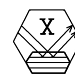

A41

PROTEIN VARIANTS OF ABD DOMAIN TARGETING HUMAN IL-23 RECEPTOR

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A42

ION TRANSFER IN G4-WIRES

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G4-wires are guanine quadruple helices of various length studied both experimentally and theoretically [1, 2]. In this work, we present results of molecular dynamics simulation of infinitively long G4-wires. The infinitive length is achieved by a periodic boundary condition applied on 12-steps long G4-fragment, which is covalently bound to its neighboring images. The main purpose of such setup is to disable possible penetration of interior channel by water or cations from bulk solution. Under these well defined conditions, the transfer of cations in various scenarios through the channel was studied by the means of potential of mean force calculations using the Adaptive Biasing Force method [3].

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A43

ISOLATION OF THE RECOMBINANT PSBO PROTEIN FROM HIGHER PLANTS

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Photosystem II (PSII) is a large protein complex in a thylakoid membrane of higher plants, algae and cyanobacteria that catalyses light-driven oxidation of water and produces molecular O_2, electrons and protons. The water splitting and oxygen releasing occur in the catalytic centre of PSII – the oxygen-evolving centre (OEC) that contains manganese-calcium cluster (4:1 Mn:Ca) located close to the luminal surface of the transmembrane domain and surrounded by intrinsic and extrinsic components in thylakoid membranes. PsbO (33kDa), PsbP (23kDa), PsbQ (17 kDa), PsbR (10 kDA) are extrinsic proteins associated to the luminal side of PSII in higher plants, which maintain stability of water oxidation site and correct ionic environment during water oxidation [1, 2, 3]. The knowledge of the structure of PSII and its proteins plays an important role in understanding of the water splitting.

The objective of the project is to obtain recombinant PsbO protein from *Pisum sativum* and *Spinacia oleracea*. The extraction of mRNA from leaves and synthesis of cDNA are the first steps to get the *psbO* gene. The conditions for polymerase chain reaction (PCR) were optimized and DNA fragments encoding the *psbO* gene were obtained. pET-28b(+) vector was used for overexpression of recombinant PsbO/HisPsbO proteins. Purification, crystallization and structure determination of the recombinant proteins PsbO/HisPsbO of the oxygen-evolving complex from *Spinacia oleracea* and *Pisum sativum* are underway.

This research was supported by the ME CR (COST LD11011, CZ.1.05/2.1.00/01.0024), by the AS CR (AV0Z 60870520) and GAJU 170/2010/P for DK.

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A44

PROTEIN CRYSTALLIZATION STUDIES – COMPLEX OVERVIEW

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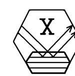
The research at the Laboratory of crystallogenesis and biomolecular crystallography of Institute of Nanobiology and Structural Biology GCRC AS CR joined with Laboratory of molecular structure and dynamics of School of complex systems of FFPW University of South Bohemia in Ceske Budejovice is aimed at crystallization and crystallographic studies of soluble and membrane proteins and protein complexes. Since y. 2000 when the research in this field has begun, more than 20 proteins, protein complexes and mutant variants such as HsdR subunit of restriction enzyme of EcoR124I from *E.coli* plus its 3 mutant forms, PsbP protein from *Spinacia oleracea*, WrbA apo and holo forms of *E.coli*, Fe-regulated protein D (FrpD) of *Neisseria meningitides*, haloalkan dehalogenases DhaAwt, DhaA04, DhaA12, DhaA13, DhaA14, DhaA15, DhaA31 from *Rhodococcus rhodochrous* NCIMB 13064 and their complexes with substrates isopropanol (IPA) and 1, 2, 3 – trichloropropane (TCP), DbeA and DbeA1 of *Bradyrhizobium elkani* USDA94 as well as two new haloalkan dehalogenases DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marynobacter* sp ELB 17 were crystallized, tested and crystals were measured at the synchrotron radiation sources. Diffraction data were used to solve and refine protein structures that have been



deposited in PDB under specific codes. Almost all enzymes were crystallized using standard, advanced and alternative crystallization techniques e.g. cross influence procedure that was directly developed and tested in our laboratory.

