Posters

P1

Eu^{3+}-INDUCED DNA CONDENSATION AND CHIRALITY TRANSFER

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Eu^{3+}-induced DNA condensation has been recently studied with several chiral optical spectroscopic techniques and microscopy imaging. Circularly polarized luminescence (CPL) of Eu^{3+} ions bound to DNA was registered through Raman optical activity (ROA) experiment (Figure 1). CPL/ROA activity was observed in both short-chain and long-chain DNA. Binding of Eu^{3+} to the long-chain DNA induced DNA condensation, as was shown by electronic circular dichroism (ECD). The DNA condensation was further visualized by microscopic images employing emitted circularly polarized light (Figure 1).

Support from the Grant Agency of the Czech Republic (grants 16-08764Y (TW), 15-09072S (PB) and 16-04902S (VA)) is gratefully acknowledged..

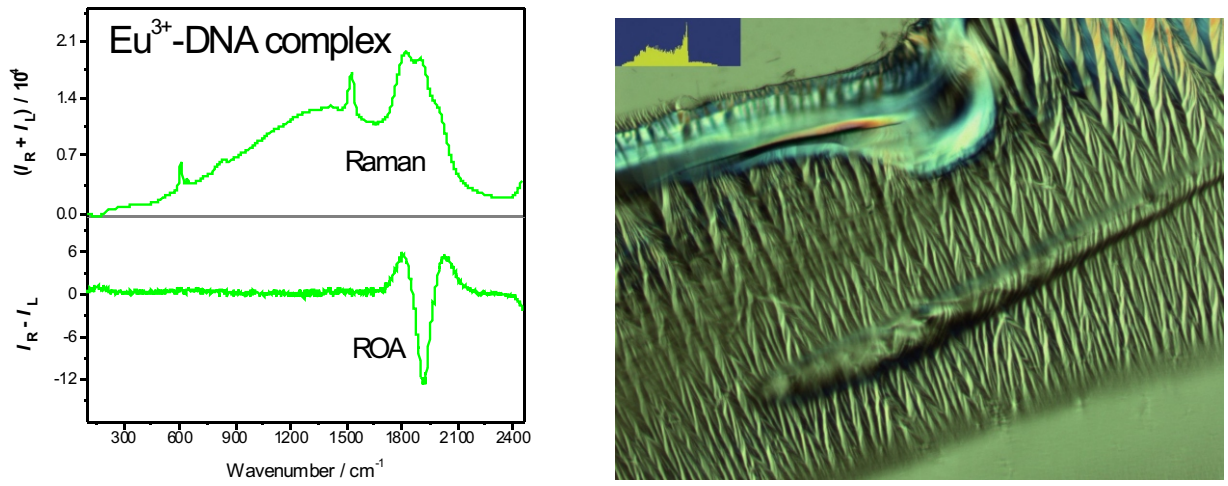

Figure 1. Raman and ROA spectra of Eu^{3+}-DNA complex (left) and circularly polarized microscopic image of DNA condensed by Eu^{3+} ions (right).

P2

TIME-RESOLVED CRYSTALLOGRAPHY OF PROTEINS AT THE LASER DRIVEN X-RAY SOURCES IN ELI BEAMLINES

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The developments of novel X-ray sources will allow for time resolved X-ray scattering and diffraction on a femtosecond to millisecond time scale. The utilization of femtosecond laser pulses for the generation of X-rays from plasma opens new opportunities in the structural studies of fast kinetical processes in proteins. This new technology is different from the principles of operation employed at free electron lasers and synchrotrons. The ELI beamlines facility that is planned to start operation by the end of 2016 in Dolni Brezany, Czech Republic will give a unique advantage for time resolved crystallography. The generated pulses will span approx. 100 fs with a repetition rate of 1 kHz. The scattered and diffracted by the protein crystal X-rays will be counted using a Dectris Eiger 1M area detector which operates at the same frame rate as the source, i.e. 1 kHz. Such setup can be combined with several pump probe lasers to study the fast kinetics for example in photoproteins. For obtaining a crystallographic image several pulses will be necessary due to the low flux, nevertheless, serial femtosecond crystallography is becoming feasible.

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P3

HUMAN HISTONE DEACETYLASE 6: PROFILING OF DEACYLASE SPECIFICITY

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In addition to acetylation, several acyl modifications at the lysine side chain were identified *in vivo* including formylation, propionylation, butyrylation, crotonylation and succinylation. Some sirtuin isoforms were reported to preferentially remove "non-acetyl" functional groups, but there are limited data on the acyl specificity of zinc-dependent histone deacetylases. We systematically mapped the acyl specificity of human histone deacetylase 6 (HDAC6) using a panel of carbamoyl phosphate synthetase 1 derived peptides featuring more than 20 acyl modifications. Our data revealed for the first time that in addition to deacetylase, HDAC6 harbors deformylase and depropionylase activities *in vitro*. Ensuing structure-function and modeling studied provided further insight into lysine deacylation by HDAC6.

P4

NOVEL INHIBITION SCAFFOLDS TARGETING HUMAN CYSTEINE CATHEPSINS

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This work is focused on novel structural motives for inhibition of two model human cysteine proteases, namely cathepsin K and cathepsin V. Cathepsin V is overexpressed in breast and colorectal adenomas and adenocarcinomas and is a potential oncology target while cathepsin K is expressed in osteoclasts and has been evaluated as a target for osteoporosis. We demonstrated that cathepsin V is effectively inhibited by PCPI3 (potato cysteine protease inhibitor), a 20 kDa protein isolated from potato tubers which belongs to the plant Kunitz-type inhibitor family. Also, we identified synthetic peptidomimetic compounds containing a nitrile reactive warhead as potent inhibitors of cathepsin K. Both cathepsins and their active-site mutants were produced in the form of recombinant proteins in the *P. pastoris* expression system and purified. Inhibitor-cathepsin complexes were prepared and subjected to high-throughput crystallization screening. A preliminary crystallographic model of the complex of PCPI3-cathepsin V is presented.

P5

ATLAS OF PROTEIN HYDRATION – CONNECTING INFORMATION FROM CRYSTALLOGRAPHY AND *AB INITIO* CALCULATIONS

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Proteins function in an aqueous environment and are evolutionarily adapted to it. Water molecules represent an integral part of protein molecules and a key determinant of protein structure, dynamics and function. Protein-water interactions govern various processes, including protein folding, enzymatic catalysis, and molecular recognition. Water does not simply fill up the available space around proteins, but occupies specific sites and forms localized clusters, determined by its hydrogen-bonding capabilities. Here we present the newly developed "Atlas of Protein Hydration" [1]. The atlas provides statistical overview of the first hydration shell of amino acid residues in different conformational states in X-ray crystal structures, and also visualization of water distribution and the location of the preferred hydration sites using JSmol applet. The positions and probabilities of the hydration sites were derived from the analysis of of a non-redundant set of 2818 high resolution protein crystal structures from the Protein Data Bank, as described in ref. [2]. Besides the crystal-based data, the atlas also contains information derived from *ab initio* quantum mechanics calculations. For each hydration site the position was optimized and interaction energy was calculated at the level of DFT-D (RI-TPSS/TZVP augmented with empirical dispersion term) with the solvation effects described using COSMO. These calculations show that the majority of hydration sites are located in local energy minima. The calculated interaction energies help to assess the preference of water for the individual hydration sites.

The atlas of conformation-specific hydration of amino acid residues in proteins could find applications in the development of more precise water placement algorithms in structural bioinformatics areas such as crystallographic refinement, protein structure prediction, computational drug design and protein-protein docking. The Atlas of Protein Hydration website is available at www.dnatco.org/atlas.

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P6

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.

P7

TOWARDS STRUCTURE OF HelD – A PARTNER PROTEIN OF RNAP IN GRAM POSITIVE BACTERIA

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The multisubunit protein complex - bacterial RNA polymerase - employs a protein partner called HelD. We have characterised this protein from *Bacillus subtilis* (1, 2). HelD stimulates transcription in an ATP-dependent manner by enhancing transcriptional cycling and elongation. Its effect can be amplified by subunit delta. Based on sequence homology HelD belongs to the superfamily of DNA and RNA helicases, possesses an ATP binding cassette but its structure and molecular detail of function cannot be easily inferred from the knowledge about the model helicases UvrD and Rep from *E. coli.* HelD interacts with the RNAP core between the secondary channel of RNAP and the alpha subunits and is highly likely capable of DNA/RNA binding. We have extensively studied the role of HelD in transcription initiation and elongation, its biophysical properties, sequence and domain structure and co-stimulatory effects (2).

In our effort to gain insights into the structure-function relationship of this RNAp partner we have recently cloned, expresed and purified several homologs from other organisms – *Bacillus atrophaeus, Geobacillus stearothermophillus and Mycobacterium smegmatis.* Biophysical characterisation and initial SAXS experiments help optimize these targets for further structural studies.

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P8

PROLYL OLIGOPEPTIDASE FROM THE BLOOD FLUKE *SCHISTOSOMA MANSONI*: DESIGNING ACTIVE-SITE INHIBITORS WITH ANTI-SCHISTOSOMAL ACTIVITY

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Blood flukes of the genus *Schistosoma* cause schistosomiasis, a parasitic disease that infects over 240 million people worldwide, and there is a need to identify new targets for chemotherapeutic interventions. *Schistosoma mansoni* prolyl oligopeptidase (SmPOP) from the serine peptidase family S9 has not been investigated in detail, so far. We demonstrated that SmPOP is expressed in adult worms and schistosomula in an enzymatically active form. By immunofluorescence microscopy, SmPOP is localized in the tegument and parenchyma of both developmental stages. SmPOP was produced in the bacterial expression system and its active site specificity was investigated using synthetic substrate libraries and a SmPOP homology model. SmPOP is a true oligopeptidase that hydrolyzes peptide (but not protein) substrates with a strict specificity for Pro at P1. Both the recombinant enzyme and live worms cleave host vasoregulatory, proline-containing hormones such as angiotensin and bradykinin. Finally, we designed nanomolar inhibitors of SmPOP that are effective against schistosomes. Our results suggest that SmPOP plays a role in host-parasite interactions and is a potential target for the development of anti-schistosomal drugs.

