Posters

P1

STUDY OF N-GLYCAN MOIETY DEPENDENCY IN THE INTERACTION OF HUMAN EARLY ACTIVATION MARKER CD69 WITH ITS PROPOSED LIGAND GALECTIN-1

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Natural killer (NK) cells are a subpopulation of effector lymphocytes of the innate immune system with cytotoxic activity and cytokine-producing effector functions. Functions of NK cells include tumour elimination, engagement into and regulation of antiviral immune responses and regulation of other immune cells such as dendritic cells, T lymphocytes, and B lymphocytes by secretion of chemokines and cytokines [1].

CD69 is a type II C-type lectin-like transmembrane receptor expressed on cells of hematopoietic origin. It is a disulphide-linked homodimer with subunits of varying molecular mass depending on glycosylation degree. Recent evidence suggests the involvement of the early-activation antigen CD69 in the modulation of cytokine secretion as well as in the homing and migration of activated lymphocytes. Several protein ligands (galectin-1, S100A8/S100A9, S1P1R, MLC9 and MLC12) have been described for CD69; however, there is no detailed mechanistic and structural description of the proposed protein:protein complexes.

Galectin-1 (Gal-1) is a prototypical protein belonging to the family of galectins. Galectins are characterized by the presence of a common structural fold and a carbohydrate recognition domain with high affinity for -galactosides. Expression of galectin-1 has been reported mostly in lymphoid organs, activated lymphocytes, and in immune-privileged sites. Physiologically, Gal-1 exists as a monomer and as a non-covalent homodimer. Gal-1 has been shown to mediate processes in cellular adhesion, motility and growth-regulation as well as in the immunoregulation of T lymphocyte homeostasis.

The interaction between CD69 and galectin-1 has been described in 2014 by de la Fuente [2]. The mechanisms associated with CD69:Gal-1 interaction along with the required biochemical and biophysical properties still remain to be elucidated. Our project aims at a better understanding of how the interaction occurs and if the interaction is protein- or glycosylation-specific. To answer such questions, we have employed MST and AUC techniques to assess the carbohydrate dependence of the binding. The obtained results show a considerable difference in the dissociation constants of the glycosylated CD69:Gal-1 and deglycosylated CD69:Gal-1 complexes, strongly indicating that the interaction is glycosylation-dependent. Unexpectedly, galectin-1 appears to be binding with similar or stronger affinity to other sequence- or family-related NK cell surface receptors, indicating that the interaction is not as specific as initially assumed.

To assess the specificity of the interaction and the biophysics involved in complex formation, complementary biophysical analyses (e.g., SPR, nanoDSF, and ITC) and protein crystallography are to be employed to provide a more detailed description of the interaction and structural mechanics of CD69:Gal-1 complex.

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P2

BIOLOGICAL ACTIVITY OF FLUORESCENT DIGITOXIN DERIVATIVES

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Cardiac glycosides are secondary plant metabolites, which are commonly used for treatment of heart failure and cardiac arrhythmias due to their positive ionotropic effect. On molecular basis, they are composed of a steroid skeleton and a sugar moiety. Their mechanism of action is mediated through an interaction with Na^+/K^+-ATPase, the inhibition of which also leads to apoptosis. Nowadays, cardiac glycosides are studies as potential therapeutics for cancer treatment. One of the most important members of cardiac glycosides is digitoxin.

The aim of this work was design, synthesis and biological evaluation of novel fluorescent digitoxin conjugates, possibly applied in theranostics, having selective cytotoxicity between cancer and primary cell lines. We prepared digitoxin derivatives conjugated to various BODIPY dyes by modification of the terminal digitoxose of digitoxin using copper-catalyzed click chemistry and studied their interactions with Na^+/K^+-ATPase (organism: wild boar) using molecular docking methods. Further, we evaluated their cytotoxicity in cancer (A549) and primary (MRC-5) cell lines after 72 h of treatment using WST-1 method. Fluorescent derivatives of digitoxin were less cytotoxic in comparison with digitoxin, which is satisfactory according to excessive digitoxin toxicity and numerous side effects when used in clinics. All tested compounds were more cytotoxic against the tested cancer cell line in comparison to the primary cell line. By means of fluorescence microscopy, we investigated intracellular localization of the digitoxin-BODIPY conjugates, which were detected in endoplasmic reticulum and lysosomes of A549, HEK 293T and MRC-5 cells. Last but not least, the effect of digitoxin-BODIPY on the cell cycle of A549 cancer cell line was studied using flow cytometry. We detected a decrease in the G0/G1 and, on the contrary, an increase in the S and G2/M phases. We also confirmed that digitoxin-BODIPY induced apoptosis as a predominant type of cell death in A549 cells. The results of this study could help us understand how the derivatization of a sugar moiety changes the behavior of digitoxin within biological compartments and also how to develop new compounds with better targeting toward cancer cells.

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P3

STRUCTURE OF CATHEPSIN K IN COMPLEX WITH HIGHLY POTENT AZANITRILE INHIBITORS

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Cathepsin K expressed in osteoclasts possesses elastolytic and collagenolytic activities and plays a critical role in the bone remodeling process. It has been validated as a chemotherapeutic target for osteoporosis treatment. Cathepsin K is effectively inhibited by synthetic peptidomimetic compounds containing an azanitrile reactive warhead forming reversible interaction at subnanomolar concentrations. We prepared azanitrile inhibitor complexes with recombinant activated cathepsin K that were subjected to high-throughput crystallization screening. Here we present two crystal structures, determined at 1.9 Å resolution and describe for the first time the binding mode of the azanitrile scaffold to the active site of cysteine proteases.

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P4

MONOMER – DIMER EQUILIBRIA OF 14-3-3 PROTEINS

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14-3-3 proteins are regulatory proteins involved in many signaling pathways. They play a significant role in the regulation of cell growth, apoptosis, cytoskeletal dynamics and transcription control of genes [1–4]. The 14-3-3 family of proteins consists of seven isoforms in mammals, which interact with a large number of binding partners containing phosphorylated site [5]. The function of 14-3-3 proteins is strongly dependent on their oligomeric state. Detailed characterization of dimer-monomer equilibrium is essential for full understanding of the function of 14-3-3 proteins.

We designed two potential monomeric mutants of 14-3-3 to understand monomer-dimer equilibria of 14-3-3 protein [6]. Two monomeric variants were firstly examined by standard biophysical methods such as Circular dichroism, Differential scanning calorimetry. The dissociation constant of monomeric mutants was experimentally investigated by NATIVE PAGE and micro-scale thermophoresis. In addition, we experimentally determined dissociation rate constant and dissociation constant of the dimeric form of 14-3-3 . In order to achieve this, we designed two fluorescence assays using Förster resonance energy transfer and self-quenching to determine thermodynamic and kinetic parameters of 14-3-3 dimerization. Moreover, we studied the stability of 14-3-3ć dimer under a variety of conditions.

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P5

STRUCTURE OF A STAPHYLOCOCCAL PHAGE CONNECTOR PROTEIN

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Antibiotic resistance of microorganisms is a major threat accounting for a rising number of deaths [1]. Bacteriophages offer an alternative to conventional antimicrobial treatment. Phage phi812 is a lytic virus from the Myoviridae family infecting up to 95 % of *Staphylococcus aureus* strains, including MRSA [2] and its structure was previously determined by cryo-electron microscopy [3]. Here we present our work towards resolving the structure of protein gp99 forming the connector region. The protein may play a role in genome release regulation during infection. Gp99 was overexpressed 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 2.2 Å were collected. To solve the phase problem, a variety of methods had been tried, including molecular replacement, heavy atom soaking, co-crystallization, and seleno-methionine incorporation. The structure of gp99 will help explain the mechanism of bacterial infection by bacteriophage.

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P6

CONFORMATIONS OF PEPTIDES FROM MICROTUBULE REPEAT REGIONS OF INTRINSICALLY DISORDERED PROTEIN TAU

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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. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeats (MTBRs) of tau. The active vaccine based on the DC8E8 epitope peptide has successfully passed the phase 1 clinical trial [1].

We have performed 300 ns long molecular dynamics simulations of 18 amino acids containing tau peptides from all four MTBRs in NAMD program with CHARMM36m force field suitable for simulation of intrinsically disordered proteins [2]. We have compared the sampled conformations with the conformations of respective peptides observed by cryo-EM in filaments isolated from cases of Alzheimer's disease and Pick's disease [3, 4]. We have also measured FTIR spectra of tau peptides used for MD simulations and compared the results. Unravelling the unique mode of recognition of DC8E8 antibody and conformational biases of tau protein repeat regions can aid to reveal the hindered structural features of tau protein biology.