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A45

PHOTOSYSTEM II REACTION CENTER, QM STUDY OF CHARGE DISTRIBUTION AND TRANSFER

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Photosystem II (PSII) is a pigment-protein complex of thylakoid membrane in cyanobacteria, algae and higher plants. PSII performs series of light driven reactions resulting in charge separation and subsequently in a reduction of an electron-transport chain and water oxidation. Primary site of the light to chemical energy conversion is located in so-called reaction center (RC).

In our previous computational studies of the PSII RC we focused on the role of the reduced pheophytin *a* (PHO) inducing conformational changes of the RC protein environment and affecting the excitonic interaction of the RC chlorophylls. Nowadays we are developing QM/MM approach to study charge separation and transfer in the PSII RC pigments. The charge distribution is calculated using QM/MM method with novel polarized embedding. The quantum-classical approach is used for kinetic study of charge transfer in the PSII RC pigments. The method is using the stochastic time evolution of charge on PSII RC pigments based on the combination of classical MD simulation with QM calculation of charge transfer rates.

In a paralell direction we are developing model of thylakoid photosynthetical membrane (glycolipid membrane instead of our previous octane layer) to build complex model of PSII RC.

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A46

ROLE OF INDIVIDUAL PHOSPHORYLATION SITES FOR THE 14-3-3 PROTEIN-DEPENDENT ACTIVATION OF YEAST NEUTRAL TREHALASE NTH1

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Trehalases are important highly conserved enzymes found in a wide variety of organisms and responsible for the hydrolysis of trehalose to two glucose molecules. Trehalose, a naturally occurring nonreducing disaccharide, serves as a carbon and energy source as well as universal protectant from various stress conditions like dehydration, temperature extremes, oxidative stress and desiccation in a wide variety of organisms ranging from bacteria to invertebrates and higher plants. In yeast and plants it may also serve as a regulatory or signaling molecule to control certain metabolic pathways or even to affect growth.

Recently it has been shown that the enzymatic activity of neutral trehalase (Nth1) in yeast is enhanced by 14-3-3 protein binding in a phosphorylation-dependent manner through unknown mechanism. In this work, we investigated in detail the interaction between *Saccharomyces cerevisiae* Nth1 and 14-3-3 protein isoforms Bmh1 and Bmh2. The mass spectrometric analysis revealed that four residues within the disordered N-terminal segment of recombinant full length Nth1 (Ser20, Ser21, Ser60 and Ser83) are phosphorylated by PKA *in vitro*. Sedimentation analysis and enzyme kinetics measurements show that both yeast 14-3-3 isoforms form a stable complex with phosphorylated Nth1 and significantly enhance its enzymatic activity. The 14-3-3-dependent activation of Nth1 is significantly more potent compared to calcium-dependent activation. Limited proteolysis confirmed that 14-3-3 proteins interact with the N-terminal segment of Nth1 where all phosphorylation sites are located. Site-directed muta-

genesis was used to decipher the importance of found phosphorylation sites for Nth1 activation.

In conclusion, our results show that *S. cerevisiae* Nth1 is phosphorylated by PKA at multiple sites out of which Ser60 and Ser83 are sites primarily responsible for PKA-dependent and 14-3-3-mediated activation of Nth1. Finally H/D exchange and cross-linking experiments coupled to mass spectrometry were used to determine the interacting surface of Nth1:Bmh1.

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A47

THEORETICAL DESCRIPTION OF CARBOHYDRATE-AROMATIC CH-Đ INTERACTIONS ADDITIVE PROPERTIES VIA DFT AND AB INITIO CALCULATIONS

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There are several ways how saccharides may interact with their receptors (e.g. classical hydrogen bonds, through metal ions as Ca(II)). The CH- interactions that occur between carbohydrates and aromatic amino-acids are also strongly involved in carbohydrate-recognition process. However, their influence to the recognition process has been underestimated for a long time. Despite the fact, that CH- interactions were recently proved to have strength (which means contribution to the recognition process) comparable to classical hydrogen bonds.