P9

NOVEL STRUCTURE OF MEMBRANE NANODISC

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Transmembrane proteins are essential part of numerous cell processes, however, the purification and structure determination of transmembrane proteins remains challenging and typically requires detergents that partially substitute membrane environment. Recently, a membrane nanodisc has been developed to over come these difficulties and provide a natural lipid environment. This attractive biotechnological tool consists of a natural bilayer patch stabilized by a membrane scaffold proteins (MSP) such as modified version of human apolipoprotein [1]. Despite the nanodisc's success in isolation, purification, crystallographic and NMR studies of transmembrane proteins, the main limitation of its use is size determined by fixed size of MSP that cannot be altered easily and thus reducing the potential utilization of membrane nanodiscs.

We present a dozen of designed sequences for amphiphilic helices that can be used as a repeating unit for MSPs whose length could be easily altered by addition of removal of the helices and thereby modify the diameter of nanodisc. Our coarse-grained simulations demonstrated that the designed helices posses necessary perpendicular orientation on the membrane edge and thus are likely to form a novel model on membrane nanodisc with so called protein fence structure. In this structure, helices tend to orient perpendicularly to the membrane and do not remain parallel to the membrane as predicted for double-belt structure [2].

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P10

CRYSTAL STRUCTURE OF HONEY BEE HEXAMERIN 70b AT 3.2 Å RESOLUTION

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Hexamerins are proteins related to hemocyanins but they lack the ability to bind copper ions and transport oxygen. Hexamerin is the most abundant protein in honey bee (Apis mellifera) larvae and pupae. It is produced by larval fat body and serves mainly as a storage of amino acids but there is an evidence that it may act also as a juvenile hormone-binding protein. There are four types of hexamerin genes in honey bees, termed hex70a, hex70b, hex70c and hex110, which differ in the structure and expression patterns. The Hex70b is a methionine-rich hexamerin and it may serve as a sulphur reserve for development toward the adult stage [1]. Here we present a crystal structure of honey bee Hex70b. Hex70b was purified from honey bee pupae and crystallized. The three-dimensional structure has been solved to 3.2 Å resolution using molecular replacement. The structure provides a basis for further studies of honey bee life cycle where participation of hexamerin is essential.

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P11

NUCLEOSIDE N-RIBOHYDROLASES AND ADENOSINE KINASES IN MAIZE (*ZEA MAYS*)

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Nucleosides and nucleobases can be recycled to nucleoside monophosphates, which is also known as salvage pathway preserving an energy, which would otherwise be needed for *de novo* synthesis of purine and pyrimidine derivatives. In plants, both uridine kinase (UK, E.C. 2.7.1.48) and uracil phosphoribosyl transferase act on the pyrimidine salvage pathway. In purine salvage, an important role has been shown for adenosine kinase (AK, E.C. 2.7.1.20) and adenine phosphoribosyltransferase. Purine and pyrimidine nucleosides are hydrolyzed by nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-) to corresponding nitrogenous bases and ribose. There are two NRH subclasses in the plant kingdom; one preferentially targets the purine ribosides inosine and xanthosine while the other is more active towards uridine and xanthosine.

In this work, we focused on purine metabolism and interconversion of plant hormones cytokinins, which are *N*6-substituted adenine/adenosine derivatives. We analyzed spatial and temporal expression of all five *NRH* and three *ADK* genes present in maize (*Zea mays*). Transcripts of *ADK2* were the most abundant of all three ADKs in all organs and developmental stages. Transcripts of *NRH1a*, *NRH2a* and *NRH3* genes are the most abundant in leaves while roots contain mainly *NRH1b* and *NRH3* transcripts. Protein sequences of ADKs comprise ~ 340 amino acids and are monomeric. Those of NRHs encompass ~ 320 amino acids and enzymes are dimeric. NRHs impose a strict specificity for the ribose moiety. In contrast, the residues interacting with nucleobase highly vary. We combined a site-directed mutagenesis approach with kinetic and structural analyses to study nucleoside binding sites in two NRHs, namely NRH2b and NRH3. The crystal structures of both NRHs were solved at 1.75 and 2.51Å resolution, respectively. Five NRH2b variants and seven NRH3 variants were studied in detail. Replacement of two active-site histidine residues by alanine led to two-fold lower specific activities. Replacement of a lysine residue, which protrudes into the active site from the second subunit and interacts with nucleobase, reduced the specific activity but increased hydrolysis of cytokinin ribosides isopentenyl adenosine (iPR) and *trans*-zeatin riboside (ZR). Replacement of the active-site tryptophan residue in NRH3 also increased hydrolysis of cytokinin ribosides.

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P12

DYNAMICS OF ClC-ec1 D417C MUTANT FORM STUDIED BY HYDROGEN/DEUTERIUM EXCHANGE

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Chloride channels belong to an extensive family of transmembrane proteins, whose dysfunction causes a wide range of illnesses [1]. A detailed study of the structural changes during transport enables us to understand the transport mechanism and can provide valuable information required for effective treatment.

Bacterial chloride channel from E. coli (ClC-ec1), whose structure has been solved by X-ray crystallography, is widely used as a model protein for studies of the transport mechanism. ClC-ec1 is a homodimer and each of its subunits consist of two topologically related domains in an antiparallel architecture arrangement [2]. This so called ‘’inverted topology repeats’’ propose existence of an ‘outward-facing’ (OF) and an ‘inward-facing’ (IF) shapes. However the crystal structure traps the protein in an occluded state.

In a recent work [3], Khantwal, Abraham *et al.* have discovered new previously uncharacterized ‘outward-facing open’ state using cross-linking of Y419C and D417C mutants studied by NMR and DEER techniques. Our aim is to obtain more information about this new state by use of hydrogen/deuterium exchange that enables us to monitor dynamic conformational changes under different conditions (pH, various concentrations of chloride anions) directly in solution.

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P13

INHIBITORY BINDERS DERIVED FROM ABD-DOMAIN SCAFFOLD TARGETING HUMAN IL-17RA RECEPTOR AS AN ALTERNATIVE FOR MODULATION OF Th17-MEDIATED PRO-INFLAMMATORY AXIS

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Interleukin 17 (IL-17) and its cognate receptor (IL-17RA) [1] play a crucial role in Th17 cells-mediated pro-inflammatory pathway and pathogenesis of several autoimmune disorders including psoriasis. Psoriasis is a chronic inflammatory skin disease with prevalence up to 3% worldwide it is characterized by hyperplasia of the epidermis, infiltration of leukocytes into both dermis and epidermis, and dilation and growth of blood vessels. IL-17 is mainly produced by Th-17 helper cells and, via binding to its receptor, mediates IL-17-driven cell signaling in keratinocytes [2].

This work was aimed to generate a novel protein binders of IL-17RA that will prevent from binding of IL-17 to this receptor expressed on the surface of keratinocytes. To this goal, we used a high-complex combinatorial library derived from a scaffold of albumin-binding domain (ABD) of streptococcal protein G [3], and ribosome display selection, to yield a collection of ABD-derived high-affinity ligands of human IL-17RA, called ARS binders. From 67 analysed ABD variants, 7 different sequence families were identified. Representatives of these groups competed with human IL-17A for binding to recombinant IL-17RA receptor as well as with IL-17RA-IgG chimera, as tested in ELISA. Five ARS variants bind to IL-17RA-expressing THP-1 and Raji cells, as tested by flow cytometry, and four variants exhibited high-affinity binding in nanomolar range to human keratinocyte HaCAT cells, as measured using LigandTracer Green Line system. Thus, we identified several ARS inhibitory variants with a blocking potential that will be further tested for their immunomodulatory function.

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P14

STRUCTURAL STUDY OF THE INTRACELLULAR DOMAINS OF THE ETHYLENE RECEPTOR ETR1 FROM *ARABIDOPSIS THALIANA*

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Ethylene acts as a gaseous hormone that controls numerous aspects of plant growth and development. In *Arabidopsis*, the ethylene signaling cascade is initiated upon ethylene binding to the ethylene receptors ETR1, ERS1, ETR2, ERS2 and EIN4, localized at the endoplasmic reticulum, and proceeds via the pathway containing the Raf-like serin/threonine (S/T) kinase CTR1 acting upstream of EIN2 and EIN3. However, ETR1 receptor possesses all sequence motifs of canonical histidine kinase (HK) domains including HK activity, necessary for the signal transduction via parallel multistep phosphorely (MSP) pathway, mediating a wide spectrum of responses including plant hormones cytokinins. Accumulating evidence suggests a role of ETR1 in integrating ethylene recognition with MSP mediated signaling.

The main objective of our work is the structural determination and understanding of ETR1 features which will help us to elucidate the structural aspects and HK activity of ETR1 in the ethylene/MSP cross-talk. We prepared ^{15}N-labelled protein samples of ETR1 HK and receiver domain (RD) at high concentrations necessary for nuclear magnetic resonance (NMR) spectroscopy. Titration trials with divalent ions (Mg^{2+}, Mn^{2+}) were performed in order to determine whether the ions affect the ETR1RD structure. Minor changes were observed in the presence of Mg^{2+} in several residues, but in region distant from the phosphorylation site.

Furthermore, we investigated possible interactions between the receiver and HK domains. Our preliminary results from NMR measurements indicate complex formation and undergoing structural changes on RD including its phosphorylation site. Because of the large complex size of approximate 75 kDa, expression and purification must be optimized for structural studies applicable to large proteins (segmental labelling, deuteration).

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P15

STRUCTURE AND DYNAMICS OF THE MEMBRANE-ANCHORED CYTOCHROME P450 1A2-CYTOCHROME B5 COMPLEX

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Eukaryotic cytochromes P450 (P450) are membrane bound enzymes oxidizing a broad spectrum of hydrophobic substrates including xenobiotics. Protein-protein interactions play a critical role in this process. In particular, the formation of transient complexes of P450 with another protein of the endoplasmic reticulum membrane, cytochrome *b5* (cyt *b5*), dictates catalytic activities of several P450s. To lay structural foundation for the investigation of these effects we used microsecond all-atom molecular dynamics (MD) simulations to identify three stable binding modes (sAI, sAII, and sB) between soluble domains of human P450 1A2 and cyt *b5* in aqueous solution, and examined these binding modes in the full-length complex (mAI, mAII, and mB) using multi-scale modeling techniques. The membrane-spanning transmembrane domains of both proteins were spontaneously assembled from the protein/phospholipid/water mixture using coarse-grained MARTINI force field, and the full-length complexes representing all three binding modes in the solvated dilauroylphosphatidylcholine bilayer were simulated using

all-atom MD. The X-shaped contact between antiparallel transmembrane domains of P450 1A2 and cyt b5 that was established during the coarse-grained MD was preserved in the all-atom MD. In the membrane environment, modes mAI and mB were retained in essentially unchanged form, whereas the mAII mode was excluded as topologically impossible. The mutual position of domains in binding mode mAI is analogous to the most favorable structure of the P450 1A2 - cyt b5 complex identified previously for soluble domains. Featuring the largest contact area, the smallest structural flexibility, the shortest electron transfer distance and the largest number of inter-protein salt bridges, the mAI mode is the best candidate for the catalytically relevant full-length complex.