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P7

BIOPHYSICAL CHARACTERIZATION OF PROTEINS AT CENTRE OF MOLECULAR STRUCTURE OF BIOCEV

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Centre of molecular structure (CMS) of BIOCEV, operated by the Institute of Biotechnology, is a member of the European project of large research infrastructure INSTRUCT. The biophysical research facilities as a part of the CMS provide shared resource of the state-of-the-art instruments and technologies for biophysical characterization of the stability and structure of proteins and their interactions with other proteins, DNA, RNA, lipids, carbohydrates and small molecules.

CMS provides access to the equipment for the study of biomolecular systems for research groups of Institute of Biotechnology, BIOCEV, and the broader community of the Czech and EU molecular biologists. The biophysical facility at CMS allows determining binding constants, re-

action stoichiometry, thermodynamic profiles, real-time affinities, specificities, and kinetics of the interactions, stability and conformation of biomolecules using surface plasmon resonance technique, label and label-free microscale thermophoresis, isothermal titration calorimetry and differential scanning calorimetry, circular dichroism and UV/Vis spectroscopy, differential scanning fluorimetry and dynamic light scattering techniques.

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P8

NK CELL ACTIVATING LIGANDS IN FUSION WITH NANOBODIES: THE ROLE OF ARRANGEMENT ON THEIR BINDING CAPACITY

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Natural killer (NK) cells are part of the innate immunity. They manifest cytotoxic effect against stressed, infected or malignantly transformed cells. The activation of this process is realized through binding of specific surface ligands of harmed cells to activating receptors of NK cells. But tumour cells strive to escape the immune system surveillance. Thus, the reconstitution of the cytotoxic recognition could be an interesting target of tumour immunotherapy.

We have prepared bivalent fusion proteins able to recognize and bind both tumour cell, and NK cell. The fusion proteins consist of VHH nanobody targeting specific tumour cell marker HER2, and of extracellular domain of MICA, a ligand for the activating NK cell receptor NKG2D. Such approach allows two possible designs of the fusion protein: the activating ligand positioned on N-terminus and the VHH nanobody on C-terminus of the protein or the inverse arrangement. The *in vitro* binding studies have revealed that the arrangement plays an important role in the binding capacity of both parts of the fusion protein. The position of MICA on the N-terminus is favourable for NK activating receptor binding, whereas the position of antiHER2 nanobody on the N-terminus is advantageous for targeting the tumour marker on the cell surface.

The same approach was used for construction of another fusion protein containing B7-H6, a ligand for the activating NK cell receptor ligand NKp30, and its binding capacity was characterized as well. The prepared fusion proteins will be further used with tumour and NK cell lines where their potential to establish the cytotoxic response will be evaluated.

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P9

CRYSTAL STRUCTURE OF KOBUVIRAL RNA POLYMERASE REVEALS THAT THE N-TERMINI HAS A UNIQUE FOLD AMONG PICORNAVIRUSES

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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 first residue of $3D^{pol}$ proteins is burned inside the protein. The correct protein folding of the N termini is the first residue dependent. The very first glycine is conserved among all the picornavirus except for kobuviruses (i. e. Aichi virus)- kobuviruses have a serine residue instead. Intrigued by this anomaly we sought to solve the crystal structure of kobuviral $3D^{pol}$ enzyme.

We determined the crystal structure of Aichi $3D^{pol}$ at 2.3Å resolution. The structure uncovered an overall conserved fold of right hand. But the structure also revealed a unique fold of the $3D^{pol}$ N-termini, however, the very first serine residue is also inserted into a charged pocket via a water bridge suggesting that throughout the evolution of picornaviruses the mechanism of $3D^{pol}$ activation after precursor cleavage remains conserved.

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P10

INFLUENCE OF GLUCOSE O3 METHYLATION ON BINDING PROPERTIES TOWARDS PLL2 LECTIN

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O-methylation of sugars is an uncommon modification with not fully understood function. Recently, it was described as a pathogen-associated molecular pattern (PAMP), since its interaction with the immune system effector lectin Lb-Tec2 was described [1]. Lectins are proteins able to reversibly bind glycoconjugates with high specificity. Besides their important role in the innate immune system, they play a crucial role in many other biological processes, including both mutualistic and parasitic interactions between microorganisms and hosts [2]. Lectin/saccharide interaction is mostly mediated via hydrogen bonds. However, the importance of non-polar interactions, such as CH- interactions between aromatic amino acids and apolar part of carbohydrate molecules, was shown recently [3].

Our research is focused on studying lectins from entomopathogenic bacterium *Photorhabdus laumondii*, which is known for its complicated life-cycle, including mutualism and pathogenicity towards two different invertebrate hosts. The contribution is focused on the PLL2 lectin, which forms a 7-bladed â-propeller with two sets of binding sites situated in between the blades. PLL2 has been confirmed to bind multiple monosaccharides, including D-glucose and 3-*O*-methyl-D-glucose. SPR measurement revealed two orders of magnitude stronger inhibition potential of 3-*O*-methyl-D-glucose compared to D-glucose. X-ray structures showed both ligands are able to occupy one set of sites ("polar" sites), but only 3-*O*-methyl-D-glucose was found in the second set of sites ("hydrophobic" sites).

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P11

ROLE OF HONEYBEE HEXAMERIN IN REGULATION OF PUPAE DEVELOPMENT

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Hexamerins are the most abundant proteins in the haemolymph of honey bee (*Apis mellifera*) larvae and pupae. They serve as an aminoacid source for development of pupa, when a bee doesn't consume food. Furthermore, there has been an evidence that hexamerins act as juvenile hormone binding proteins. The details of this interaction, however, remained unknown. We have solved a crystal structure of a native hexamerin 70b, isolated from bee pupae, to 2.0Å resolution. It shows that hexamerin is composed of six identical subunits, each possessing an enclosed hydrophobic cavity occupied by one molecule of putative juvenile hormone. We propose that the juvenile hormone is released upon proteolytic digestion of hexamerin. Therefore, the consumption of hexamerin is linked to level of free juvenile hormone in haemolymph, affecting the development of pupa. This mechanism might be conserved among holometabolous insects.

P12

NOVEL STRUCTURALLY CHARACTERIZED HAD PHOSPHATASE FROM *THERMOCOCCUS THIOREDUCENS* WITH DIVERSE SUBSTRATE SPECIFICITY

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The HAD (haloacid dehalogenase) superfamily is one of the largest known group of enzymes, the majority of them catalyze the hydrolysis of phosphoric acid monoesters into a phosphate ion and an alcohol. Despite the fact that sequence similarity between HAD phosphatases is generally very low, the members possess some characteristic features, such as Rossmann-like fold, HAD signature motifs or the requirement for Mg^{2+} ion as an obligatory cofactor. This study was focused on new hypothetical HAD phosphatase from *Thermococcus thioreducens*, that can possibly degradate phosphorylated compounds. The protein crystallized in space group $P2_12_12$ with unit-cell parameters a = 66.3, b = 117.0, c = 33.8 Å, and the crystals contained one molecule in the asymmetric unit. The protein structure was determined by X-ray crystallography and refined to 1.75 Å resolution. The structure revealed a putative active site, common to all HAD members. Computational docking into the crystal structure was used to propose substrates for the enzyme. Activity of this thermophilic enzyme towards selected substrates was confirmed at temperature 333 K.

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P13

BIOPHYSICAL CHARACTERIZATION OF NKp44: PCNA INTERACTION

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Natural killer (NK) cells are key components of innate immunity. The task of these cells is to kill virally infected cells or tumour cells. A NK cells have a variety of surface receptors to recognize target cells. Signals which the NK cell is getting from receptors could be activating or inhibitory (so they activate or inhibit cytotoxic response of the cell). Killing of an infected or otherwise damaged cell is mediated by so-called cytotoxic molecules (proteins) found in the NK cells. Recognition of the target cell induces immune synapse formation through which the cytotoxic molecules are introduced into the target cell, which leads to apoptosis.

One of the natural cytotoxicity receptors (NCRs) of NK cells is NKp44. NKp44 is a unique protein in the family of NCR. It has been observed that binding of a proliferating cell nuclear antigen (PCNA), a nuclear protein involved in DNA replication expressed on cell surface upon malignant transformation, to NKp44 leads to an inhibitory signal, even though it was assumed that NKp44 is an activating receptor.

The goal of this project is to characterise the interaction between NKp44 and PCNA and finally to try to crystallize the NKp44:PCNA complex. Both proteins are being prepared via recombinant expression in HEK293S GnTI$^-$ cell line. Purification is performed by affinity chromatography using TALON or protein A columns followed by gel filtration. PCNA has been already successfully prepared by intracellular expression in milligram amounts. Recombinant expression of extracellular domain of NKp44 failed so far, however, we are able to produce NKp44 in fusion with Fc fragment of human IgG by secreted expression to cell culture media. Although the yield of this NKp44 fusion construct is not sufficient for crystallization and optimisation of NKp44 production is still ongoing, it enabled us to perform preliminary biophysical characterization of

NKp44:PCNA interaction using microscale thermophoresis and analytical ultracentrifugation.