In previous study [1], we have introduced systematic DFT and high-level *ab initio* study of CH- interaction features between benzene as the simplest representative of aromatic moiety in proteins and three saccharides – namely -D-glucopyranose, -D-mannopyranose and -L-fucopyranose. Nevertheless, also condensated aromatic moieties as Trp residues are responsible for the CH- mediated recognition of carbohydrate molecules. Lutteke et al. [2] have shown, that Trp is the most common residue found in direct contact with -D-glucopyranose molecules.

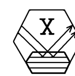
Introduced computational study [3] aims to describe the degree of additivity of the CH- interaction analyzing the interaction energy of carbohydrate-benzene complexes with monodentate (one CH- contact) and bidentate (two CH- contacts) carbohydrate-naphtalene complexes. For illustration of carbohydrate-naphtalene complexes structure, see Figure 1A. All model complexes were optimized at DFT-D BP/def2-TZVPP level of theory, followed by refinement of interaction energies at highly-correlated and accurate CCSD(T)/CBS level. Also Boltzmann-weighted populations of naphtalene-carbohydrate complexes were calculated for each carbohydrate apolar face (see Figure 1B).

Bidentate carbohydrate-naphtalene complexes exhibit very high interaction energy values ranging from -7,15 kcal mol^{-1} to -10,79 kcal mol^{-1} for DFT-D level, and from -6,14 kcal mol^{-1} to -8,20 kcal mol^{-1} for CCSD(T)/CBS level. Values of interaction energy for bidentate carbohydrate-naphtalene complexes were compared with values for monodentate carbohydrate-naphtalene complexes and carbohydrate-benzene complexes.

The analysis unravels that the CH- is not completely additive, because the interaction energy of bidentate complexes is higher (the interaction is weaker) than the sum of interaction energies of two corresponding monodentate complexes. However, deeper analysis discovers certain measurable degree of additivity. More precisely, the interaction energy of bidentate complex is 2/3 of the sum of interaction energies of appropriate monodentate complexes. Similarly, the interaction energy value for bidentante carbohydrate-naphtalene complexes is comparable to 4/5 of the sum of interaction energies of corresponding carbohydrate-benzene complexes.

Geometries of bidentate complexes are characteristic with shortening of H- distance to the narrow range about 2,3 Å compared to monodentate complexes. The C-H bond line forms almost normal of the naphtalene plane with minimal deviations for all bidentate complexes. Taking into consideration calculated interaction energies, we may conclude that bidentate complexes of carbohydrates with condensed aromatic moiety (naphtalene) are very stable and rigid.

This study also serves as illustration that DFT-D methods describe CH- interactions in qualitatively similar manner as more computationally demanding CCSD(T)/ CBS method. Based on both performed studies, we may