P16

PREPARATION OF HUMAN NK CELL ACTIVATION RECEPTOR NKp80 AND ITS LIGAND AICL

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Natural killer cells (NK cells) play a key role in innate immunity. Their function is to recognize and kill infected, stressed or malignantly transformed cells. A range of surface receptors promotes this recognition. Cytotoxic mechanisms lead to induction of apoptosis in the target cell [1]. Activating and inhibitory NK cell receptors (NCRs) can be subdivided into immunoglobulin-like family and C-type lectin-like family. NCR NKp80 and its myeloid-specific activating ligand AICL are both C-type lectin-like receptors (CTLRs) with C-type lectin-like domain (CTLD) [2]. Immunocomplex NKp80-AICL promotes lysis of malignant myeloid cells, mediates crosstalk between NK cells and monocytes, is engaged in cytokine release and contributes to initiation of immune response during inflammation [3].

AICL ectodomain contains odd cysteine which is believed to be responsible for formation of dimers on the cell surface. However, this cysteine in position 87 is not conserved, compared to other CTLD receptors. AICL ectodomain was expressed in *E. coli* BL21 Gold (DE3) strain as inclusion bodies and we have found out that upon mutation of this cysteine to serine (C87S mutation) the yield of subsequent *in vitro* refolding and purification as well as the stability of prepared protein are greatly enhanced compared to wild-type construct.

We used mammalian expression system of modified human embryonic kidney cell line 293 (HEK293) to produce glycosylated NKp80 ectodomain. Unfortunately, transient transfection was not successful. Using pOPINTTGneo plasmid as well as PiggyBac cloning system we were able to create stable cell lines expressing soluble extracellular parts of NKp80 in constitutive or inducible way. Thanks to these approaches, we are able to produce both proteins in sufficient amount to initiate structural studies using analytical ultracentrifuge, dynamic light scattering and finally crystallization of this immunocomplex.

This study was supported by Czech Science Foundation (15-15181S), Charles University (UNCE 204025/2012, SVV 260079/2014), BioStruct-X and Instruct European infrastructure projects.

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P17

ARTIFICIAL PROTEINS MODULATE ALLOSTERY OF PDZ3 AND SH3 IN TWO DOMAINS CONSTRUCTS. A COMPUTATIONAL CHARACTERIZATION OF NOVEL CHIMERIC PROTEINS

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Artificial multi-domain proteins with increased structural and functional properties can be utilized in a broad spectrum of applications. The design of chimeric fusion proteins utilizing protein domains or one-domain miniproteins as building blocks is an important advancement for the creation of new biomolecules for biotechnology and medical applications. However, we critically miss computational studies to describe in details dynamics and geometry properties of two domain constructs made from structurally and functionally different proteins. In this article we attempt to test our *in silico* design strategy using all-atom explicit solvent molecular dynamics simulations. The well characterized PDZ3 and SH3 domains of human zonula occludens (ZO-1) (3TSZ) along with other 5 artificial domains and two kind of molecular linkers were selected to construct chimeric two domains molecules. The influence of the artificial domains on the structure and dynamics of the PDZ3 and SH3 were determined using a range of analysis. This approach allowed us to describe the artificial domains as allostery modulators of the PDZ3 and SH3 domains.

P18

HOW Min SYSTEMS FROM *CLOSTRIDIA* SPECIES HELPS TO FIND THE MID-CELL SITE DURING CELL DIVISION?

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Bacterial cell division begins with polymerization of FtsZ protein and formation of Z-ring, which marks the future site of the septum at the mid-cell. Z-ring serves as a scaffold for division proteins, and its proper placement is crucial for subsequent steps in cell division. In rod-shaped bacteria, one of the mechanisms responsible for correct placement of the division septum is the Min system. The action of Min system creates a concentration gradient of division inhibitor MinC, which is highest at the cell poles and lowest at the midcell. The underlying mechanism fundamentally differs between *Escherichia coli* and *Bacillus subtilis* though, the core components of the Min system, MinC and MinD, are evolutionary conserved. MinC, the direct inhibitor of FtsZ polymerization, is attracted to the membrane via MinD, whose localisation is in turn dependent on another protein/s called topological determinant. While in *E. coli* MinD protein undergoes rapid pole-to-pole oscillation driven by MinE protein, in *B. subtilis* MinD is anchored to the cell poles via MinJ/ DivIVA complex.

In our previous study, we have shown that oscillating Min system of *E. coli* can be transplanted into *B. subtilis*. Interestingly, strains in which oscillation was observed were impaired in sporulation at the step of asymmetric septum formation [1]. The finding that oscillating Min system is not compatible with sporulation might partially explain why two Min systems with such distinct mechanism evolved for two different life cycles, vegetative growth and sporulation. Genome-wide search for Min system homologues in selected Gram-positive endospore-forming bacteria revealed that they harbour various combinations of homologues from both Min systems.

Two of the species carrying a combination of Min proteins from both systems *Clostridium difficile* (MinCDE, DivIVA) and *Clostridium beijerinckii* (MinCDE, MinJ/ DivIVA) have been chosen for our further studies. Here we show the oscillatory behaviour of MinDE proteins of the two clostridial species in *B. subtilis* cells and investigate protein-protein interactions between Min proteins of these organisms [2]. We evaluated the effects of expression of these proteins on sporulation of *B. subtilis*.

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P19

STRUCTURAL DETAILS OF THE INTERACTION BETWEEN pTyr1-CTD AND THE ELONGATION FACTOR Spt6

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Post-transcriptional modifications of the consensus motif Y1-S2-P3-T4-S5-P6-S7 of the C-terminal domain (CTD) of RNA polymerase II (RNAPII) are since last decade known as "CTD code" [1]. These modifications are dynamic and specific for each phase of the transcriptional cycle [2]. However, how does the CTD code control the recruitment, (de)activation, and displacement of relevant processing factors, remains still poorly understood. Increased phosphorylation level of Y1 of the CTD during the early elongation inhibits binding of termination factors, yet it stimulates binding of the elongation factor Spt6 through its tandem SH2 (tSH2) domain [3]. The limited structure information for the recognition of phospho-tyrosine modified CTD that is currently available prevents deciphering of how this important CTD modification mark is read out by transcription factors [4, 5]. We set out to investigate this recognition phenomenon using integrative approach to structural biology. Here, we will show our combined structural data from NMR, X-ray, and SAXS along with binding and biochemical assays on the study of recognition Y1-phosphorylated CTD by the tSH2 domain of the elongation factor Spt6.

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P20

TRACKING OF PROTEIN FOLDING BY CHIRAL SPECTROSCOPIC METHODS

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The fibrous protein aggregates, so-called -amyloids, are implicated in a variety of neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's disease or cerebral â-amyloid angiopathy. Study of protein folding and misfolding is therefore crucial for further diagnostics and treatment of these diseases. Traditional techniques for protein structure determination such as X-ray or NMR are often not suitable to quickly track changes in 3D protein structures in solutions. Moreover, limited solubility and molecular size of -amyloids represent another problem.

In the presented work, chiral spectroscopic methods based on Vibrational Optical Activity (VOA), were used to study fibrous protein aggregates formed from model proteins *polyglutamic acid* (PGA) and *hen-egg lysozyme* (HEL). Infrared absorption and vibrational circular dichroism (VCD) proved to be very valuable in detection of such extended structures. The amide I band provides information on the peptide secondary structure, while enhanced VCD signal and its sign indicate a longer range arrangement. On the other hand, Raman optical activity (ROA) was not sensitive enough to study -amyloid structures alone. Therefore $EuCl_3$ and water soluble europium complexes were used as probes of molecular chirality in ROA measurements. It was shown that -amyloids can interact with solvated Eu^{3+} ions or their complexes inducing a strong ROA signal due to chirality transfer.

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P21

STRUCTURE AND DYNAMICS OF THE METHYL-TRANSEFERASE SUBUNIT OF THE *E. COLI* RESTRICTION-MODIFICATION SYSTEM EcoR124I

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EcoR124I belongs to the IC family of type I restriction-modification (R–M) enzymes of *E. coli*. Type I R–M enzymes are pentameric proteins comprised of 2 motor subunits (HsdR), and a trimeric methyltransferase (MTase) that includes the specificity subunit. On hemimethylated dsDNA, the enzyme complex acts solely as conventional adenine methylases. On unmethylated DNA translocation occurs independently on the motor subunits that translocate dsDNA towards the stationary enzyme. Recombination between Target Recognition Domains (TRDs) generates new sequence specificities and is a powerful driver of Type I R–M system diversification. Due to the lack of structural information, the overall atomistic mechanism of DNA recognition and modification is unknown. Based on early genetic studies a paradigm was established that mutations within the *hsdS* gene produce an r- m- phenotype, as do mutations within the *hsdM* gene while mutations in the *hsdR* caused Mod+ Res- (1). Res- Mod- phenotype was presumed to reflect a loss of DNA-binding ability of the HsdS subunit responsible for DNA recognition which than must prevent both restriction and modification functions. However, two point mutants (K184N) (2) and (K384N) that are mutated in the conserved helical domain of the HsdS subunit were found to have an r- m+ phenotype in vivo (including the double substitution), which could only be a result of subunits assembly defect. We generate and refine three-dimensional homology model(s) of the EcoR124I HsdS subunit and study of dynamics of WT and mutant HsdS to predict the overall affect of the experimental mutations on the dynamical behaviour and the conformational space sampled.

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P22

TOWARDS STRUCTURAL CHARACTERIZATION OF SmSP2 PROTEASE FROM PARASITIC BLOOD FLUKE *SCHISTOSOMA MANSONI*

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Schistosomiasis is a parasitic disease caused by several blood flukes of the genus Schistosoma. It is considered the second most important parasitic infection after malaria with more than 200 million people infected worldwide and many more at risk. Treatment relies on a single drug – praziquantel. Hence, there is a pressing need to develop additional therapeutics. Proteolytic system of schistosomes is a promising target for the development of new antischistosomal drugs. In our research we focused on newly discovered trypsin-like protease SmSP2 from *S. mansoni*, predominant serine protease in schistosmula and adults. Recombinant SmSP2 was expressed in the *P. pastoris* expression system and purified using chromatographic methods. Polyclonal antibodies against SmSP2 were produced and used for immunolocalization experiments. Substrate cleavage specificity and inhibitor sensitivity were identified and explained using 3D homology model of SmSP2. We demonstrated that SmSP2 is capable of activate plasminogen to plasmin and release bradykinin from kininogen, therefore it could serve schistosomes as anticoagulant and vasodilatation agent, respectively. We prepared sufficient amount of recombinant SmSP2 for high-throughput crystallization screening, which is currently in progress. 3D structure model of SmSP2 will be employed for rational designing of specific inhibitors as potential drugs against schistosomiasis.

