Ap to G [3, 4]. The calculated coupling integrals indicated that guanine is better quencher than OxoG or FapyG. The results indicated that experimental detection of damaged nucleosides within DNA duplex employing fluorescence spectroscopy is possible.

This work was supported by the Grant Agency of the Czech Republic 18-14990S. The access to the MetaCentrum computing facilities is appreciated.

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P48

STRUCTURE OF VIRUS LIKE PARTICLES OF MERKEL CELL POLYOMAVIRUS

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Polyomaviruses are small, non enveloped, dsDNA viruses. Their capsids are formed by major capsid protein (VP1) and two minor capsid proteins (VP2 and VP3). Merkel cell polyomavirus (MCPyV) is a human pathogen form the family *Polyomaviridae*. MCPyV is widespread in humans and causes rare but aggressive skin tumors - Merkel cell carcinomas, in immunosuppressed individuals. No vaccines or antivirals against MCPyV are known. We determined the structure of the MCPyV virus like particle (VLP) by cryo-electron microscopy and single particle analysis to the resolution of 3.7 Å. Previously, structure of MCPyV VP1 pentamer, truncated from C and N terminus, was solved. Our reconstruction of whole VLPs reveals how pentamers form the capsid through interactions of C- and N-terminal parts of VP1s. Capsid stability is strengthened by disulfide bonds between VP1s from two adjacent pentamers.

P49

THE STUDY OF THE ROLE OF FANCI PHOSPHORYLATION IN FANCD2 MONOUBIQUITYLATION AND DNA CROSSLINK REPAIR

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Interstrand crosslinks (ICLs) are cytotoxic form of DNA damage. ICLs precipitate Fanconi anaemia (FA) a rare genetic disease with patients carrying biallelic mutations in one of some 22 genes (FANC genes). The patients suffer from developmental defects, small stature, bone marrow failure and often succumb to cancer. Cell lines derived from FA patients are hypersensitive to DNA crosslinking agents, such as Cisplatin. Leading to implication of FANC genes in ICL repair and further postulation of FA DNA repair pathway [1]. The hallmark of FA dependent ICL repair is FANCD2 monoubiquitylation, a crucial reaction required for further ICL excisions by DNA nuclease complex SLX4-XPF-ERCC1 [2-4]. Additinally, genetic studies have shown that phosphorylation of FANCI triggers FANCD2 monoubiquitylation. In this work we investigate the role of phosphorylated FANCI in the process of initiation and progression of ICL repair.

To dissect the molecular mechanisms FANCI phosphorylation, we generated mutants of five known ATR phosphorylation. These sites were mutated to Asp as a phosphorylation mimic and Ala as a control. To test the influence of FANCI phosphorylation we used various DNA substrates mimicking DNA replication including single stranded and double stranded DNA. We have measured fluorescent anisotropy, gel based DNA binding assays and kinetics of FANCD2 monoubiquitylation reaction. We have validated DNA binding of FANCD2 and FANCI in comparison with FANCD2-FANCI complex. Here FANCD2 bound to DNA with lower affinity then FANCI. Although we did not observe striking differences between

individual DNA structures, combining the two proteins led to significant increase of DNA binding affinity. This is in accordance with the fact that FANCD2 is monoubiquitylated on DNA. Although the presence of monoubiquitylated FANCD2 has not revealed significant changes in DNA binding affinities we have observed significant stimulatory effect of DNA binding using phosphomimetic mutants of FANCI. Our data bring the evidence that ATR phosphorylation triggers the FANCI and FANCD2 ubiquitylation by its localisation on DNA.

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P50

STRUCTURAL CHARACTERIZATION OF THE COMPLEX BETWEEN THE PROCASPASE-2 AND THE 14-3-3 PROTEIN

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The process of programmed cell death, apoptosis, is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Caspases were identified to play a crucial role in apoptotic pathways. It was described previously that sufficient NADPH level induces phosphorylation of procaspase-2 (proC2) and 14-3-3 protein binding preventing proC2 maturation. The nutrient depletion promotes the 14-3-3 protein release and caspase-2 activation as was described previously in *Xenopus laevis* [1]. Our recent research suggested that two phosphorylation sites are required for proC2 binding to 14-3-3 [2].