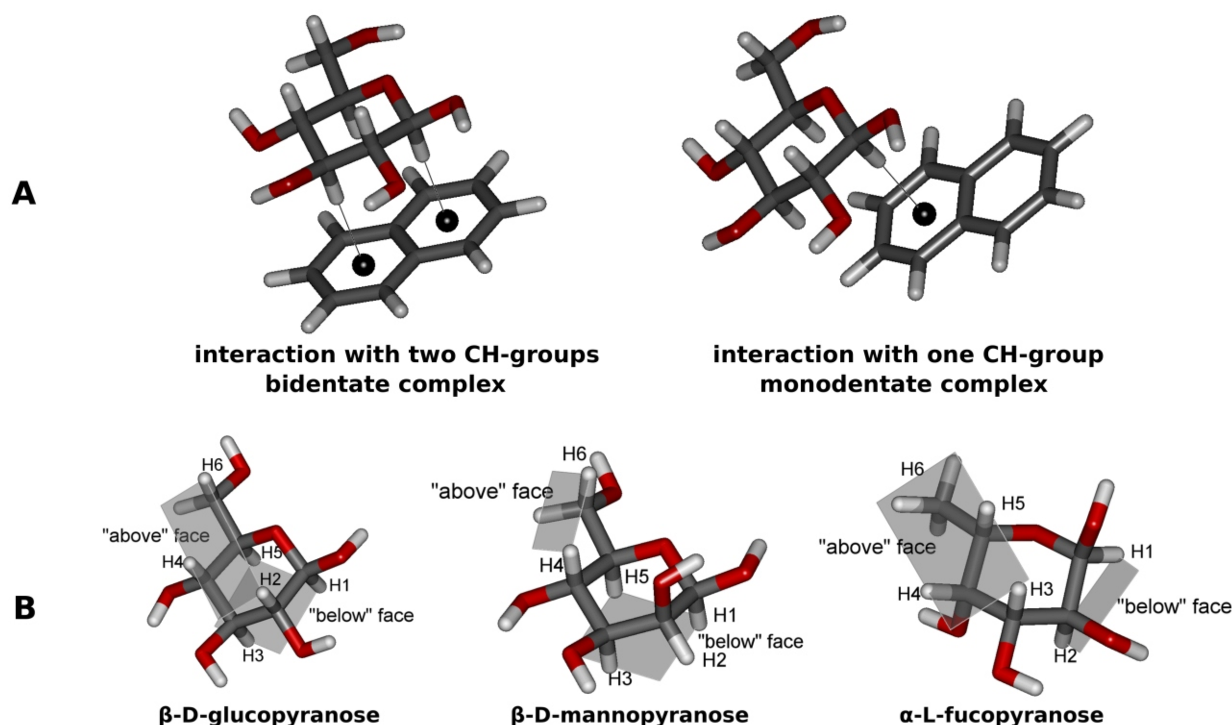


Figure 1. (A) Schematic depiction of monodentate (one CH- contact) and bidentate (two CH- contacts) carbohydrate-naphtalene complexes. (B) Structures and their apolar faces involved in CH- additivity study.

state that DFT-D approach may be utilized for computational treatment of larger complexes of biological interest, where CH- dispersion interactions play non-negligible role.

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A48

SPOIISA-SPOIISB PROTEINS AS SUICIDE COMPONENTS OF *BACILLUS SUBTILIS*

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The bacterial cell is capable of suicide in response to a several environmental stresses or developmental processes. This response is called programmed cell death (PCD). We study one of such PCD system present in bacilli, in which the process is mediated by a pair of toxin/antitoxin (SpoIISA/SpoIISB).

This newly indentified system is coded by *spoIIS* locus consisting of two genes. The gene *spoIISA* is coding a toxin, while *spoIISB* is a gene for an antitoxin. It has been shown, that inactivation of toxin gene has no effect on sporulation. Inactivation of antitoxin gene decreases sporulation efficiency. SpoIISB antitoxin is required for sporulation only if SpoIISA is present in the cell [1]. Morphological effect of forced toxin production is presented by formation of the holes in cytoplasmic membrane leading to cell death. SpoIISA toxin is a membrane protein with putative three transmembrane domains and cytoplasmic domain. When the domains are separated, the toxic effect is eliminated [2]. Antitoxin SpoIISB is a small cytoplasmic protein. Despite that the maximum level of SpoIISA production was observed in *B. subtilis* during the entry into stationary phase, it has been shown, that its expression is not dependent on the key early sporulation-specific transcription factor, Spo0A [3]. Mechanism of inactivation of SpoIISA occurs via formation of tight heterocomplex with SpoIISB. Resolved crystal structure of cytoplasmic domain SpoIISA with SpoIISB revealed that both proteins form heterotetrameric complex in the stoichiometry C-$SpoIISA_2$:$SpoIISB_2$. The toxin contains a putative GAF domain which is present in many proteins involved in the cell signaling [4].

TA systems are wide distributed in-between many bacterial species. However, homologues of SpoIISA and SpoIISB proteins were identified only among different bacilli species [2]. These proteins often display a low level of homology. SpoIISA of *B. subtilis* as well as of *B. cereus* has inhibitory effect on *E. coli* growth and antitoxin SpoIISB of these *Bacilli* is able to neutralize the SpoIISA toxicity in *E. coli* [2].