P23

THE KINETICS OF PHOSPORYLATION OF TYROSINE HYDROXYLASE AND ITS INTERACTION WITH 14-3-3 ZETA PROTEIN STUDIED BY NMR

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

We also measured kinetic rates of phosphorylation of RD-hTH1 by PKA and PRAK kinase to gain better understanding of phosphorylation process. Kinetic series were derived from intensity changes of peaks of selected residues close to phosphorylation site. To reveal interactions between RD-hTH1 and protein 14-3-3 zeta we performed titration of doubly phosphorylated RDhTH1 with highly concentrated 14-3-3. Changes in the peak intensity indicated the regions mostly involved in the interaction.

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P24

ELECTRONIC SCULPTING OF AT2R LIGANDS BY 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/p 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 150 - 200 ns metadynamics simulations were predicted as: 25 ± 18 % (exp. 40 %) for tyrosine, 32 ± 8 % (exp. 25 %) for O-phosphotyrosine, 18 ± 6 % (exp. 20 %) for phenylalanine and 9 ± 6 % (exp. 5 %) for 4-nitrophenylalanine, respectively (mean ± s.d.) as Xxx. The accuracy of these result are currently being improved by prolongation of simulations.

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P25

COMPUTATIONAL STUDY OF SPECIFIC ION EFFECT ON THE GRAPHENE OXIDE SURFACE

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Graphene oxide (GO), which is a monolayer of graphene with various oxidized groups, has wide application in materials technology, biomedicine, catalysis, as well as a surfactant. Due to it's amphiphilic nature GO can be dispersed in both water and organic solvents. This makes it a suitable carrier for isolation of hazardous chemicals such as dyes from industial waste water. The efficiency of graphene oxide as a carrier depends on pH [1] as well as the presence of different salts in solution [2].

MD simulations enable to understand the pH dependece of the interactions of different ions with GO surface on molecular level. The influence of ions to the surface charge of GO has been quantified by radial distribution function, which calculates the distribution of ions around a single molecule of GO. The observed interaction modes of ions with the surface of GO support experimental results from zeta potential measurements of graphene oxide in different salt solutions, as well as the efficiency in extraction of methyl blue from aqueous solutions [2].

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P26

EFFECT OF THE RIBOSE *VERSUS* 2'-DEOXYRIBOSE RESIDUE IN GUANOSINE-5'-MONOPHOSPHATES ON THE FORMATION OF G-QUARTETS STABILIZED BY K^+ AND Na^+

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Guanine quadruplexes [1] are widely studied because of their role in various biological processes including gene expression and cell immortalization, and possible nanotechnological applications [2]. G-quadruplexes can be formed under specific physico-chemical conditions from DNA as well as RNA oligo- or polynucleotides containing repetitive sequences of several consecutive guanosine residues. DNA quadruplexes exhibit greater structural polymorphism than RNA, as they can adopt antiparallel, various hybride (3+1) and parallel folds, whereas RNA quadruplexes seem to be restricted simply to parallel form. Basic building blocks of G-quadruplex consist of four guanines interconnected by Hoogsteen hydrogen bonding to form G-quartet, stabilized by coordinated metal cation, especially Na^+ or K^+. G-quartets are stacked one upon the other.

However, G-quartets and their supramolecular self-associates resembling G-quadruplexes can be formed also from monomeric guanosine-phosphates. Although G-quartets and self-associates of 5'-rGMP have been thor-

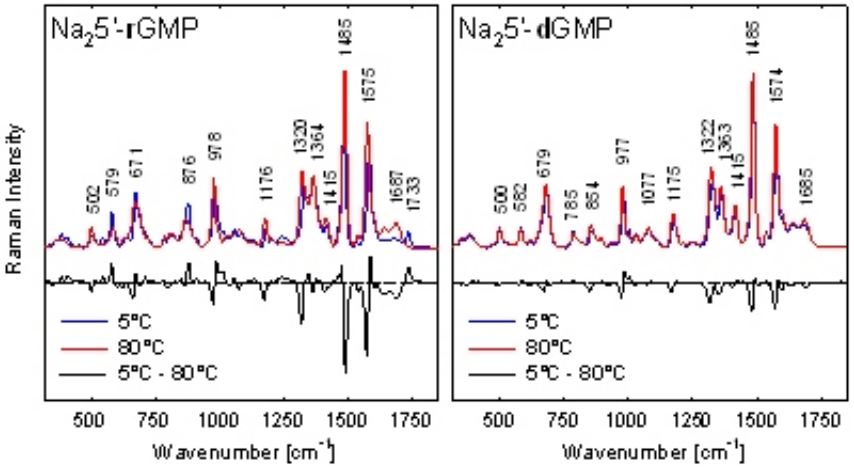

Figure 1. Raman spectra of 800 mM $Na_2$5'-rGMP and $Na_2$5'-dGMP at 5 and 80 °C.

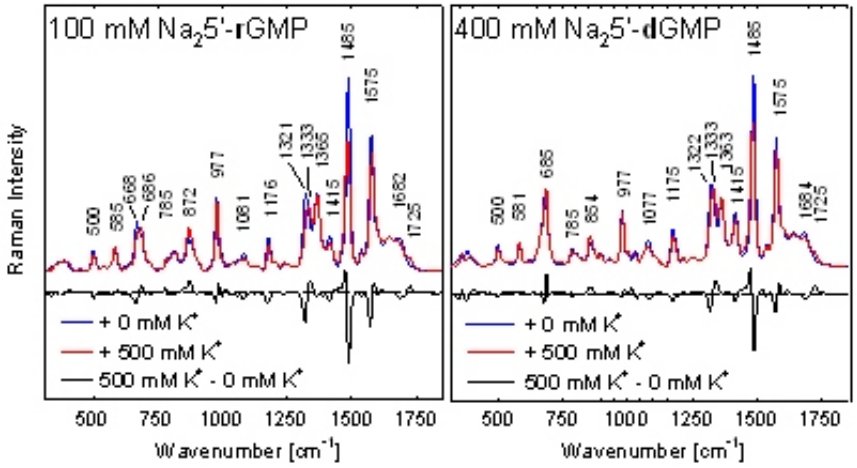

Figure 2. Raman spectra of 100 mM $Na_2$5'-rGMP and 400 mM $Na_2$5'-dGMP in the absence and presence of additional 500 mM K^+. T = 10°C.

oughly studied in the presence of various alkali metals by various methods, notably NMR [3], similar studies of deoxyribonucleotide 5'-dGMP are still rare. In particular a direct comparison of the ability of monomeric 5'-rGMPs and 5'-dGMPs to constitute G-quartets and self-associates stabilized by the most common alkali ions Na^+ and K^+ is missing, although it may contribute for better understanding of the reasons of different polymorphism of DNA and RNA G-quadruplexes in Na^+ and K^+ solutions.

Here we report results of a systematic study of the effect of the ribose *versus* 2'-deoxyribose on the 5'-GMP self-assembling in pH-neutral or slightly basic Na^+ and K^+ solutions by means of Raman and ^{1}H and ^{31}P NMR spectroscopy. Although not yet frequently used in the G-quadruples research, Raman spectroscopy is especially suitable for the task, since it can be conveniently used at high 5'-GMP concentrations favorable to self-aggregation. Moreover, Raman spectra provide well-established spectral markers sensitive to nucleotide conformation, formation of hydrogen bonds and stacking interactions [4, 5]. In the present study, the nucleotide concentration, nature, concentration and stochiometry of alkali cations, and temperature were systematically varied to find that ability of 5'-dGMP to constitute G-quartets and ordered supramolecular structures stabilized by Na^+ is substantially lower than that of 5'-rGMP. As shown on Fig. 1, 5'-dGMP remained in Na^+ solution as monomer even at 800 mM concentration where $Na_2$5'-rGMP clearly form firmly stacked G-quartets. On the other hand, after introduction of K^+ ions both guanosine-monophosphates readily form self-stacking G-quartets, although self-association of 5'-rGMP was still greater than that of 5'-dGMP (Fig. 2). Possible consequences for G-quadruplex polymorphism will be discussed.

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P27

CHARACTERIZATION OF A NOVEL BIDIRECTIONAL NK ACTIVATING LIGAND CD160

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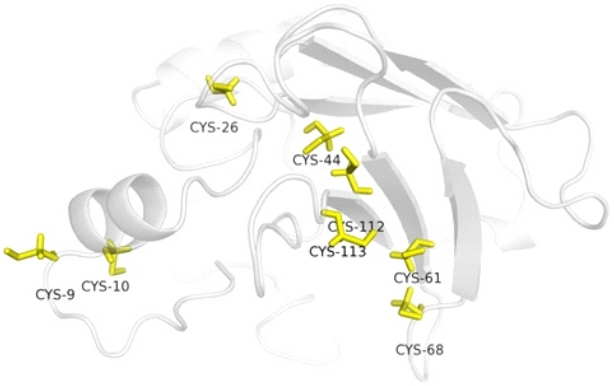
Natural killer (NK) cells require cytokine signals to differentiate into fully functional effector cells that initiate protective antiviral responses [1]. Many pathogens have evolved countermeasures to avoid interferon-mediated detection and clearance by NK cells [1, 2], however, regulatory mechanisms limiting cytokine activation of NK cells that reduce non-specific tissue damage remain poorly defined. CD160 is a 27 kDa glycoprotein which was initially identified with the monoclonal antibody BY55 [2]. Its expression is tightly associated with peripheral blood NK cells and CD8 T lymphocytes with cytolytic effector activity [2,3,4]. The cDNA sequence of CD160 predicts a cysteine-rich, glycosylphosphatidylinositol-(GPI)-anchored protein of 181 amino acids with a single Ig-like domain weakly homologous to KIR2DL4 molecule. It was found that TNF receptor herpesvirus entry mediator (HVEM) preferentially engages CD160 trimer to costimulate activation, while a viral ortholog of HVEM specifically binds to B and T lymphocyte attenuator (BTLA) to suppress this signaling. CD160 antigen is a protein that in humans is encoded by the CD160 gene. We have found that CD160 is expressed at the cell surface as a tightly disulfide-linked multimer. The homology model of CD160 antigen domain [Figure 1] shows cysteine-rich region that was found to be responsible for CD160 tight-timer formation even under reduced conditions. CD160 trimer forms stable complex with HVEM, while monomeric form refused to binds its cognate ligand. Thus, regulation of CD160 bidirectional binding may represent a common mechanism of immune regulation targeted by multiple pathogens, which by extension is a potential target for therapeutic manipulation.