Conformational behavior of phosphorylated proC2 and its changes upon 14-3-3 protein binding was studied next by time resolved fluorescence intensity and decay measurements and acrylamide quenching. Four proC2 mutants containing single tryptophan residue at positions 151, 188, 218 and 426 and wt (containing Trp^{385}), were prepared to sample various regions of proC2. Values of mean fluorescence lifetimes clearly show the different vicinity in individual mutants after 14-3-3 binding with exception of Trp^{188} which seems to be buried within the structure of proC2. Data obtained from fluorescence anisotropy determined Trp^{151} and Trp^{426} exhibit significantly lower fast local motions of Trp upon complex formation, indicating lower mobility of this region upon 14-3-3 protein binding.

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P51

BIOMOLECULAR SIMULATIONS BY COMBINATION OF FLYING GAUSSIAN METHOD AND PARALLEL TEMPERING

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Biomolecular simulations can simulate nano- or microsecond time scales. Unfortunately, many biological processes take place in significantly longer time scales. In order to make such simulations possible it is necessary to apply enhanced sampling methods. In our group we developed a method called flying Gaussian method [1]. The studied system is simulated in multiple replicas by this method. Replicas with similar states of the system (e.g. similar conformations) repulse each other forcing the system to sample various states. Free energy of the simulated system can be obtained from sampling and from onergis by which replicas influence each other.

In order to further extend sampling we combined flying Gaussian method with parallel tempering. Each replica is simulated at different temperature. Every 1 ps a exchange of coordinates is attempted in a pair of replicas. Here we present the design of the method as well as preliminary results of its application.

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P52

STRUCTURAL CHARACTERIZATION OF A NOVEL TYPE OF REACTIVE SITE FOR INHIBITION OF SERINE PROTEASES

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Protease inhibitors from the Kunitz family are widely distributed in plant kingdom. They share a conserved -trefoil fold in which variable loops are involved in interactions with proteases. The majority of the Kunitz inhibitors are targeting serine proteases using canonical (Laskowski) mechanism based on a single binding loop with conserved structure. Here, we present high-resolution crystal structures of two potato Kunitz inhibitors in complexes with the serine protease trypsin. We identified a new, non-canonical type of a reactive center on these inhibitors that is formed by two loops. Furthermore, we employed cross-linking mass spectrometry approach to demonstrate that the non-canonical reactive site interacts also with chymotrypsin.

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FLYING GAUSSIAN METHOD: NEW APPLICATIONS

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The time necessary to overcome significant energy barriers in complex free energy surfaces (FES) can be considered as a major drawback in molecular dynamics simulations. This issue can be tackled by developing enhanced sampling techniques. Metadynamics is one of those methods and it has been successfully used for more than 15 years now [1]. In metadynamics, the history-dependent bias potential acts on preselected collective variables (CV) and by doing so, it discourages the system from visiting previously sampled states. The bias potential is defined as a sum of Gaussian

"hills" (with preselected height and width) which are added to the system and accumulate during the simulation, forcing the system to visit new areas of FES. Selection of CVs is of crucial importance primarily in distinguishing important states of the studied system.

Flying Gaussian method [2] was inspired by multiple walkers metadynamics. The system is simulated in multiple walkers (replicas), but the bias potential does not accumulate during the simulation. Instead, with the new value of CV being calculated for each walker in every microscopic step of the simulation, the position of the "hill" for each walker is only updated. This means that during the whole simulation, the number of "hills" is the same as the number of the walkers. The filling of the free-energy minima is achieved by walkers concentrating in them.

This method was successfully applied to exploration of CV space of selected biomolecules, showed good performance in the terms of time saving and efficiency in exploring FES. FES were reconstructed using on-the-fly reweighting, as the bias potential is too dynamic to directly estimate it. Furthermore, the method was tested for cases where choice of CVs is particularly difficult, by aligning the starting replicas to a reference structure and applying the bias based on the calculations of components of position of preselected atoms of the replicas. We also present preliminary results of using flaying Gaussian method in docking simulations.