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A49

INCREASING AFFINITY BETWEEN INTERFERON GAMMA AND ITS RECEPTOR BY COMPUTER DESIGN OF RECEPTOR MUTATIONS. II. AFFINITY MEASUREMENTS

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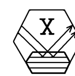
Molecules of living systems form a complex maze of interdependent networks and their proper functioning depends on intermolecular interactions. We study protein-protein interactions of cell signaling on a model of human interferon gamma (IFNg) and its cellular receptor (IFNgRec1). In this study, we show successful expression, purification and binding analysis of eighteen mutants of the recombinant IFNgRec1 where mutations were designed by computer modeling (see presentation by Schneider et al.) to increase (14 mutants) and lower (4 mutants) the affinity. The measurements of kinetic (k_{on}, k_{off}) and thermodynamic (K_D) constants of the IFNg/IFNgRec1 interaction were performed by surface plasmon resonance. The measured K_D value of recombinant wild type receptor is in agreement with the literature value. Of the selected positive mutants, four have slightly better affinity to IFNg than the wild type receptor and two have their affinity increased significantly. Therefore, our results indicate that rational design of mutations of proteins based on relatively simple and cheap computer models can predict replacement of amino acid residues that change the interaction in the desired direction. Such an approach can lead to creating mutants useful for better understanding of protein-protein interactions and may also have practical use in detection of IFNg.

Support from grant P305/10/2184 from the Czech Science Foundation is greatly acknowledged. All authors are supported by institutional grant AV0Z50520701.



A50

EXPERIMENT BASED DETERMINATION OF FRACTAL DIMENSIONALITY OF BELOUSOV-ZHABOTINSKY REACTION

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Belousov-Zhabotinsky (BZ) reaction represents a simple model of citric acid cycle. The BZ reaction is one of oscillating chemical reactions also called as chemical clock. The non-equilibrium thermodynamics behind BZ reaction creates oscillating structures between colors. Easy setup of the experiments allows us to capture the oscillations by ordinary RGB digital camera. The captured images are transformed by Rényi entropy using different alpha parameters. The Rényi entropy is generalization of Shannon's information entropy. The alpha parameter is related to weights of probability events. In other words, the alpha parameter describes the inner dimension of observable structures. The evaluations of different Rényi entropies in each color channel and therefore events of different probabilities are our state variables. The aim of the research is to develop dynamical model of BZ reaction with respect to its fractal dimension. We define two approaches how to use the Rényi entropy for image transformation: (a) computation from the whole image; (b) computation from the cross neighborhood. Our 39 state variables (13 from each color channel) obtained from image transformation are not independent to each other. To obtain uncorrelated state variables, we carry out the Principal Component Analysis (PCA). The first five principal components (PC) from transformation (a) are able to describe 94.15% of the original state space. Via transformation (b), only the first three PCs are necessary to describe 97.25% of the original state space. Uncorrelated state space (PC, (b)) is used for description of the BZ reaction trajectory. In this new state space, the trajectory is observably segmented into distinguishable clusters. According to the Rényi and Theiler definitions, the alpha parameters which contributed most to the PCs should be used for determination of the multifractal dimensionality of BZ reaction. Back to the original experimental data, we can pick up a typical image related to every single cluster. Therefore, we conclude that linear combination of several Rényi entropies is enough to describe properly and sufficiently the state space as well as the dimensionality of BZ reaction trajectory. Moreover, this uncorrelated state space is also directly suitable for the decomposition of BZ reaction state trajectory. The property of clusters distinguishability is caused by weighting of different probability events by alpha parameter in Rényi entropy equation. The theoretical presumption was also confirmed by several simulations of different common probabilistic distributions.

A51

CHEMICAL CROSS-LINKING AND HIGH RESOLUTION MASS SPECTROMETRY FOR MNKR-P1C PROTEIN STRUCTURE MODELING

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Determination of the three-dimensional structures of proteins has traditionally been realized by X-ray crystallography and NMR spectroscopy. Although these techniques provide high resolution atomic data, they have some limitations. Both NMR spectroscopy and X-ray crystallography require large amounts of pure analyte and are time-consuming techniques. Mass spectrometry combined with chemical cross-linking offers alternative approach to identify the protein fold. This method is fast, is general and uses small amounts of material.

Our aim was to gain insight into low-resolution structure of NKR-P1C protein, important activating receptor which plays a key role in eliminating virally infected and tumor cells, using unambiguous distance constraints derived from the chemical cross-linking data in combination with computational methods.