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P28

RECOGNITION OF DIFFERENT METHYLATION STATES ON HISTONE H3 LYSINE 4 BY Set3 PROTEIN

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Nucleosomes form the fundamental repeating units of eukaryotic chromatin, which are used to pack the large eukaryotic genome into the nucleus while still ensuring appropriate access to it. They are thought to carry epigenetically inherited information in the form of covalent post-translational modifications of core histones, such as acetylation, methylation, phosphorylation, sumoylation and ubiquitynation. These modifications are possible due to the N-terminal tails of the histones, which are unstructured and protrude outward from the nucleosome core. Some of those modifications can directly influence chromatin structure or can be bound and 'read' by histone recognition modules found in many proteins and protein complexes acting on chromatin. It is now well known that

many of the histone modifications play a crucial role in regulation of a diverse set of biological processes [1-2]. For example, lysine methylation is coupled to the regulation of transcription by RNA polymerase II (RNApII), X-chromosome inactivation, heterochromatin formation and gene silencing. Methylations at particular histone residues are mostly correlated with either activation or repression of transcription. In our study we are interested in histone H3 modifications, particularly of lysine 4 methylations, which are enriched at actively transcribed gene regions [3-5]. Here we would like to present our structural data and binding assays, which characterize the recognition of the H3K4me2 and H3K4me3 by Set3PHD.

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P29

SPECTROSCOPIC STUDY OF PROTEIN FIBRILS

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Understanding of processes of amyloid fibril formation is one of the key tasks in searching for the role of protein structure changes in human neurodegenerative diseases [1]. Many proteins have been found to form amyloid deposits and it has been shown that this might be a general property of a peptide chain [2]. Despite intensive research, the detailed mechanism of fibril formation is still far from clear. Previously, it was discovered that infrared vibrational circular dichroism (VCD) possesses unusual sensitivity to the growth and development of amyloid fibrils, with hen egg lysozyme and bovine insulin as examples [3]. Various homologs of bovine insulin can serve as a model for studies of amyloid fibril formation.

Here we combine vibrational circular dichroism (VCD), atomic force microscopy (AFM) and deep-UV resonance Raman spectroscopy (DUVRR) in an attempt to understand the structure and stability of insulin fibrils under different conditions. Differences in structure and fibril stability for different species and growth conditions will be presented.

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P30

RELATION OF NKp30 GLYCOSYLATION AND C-TERMINAL CHAIN LENGTH TO ITS STRUCTURE

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Immune system is able to recognize tumor cells and subsequently to eliminate them. Special sort of lymphocytes –natural killer (NK) cells are able to provide this function by causing apoptosis of tumor cells using Fas ligand binding or granzymes. These processes are activated when signals from their inhibitory receptors are decreased and on the other hand, signals from activating receptors are increased. NKp30 is an activation receptor of NK cells with

one Ig-like extracellular domain. Out of its ligands, two of them are stimulating NK cells through NKp30 when bound to membrane: B7-H6, a membrane protein with two Ig-like domains, which is present on the surface of some tumor cells; and BAG-6, a large multifunctional protein normally found in cytoplasm or nucleus which is transported onto the cell surface under stress condition by an unknown way [1].

The structure of the B7-H6 ligand bound to NKp30 produced in *E. coli* has already been solved [2]; however, the structure of the BAG-6 ligand is yet to be elucidated. Moreover, the glycosylation and length of C-terminal chain of NKp30 extracellular domain as well as its oligomerization status influence its ability to bind ligands [3]. The structural basis of these effects is not known.

For our studies the extracellular domains of NKp30 and B7-H6 have been cloned into the pTW5sec vector with C-terminal histidine tag. To study the effect of C-terminal region of extracellular domain of NKp30, shorter and longer constructs have been cloned. Both proteins have been produced in human HEK293S GnTI$^-$ cell line possessing homogeneous N-glycosylation profile, purified by TALON affinity column and size exclusion chromatography. Glycosylation of NKp30 was confirmed by mass spectrometry and formation of its oligomers was observed by analytical ultracentrifugation and transmission electron microscopy. Impact of glycosylation and C-terminal length of NKp30 construct was measuredusing analytical ultracentrifugation. Crystallization screens of the complex with glycosylated NKp30 constructs have been set up, too.

Library of 47 plasmids of BAG-6 was generated by cloning constructs of various lengths into vectors with TriEx plasmid backbone. Production of these constructs is to be screened in *E. coli*, as well as in Sf9 insect and HEK293S GnTI$^-$ human cell lines.

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P31

ROA STUDY OF CHONDROITIN SULPHATE

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Glucoaminoglycans (GAGs) represent a class of linear carbohydrate polymers with essential roles in many biological processes, such as cell signalling and proliferation, angiogenesis and tumorigenesis etc. One of the most common GAGs is chondroitin sulphate, an important structural component of cartilage that provides much of its resistance to compression. Chondroitin sulphate is composed of repeating sulphated disaccharide units, formed by -D-glucuronic acid (GlcA) and 2-acetyl- -D-galactosamine (GalNAc), joined together by (1 › 4) and (1 › 3) glycosidic linkages [1]. Despite its biological importance little is still known about the secondary and tertiary structural properties of chondroitin sulphate and any effects related to the sulfation of its chain, as X-ray crystallography and NMR are difficult to apply to these samples and provide only limited information.

In our experiment, we measured the Raman and Raman optical activity (ROA) spectra of chondroitin A sulphate in a wide frequency range between 250 cm^{-1} and 1800 cm^{-1} and analyzed these with respect to the occurrence of specific spectral marker bands and the influence of sulphation. Furthermore, we have studied changes in the chondroitin structure induced by variation of chemico-physical properties as concentration, pH, temperature and cations countering the charge of the polyanionic chondroitin chain. The technique of ROA [2,3], which is based on a different interaction of a specimen with right- and left-handed circularly polarized laser light, represented an ideal methodology for this type of observation due to its high sensitivity to the conformational stability and rigidity of pyranose rings of the saccharides, orientation of sugar hydroxyl groups and also secondary structure of the GAG's backbone. There is also a strong link to previous experiments [4], which focused on the characterization of hyaluronan, another important GAG.

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P32

STRUCTURE-FUNCTIONAL CHARACTERIZATION OF HALOALKANE DEHALOGENASES

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Haloalkane dehalogenases (EC 3.8.1.5) are bacterial enzymes cleaving a carbon-halogen bond by a hydrolytic mechanism in a broad range of halogenated aliphatic compounds [1]. The enzymes can be potentially applied in bioremediation, biosensing, biosynthesis, cellular imaging and protein immobilization [2]. Structurally haloalkane dehalogenases belong to the -hydrolase superfamily with two domain organization: an -hydrolase core domain and -helical cap domain, which lies on the top of the core domain. Active site residues are located in a hydrophobic cavity at the interface between the two domains and are connected to the protein surface by several tunnels. Nowadays more than 20 proteins and their mutant variants from haloalkane dehalogenases family are systematically studied. The main target is focused on research of proteins such as DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DbeA of *Bradyrhizobium elkanii* USDA94, LinB of *Sphingobium japonicum* UT26 or novel haloalkane dehalogenases DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marynobacter sp*. ELB 17, etc.

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P33

ROLE OF CYS RESIDUES ON THE STABILITY OF THE COMPLEX BETWEEN PROTEINKINASE ASK1 AND THIOREDOXIN

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis in various cells and plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin 1 (TRX1) and the 14-3-3 protein that both function as inhibitors of ASK1 [1]. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear. We have previously shown that the TRX1 binding domain of N-terminal ASK1 (ASK1-TBD) is a compact monomeric and rigid domain that under reducing conditions forms with TRX1 a stable and well defined complex with 1:1 molar stoichiometry. We have also showed that the complex formation does not involve the formation of intermolecular disulfide bonds and that residues from the catalytic WCGPC motif of TRX1 are essential for complex stability with Trp31 being directly involved in the binding interaction [2].

The aim of this study was to investigate the role of individual cysteine residues from TRX1 as well as from ASK1-TBD in the interaction between these two proteins. Sedimentation velocity analysis together with the site-directed mutagenesis revealed that from five cysteine residues in human TRX1 molecule, the residue Cys32 is crucial for TRX1 binding to ASK1-TBD in reducing conditions. Formation of disulfide bond between Cys32 and Cys35 is the main factor responsible for complex dissociation under oxidative stress. The sequence of ASK1-TBD contains seven Cys residues from which six seem to be well accessible at the surface of the ASK1-TBD molecule. Oxidation of ASK1-TBD leads to the formation of one intramolecular disulfide bond between cysteines Cys225 and Cys226. Site-directed mutagenesis of ASK1-TBD Cys residues suggested that some of these residues play an important role in the stability of the ASK1-TBD:TRX1 complex.

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P34

CALCIUM ION IS REQUIRED FOR PROPER FOLDING, DIMERIZATION AND ENZYMATIC ACTIVITY OF HUMAN GLUTAMATE CARBOXYPEPTIDASE II

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Glutamate carboxypeptidase II (GCPII) is a transmembrane dimeric metalloprotease harboring two zinc ions indispensable for its enzymatic activity in the active site. In addition, a calcium ion of an unknown function is observed at the dimer interface in the distance of 20 Å from the active site. The present work is aimed at elucidating the structural and functional role of the Ca^{2+} ion *in vitro*.

To this end, we designed, expressed and purified a panel of GCPII variants with mutated amino acids coordinating Ca^{2+} and/or positioned at the homodimer interface. Size-exclusion chromatography reveales that a significant portion of the protein is either aggregated or eluted in fractions corresponding to the monomeric species that have not been described in the literature. Activity measurements showed no or very limited activity of the mutant enzymes. At the same time circular-dichroism spectra didn't reveal any significant structural changes in comparison with wtGCPII. Based on these findings we assume that Ca^{2+} is crucial for enzyme dimerization that is in turn required for GCPII enzymatic activity.