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P14

STRUCTURAL STUDY OF KIX DOMAIN BY NMR

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The KIX domain of transcriptional co-activator CREB binding protein (CBP) mediates transcription by recruiting transcription factors such as oncogenes c-Myb and mixed lineage leukemia (MLL) [1-2]. These binding partners are largely disordered and attain secondary structure after binding to KIX domain. The KIX domain can bind transcription factors simultaneously to its two opposite binding sites which communicate allostericaly through hydrophobic core [3]. Many transactivation domains for transcription factors interacting with KIX domain belong to established Nine amino acid Transactivation Domain (9aaTAD) family [4].

Standard triple resonance experiments (HNCACB, HN(CO)CACB, HNCO, HNCA, HN(CO)CA) were measured on double labelled ^{13}C,^{15}N KIX domain in order to assign protein backbone. Subsequently, ^{15}N KIX domain was titrated with c-Myb and MLL ligands to observe structural changes and dynamics using 2D HN-HSQC experiment. Dissociation constant for both ligands is in micromolar range.

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P15

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE ACBD3-3A COMPLEXES IN ENTEROVIRUS INFECTION

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Enteroviruses, members of the family *Picornaviridae*, are small single stranded RNA viruses with positive-strand polarity and non-enveloped icosahedral capsids. Depending on species enteroviral infections lead either to the asymptomatic or mild respiratory diseases, but also cause severe illnesses as acute hemorrhagic conjunctivitis, meningitis, myocarditis, encephalitis, or poliomyelitis [1].

Viral infection affects a lot of cellular processes and enteroviruses need host protein machinery for their successful replication. ACBD3 was described to be in different cases of picornaviruses a crucial player in viral RNA replication due to its influence on reorganization of intracellular membranes. The C-terminal GOLD domain of ACBD3 has been reported to interact with the golgin B1, which results in the Golgi localization of ACBD3 [2]. However, in enterovirus-infected cells, the ACBD3 GOLD domain interacts preferentially with viral non-structural 3A proteins, which causes re-localization of ACBD3 to the sites of virus replication [3]. The structural determinants of its recruitment to the viral replication sites are poorly understood.

Here we describe structures of GOLD:3A complexes from four representative enteroviral species. Using mutation analysis we identified amino acid residues important for the ACBD3:3A interaction, co-localization, stimulation of PI4KB recruitment, and facilitation of virus replication in human cells. Interestingly the enterovirus and previously

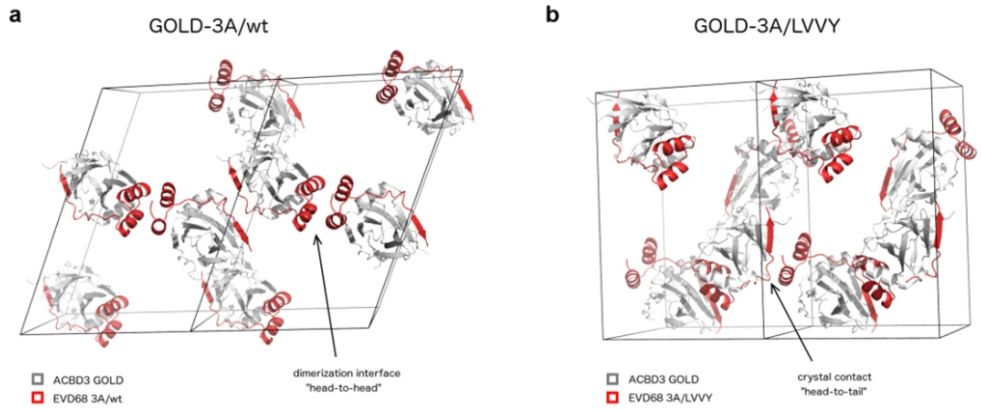

Figure 1. Analysis of the dimerization interface of the GOLD-3A complexes. Crystal packing of the wild-type GOLD -EVD68 3A fusion protein (**a**) and its LVVY mutant (**b**).

described kobuvirus 3A proteins bind to the same regions of the ACBD3 GOLD domain but in opposite orientation.

We found that ACBD3: enterovirus 3A complexes form heterotetramers consisting of two molecules of the viral 3A protein and two molecules of host ACBD3. We identified also that LVVY 3A mutants remain monomers (Fig. 1).

In conclusion, in our study we analysed in detail molecular interactions between entreroviral 3A protein and human ACBD3 GOLD domain and we showed a conserved mechanism how diverse enterovirus species recruit the ACBD3 protein. In comparison with kobuvirus 3A we also found nice example of convergence in picornavirus evolution.

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P16

RECOMBINANT EXPRESSION OF PROTEIN NKp46 AND ITS LIGAND Epa1 AND STUDY OF THEIR INTERACTION

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One part of innate immunity is natural killer cells that play an essential role in the immune response of an organism. In contact with infected, stressed or tumour cells, the NK cells can trigger a cytotoxic response. Initiation of this mechanism depends on the presence of activating or inhibiting ligands on the surface of the target cells. NKp46 is one of the activation receptors. This receptor belongs to the group of natural cytotoxicity receptors and has many ligands. One of them is Epa1, a lectin-like epithelial adhesin occurring on the surface of yeast *Candida glabrata*. That is one of the most common agents of invasive candidiasis in the world. The interaction between NKp46 and its ligands in some cases (e.g., for hemagglutinin) depends on sialic acid O-glycosylation on the surface of NKp46. It was found that the interaction of adhesin of *C. glabrata* depends on glycans located on the host cell surface.

One of those glycans is very similar to glycosylation on the NKp46 surface [1, 2]. The short-term objectives of this project were recombinant expressions of both Epa1 and NKp46 proteins. Protein Epa1 was successfully produced in bacterial expression system and purified using lactose affinity chromatography. Production of protein NKp46 is now being optimised. The protein is produced via recombinant expression in HEK293S GnTI$^-$ and purified using TALON affinity a gel permeability chromatography. Building on that, the long-term goals are (i) biophysical description of the interaction of these proteins and (ii) crystallization of the NKp46:Epa1 complex. These results may contribute to the understanding of innate immune mechanisms and could be used in the design of immunotherapeutics.

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P17

THE FANCONI ANAEMIA REPAIR PATHWAY: „STAY WITH ME, UBIQUITIN.“

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Due to the action of endogenous and exogenous agents, DNA is subject up to 70,000 lesions per day, thus the existence of repair mechanisms and enzymes is more than necessary. We already know basic mechanisms of several specific DNA repair pathways include the base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), and among others, Fanconi anaemia (FA) repair pathway. FA is a rare, autosomal-recessive disorder characterized by early onset bone marrow failure, developmental defects, genomic instability and predisposition to acute myeloid leukaemia and solid tumours. The primary diagnosis of FA is a hypersensitivity to cross-linking agents such as mitomycin C or cisplatin. The molecular defect in FA is an impaired repair of DNA interstrand cross-links (ICLs). The ICLs are cytotoxic lesions that inhibit process of DNA replication and transcription. Crucial step in FA pathway that initiates ICL repair is a mono-ubiquitination of FANCD2 and FANCI. Ubiquitinated FANCD2-FANCI is a base for the recruitment of additional proteins that coordinate DNA repair. Ubiquitin (Ub) is recruited via activating enzyme E1 (UBA1), ubiquitin conjugating enzyme 2T (UBE2T) and transferred onto FANCD2 resp. FANCI by multisubunit E3 ligase (FA core complex). There is upto 11 different proteins composing FA core complex whilst most important are FANCB, FAAP100 and FANCL. However, minimal ubiquitination system for proper mono-ubiquitination of FANCD2 and FANCI is ubiquitin, UBE2T, FANCL, UBA1 and DNA. In this work we study molecular architecture and mechanistic properties regulating the ubiquitination and entire FA pathway. We have produced covalent complex of UBE2T with ubiquitin bound by thioester bond.

In order to investigate the molecular mechanisms of mono-ubiquitination of FANCD2 we have prepared variants of constructs of all three different domains of FANCL. These were expressed, purified and we have assayed their enzymatic activities and binding properties. We obtained minimal components for sufficient FANCD2 mono-ubiquitination. These were put forward in experiments focused on crystallization of complex UBE2T-Ub with FANCL. Our aim is to obtain structural information about interaction sites of ubiquitin in complex with UBE2T and FANCL for better understanding of molecular mechanism of activation step in FA repair pathway. Since ICL-forming agents are used in cancer therapy, our structure could help in development of therapeutic targets for inhibiting repair mechanism in cancer cells.