We used homobifunctional cross-linking reagents disuccinimidyl suberate (DSS) and disuccinimidyl glutarate (DSG), which react with primary amino groups, and heterobifunctional "zero-length" cross-linking reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), which couples carboxyl groups to primary amines. After cross-linking reaction, the SDS-PAGE of the cross-linking reaction mixture was performed and the band of cross-linked protein was excised and subjected to *in gel* digestion by endoproteinases Asp-N, Glu-C and trypsin.

The peptide mixtures from the enzymatic digest were separated by HPLC system coupled online to an Apex-ULTRA FT ICR mass spectrometer equipped with 9.4 T superconducting magnet. Cross-linking products were identified using MS3D software.

In order to build the protein structure model of NKR-P1C receptor, we have prepared recombinant NKR-P1C protein encompassing amino acids Ser89 – Ser223 and performed optimization of conditions for the chemical cross-linking reactions. Mass spectra of the crude product mixtures from the cross-linking reactions showed that 3× molar excess of DSS and DSG and 10× molar excess of EDC to the protein concentration gave the highest relative yield of protein containing a single internal cross-link. Optimal duration was chosen so that all unreacted cross-linkers were hydrolyzed. After LC-ESI-FT MS analysis, the experimentally obtained monoisotopic masses were compared with calculated masses of peptides and cross-linking products employing MS3D software. Masses corresponding to cross-linked intramolecular peptides were found. These cross-links provided new and very valuable throughspace distance information. It is the type of throughspace distance constraint that is the key to build the protein structure model of the NKR-P1C receptor.

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A52

CRYSTALLIZATION AND PRELIMINARY STRUCTURE ANALYSIS OF DHAA57 AND DHAA80 MUTANTS FROM *RHODOCOCCUS RHODOCHROUS*

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Haloalkane dehalogenases catalyze a reaction of great environmental and biotechnological significance: conversion of halogenated aliphatic hydrocarbons to their corresponding alcohols [1]. Practical use of these enzymes could be significantly improved by the availability of biocatalysts stable in the presence of organic co-solvents and at elevated temperature. New mutant variants of DhaA from *Rhodococcus rhodochrous* DhaA57 and DhaA80 with enhanced structural and kinetic stability in the presence of dimethyl sulfoxide and elevated temperature were recently constructed by directed evolution and site-directed mutagenesis. The main goal of presented project is to determine the 3D structure of the DhaA57 and DhaA80 mutants at atomic resolution in order to explore the effects of mutations on the enzymatic activity of modified protein from a structural perspective.

Crystallization experiments were performed using the sitting-drop vapor-diffusion method at temperature of 4°C. Crystals of DhaA57 grown from the precipitant containing 35% PEG 4000; and DhaA80 crystals were appeared in the solution consisting of 20% PEG 3350 and 0.2M sodium fluoride. Both crystals were tested on the home diffractometer at the IOCB & IMG AS CR in Prague. Diffraction data sets for DhaA57 and DhaA80 were collected at the synchrotron EMBL/DESY in Hamburg (Germany). Crystals diffracted to the resolution of 1.8 Å for DhaA57 and 1.9 Å for DhaA80, respectively. The DhaA57 crystals belong to the primitive triclinic space group P1, while the DhaA80 crystals to the primitive orthorhombic space group P222. The known structure of the haloalkane dehalogenase from *Rhodococcus sp.* [3] will be used as a template for molecular replacement. The process of the DhaA57 and DhaA80 structure refinement is currently in the progress.

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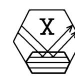

A53

THE STRUCTURE OF ENTEROVIRUS 71 AND ITS IMPLICATIONS FOR THE DESIGN OF CAPSID BINDING ANTI-VIRAL COMPOUNDS

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Enterovirus 71 is a picornavirus associated with fatal neurological illness in infants and young children. Stability of most picornaviruses is regulated by a pocket factor, a lipid molecule, located within a pocket of one of the capsid proteins. Binding of an immunoglobulin-like receptor molecule into the canyon, a depression on capsid surface, induces release of the pocket factor, resulting in particle destabilization and genome release. Nevertheless, the two EV71 receptors that have been identified do not belong to the immunoglobulin family. Here we report that EV71 retains the pocket factor suggesting that the destabilization of particles is regulated by its expulsion. Unlike in other picornaviruses, the pocket factor of EV71 is partly exposed on the canyon floor and interacts with polar residues. Thus the structure of anti-EV71 compounds will need to include a hydrophilic head group designed to interact with residues at the entrance of the pocket.