P35

THEORETICAL STUDY OF CRYSTAL STRUCTURE OF WrbA FROM *E. COLI* IN COMPLEX WITH BENZOQUINONE USING QM CALCULATIONS OF CHARGE TRANSFER RATES

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The computational methods are applied to compare the electron-transfer probability for two distinct crystal structures of the *Escherichia coli* protein WrbA, an FMN-dependent NAD(P)H:quinone oxidoreductase, with the bound substrate benzoquinone. The computational methods were based on the combination of quantum mechanics/molecular mechanics approach, semi-empirical methods and quantum mechanical (QM) calculations of charge transfer rates using Marcus equation. The calculations indicate that the position of benzoquinone in a new structure reported here and solved at 1.33 Å resolution is more likely to be relevant for the physiological reaction of WrbA than a previously reported crystal structure [1] in which benzoquinone is shifted by ~5 Å. Because the true electron-acceptor substrate for WrbA is not yet known, the present results can serve to constrain computational docking attempts with potential substrates that may aid in identifying the natural substrate(s) and physiological role(s) of this enzyme. The approach used here highlights a role for QM calculations in crystal structure interpretation.

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P36

OLIGONUCLEOTIDE ANALYSIS AND SEQUENCING USING MALDI-TOF MASS SPECTROMETRY

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Since its discovery, matrix-assisted laser desorption/ionization (MALDI), which is usually associated with a time-of-flight (TOF) mass analyzer in commercial instruments, has become a common technology in biological mass spectrometry (MS). It is useful for measurements with peptides and proteins, oligonucleotides, oligosaccharides, technical polymers, small polar compounds, viruses or intact microbial cells. The well-known possibility of fast identification or biotyping of microorganisms (the latter representing a differentiation of strains or species by distinctive biochemical characteristics – this is in principle applicable to any group of biological samples of the same type) makes MALDI-TOF MS more common and increasingly popular in biochemical, bioanalytical, medicinal or biotechnological laboratories. Both matrix choice and optimization of the sample preparation protocol are the most important steps in MALDI experiments. Matrix is indispensable in the ion formation process. The applications of MALDI-TOF MS to nucleic acid research include quality control of synthetic oligonucleotides, oligonucleotide sequencing, single-nucleotide polymorphism genotyping and microsatellite analysis. Oligonucleotides, which are largely utilized as gene probes in molecular biology, can efficiently be measured for example using 3-hydroxypicolinic acid (HPA) as a matrix but there also other matrix compounds available for this purpose: 2',4',6'-trihydroxyacetophenone, 6-aza-2-thiothymine, picolinic and anthranilic acids. A major obstacle is the relatively poor mass resolution, which increases in parallel with the increasing molecular mass accompanied by the decrease in signal intensity and sensitivity. Solid mixed matrices have been introduced such as HPA/picolinic acid or HPA/pyrazinecarboxylic acid to cope with these obstacles. Additives (e. g. diammonium hydrogen citrate) are then used to reduce or prevent from the formation of adducts between the phosphate groups of DNA and counter ions (Na+, K+). In this work we have used synthetic oligonucletotides up to a 60-mer to look for the most optimal sample preparation and data acquisition procedure. The ionophore antibiotics nigericin and monensin were checked as possible additives together with 1,4-bis(3-aminopropyl)piperazine. For oligonucleotide sequencing based on the fragmentation process of ion-source decay, combining HPA with different co-matrices based on aromatic carboxylic acids was evaluated to achieve excellent results with the optimized protocol.

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P37

STRUCTURAL STUDY OF THE REACTION MECHANISM OF 3' DNA PHOSPHATASE FROM *NEISSERIA MENINGITIDIS*

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Spontaneous base loss and oxidative DNA damage give rise to a large number of abasic sites (AP sites). These AP (apurinic/apyrimidinic) sites are generally recognised and repaired by a class of DNA AP endonucleases. Unrepaired AP sites as well as some products from other oxidative DNA repair processes can result into more toxic 3' DNA lesions including 3' phosphate. The 3' lesions block polymerization and ligation and impair the repair of single strand break. Previously, we have identified meningococcal AP endonuclease homologue NExo to be solely responsible for removal of the 3' phosphate in *Neisseria meningitidis*. In order to understand the mechanism of the reaction of this specialized enzyme we have crystallized NExo complexed with DNA substrate containing 3' phosphate. We also crystallized inactive point mutant with coordinated metal ion both in presence of the substrate and the product. Here we present three structures that allowed us to outline the reaction mechanism of meningococcal DNA 3' phosphatase.

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P38

RECOMBINANT EXPRESSION OF RAT NATURAL KILLER CELL INHIBITORY RECEPTORS NKRP1B-Clrb

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Natural killer (NK) cells are an intensively studied part of immune system, possessing unique ability to recognize and induce death of tumor and virus-infected cells without prior antigen sensitization [1]. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell.This can be illustrated on the homodimeric rat inhibitory receptor NKRP1B and its ligand Clrb, which play a crucial role in the immunological response of NK cells to the infection with rat cytomegalovirus (RCMV), one of the most studied NK cell function model in rat model organism [2].

During the infection of RCMV the target cell down-regulates cell surface expression of Clrb, thus decreasing the inhibitory signal transmitted through NKRP1B to the NK cell, which would ideally lead to NK cell activation and lysis of the infected cell. However, RCMV carries a gene for "decoy" surface receptor – RCTL that mimics Clrb and thus escapes the immunological response of NK cells. Moreover, while this escape strategy was demonstrated in the rat strain WAG, it has been shown that the NKRP1B homologue from rat strain SD ligates only Clrb and does not recognize RCTL. Thus the SD rat strain is less susceptible to the RCMV infection [2].

This research aims to elucidate the molecular basis of the NKRP1:Clr receptor ligand recognition, based on previous successful results with homologous human NKRP1:LLT1 receptor ligand pair[3]. For protein crystallization, it was found out that the best recombinant expression system for production of soluble extracellular domains from this family of receptors is transiently or stably transfected HEK293S GnTI$^-$ human cell line possessing homogeneous N-glycosylation profile. To increase the yields of recombinant proteins, we have optimized transposon-based doxycycline inducible mammalian cell expression system piggyBac [4] within HEK293S GnTI$^-$ cell line using Clrb soluble expression construct as target protein.

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P39

MOVING HILLS METHOD

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Many interesting biological processes cannot be modelled by molecular simulations because they take place in time scales inaccessible for current computers. This can be addressed either by parallelisation, i.e. splitting the task to be calculated on a large number of computers, or by application of enhanced sampling techniques. Here we report a new approach combining both strategies. Moving hills method inspired by metadynamics [1] simulates a series of replicas of the studied system in parallel with a shared history-independent bias potential. The bias potential depends on the distribution of selected low-dimensional degrees of freedom (collective variables) among all replicas. The method was tested on model energy profiles, alanine dipeptide (Ace-Ala-Nme) in water and vacuum, *cis*/*trans* isomerisation of Ace-$(Pro)_n$-Nme and other molecular systems.

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P40

CRYSTAL STRUCTURE OF PLANT DEFENSE PROTEIN IN COMPLEX WITH SERINE PROTEASE

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Protease inhibitors from the Kunitz family (I3 in MEROPS) of 20-25 kDa are widely distributed in plant kingdom. They share a conserved -trefoil fold in which a set of variable loops forms reactive centers for the interaction with target proteases. The majority of the Kunitz inhibitors are targeting serine proteases; however, inhibitors of cysteine and aspartic proteases have also been described. PDI (potato cathepsin D inhibitor) from the Kunitz family is a wound inducible plant defense protein against insect herbivores and pathogens. PDI is a unique bifunctional inhibitor of serine proteases as well as aspartic proteases; its binding mode has not been structurally studied so far. Here, we present the crystal structure of the complex of PDI and the serine protease trypsin at 1.95 Å resolution. The binding mode of PDI is compared with that of other Kunitz inhibitors, and structural variability of their reactive centers is discussed.

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A PARAMETER OF DIFFRACTION EXPERIMENT WITH LOW ATTENTION: EXPOSURE

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Speed and sensitivity of new pixel array detectors bring new possibilities to the measurement of X-ray diffraction data. Due to their point spread function and their ability to measure weak reflections, reliable estimation of exposition parameters by human eye is made more difficult. However, a correct exposure helps increase the information gain of the measurement (more precise measurement of anomalous differences or weak reflections for improvement of electron density maps) and at the same time reduce radiation damage.

As a signal to noise ratio of individual reflections ($I/$) has, with a chosen error model [1], an upper limit (*ISa*), the optimal exposure can be estimated from the relation of $I/$ of strong reflections to *ISa*. The relation can be analyzed *e.g.* in a graph of $I/$ to intensity [2], which is informative even for initial data containing few frames. Therefore, a script for fast analysis was developed. The script produces graph for optimal exposure estimation before the final data collection.

A preliminary analysis of earlier measured datasets shows that majority of datasets measured with a PILATUS detector is underexposed. In comparison, datasets measured with a CCD detector (marCCD) show correct exposure or overexposure.

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INTEGRATED STRUCTURAL BIOLOGY STUDY OF THE FrpD PROTEIN FROM *NEISSERIA MENINGITIDIS*

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FrpD is a highly conserved lipoprotein of *Neisseria meningitidis* anchored to the bacterial outer membrane. The *frpD* gene sequence contains two translation initiation sites, which give rise to production of the full-length FrpD protein ($FrpD_{271}$) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein ($FrpD_{250}$) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported $FrpD_{271}$ precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys_{25} residue, and sorted to the outer bacterial membrane [1]. The biological function of FrpD appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity (K_d = 0.2 nM) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of any structural information on these proteins.

We present here the crystal and solution structures of the FrpD protein and the NMR spectroscopy identification of the $FrpD_{43-271}$-$FrpC_{1-414}$ interaction interface. According to the detailed structure analysis, the atomic structures of FrpD reveal a novel protein fold. This structural information provides the first step in deciphering of the mechanism of $FrpD_{43-271}$-$FrpC_{1-414}$ interaction and sets the base for further investigation of the role of FrpD in the *N. meningitidis* lifestyle.

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P43

STRUCTURE-FUNCTIONAL ANALYSIS OF THE HYDROLASE FOLD SUPERFAMILY MEMBER

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

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

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

Crystal of DmxA belonged to $P2_12_12_1$ space group (a = 43.371 Å, b = 78.343 Å, c = 150.51Å; = = = 90.0) and contained 2 molecules in the asymmetric unit. The structure was solved by molecular replacement with *MOLREP* from the CCP4 software suite by using the coordinates of *Rhodococcus rhodochrous* (PDB entry 4E46; 48% sequence identity for 142 residues and 63% sequence similarity).