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INTO THE WILD: EXPRESSION AND CHARACTERIZATION OF RANDOM PROTEIN LIBRARIES

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Modern protein science heavily relies and benefits from the data generated from experimental characterization of natural protein sequences. Our study moves beyond the natural world in attempt to construct and describe the behaviour of random protein libraries without any evolutionary background. In order to investigate the structure-forming potential of random proteins we designed and applied a novel random library construction and purification methodology. Our libraries, although being random in sequence, are customized in amino acid content and ratios. This approach allowed us to study the secondary structure content of (i) natural-like random proteins composed of all 20 amino acids, library of proteins built from amino acids present in (ii) prebiotic and (iii) early biotic world and protein library made from (iv) minimal set of amino acids from the rational protein design point of view.

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THERMODYNAMIC AND KINETIC STUDY OF 14-3-3 PROTEIN

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14-3-3 proteins are regulatory proteins involved in many signaling pathways. They play a key role in nervous system and neurodegeneration [1–3]. The 14-3-3 family of proteins consists of seven isoforms in mammals, which interact with large number of binding partners containing phosphorylated Serine or Threonine [4]. The X-ray three-dimensional structure showed dimeric form of 14-3-3. Each monomer consists of 9 -helices in an antiparallel arrangement. Dimer interface is stabilized by multiple conserved hydrophobic interactions (e.g. L12), polar contacts and by several isoform-specific salt bridges (e.g. K78) [5–6]. However, dimer dissociation constants as well as fundamental kinetic rate constants remain unknown.

In order to study thermodynamics and kinetics of 14-3-3 dimer, we prepared a new construct with a single accessible cysteine at the N terminus of protein. This construct was used in our recently developed assays based on the Förster resonance energy transfer (FRET) and self-quenching (SQ) phenomena and in microscale thermophoresis (MST) methodology. We determined the

thermodynamic parameter (dissociation constant, K_d) and kinetic parameter represented by life-time of 14-3-3 dimer. Moreover, we studied the stability of 14-3-3 dimer under variety of factors.

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INTRINSICALLY DISORDERED MICROTUBULE ASSOCIATED PROTEIN 2c (MAP2c) STUDIED VIA COMPUTATIONAL METHODS AND NUCLEAR MAGNETIC RESONANCE

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The microtubule dynamics in the neuronal cells is mainly modulated by two members of the structural MAPs, MAP2 and tau, which play fundamental roles in the development of dendrites and axons. The MAP2 proteins are present mainly in the dendrites of neuronal cells, while tau is present in axons.

MAP2c is a 49 kDa intrinsically disordered protein (IDP). IDPs are macromolecules interesting both from biophysical and physiological point of view, but difficult to study by the current biophysical methods. Nuclear magnetic resonance (NMR) is a key technique for atomic-resolution studies of IDPs, but its applicability is limited by a spectral overlap in case of long or highly repetitive amino-acid sequences. Our group developed high-resolution NMR methodology that overcomes this limitation and makes studies of large IDPs possible.

We present an atomic-resolution conformational analysis of MAP2c based on chemical shifts obtained from 5D NMR experiments, paramagnetic relaxation enhancement providing information about intramolecular distances, and small angle x-ray scattering experiments reflecting the overall size and shape of the MAP2c molecule in solution. Ensembles of structures of phosphorylated and non-phosphorylated MAP2c reproducing the experimental data were selected by the program ASTEROIDS. The ensembles were used to evaluate local conformation and long-range contacts in the MAP2c molecule.

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BLI TECHNOLOGY AS A TOOL FOR BIOMOLECULAR INTERACTIONS

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United Kingdom io-Layer Interferometry (BLI) is a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time.

The binding between a ligand immobilized on the biosensor tip surface and an analyte in solution produces an increase in optical thickness at the biosensor tip, which results in a wavelength shift, , which is a direct measure of the change in thickness of the biological layer. Interactions are measured in real time, providing the ability to monitor binding specificity, rates of association and dissociation, or concentration, with precision and accuracy.

Only molecules binding to or dissociating from the biosensor can shift the interference pattern and generate a response profile on the Octet® System. Unbound molecules, changes in the refractive index of the surrounding medium, or changes in flow rate do not affect the interference pattern. This is a unique characteristic of BLI and extends its capability to perform in crude samples used in applications for protein:protein binding, quantitation, affinity, and kinetics.

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