A54

ELI BEAMLINES FACILITY AS A NEW PROMISING TOOL FOR STRUCTURAL STUDIES OF MACROMOLECULES

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Knowledge of structure and dynamics of molecular systems is the prerequisite for understanding of function. While static structures of molecules are usually obtained from methods like X-ray crystallography or NMR spectroscopies, information about dynamics is typically obtained from various forms of time-resolved spectroscopies. Yet, the past decade has witnessed development of new experimental approaches combining ultrafast time resolution with detailed structural information. These methods are usually based on ‘standard’ time resolved methods, but ultrashort X-ray pulses are used for probing the structure of the studied system. One of the main obstacles preventing further development of these methods is lack of equipment producing sufficiently bright and short X-ray pulses at higher repetition rates. The Extreme Light Infrastructure (ELI) facility whose Czech pillar ELI Beamlines is being built near Prague, holds promise to meet requirements for experiments probing time-resolved structural dynamics. This contribution will provide overview of the ELI Beamlines facility and will discuss various options which this facility offers to study biological systems.

A55

COMPUTATIONAL METHODS FOR DETECTING LIGAND ACCESSIBLE PATHWAYS – APPROACHES AND BENCHMARKING STUDY

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The number of protein and nucleic acid structures stored in the RCSB Protein Data Bank has risen exponentially in recent years. This offers the possibility to study the relationship between the structure of proteins and their function. Information about empty spaces in proteins, i.e. pockets, cavities, pores or tunnels, can provide valuable insight into protein behaviour and properties. Finding and characterizing tunnels is fundamental to understanding the mechanism and intensity of many biochemical processes, such as the interaction of these proteins with their substrates or with drug molecules. This knowledge can find immediate applications in rational drug design, protein engineering, enzymology, etc.

Numerous algorithms for empty space detection in proteins have been developed and implemented. These algorithms are generally specialized for different types of volumes, such as shallow clefts called pockets, buried active sites with accessible paths called tunnels, buried volumes called cavities, or simply pores in membrane proteins. With respect to the applied approach for detecting different structural features, algorithms can be divided among a few classes [1]: grid-based methods, probe sphere filling methods, methods which utilize Voronoi diagrams and slice and optimization methods.

The software tools developed based on these various principles differ dramatically in time complexity, efficiency, and interactivity. Here we present an overview of the main approaches for finding tunnels, as well as a benchmarking study of the related software tools (i.e., Mole 2.0, Mole 1.4 [2], MolAxis [3], Caver [4]). The proteins used for benchmarking have been chosen as representatives of the classes of proteins which are generally accepted as interesting for the research of tunnels. The presented software tools are compared with emphasis on the speed, accuracy and user experience.

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A56

STRUCTURE OF MYRISTOYLATED MATRIX PROTEIN OF MASON-PFIZER MONKEY VIRUS AND THE ROLE OF PHOSPHATIDYLINOSITOL-(4,5)-BISPHOSPHATE IN ITS MEMBRANE BINDING

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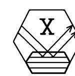
During the late phase of the Mason-Pfizer Monkey Virus (M-PMV) life-cycle, its immature virus particles are transported to the plasma membrane and bud out of a cell. Binding to the membrane is facilitated by an N-terminal domain of Gag, the matrix protein (MA), which is N-terminally myristoylated. Structural studies on HIV-1 and HIV-2 MAs have proven that the myristoyl serves as an anchor in the membrane, but while MA is in an unbound state the myristoyl is sequestered in MA. The change between these two states is called a myristoyl-switch. Saad at al. reported that the myristoyl-switch of HIV-1 MA is triggered by a phosphatidylinositol-4,5bisphosphate (PIP), which is a phospholipid found exclusively at cytoplasmic membrane.