Structurally DmxA is represented by the two domains: a highly conserved - hydrolase main domain, composed of eight - strands, within antiparallel (2) and are flanked by - helices: four are on the one side and two are on the other side of the – sheet; and the second - is smaller helical cap domain, composed of - helices, covering the active site, which has revealed the catalytic pentad essential for the S_N2 reaction mechanism: D105, H273, E129, W106 and uniquely Q40 halide-stabilizing residue among of the other HLDII subfamily members.

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SOLVATION OF ENGRAILED HOMEODOMAIN MUTANT K52E

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The homeodomain fold is a relatively simple DNA/RNA-binding protein structure consisting of 60 aminoacids which form three -helices and bind to the nucleic acid with a helix-turn-helix motif. The domain was found in many transcription factors involved in several aspects of embryonic development, regulating cell fate and development plan of the body [1].

Our main goal consisted in searching for water-protein contacts observable by NMR. A doubly labeled mutant K52E of the drosophila Engrailed homeodomain was prepared for an NMR analysis. Standard 3D NMR spectra (HNCACB and CBCACONH) were measured for the backbone assignment. Protein solvation was monitored at various temperatures by recording homonuclear 2D NOESY spectra with high resolution in the indirect dimension. Several NOESY cross peaks were observed at the water frequency, indicating water molecules tightly bound to protein residues [2]. The movement of these water molecules is significantly slowed down, giving rise to an NOE signal. In order to distinguish exchangeable water molecules from bulk water and from tightly bound water molecules, a CLEANEX-PM experiment was conducted [3].

NOESY experiments showed that residues S13, R18, R32, R56 are tightly bound to water molecules. Several residues in exchange between bulk and bound water (R6, R21, R27, R33 and R34) have been revealed by the CLEANEX-PM experiment.

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P45

PRODUCTION OF READILY CRYSTALLIZABLE GLYCOPROTEINS IN HEK293S GnTI$^-$ CELL LINE: A CASE STUDY OF HUMAN NATURAL KILLER CELL RECEPTORS

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Recombinant protein expression can be a costly enterprise, especially for proteins that are not easily expressed in prokaryotic cells and are sometimes labelled as „difficult targets“. Here we would like to show a case study of a recombinant expression of such a difficult target – human natural killer (NK) cell receptor protein 1 and its binding partner lectin-like transcript 1, both belonging to the NK cell receptor C-type lectin-like family.

Human embryonic kidney 293 cell line deficient in N-acetylglucosaminyltransferase I (HEK293S GnTI$^-$) is well known tool for expression of proteins with homogeneous and deglycosylatable N-glycosylation, a feature crucial especially for protein crystallography [1]. However, production protocol using this cell line based on transient transfection of adherent cell culture is costly to scale-up and has reportedly lower expression yields [2].

In this work we have adapted HEK293S GnTI$^-$ cell line to growth in suspension and optimized its transient transfection. While transfection at standard cultivation cell density proved very little success we have found out that concentrating the cells to high cell density substantially increases transfection efficiency, greatly enhancing protein yields and creating fast and scalable production process.

We demonstrate this on the production of soluble lectin-like transcript 1 [3] naturally present on natural killer and T-lymphocytes, but upregulated in glioblastoma cells, one of the most lethal tumours, where it acts as a mediator of immune escape [4]. Furthermore, lectin-like transcript 1 has been recently suggested as a biomarker for B-cell non-Hodgkin's lymphomas and connected with other cancerous manifestations [5]. The prepared soluble domain of lectin-like transcript 1 with homogeneous glycosylation was readily crystallized and following optimization of crystal conditions this protein preparation ultimately led to the first structure determination of this receptor [6].

However, both of these receptors are present on NK cells and also on circulating T and B lymphocytes, with lectin-like transcript 1 being also expressed on antigen presenting cells, suggesting a wider role of these receptors in immune regulation and response. NK cell receptor protein 1 positive lymphocytes are thus being implicated to have role in diseases connected with immune malfunctions – e.g. multiple sclerosis [7], rheumatoid arthritis [8] or Crohne's disease [9]. In order to improve on the productivity for NK cell receptor protein 1 we are now using a stably transfected HEK293S GnTI$^-$ cell pool with a tenfold yield improvement.

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CRYSTAL STRUCTURE OF A GLYCEROL TRINITRATE REDUCTASE XdpB FROM *AGROBACTERIUM* SP. R89-1 REVEALED INHIBITION BY REVERSIBLE AGGREGATION

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The Old Yellow Enzyme family members (OYEs) are NAD(P)H dehydrogenases containing noncovalently bound FMN. These enzymes have been reported in bacteria, yeasts, fungi, plants and in animals. Since 1932, when the first OYE protein was isolated and characterized as the first flavoprotein in general, the physiological role of OYEs is still under discussion. So far, more than 100 structures of OYEs have been published. It is hypothesized that these enzymes may play certain role in cell response to oxidative stress. Moreover, these enzymes have biotechnological and biodegradatory potential and are, therefore, subjects of biochemical and crystalografic studies.

The crystal structure of glycerol trinitrate reductase from *Agrobacterium* sp. R89-1 (XdpB) has been determined at 2.1 Å resolution in its Apo form. In analogy to proteins homologous to XdpB, the enzyme has the TIM barrel fold with the N-terminal -hairpin lid. Unexpectedly, XdpB C-terminus interacts with the active and FMN binding sites of a symmetry-related protein. This interaction contributes to stabilization of the crystal lattice and represents a unique structural feature among analogous bacterial enzymes which has possible functional consequences. There was no crystallographic evidence for the presence of natural, noncovalently bound co-factor FMN. The crystallographically well defined C-pentapeptide binds to the amino acid residues lining the FMN site by an extensive network of interactions: 10 hydrogen bonds, 4 water bridges, one CH-pi interaction with the total interaction area is about 86 Å^2. Conceptually similar autogenic blocking as reported here has been observed in the crystal structure of an old yellow enzyme OPR3 from the plant species Solanum lycopersicum (PDB code 2hsa).

We expect the same behavior of other OYE proteins with known 3-D structures because their natural interactions may be disrupted with His tagging of their C-terminuses (e.g. in PDB 4qnw(20)). Currently we work on detailed explanation of these intermolecular interactions of members of OYE.

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P47

COMPARISON OF COMPUTATIONAL AND EXPERIMENTAL RESULTS FOR INTRINSICALLY DISORDERED PROTEINS (IDPs)

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The discovery of intrinsically disordered proteins (IDPs) has challenged the traditional protein structure paradigm, that protein function depends on a fixed three-dimensional structure. The various branches of structural biology developed new approaches for the study of IDPs which are compared in this work. Delta subunit of RNA polymerase from *Bacillus subtilis* and a regulatory domain of human tyrosine hydroxylase 1 were chosen for studies because they consist of structured and disordered regions of a similar size. RNA polymerase plays a fundamental role in the process of protein synthesis and tyrosine hydroxylase catalyzes hydroxylation of L-tyrosine to L-DOPA and it is a

key enzyme in biosynthesis of dopamine. Experimental data such as NMR chemical shifts, residual dipolar coupling (RDC), paramagnetic relaxation enhancement (PRE), and SAXS data are compared with the theoretical values calculated from the MD trajectory. This comparison can be used to determine the accuracy of the calculated structural ensembles and to guide design of additional experiments.

The project is financed from the SoMoPro II programme. The research leading to this invention has acquired a financial grant from the People Programme (Marie Curie action) of the Seventh Framework Programme of EU according to the REA Grant Agreement No. 291782. The research is further co-financed by the South-Moravian Region. The article/paper reflects only the author´s views and the Union is not liable for any use that may be made of the information contained therein. In addition, this work was also supported by Czech Science Foundation (15-34684L). The computational simulations were realized in the National Supercomputing Center IT4Innovations, which is supported by The Ministry of Education, Youth and Sports from the Large Infrastructures for Research, Experimental Development and Innovations project „IT4Innovations National Supercomputing Center – LM2015070". Further computational resources were provided by the MetaCentrum under the program LM2015042 and the CERIT Scientific Cloud under the program LM2015085, provided under the programme "Projects of Large Infrastructure for Research, Development, and Innovations". This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II. This work was supported by Czech Science Foundation, grant number GA 13-16842S.

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STRUCTURAL AND BIOPHYSICAL CHARACTERIZATION OF THE PROJECTION DOMAIN OF NEURONAL TAU PROTEIN

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Tau protein belongs to intrinsically disordered proteins (IDPs). IDPs do not have well defined secondary and tertiary structure. Under physiological conditions, IDP tau promotes tubulin monomers assembly, binds to axonal microtubules and stabilizes them. However, in a group of neurodegenerative diseases called tauopathies, tau protein aggregates and accumulates in the entire neuron forming insoluble fibrillary deposits – neurofibrillary tangles. The most prevalent tauopathy is Alzheimer's disease, which today is one of the most serious health problems in the world.

It is little known about the structure of tau protein and its pathological transformations. Recent evidences suggest that N-terminal region of tau protein is critical for the stabilization and organization of certain types of axons. In this work we are focusing on structural and biophysical characterization of N-terminal projection domain of tau protein to bring some new insights about its conformational and dynamic features. Monoclonal antibody AX-N1, which recognizes an epitope in the first alternatively spliced N-terminal insert of tau was used as a molecular probe.

We have performed a crystallization study of AX-N1 antibody Fab fragment alone and in the complex with tau peptides. We have found crystallization conditions for all tested systems; crystals of Fab fragment alone diffracted to 3.8 Å on a laboratory X-ray source. For biophysical characterisation we used ELISA and surface plasmon resonance (SPR). With SPR we have determined binding rate constants of tau polypeptide 31-441 and tau protein 1-441 with AX-N1 Fab fragment. The results show that full-length tau protein isoform has higher association rate constant and lower dissociation rate constant then truncated tau. cELISA revealed that the AX-N1 affinity is enhanced in the presence of the second alternatively spliced N-terminal insert and in the absence of the second alternatively spliced repeat in the C-terminal part. Therefore, the three-repeat tau protein isoforms are more prone to bind the antibody then the four-repeat isoforms. Obtained results suggest different configuration of the first tau insert in the context of various tau protein isoforms.