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P18

CYTOCHROME P450 1A2 FEATURES SIMILAR STRUCTURAL PATTERN OF THE TRANS-MEMBRANE SEGMENT IN MEMBRANES WITH A DIFFERENT THICKNESS

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Cytochrome P450 (P450) enzymes are components of a mixed-function oxidase system located in the membrane of endoplasmic reticulum. Using multiscale computational methods, we investigated the structure and dynamics of the full-length membrane-anchored P450 1A2 enzymes. The absence of the structural information on the trans-membrane (TM) domain of these two proteins was surpassed by employing a spontaneous self-assembly molecular dynamics (MD). The simulation was performed in randomized dilauroylphosphatidylcholine (DLPC)/water/salt mixture

and also in randomized palmitoyloleoylphosphatidylcholine (POPC)/water/salt mixture. The resulting membrane-bound full-length structures of P450 1A2 in different membranes were then optimized using coarse-grained and all-atom MD. The resulting models show that, despite of the different membrane thickness, the upper part of the TM helix in both cases directly interacts with a conserved and highly hydrophobic *N*-terminal proline-rich segment of the catalytic domain. The shallow membrane immersion of the catalytic domain appears to induce a depression in the opposite intact layer of phospholipids, which may help in stabilizing the position of the TM helix directly beneath the catalytic domain. The phospholipid membrane thickness has a direct impact on the TM domain tilt being more inclined in case of the thinner DLPC membrane.

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P19

EFFECT OF PHOSPHORYLATION ON PDZ DOMAIN IN DISHEVELLED

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Wnt signaling pathway belongs among ancient and evolutionarily conserved pathways guiding cell proliferation and cell-cell communication in tissues. The dysfunction of Wnt pathway is connected to development defects, inherited diseases, and cancer [1]. Dishevelled (DVL) protein plays a pivotal role in the Wnt pathway as a key signaling hub [2], however, the regulation of DVL function still remains largely unknown [3]. Here, we investigated the effects of posttranslational modifications on DLVs PDZ domain, a key domain common to all Wnt signaling pathways [1]. In particular, we focused on recently determined phosphorylation sites in the third isoform of human DVL protein (hDVL3) using molecular dynamics simulations. We discovered that the four biologically relevant phosphorylations can be split into two groups with a distinct mode of action. The first group situated on the 2 strand caused strong electrostatic interaction across the canonical binding cleft, effectively closing it. While phosphorylations in the second group induced stabilization of the long 2- 3 loop, a typical secondary binding site [4]. We also investigated the behavior of phosphomimetics, a frequently used mimic of the phosphorylated residue by their mutation to either glutamic or aspartic acid, which effects were similar but weaker compared to phosphorylated residues. Our findings provide a molecular understanding of how phosphorylation influence PDZ structure in DVL protein and may be applied to other PDZ domains which are frequently occurring protein-protein recognition motif [5].

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P20

VIBRATIONALLY-RESOLVED UV-ABSORPTION AND MAGNETIC CIRCULAR DICHROISM OF NUCLEOSIDES

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Nucleobase pairing enables the encoding of genetic information in DNA and RNA. Thus far, the interaction of (deoxy)ribose with the nucleobases has been hard to explain from optical spectra, which is compounded by the lack of understanding of sugar conformations. Circular dichroism (CD) spectra provide clear understanding of molecular conformations. Despite of numerous experimental and theoretical CD studies on the base components of nucleic acids reported earlier [1-3], transitions in the UV-vis region that are too weak to be seen in normal CD require further elucidation. For example, the experimental absorption spectrum of adenosine (Figure 1) shows a strong absorption apparently broad at 259.2 nm. The recent studies on the adenine nucleobase [1] and deoxyadenosine [4] reported the band at 260 and 259.7 nm, respectively. This implies that the effect of sugars on the absorption spectrum of adenine seems to be insignificant.

Magnetic circular dichroism (MCD) is a useful tool for precise analysis of the electronic states of a molecule, and it resolves bands that are not obvious from absorption spectrum. The present work attempts to investigate the magnetically induced chirality of electronic transitions of the nucleosides, and to assign spectral bands more precisely. The effect of the sugar components on the electronic transition of nucleobases is interpreted. The possible conformations along the -glycosidic bond were evaluated using DFT theory. TDDFT calculation and MCD simulation

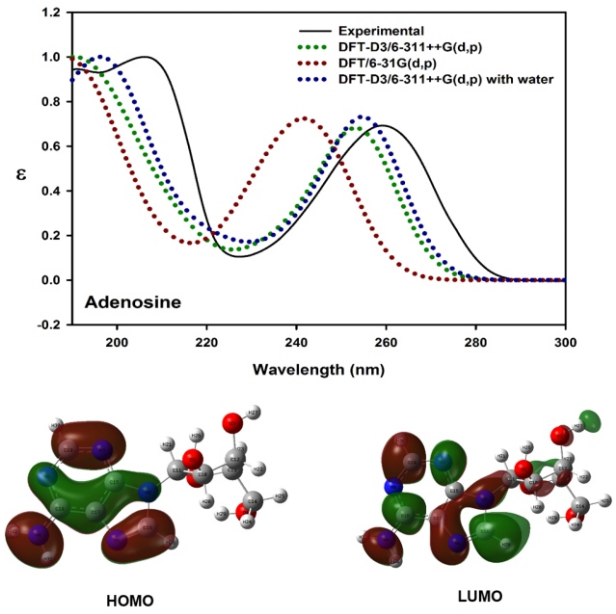

Figure 1. UV absorption spectrum (Top) and frontier molecular orbitals (Bottom) of adenosine

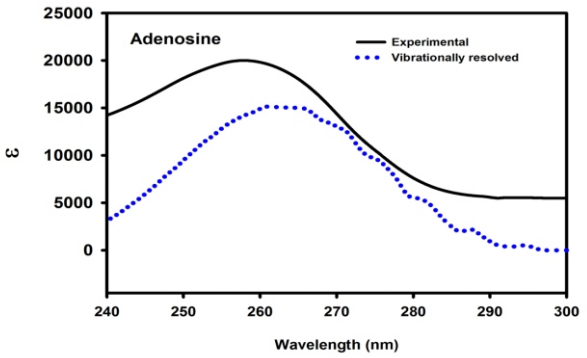

Figure 2. Vibrationally-resolved absorption of adenosine

results were used to interpret the experimental spectra. Simulation of MCD spectra is obtained from TDDFT using sum over state (SOS) summation [5,6]. SOS involves a quadruple sum over the virtual and occupied molecular orbitals [6]. In general, MCD intensities are described by Faraday terms A, B and C in which origin-independent expression (localized orbital/localized origin) of the MCD tensor is used to obtain the B term. In addition, vibrationally-resolved absorption and MCD spectra of nucleosides such as adenosine and methyluridine were generated in order to consider the dynamic effects during the electronic transition. The band shape of the prominent absorption of adenosine corresponding to HOMO LUMO transition is reproduced by theoretical vibronic spectrum (Figure 2). For simple systems, the experimental and simulated MCD spectra were nearly matching.

In Figure 3, the absorption, CD and MCD spectrum of methyluridine is presented. Methyluridine produces two broad bands; one at 267.2 nm responsible for two electronic transitions (H L and H 1 L) and another at 205.9 nm. It is obvious from the theoretical UV (Figure S1, Supplementary Material) and CD spectrum of methyluridine, the absorption around 200 nm in the UV spectrum is a composite and it corresponds to the peaks having small rotatory strengths near 205 and 194 nm in CD spectrum. MCD spectrum shows the magnetically enhanced CD bands in opposite orientations of the samples with respect to the magnetic poles. The simulated MCD reproduces experimental MCD spectrum. However, over all blue shift is observed in most of the cases.

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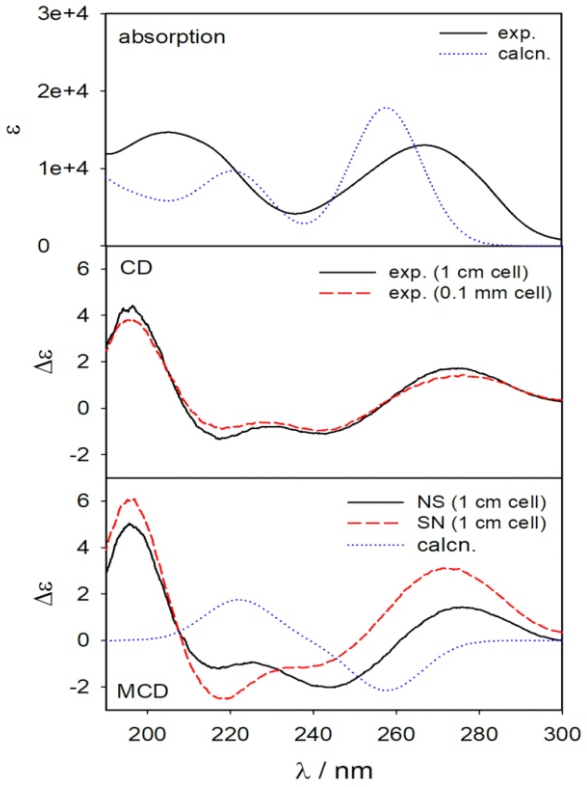