We used NMR spectroscopy to determine the structure of myristoylated M-PMV MA and to observe its interaction with PIP. The structure of myristoylated M-PMV MA is generally similar to the structure of nonmyristoylated M-PMV MA, but shows some differences mainly in the orientation of the first helix and in its C-terminal part. The myristoyl is sequestered deeper than in HIV-1 MA. PIP interacts with myristoylated M-PMV MA. It binds to a similar part of M-PMV MA as to HIV-1 MA, but the interaction is weaker and PIP with shorter fatty-acid chains is not sufficient to trigger the myristoyl-switch. These results show that M-PMV MA interacts with the membrane in a similar way as HIV-1 MA, but shows some significant differences that can be contributed to distinctions in the life-cycle of these retroviruses.



A57

ROA STUDY OF CHONDROITIN SULPHATE AND ITS BUILDING BLOCKS

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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.

In our experiment, we measured the Raman and Raman optical activity (ROA) spectra of chondroitin A sulphate and its building blocks; GlcA, 1-O-methyl-GlcA, GalNAc, GalNAc-4-O-sulphate and GalNAc-6-O-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. 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.

Focus was placed on the interconnection between experiments and simulations: We performed *ab initio* calculations of Raman and ROA transitions and their intensities in order to obtain a more accurate interpretation of recorded spectra and observed phenomena. The initial geometries of most of the probable conformers were acquired from molecular dynamic simulations and quantum mechanical computations were performed using the Gaussian09 program suite, where implementation of analytical gradient calculations of optical activity tensors has lead to a significant decrease of computational time.

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A58

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF THE Folyl-poly- -L-glutamate HYDROLYSING ACTIVITY OF HUMAN GLUTAMATE CARBOXYPEPTIDASE II

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Human glutamate carboxypeptidase II (GCPII) acts as folate hydrolase in proximal small intestine, participating thus in the absorption of dietary folates by humans. Additionally, there are conflicting genotype-phenotype association data linking the GCPII H475Y variant to changed blood-levels of folate or homocysteine and to various pathologies. Despite the importance of GCPII as a folate hydrolase there is limited the knowledge of folate recognition and processing by the wild-type enzyme as well as virtually no information on the structural and functional consequence of GCPII H475Y polymorphism on folate hydrolysis. Here we present a series of detailed structural, kinetic and mutagenesis studies aimed at dissecting GCPII role in folate metabolism.

We first determined the X-ray structure of the GCPII H475Y variant at 1.70 Å resolution and using a newly introduced UPLC assay we compared the H475Y folate hydrolyzing activity on a panel of folyl-poly- -L-glutamates to the wild-type enzyme. Combined structural and kinetic data suggest near identity of both GCPII variants, putting into question a direct link between GCPII enzymatic activity and physiological levels of folate metabolites. To gain a deeper insight how GCPII recognizes and interacts with different folate species, we also determined X-ray structures of complexes between the inactive GCPII(E424A) mutant and a panel of naturally occurring polyglutamylated folates. The structural data revealed details of GCPII-folate interactions including the surprising engagement of the previously identified "arene-binding site" by the pteroate group of folates. To support and extend our structural observations, mutants of the GCPII "arene-binding site" W541A, R511L and R463L were produced and characterized in terms of the hydrolysis of the folyl-poly- -L-glutamates and inhibition profiles of pteroic acid.

A59

CELL STATES ON THE BASIS OF MANUAL DETECTION

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Living cells are dynamic objects which occupy certain region of space and time. The time-lapse microscopy experiment is the best way how to observe dynamic of living cells during their life. The main aim of investigation was analysis of cell states during cell cycle using method which was discovered in our Institute. As an object of our experiment we observed cell line MG63.

For our analysis we need to extract cells from time-lapse images. This was done manually, using software "Expertomica CellMarkerSci". We calculate Rényi entropy for every image using different Rényi coefficient values. The colour channels and different Rényi entropy coefficients may be combined to best discriminate individual states. For the processing of data was used software Unscrambler X 10.1, which provided principal component analysis (PCA) and clustering analysis for divided obtained data into several clusters. Number of cells and position in clusters depends on number of clusters, so each cluster contains different images. We were able to find cell state corresponding to each of the clusters. Such detailed analysis is extremely computationally intensive; however, it might be of high value for rapid diagnostics in medicine, biotechnology and any other discipline utilizing cell biology results.