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IMPROVED INTERPRETATION OF 14-3-3 FLUORESCENCE MEASUREMENTS BY MOLECULAR DYNAMICS SIMULATIONS

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Förster resonance energy transfer (FRET) is a spectroscopic method widely used to determine inter- and intramolecular distances of biomolecules through distance dependent energy transfer between two fluorophores. Apart from distances, the efficiencies of FRET also significantly depend on the mutual orientation of the fluorophores which are generally not accessible to experiments and often crudely assumed to be isotropic. This assumption might become especially invalid for dyes attached to protein surfaces where special interactions can be formed and cause the dye orientations deviate far from isotropic. In order to improve interpretation of FRET measurements, overall dynamics of the dyes attached to the protein can be probed by molecular dynamics (MD) simulation. Obtained ensemble averaged values of FRET efficiency can be directly compared to the experimental values.

In this study we simulated set of selected donor and acceptor fluorophores connected to the 14-3-3 protein. For that purpose we prepared force-field parameters for the simulated labels compatible with the 54A7 GROMOS force-field. Our MD simulations indicate strong tendency of the dyes to interact with protein surface what significantly restricts the range of possible orientations. However the transitions between potential interaction sites are very slow, what may hinder proper sampling. We note that for two FRET pair variants the donor-acceptor distances and orientation factors differ considerably which results in significant differences in calculated FRET efficiencies.

The project is financed from the SoMoPro II programme. The research leading to this invention has acquired a financial grant from the People Programme (Marie Curie action) of the Seventh Framework Programme of EU according to the REA Grant Agreement No. 291782. The research is further co-financed by the South-Moravian Region. The article/paper reflects only the author´s views and the Union is not liable for any use that may be made of the information contained therein. In addition, this work was also supported by Czech Science Foundation (15-34684L) and MOBILITY grant CZ 14/2015, WTZ Tschechien 2015-16. The computational simulations were realized in the National Supercomputing Center IT4Innovations, which is supported by The Ministry of Education, Youth and Sports from the Large Infrastructures for Research, Experimental Development and Innovations project „IT4Innovations National Supercomputing Center – LM2015070". Further computational resources were provided by the MetaCentrum under the program LM2015042 and the CERIT Scientific Cloud under the program LM2015085, provided under the programme "Projects of Large Infrastructure for Research, Development, and Innovations". This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II.

P50

THE ROLE OF THE S1-S4 SENSOR DOMAIN IN THE ACTIVATION AND MODULATION OF THE TRPA1 ION CHANNEL

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Transient receptor potential ankyrin 1 (TRPA1) is a polymodal ion channel sensitive to noxious chemical agents. It is mainly expressed in peripheral nociceptor neurons. TRPA1 agonists include a broad class of electrophiles that activate the channel through covalent modification of reactive cysteines. It has been generally believed that the N-terminus is a key target for electrophiles [1, 2]. However, recent results of Moparthi et al. [3] showed that human TRPA1 is activated by electrophilic compounds even in the absence of the N-terminal domain. When activated, TRPA1 is partially calcium selective and permeating Ca^{2+} ions critically regulate its gating by promoting both potentiation and inactivation [4]. The molecu-

lar details behind these processes remain unknown as well as the nature of weak TRPA1 voltage sensitivity.

Here, we investigate the involvement of TRPA1 sensor domain, the bundle of S1-S4 helices, in electrophile sensitivity. Transmembrane location of this domain requires careful consideration of the contribution of voltage- and Ca^{2+}- dependent modulation of the channel gating. There are 6 cysteine residues within the transmembrane part of each TRPA1 subunit. All of them represent possible targets for covalent modifications during stimulation by membrane permeable electrophilic agonists. Using the site directed mutagenesis and whole-cell electrophysiology we identified C856A mutant to have detrimental effect on TRPA1 gating with remarkable dependence on membrane voltage polarity and agonist character. Moreover, we were able to characterise two more alanine mutations that share very similar unique phenotype. Both are located at the intracellular side of S1-S4 bundle and both are approximately equally embedded in membrane yet probably spatially distant of each other. Thanks to the recently published 3D structure of the TRPA1 channel [5] we were able to build a reliable homology model, which showed that both residues are oriented into the splayed open lower vestibule of the S1-S4 sensor domain. The results allow us to suggest that the shape of these solvated vestibules of TRPA1 channel tetramer is a critical determinant of its voltage sensitivity and, by extension, an essential component of TRPA1 gating machinery.

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CHARACTERIZING NANOPARTICLES IN LIQUIDS: PROTEIN AGGREGATION STUDIES

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Many patients' lives have been improved with the use of monoclonal antibodies and recombinant proteins since their commercialisation some thirty years ago. Work in the field of therapeutic proteins continues to be attractive for academia, pharma and biotechs with the global therapeutic proteins market forecast to reach an estimated $141.5 billion in 2017 with monoclonal antibodies, insulins, interferons, growth hormones and blood factors being just some of the areas of interest1. As large, macromolecules, working with proteins brings up an array of challenges that are not generally encountered when working with traditional, small molecule therapeutics. They have complex 3-D structures that need to be maintained for binding efficacy, are generally more prone to degradation in the body and their synthesis is typically achieved in a biological host thus requiring purification from complex media.

For a protein therapeutic to be efficacious it needs to be available in its monomeric form, but a further key feature of protein molecules is their tendency, to varying degrees, to aggregate. This aggregation can progress more quickly or even be initiated when the protein is subjected to stress such as exposure to interfaces (air-liquid or solid liquid, for example), light or changes in temperature, ionic strength or pH [1, 2]. Unfortunately these stresses are often inherent in the synthesis, purification, packaging, transport, storage and use of proteins. The formation of aggregates produces a wide spectrum of sizes, types and lifetimes [3]. The mechanisms or pathways involved in aggregation, in many cases, are not well understood and are numerous, varying from protein to protein and multiple mechanisms can occur within the same sample [4].

When prescribed to a patient, therapeutic proteins are dosed as liquid formulations, often being produced prefilled in syringes which are usually lubricated with silicon oil. The oil, along with other particulate debris that may be introduced with the processing of the protein can also serve as sites for aggregate formation due to heterogeneous nucleation [5].

As with all therapeutics, liquid formulations are regulated and are subject to US Pharmacopoeia <788> light obscuration test which since 1995 have set limits on the allowable number of sub-visible particles that are > 25 μm and > 10 μm as ≤ 600/container and ≤ 6000/container, respectively. These sizes are more to control levels of process debris that could potentially block blood vessels, whilst the risks associated with the administration of large aggregated protein particles were not known at the time of in the establishment of USP light obscuration test <788> [2]. Sub-visible protein particles 100 nm -10 μm, as well as those that are larger have the potential to impact the safety and efficacy of the therapeutic over its shelf life [2]. Furthermore, small aggregates can grow into larger ones and eventually become the size and number to exceed the limits set out USP<788>. When working with proteins at any stage of research, development and production, it is important to understand and control the profile of the

smaller aggregates in order to identify the point of aggregation onset. The measurement of small aggregates has historically been investigated using Size Exclusion Chromatography (SEC). However this technique gives a readout of mass fraction and relies on having 100% sample recovery to be sure of the data profile; even 99% recovery means 1% of a particularly large aggregate may have been lost [3]. Sub-visible particles usually do not constitute a sufficient mass fraction to be quantified [2]. In addition, the method of SEC required substantial dilution of the sample which itself can change the aggregation profile. Techniques that can count and size individual species in undiluted therapeutic proteins may be more appropriate than mass fraction methods when studying protein aggregation.

Nanoparticle tracking analysis (NTA, **Malvern Instruments Ltd.**, Wiltshire, is a method of visualizing and analyzing particles in liquids that relates the rate of Brownian motion to particle size. The rate of movement is related only to the viscosity of the liquid, the temperature, and the size of the particles, and generates a high resolution particle size distribution by sizing each particle individually along with giving an estimation of the concentration of particles present in the sample [6]. Due to the low refractive index of protein, the limit of detection in NTA measurement is approximately 30 nm. This means that the protein monomer units which are typically in the range 3-10 nm are not measured by NTA, but aggregates comprised of a few monomers to many thousands of units can be sized and counted. As it is not necessary to dilute the sample to obtain the particle size distribution the aggregation profile is not changed due to sample processing.

Dynamic light scattering (DLS) or photon correlation spectroscopy (PCS), is a commonly used sizing technique that also has its basis in Brownian motion, but unlike NTA, the light scattered from all the particles in the sample is measured as a whole, thereby giving a single average size measurement for the sample, along with a guide to the level of polydispersity of the sample [7]. As with NTA, the smaller particles scatter smaller amounts of light and larger particles scatter more light. The overall fluctuations in the scattered light over time are used to calculate the size of the particle population. Since the intensity of the scatter is of a factor r^6, this allows the protein monomer to be sized but a few larger particles in a population can greatly skew the data obtained, and the technique struggles to resolve very poly dispersed mixtures which, as discussed above, are often observed when measuring protein solutions.

When NTA and DLS technologies are used with the sample protein sample the data from both can be used to analysis of both monomer and aggregates. Using heat (50 ^{o}C), 1mg/mL IgG has aggregated over time, with the particles scattering light increasing in number and intensity when observed in the NanoSight NS500 fitted with a 635nm laser. At each time point, the NTA and DLS measurements were taken and size data for both the monomer and the aggregates could be observed. After 20 minutes of thermally induced aggregation, the monomer peak described with DLS showed a sphere equivalent hydrodynamic radius of approx 10 nm, with NTA measuring aggregate particles starting approximately 30 nm, with peaks observed at 50 and 85 nm and the largest aggregates being approximately 300 nm. Since NTA also gives an estimation of particle concentration, the increase in particle number during the time course of the thermal aggregation could also be tracked. These data suggest that for thirty minutes there are minimal aggregates above 30 nm in size. From thirty to 100 minutes, the numbers of aggregates at 30 nm or larger remains stable and after this time the number of aggregates appear to increase in a more exponential manner. Using NTA and DLS size data and NTA concentration data from the same sample gives an information rich solution when studying the complex area of protein aggregation.

To prevent the presence of large aggregates rendering a protein therapeutic unsuitable for patients, scientists need to have an understanding of where in the process of synthesis, purification, packaging, transport, storage and use the proteins monomer units begin to aggregate together. By taking size distribution measurements with NTA and DLS at different points in the process enables scientists to identify the point where aggregation begins. This point/step can then be reviewed and potentially modified to prevent or slow the formation of protein aggregates.

More information on the mentioned products can be obtained from company Anamet, www.anamet.cz.

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