Figure 3. Absorption, CD and MCD spectrum of methyluridine

P21

MOLECULAR-BIOLOGY AND STRUCTURAL STUDY OF AVIAN ORTHOREOVIRUS PROTEIN NS

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The fusogenic avian Orthoreoviruses of Reoviridae family are important pathogens of birds that can cause considerable economic losses in the poultry industry. Avian reoviruses have been associated with a variety of disease conditions in poultry, including enteric and respiratory diseases, myocarditis, hepatitis, arthritis syndrome and the so-called stunting/malabsorption syndrome [1]. The avian reovirions are non-enveloped icosahedral particles of 85 nm external diameter with 10 dsRNA genomic segments (23.5 kb) encased within two concentric protein shells, forming the outer capsid and the core [2]. The RNA replication and morphogenesis of reoviruses occurs exclusively within cytoplasmic inclusion bodies, also known as viral factories, or 'viroplasms'. The viroplasms are formed by non-structural protein NS in association with non-structural protein NS [3]. The NS acts as RNA chaperone and destabilizes helical regions of RNAs. The structure is not known yet. The NS protein was constructed in order to study the process of the viroplasm formation in details. The NS is a non-structural protein approximately 41 kDa large and is composed of 367 amino acids. The homology modelling by Phyre2 prediction server estimated a high -helical structure [4]. The SAXS experiments revealed the elongated pear-shaped structure. The NS protein is homodimer as a biological unit with high probable further hexamerisation. It forms likely octamers in the presence of bound ssRNA in solution by hydrophobic interactions. The óNS rapidly binds ssRNA in a sequence-independent manner and then form large nucleoprotein complex [5].

The *NS* gene was amplified by Q5® Polymerase (New England Biolabs, UK) and cloned into the pET SUMO expression vector. Recombinant NS was produced in *E. coli* BL21 (DE3) cells at a constant temperature of 37 °C and 220rpm for 4 hours. Cells were subsequently harvested by centrifugation, lysed using French press and the cell lysate was clarified by ultracentrifugation at 25000 rpm for 1

hour. Recombinant NS was purified from collected supernatant by various chromatography methods including affinity chromatography, anion exchange chromatography size exclusion chromatography. The purity and homogeneity of NS protein was analyzed via SDS-PAGE and MALS analysis, respectively. The electron microscopy (EM) negative staining was used to clarify the oligomeric state of the purified samples. The purified samples were used for the crystallization experiment by the sitting-drop vapor-diffusion procedure. For the initial screening several commercial precipitant kits were applied to grow 3D crystals suitable for the diffraction measurement (Morpheus II, Molecular Dimensions, UK; PEG/Ion, Hampton Research, USA).

Here we report the results of the expression, purification and further crystallization experiments of the NS protein. Subsequent monitoring of purified samples revealed that protein is quite stable and in presence of ssRNAs forms octamers. These afterwards forms pseudocapsids that were observed by EM negative staining.

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P22

Hsp70 ATP-DEPENDENT DIMERIZATION AND INTERACTIONS

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The 70kDa heat shock protein (Hsp70) is the central component of nanomachinery maintaining the cellular proteostasis [1]. Sequence and structural conservation of Hsp70 across all domains of life suggest the evolutionary importance and conserved, but protean mechanism of action [2]. Hsp70 system is able to bind nascent polypeptide chains at ribosomes or translocons [3, 4]; misfolded, stress-denatured proteins or solubilize protein aggregates or amyloid fibrils [5]. Hsp70 proteins are composed an nucleotide-binding domain (NBD) and substrate-binding domain (SBD) connected via highly conserved linker [6]. SBD interacts with short degenerative sequence motifs present in virtually every globular protein, while NBD covers the SBD as a helical lid and stabilizes substrate binding [7]. Beside this allosteric opening of the polypeptide binding site upon ATP binding [8], Hsp70 proteins were reported to form antiparallel dimers facilitated by Hsp40, which is part of Hsp70 system [9].

In this study, we inspect the human Hsp70 by hydrogen/deuterium exchange mass spectroscopy, native electrospray ionization mass spectroscopy and small angle X-ray scattering, showing the Hsp70 to form an antiparallel dimer in solution in ATP-dependent manner. Dimerization of Hsp70 is essential for interaction with Hsp40 as was shown by dimerization-deficient mutatnts. Formation of multichaperon complexes of Hsp70 with two tetratricopeptide repeat domain co-chaperones Chip and Tomm34 was inspected by size exclusion chromatography in presence and absence of ATP. Dimeric co-chaperone Chip interacts with ATP-induced Hsp70 dimer and forms mutichaperon complex with stoichiometry 2:2, while monomeric co-chaperon disrupts the ATP-induced Hsp70 dimer and forms 1:1 complex with Hsp70.

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P23

NANOBODIES AS VERSATILE PURIFICATION AND DETECTION SYSTEM

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Nanobodies (Nbs) are the small (15 kDa) and stable single domain fragments derived from variable region of heavy chain antibodies produced by camelids, which harboring the full antigen-binding capacity of original antibodies. The high stability and easy production in various micro-organism makes nanobodies convenient tools for numerous bioanalytical and biotechnical applications. Recently was reported a nanobody reffered to as BC2 (nanobody against beta catenin) which recognizes a short linear epitope (PDRKAAVSHWQQ, reffered to as BC2 tag) with high affinity (K_D ~1.4 nM) and selectivity [1,2]. This type of binding interaction was used in our work for preparing the protocol for purification and detection of selected RNA dependent RNA polymerases (RdRp). We cloned BC2 Nb and recombinant RdRp with BC2 tag on C-terminus into the pRSFDuet vector and pSUMO vector, respectively, and expressed in *E. coli* BL21-CodonPlus-RIL cells. Firstly, we purified both using classical IMAC techniques and after that we tested binding affinity and influence of BC2 tag on RdRp. We were fighting with misfolding of BC2 Nb, but at the end we solved the problem using denaturating buffer with urea and refolding of nanobody. Finally, we established purification protocol of BC2 Nb and confirmed the activity of BC2 tagged RdRp alone and also in complex. Our next aim is use BC2 Nb as main purification system for RdRp, because purification by IMAC in many cases leads to the precipitation of this type of proteins.

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P24

FLAVIVIRUS METHYLTRANSFERASE AS A TARGET IN ANTIVIRAL TREATMENT

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Viruses of the Flaviviridae family are widespread vector-borne pathogens causing large epidemics. Members of the Flaviviridae family consist of a large group of enveloped viruses with a +RNA genome. Dengue virus (DENV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV) and Zika virus (ZIKV) are all emerging or reemerging pathogens.

Their NS5 protein consists of RNA-dependent RNA polymerase (RdRp) subunit and methyltransferase (MTase) subunit that is responsible for N-7 and 2'-O methylation of the viral RNA cap which protects the RNA from being recognized by host sensors. We have already shown that the RdRp can be targeted by nucleotide analogs [1] and we performed structural analysis of the Zika MTase [2]. Now, we explore the possibility to target different (ZIKV, TBEV, DENV and YFV) MTase domains by the same compound.

We produced recombinant MTase domains in *E. coli* and assayed them enzymatically. A key step was the removal of S-adenosyl-L-methionine (SAM) - the methyl donor that co-purifies with the MTases. We have already designed several analogs of SAM and using thermalshift assay determined which of them bind into SAM-binding pocket. The best compound was crystallized with DENV Mtase and the crystal structure helped us with the design of new series of ligands.

In order to characterize dissociation constant of our compounds we developed a fluorescence anisotropy assay.

For this purpose, we first had to prepare a fluorescently labeled analog of SAM and determine its affinity to the Mtase domain. Now ligand displacement assay (fluorescent ligand is displaced by a non-fluorescent ligand and the anisotropy of fluorescence goes down) can be used to measure affinity of any ligand towards the MTase [3].

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P25

THE ROLE OF FANCI PHOSPHORYLATION IN FANCONI ANEMIA PATHWAY REGULATION

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Fanconi anemia is an autosomal recessive disorder caused by mutation in one of Fanconi genes and it is manifested by developmental abnormalities, bone marrow failure, predisposition to cancer, cellular sensitivity to cross-linking agents and many other symptoms [1]. Proteins encoded by Fanconi genes and some other proteins are part of Fanconi anemia pathway (FA pathway), which is responsible for DNA repair of an interstrand cross-link (ICL)[2]. The repair by this pathway requires monoubiquitylation of FANCD2, which is induced and regulated by ATR dependent FANCI phosphorylation. The FANCI phosphorylation initiates the FA pathway but the molecular mechanism of this initialization is not known. Furthermore the proper function of entire pathway requires both: sequence of phosphorylation events of FANCI and monoubiquitylation of FANCD2:FANCI complex [3].

In this project we study molecular mechanisms of initiation and regulation of FA pathway by FANCI phosphorylation. Therefore we have created phosphomimetic mutants of FANCI to investigate their role in processes leading to FANCD2 monoubiquitylation. Our aim is to reveal how the phosphorylation of FANCI affects DNA binding and also DNA binding of the FANCI:FANCD2 complex. Since both DNA and FANCI phosphorylation are required for proper FANCD2 monoubiquitylation, we measured and compared these individual phosphorylation mimetic and phosphorylation dead mutants of FANCI in DNA binding assays. In ongoing experiments we are testing the role of FANCI phosphorylation in FANCD2 binding and in monoubiquitylation assays. Although FANCI dramatically improved the binding of its partner FANCD2 to DNA, mutations or phosphomimetic had only marginal effect on the DNA binding. Therefore, we need to investigate further on how exactly the phosphorylation of FANCI triggers the FANCD2 monoubiquitylation and regulates entire FA pathway progression.

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P26

THE STUDY OF FORMATION, STABILITY AND REPAIR OF SPONTANEOUSLY FORMED ABASIC SITE INTERSTRAND CROSS-LINK

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Abasic sites (Ap site, from *apurinic*/*apirimidi*nic) are one of the most common lesions generated in DNA by sponaneous base loss or DNA repair processes. There are two equalibrating conformations of Ap site – ring-open aldehyde and cyclic hemiacetal. Ring-opened aldehydes are electrophilic funcional groups capable of formation covalent aduct with nucleophilic sites in DNA. DNA interstrand cross-link (ICL) is a lesion resulting from Ap sites by spontaneous formation of covalent bond between ring-open aldehyde and amin group of adenin residue in the opposite strand of double stranded DNA. ICLs block DNA replication and transcription. The formation of Ap site derived ICL is relatively long process taking several hours [1]. We presume that the ring-openig of an abasic site is the rate-limiting step in the formation of the thermodynamic ICL. There are two types of ICLs. The ratios and the yields of the ICLs are highly depend upon a local sequence [2]. Here we have set up mechanistic experiments *in vitro* to reveal and calculate the probability of Ap-ICl formation *in vivo*. In more detail we work on characterization of rates of formation of Ap-ICLs in dependence of sequence of neighbouring nucleotides in the vicinity of freshly formed covalent bond of ICL. We focus on sequence preference, the influence of AT/ GC rich regions and the length of oligonucleotides.

Our experiments extended the understanding of ICL formation and stability to roughly estimate the occurrence of this lesion *in vivo*. We proved dependence of ICL formation on the bases near the covalent bond of ICL. We also show that in AT rich regions are yields of ICL much higher than in GC rich regions.

Recently, it has been shown that N-glycosidic bond forming ICL is cleaved by NEIL3 glycosylase [3]. Close orthologue of NEIL3 is Formamidopyrimidine DNA glycosylase (Fpg), a DNA base excision repair enzyme with N-glycosylase activity where it removes a wide range of oxidatively damaged bases or Ap lyase activity where it cleaves both 3'- and 5'-phosphodiester bonds of the resulting apurinic/apyrimidinic site. The focus of this part of the project is the preparation of Ap-ICL and its structural characterization using crystallization in complex with Fpg or NEIL3. In contrast with NEIL3 we show that Fpg binds avidly to double-stranded DNA with ICL. We have crystallized both Fpg and Fpg in complex with Ap-ICL and we have already solved structure of Fpg.

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P27

TIME-RESOLVED INFRARED STRUCTURAL BIOLOGY AT IBT AND ELI BEAMLINES

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Vibrational spectroscopy is widely used to characterize molecular structures in different phases. At the Institute of Biotechnology (IBT) and ELI Beamlines, two state-of-the-art time-resolved infrared spectroscopy instruments are under construction, with the goal of measuring non-equilibrium (bio)-molecular dynamics upon photoexcitation. The one at IBT is based on the step-scan and fast-scan methods and uses a nanosecond optical parametric oscillator (OPO) laser as the pumping source and a Fourier-transform infrared (FTIR) spectrometer as the probe. The OPO laser has a tunable frequency range from 210 nm to 2600 nm and is therefore suitable for triggering many biological processes. This setup can be used to trace conformational dynamics from a few nanoseconds to several seconds. On the other hand, the setup at ELI Beamlines uses a femtosecond laser, optical parametric amplifiers (OPAs) and difference frequency generation (DFG) system. The introduction of a second amplifier will permit us to track dynamical processes from sub-picoseconds to milliseconds in a single measurement. By inserting a pulse shaper in the IR pump beam, the IR-pump IR-probe setup will be transformed into a two-dimensional (2D) IR spectrometer. [1] 2D IR spectroscopy spreads the contribution of components of the pumping pulse with different frequencies into a second axis, like 2D NMR but with sub-picosecond time resolution [2], allowing more detailed structural information about fast molecular events (e.g. solvation effects) to be obtained. 2D IR spectroscopy in combination with vibrational labels introduced into proteins at specific locations will be used to follow the fluctuations of the chemical environment around the probes and their coupling. A transient 2D IR experiment will also be assembled by introducing an actinic pump that initializes photoactive reactions. In order to suppress systematic errors, single-shot Fourier transform 2D IR spectroscopy is proposed by introducing front-tilted infrared pulses.

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P28

INVESTIGATION OF DIMER-MONOMER EQUILIBRIUM OF THE REGULATORY DOMAIN OF TYROSINE HYDROXYLASE

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the formation of dopamine. It catalyses the hydroxylation of L-tyrosine to form L-DOPA, an intermediate of catecholamine biosynthesis. [1] The catecholamines dopamine, adrenalin and noradrenalin play important roles in the human organism as neurotransmitters and hormones. [2]

Tyrosine hydroxylase consists of three domains: N-terminal regulatory domain, catalytic domain and C-terminal tetramerization domain. Activity of TH rapidly increases after phosphorylation of S19 and S40 situated within the regulatory domain and its subsequent interaction with 14-3-3 proteins. [3] Recently, the regulatory domain of TH was described as a stable dimer at physiological conditions. [4]

In order to describe dimer-monomer equilibria of regulatory domain of human TH, we designed an assay based on the self-quenching phenomenon for determination of dissociation rate constant. The temperature dependency of the dissociation rate constant allowed us to obtain an estimated value for the energy barrier of dissociation of the regulatory domain.

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P29

CRYSTALLIZATION OF THE ANTIBODY DC25 Fab AND ITS COMPLEX WITH RECOMBINANT TRUNCATED TAU PROTEIN FROM ALZHEIMER'S PHF

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Crystallization process requires supersaturated conditions in the macromolecule, which should not significantly perturb its natural state [1]. To solve structure using X-ray crystallography, it is necessary to use high quality crystals. As a first step the initial hits are obtained using different screening conditions in pre-assembled collections. On the market, more than 15 000 conditions from at least 13 vendors are available, therefore it is challenging to find suitable condition to achieve crystal growth [2].

Disordered regions in proteins suppress the regular ordering of protein molecules required for crystal nucleation and growth. Therefore, intrinsically disordered proteins (IDPs) are reluctant to the crystallization; however, it has been shown that complexes of IDPs with binding partners like antibody Fabs are crystallisable [3, 4]. IDP tau is principal to neurodegeneration in Alzheimer's disease. For our work we chose the anti-tau antibody DC25 with epitope on the Lys347-Lys353, which lies in the fourth microtubule-binding repeat of the tau protein [5].

Aim of this work was preparation of crystals suitable for X-ray crystallography. We used recombinant DC25 Fab and truncated form of tau dGAE (contains residues 297-391). For initial crystallization we used Structure screen 1+2 HT-96 (Molecular Dimensions) and JCSG$^+$ screen (Molecular Dimensions). We obtained initial hits for DC25 Fab alone and its complex with tau dGAE, which were further optimised with varying concentration of protein and precipitant. We successfully prepared single crystals larger than 50x50x50 μm^3 in different conditions for the DC25Fab as well as for its complex with tau dGAE. We found one condition where both DC25Fab and its complex with tau dGAE crystals grew, which would facilitate interpretation of structural data.

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P30

INTERACTION OF MAP2c WITH SH3 DOMAIN OF PLECTIN

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Microtubule associated protein 2c (MAP2c) is an intrinsically disordered protein (IDP) which belongs to a MAP2 subfamily expressed in the developing neurons and can be found mainly in their dendrites [1]. MAP2c binds to microtubules regulates their dynamics 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 [2-4] .

Plectin, the cytolinker between three main cytoskeletal components (actin microfilaments, microtubules (MT) and intermediate filaments), act as an MT destabilizer [5] . Its SH3 domain seems to interact with several regions of MAP2c, which show interesting conformational behaviour.

In our work we used various NMR experiments in order to investigate the interaction between MAP2c and the SH3 domain of plectin and dynamic properties of the binding site. In order to understand the nature of this interaction we applied HNCO NMR experiments on full-length MAP2c and its shorter constructs to describe also the effect of absence of other functional domains to this interaction.

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P31

CRYSTAL STRUCTURE OF *BACILLUS SUBTILIS* TRANSCRIPTION REPRESSOR DeoR IN COMPLEX WITH ITS OPERATOR DNA

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DeoR is involved in *B. subtilis* carbon catabolism as the local repressor for transcription of enzymes digesting deoxyribose and deoxyribonucleosides. This repressor binds an operator DNA by its N-terminal DNA-binding domain (DBD) and blocks transcription of catabolic enzymes in the absence of an effector molecule. If the effector is present it binds to the C-terminal effector-binding domain (EBD) of the repressor and causes its release from the DNA binding site. The structures of the C-terminal EBD of DeoR in free form and in the complex with the effector deoxyribose-5'-phosphate have already been reported [1]. Nevertheless, for the understanding of the allosteric effects of the repressor during the metabolic regulatory process, it is necessary to know the 3D structure of the full-length protein in complex with its operator DNA. To achieve this, we initiated structural studies DeoR in complex with DNA operator.

Recombinant DeoR was prepared by heterologous expression in *E. coli* BL21 (DE3) and purified with yield of 3.4 mg per 1 L of bacterial culture. Crystallization of DeoR in complex with 18-base pair DNA duplex derived from the operator sequence was carried out using the vapor diffusion sitting- and hanging-drop techniques. Needle-shaped crystals were obtained and diffraction data were collected to maximal resolution of 3.6 Å. Crystal structure was determined by molecular replacement and

confirmed the presence of DeoR-DNA complex. At this resolution, however, DBD and DNA could not be unambiguously modelled into the electron density maps. Optimization of crystal quality to achieve higher resolution failed. As an alternative, we chose to perform structural study on DeoR DBD in complex with DNA to be used as a model for low-resolution structure.

DeoR DBD was prepared by heterologous expression in *E. coli* BL21 and purified with yield of 5.8 mg per 1 L of bacterial culture. Crystallization of DBD in complex with 15-base pair DNA duplex yielded crystals diffracting to 2.3 Å resolution. Crystal structure of DBD-DNA complex was solved by molecular replacement using the preliminary model from full-length DeoR structure. Refinement of crystallographic model is currently in progress.

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P32

THE LEVANSUCRASE PUZZLE: HOW TO COMBINE STRUCTURE AND BIOCHEMISTRY TO UNDERSTAND THE BIOLOGICAL FUNCTION

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The Gram-negative bacterium *Erwinia amylovora* is the etiological agent of fire blight, a devastating disease which affects Rosaceae. Activity of levansucrase has been correlated with the virulence of *E. amylovora*. *E. amylovora* levansucrase defective mutants, showed retarded development of necrotic symptoms on pear seedlings inoculated with the mutated strains [1]. The plant pathogen *E. amylovora* is related to the epiphytic bacterium *Erwinia tasmaniensis* [2, 3].

To understand the role of this enzyme in the two bacteria, the levansucrase from *E. tasmaniensis* (EtLsc) has been compared with the homologous enzyme from *E. amylovora* (EaLsc) [4]. The enzymatic activity was characterized by High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detector (HPAEC-PAD). The comparison of the products profile highlights an increased efficiency of EtLsc than EaLsc. The crystal structure of EtLsc reveals structural peculiarities causing the different product profiles of the two homologues.

Based on our results, we propose that the role of this enzyme in the life cycle of the two bacteria is most likely related to survival, rather than linked to the virulence of the plant pathogen *E. amylovora* [5].

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P33

INTERACTION OF NATURAL KILLER CELL RECEPTOR NKR-P1 WITH ITS LIGAND LLT1

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Natural killer cells (NK cells) belong to innate immunity lymphocytes. They were discovered in 1970s and comprise 5-10% of lymphocytes circulating in blood. Their role in the immune system is to discover and kill cancer cells, stressed cells and cells infected by viruses. NK cells have a number of receptors on their surface, which are used for contact with other cells and for initiation of the cytotoxic response.

NKR-P1 is a C-type lectin like-receptor on surface of human natural killer cells and LLT1 is its binding partner belonging to the same structural family.

Recently, we have expressed, purified and solved four crystal structures of the extracellular part of LLT1 in monomeric, dimeric and hexameric form [1, 2]. In this contribution, we present three more structures characterizing this receptor-ligand binding pair: structures of the extracellular part of NKR-P1 in the fully glycosylated and deglycosylated form and a structure of the NKR-P1:LLT1 complex. Expression and purification of NKR-P1 was described by us recently as well [3].

All three crystal structures show NKR-P1 in a dimeric form with an unexpected dimerization mode. Unlike LLT1, which has the 2 helix in the dimerization interface, NKR-P1 dimer has the 1 helix in its dimerization interface. This different dimeric arrangement of both proteins enables spatial connection of NKR-P1 with LLT1 not only in a single molecular complex, but in a periodical chain of alternating receptor/ligand molecules. Such chain we really observe in the presented crystal structure.

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STRUCTURE OF STING WITH FLUORINATED AGONISTS

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STING (stimulator of interferon genes) is a dimeric protein localized in the membrane of endoplasmatic reticulum (ER) where it acts as a receptor of cyclic dinucleotides (CDNs) second messengers involved in the innate immune system. After triggering by CDNs of bacterial or host origin, it is capable to activate both NF B and IRF3 transcription pathways to induce type I interferons (INF- and INF-), which leads to potent antibacterial and antiviral state of organism. Therefore, STING is being investigated as a potential pharmacophore for the treatment of viral diseases. A natural endogenous agonist of STING is 2'3' cycloGMPAMP (2'3' cGAMP). This CDN is produced by cytosolic DNA sensor cyclicGMPAMP synthase (cGAS). On the basis of 2'3'-cGAMP and also CDNs of bacterial origin (c-di-AMP, c-d-GMP or c-di-IMP), novel STING agonists were designed. Among the very interesting ones are fluorinated analogues of CDNs, which exhibit higher potency in induction of type I interferons than their non-fluorinated parent compounds such as fluorinated 3'3'-cGAMP. We have successfully co-crystallized human wild type STING with fluorinated and non-fluorinated 3'3'-cGAMP and 3'3'cdiAMP. Surprisingly, our structural analysis revealed just slight differences between these structures. Subsequent computational analysis suggested that the hydrophobic effect together with conformational strain energy are responsible for the increased activity.

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THEORETICAL STUDY OF *CANDIDA ANTARCTICA* LIPASE B IN ORGANIC SOLVENTS

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Enzymes are extraordinary catalysts satisfying the needs of living organisms. Catalytic efficiency of enzymes and their selectivity is also appealing for utilizing them in technological applications [1]. Perhaps surprisingly, a wide range of enzymes do not denature and moreover retain catalytic activity in organic solvents. This opens a path for carrying out new unnatural reactions. Furthermore, the change of reaction medium significantly alters enzyme activity, chemo- and regio- and enantioselectivity. In spite of achirality of the solvent its effects on enantioselectivity are complex; different types of enzymes exhibit qualitatively different behaviour [2, 3] and simple rules for rationalization are still missing.

Our aim is to systematically investigate enzyme activity of *Candida antarctica* lipase B – mainly its intriguing non-monotonic dependence of enantioselectivity on composition of reaction medium. Specifically, we study a transesterification reaction catalysed by this enzyme in acetonitrile, toluene and their mixtures. First, we examine the detailed nature of the rate-limiting step using QM/MM calculations including a test of QM region size. Next, we sample the conformational space of the most important reaction intermediates for both enantiomers by classical molecular dynamics simulations and characterize the productive binding modes. We also inspected the behaviour of the helix 5 located in the vicinity of the active site which could act as a lid and therefore influence the reactivity. The gained molecular level insight will help to understand the puzzling dependence of enantioselectivity on solvent properties.

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NON-CANONICAL REACTIVE SITE AGAINST SERINE PROTEASES INVENTED BY PLANT KUNITZ INHIBITORS

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Protease inhibitors from the Kunitz family (I3 in MEROPS) are 20-25 kDa proteins widely distributed in plant kingdom. They share a conserved -trefoil fold in which variable loops are involved in interactions with proteases. Kunitz inhibitors target serine proteases using the canonical (Laskowski) mechanism based on a single binding loop with conserved structure.

Here, we present a set of high-resolution crystal structures of two potato Kunitz inhibitors in complex with trypsin and chymotrypsin. We identified a new, non-canonical type of reactive site that binds both serine proteases. It is formed by two separate loops interacting with the S1 and S1′ pockets. Through this structural mechanism, the non-canonical reactive site is stabilized against proteolysis by the target proteases, providing a functional advantage over the canonical design.

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CENTRE OF MOLECULAR STRUCTURE IN BIOCEV – CURRENT STATUS

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The Centre of Molecular Structure offers wide range of methods of structural biology. It operates in BIOCEV as part of Institute of Biotechnology, AS CR. CMS consists of facilities devoted to crystallization of macromolecules, X-ray diffraction, biophysical characterization and advanced mass spectrometry. Services are provided via Czech Infrastructure for Integrative Structural Biology (CIISB) and Instruct. The latest improvements of our instrument portfolio are SAXS Point 2.0 for small angle X-ray scattering studies of biomolecules in solution, MALDI TOF mass spectrometer, excimer laser for induced protein modification, and newly equipped room for spectroscopy.

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STRUCTURE OF BACTERIOPHAGE SU10 FROM THE FAMILY *PODOVIRIDAE*

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Bacteriophage SU10, from the family *Podoviridae*, can infect a wide range of *E. coli* strains. The phage has 77kbp dsDNA genome, prolate capsid, with the length of 135 nm and the diameter of 42 nm. Contrary to what was observed in most *Podoviridae* phages, bacteriophage SU10 has 27 nm long tail. Furthermore, the baseplate of SU10 changes conformation upon infection.

We used Cryo-electron microscopy and localized single particle reconstruction approach to determine the structure of portal, tail, base plate of SU10. Furthermore, we also characterized their conformational changes associated with cell-wall binding and genome ejection. The dodecameric portal complex has prolonged crown-barrel, similar to that of phage P22. In contrast to phage P22, the crown-barrel of SU10 portal is stable even after the DNA ejection. Description of structural changes of tail and base plate will improve our understanding of the mechanism of host recognition and genome delivery of bacteriophage SU10.

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P39

SUBSTRATE SPECIFICITY OF NEIL3 GLYCOSYLASE IN DNA INTERSTRAND CROSS-LINK REPAIR

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DNA interstrand cross-links (ICLs) are among the most toxic lesions. They form impenetrable barrier for the DNA replication machinery and transcription. ICLs can be ge.nerated spontaneously or induced by environmental agents *e.g.* aldehydes or cisplatin. Different DNA repair pathway have been identified for repair of ICL [1]. One type of ICL is formed from an abasic site (Ap, from apurinic/apyrimidinc). Ap sites are very frequent DNA lesions arising spontaneously in genomic DNA by base loss. Two equilibrating forms of site cyclic hemiacetal and ring-opened aldehyde characterize Ap site and undergo amino-catalyzed strand cleavage generating an electrophilic 2,3-didehydro-2,3-dideoxyribose sugar remnant. A ring-opened aldehyde of Ap sites can react with primary amines of the residues on the opposing DNA strand [2]. ICL formed from the Ap site (Ap-ICL) is known to be repaired by Endonuclease VIII-like 3 (NEIL3). This repair has been demonstrated in DNA replication profi-

cient Xenopus cell extracts and also in biochemical assays *in vitro*. During the replication fork cessation upon the Ap-ICL NEIL3 glycosylase is recruited to cleave the N-glycosidic bond unhooking the crosslink and allowing replication fork to proceed and extended the leading strand beyond the repaired lesion [3]. This work have revealed unprecedented repair machinery of Ap-ICLs. However, the mechanisms of crosslink recognition by the NEIL3 glycosylase remains unclear.

The focus of this work is the repair of spontaneously formed DNA crosslink formed within the DNA duplex. Our aim is determination of substrate specificity of NEIL3 glycosylase in order to understand the principles of substrate recognition and molecular mechanisms of the Ap-ICL repair. NEIL3 glycosylase contains three different domains. We have expressed and purified full-lenght and c-terminally truncated versions of NEIL3 glycosylase to reveal involvement of these domains in ICL repair. For enzymatic analysis we have prepared Ap-ICL in form of the DNA duplex and also different structures mimicking replication fork. We characterize structural and mechanistic features essential for the recruitment and Ap-ICL repair by NEIL3.

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P40

CRYSTALLOGRAPHY OF *FUZZINESS* IN THE COMPLEXES OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) and disordered protein regions have an intriguing property: their heterocomplexes with other cellular partners are often very stable, albeit having a large degree of structural heterogeneity (fuzziness, [1, 2]). The *fuzziness* is perceived as the inability to define a unique 3D-configuration of interacting interfaces. Prevalent methods for the fuzziness detection are ensemble and time averaging techniques like NMR, CD or single molecule fluorescence.

Studying complexes of IDP tau with antibodies by X-ray crystallography, we have noted that independently refined multiple copies of the complex in asymmetric unit may display contrasting details in their binding interfaces (**Fig. 1**). Crystallography thus may confer mostly unexpected contribution to the definition of binding contacts in fuzzy complexes, and, by implication, it may suggest the propensity of IDP to certain conformations already in the solution monomeric state.

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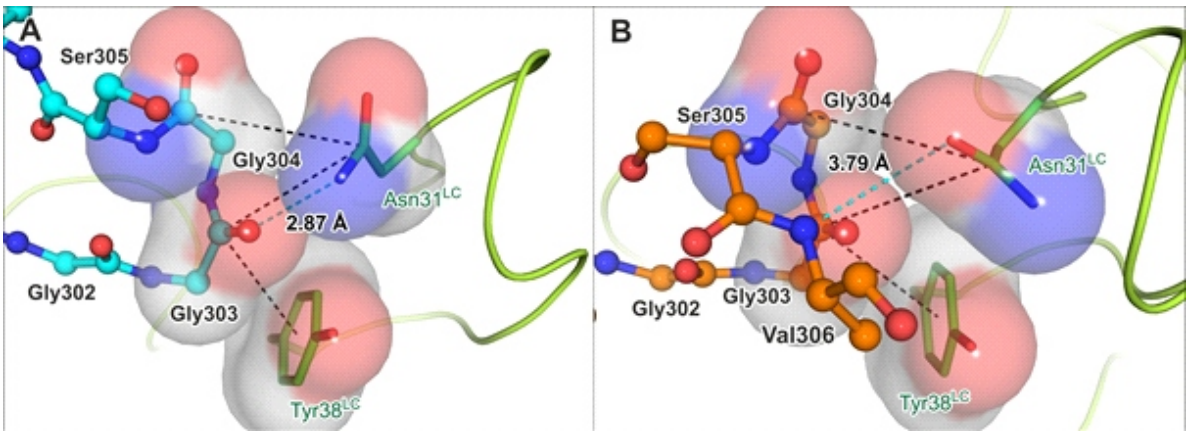

Figure 1. Different copies of antibody-tau complex in asymmetric unit exhibit contrasting conformations (PDB ID 5MP3). (A) Tau chain C (cyan) in the Fab-p5 crystal structure. (B) Tau chain D (orange) in the Fab-p5 crystal structure. Hydrogen bonds are indicated by cyan dashes with the length in angstroms, -interactions are in black dashes and -electron interacting systems are visualized as space-filling models.

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APPROXIMATING COLLECTIVE VARIABLES USING ARTIFICIAL NEURAL NETWORKS

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Artificial neural networks (ANN) are one of the main tools used in machine learning. ANN have been used in variety of fields, including computer vision, speech recognition, medical diagnosis, playing board games and also computational chemistry. Here we present a new ANN for deriving complex collective variables (CVs) just from atomistic coordinates. This ANN takes a molecular trajectory (a real or an artificial one) and a set of CVs, which are (by their nature) difficult to calculate from atomistic coordinates. The output is a series of mathematical transformations starting with positions of atoms and ending with the value of this complex CV. Everything is produced in the form of Plumed syntax and can be directly used for metadynamics simulations using popular Plumed package. Solvent accessible surface area, distances calculated by Dijkstra's algorithm and Rosetta score were tested as examples of complex CVs. The correlation between the values of original CVs and values encoded by ANN was above 0.90, in case of Rosetta score, and above 0.99, in case of two other CVs. Our new ANN is freely available in the form of Python code on GitHub (https://github.com/spiwokv/anncolvar).
In future, we would like to investigate the performance of the above mentioned collective variables.

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STRUCTURE OF *TRIBOLIUM CASTANEUM* HEXAMERIN SOLVED BY CRYO-ELECTRON MICROSCOPY

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Hexamerins are the most abundant proteins in larval stages of *Holometabola* insects. In the pupae haemolymph hexamerins constitute up to 50 % of all proteins [1]. Hexamerins evolved from crustacean hemocyanins but lost the ability to bind copper ions and to transport oxygen [2]; instead, they serve as amino acid source during the non-feeding period, when pupae undergo the development toward the adult stage. There is also evidence that hexamerins are able to bind small organic compounds and hormones such as juvenile hormone, which is involved in cuticule formation and humoral immune defence [1]. *Tribolium castaneum* known as a red flour beetle is a worldwide pest which causes damages of cereals, legumes and dried stored products. It has evolved resistance against a variety of insecticides [3].

Here we present the structure of native *Tribolium castaneum* hexamerin solved by cryo-electron microscopy. Hexamerin was purified from *T. castaneum* pupae and vitrified on holey carbon coated copper grids. Data were collected on FEI Titan Krios microscope operated at 300 kV. The final data analysis led to an electrostatic potential map with the resolution of 3.9 Å. Molecular structure was built into this map. Structure of hexamerin provides a basis for further studies of *Tribolium* life cycle.

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