Posters

P1

CALCULATION OF THE EFFECTIVE CHEMICAL SHIELDING ANISOTROPY IN L-ALANYL-L-ALANINE, CONFORMATIONAL AND CHARGE DEPENDENCE STUDY

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Biomolecular structure can be determined by the high-resolution NMR spectroscopy. Theoretical modeling of NMR parameters via modern quantum chemistry methods allows accurate predictions of structural parameters and dynamical behavior of important biomolecular compounds. Recently we determined the structure of the L-Alanyl-L-alanine di-peptide on the basis of complete assignment of isotropic chemical shifts and scalar coupling constants [1]. Another important NMR parameter, the cross-correlated relaxation rate, still requires theoretical modeling, in particular the dependence of the chemical shielding anisotropy (CSA) on the molecular structure [2, 3].

In this work we correlated the effective CSA calculated for atoms along the di-peptide backbone with its major descriptors, the torsion angles , . Further we investigated the dependence of the effective CSA on total charge of the di-peptide, i.e. for its anion, zwitterion, and cation forms that can be experimentally accessed at different pH. The geometry was optimized at the BPW91 / 6-311++G** level employing the PCM solvent model for all forms of the di-peptide. The NMR parameters were calculated using the B3LYP / IGLO-III / PCM approach. The calculated surfaces of the effective CSA can be readily utilized for more accurate interpretation of the cross-correlated relaxation rates.

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P2

COMPUTATIONAL SIMULATIONS STUDY OF HELIXES 90-92

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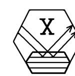
The Helix 90-92 system is a monomeric part of a 50S large ribosomal subunit from domain V of 23S rRNA. It is classified as a 3-way junction family, type C. Helix 92 represents crucial component of ribosomal peptidyltransferase center. Its most important part is a hairpin loop known as A-loop. A-loop directly interacts with aminoacyl-tRNA within the protein synthesis. These interactions, orienting tRNA properly and correctly to ribosome, involve Watson–Crick interactions between rRNA and universally conserved nucleotides 3´-end tRNA (C75). Kinetically slowest step by the selection of tRNA is the accommodation of aatRNA to A-site, followed by the release of Tu-factor from aatRNA and subsequently resulting to the rise of rapid peptide bond. This accommodation step includes the recognition of aatRNA by A-loop and the movement of A-site tRNA to proper position in the active site. Unfortunately, the exact nature of rearrangements occurring during accommodation of tRNA into the peptidyltransferase center remains still unclear.

Nowadays, posttranscriptional modification of this rRNA system was observed and shows the metylation of U2552 (2’-O-methyl). Thus the Helix 90-92 simulations can be divided into two main groups: Systems without and with methyled residue.

The project is devoted to three major subjects:

- to describe the methylation impact on Helix 92 conformation – mainly on U2552-C2556 base pair
- to characterize the overall structural dynamics and motions of the whole system and 3)to input simulated structures of Helices 90-92 into the context with the ribosome.



P3

CRYSTAL STRUCTURE OF RGS3 RGS DOMAIN

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RGS3 protein is a member of **R**egulator of **G** protein **S**ignaling (RGS) family. RGS proteins are primarily known as negative regulators of G protein signalling pathways due to their function as GTPase activating proteins (GAPs) for the subunit of heterotrimeric G proteins. The modulation of RGS action remains unclear but includes post-translational modifications, subcellular localization, and interactions with protein binding partners e.g. 14-3-3 protein.

The crystal structure of RGS3 RGS domain was determined at 2.3 Å resolution. It crystalized as a dimer connected by S-S bridge. The existence of the dimer was also proven in solution. The RGS domain is composed of nine -helices that fold into two small subdomains. The terminal subdomain contains the N and C termini of the box and is formed by 1, 2, 3, 8, and 9. Helices 1 and 9 lie in antiparallel orientation, juxtaposing the N and C termini of the box. The larger bundle subdomain, formed by 4, 5, 6, and 7, is a classic right-handed, antiparallel four-helix bundle. Helices 7, 8, and 9 form essentially one continuous helix with two bends. The RGS domain comprises of aproximately 130 AA. Thirty of these AA are conserved within the RGS family. The RGS domain 3D structure is absolutely conserved within the RGS family. Our structural study supplies another prove of this fact.

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P4

CRYO-SCANNING ELECTRON MICROSCOPY OF *Bordetella pertussis* ADENYLATE CYCLASE TOXIN EMBEDDED IN LIPID VESICLES

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The adenylate cyclase toxin (CyaA, ACT or Hly-AC) of *Bordetella pertussis* is a protein with a molecular mass of 200 kDa that belongs to RTX (repeat in toxin) protein family of bacterial pore-forming toxins. The CyaA toxin spontaneously inserts into eukaryotic membranes and it translocates its catalytic N-terminal domain into the cytosol, where catalyzes unregulated conversion of cellular ATP to cAMP. The C-terminal RTX hemolytic moiety accounts for binding of CyaA to target cells, for translocation of the adenylate cyclase domain and for making the cell membrane permeable by cation-selective pores. There are indirect evidences that CyaA could act either as a monomer or dimer/oligomer. In order to determine functional unit of CyaA in lipid bilayer we used high resolution cryo-scanning electron microscopy to visualize CyaA on the surface of lipid vesicles. CyaA was incubated with the suspension of liposomes and washed in bicarbonate buffer to remove non-specifically bound proteins. The CyaA- bound liposomes were quickly frozen in liquid nitrogen and left to evaporate in a Gatan Alto 2500 cryo-holder connected to a Jeol JSM-7401F field emission scanning electron microscope. CyaA molecules were seen as single protrusions with homogenous distribution on the surface of liposomes. The protrusions had an approximate diameter of about 15 nm indicating CyaA to be in monomeric form.

P5

STRUCTURE OF *Bombyx mori* CHEMOSENSORY PROTEIN 1 IN SOLUTION

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In insects, two different soluble protein groups have been identified in the antennal lymph. The first group comprises the Odorant Binding Proteins (OBPs). These proteins are approximately 150 residues long and contain six highly conserved cysteines forming three disulfide bridges. They are implied in the binding of pheromones and odorant molecules. The second group is formed by Chemosensory Proteins (CSPs). CSPs are smaller proteins of around 110 amino acids that contain only four cysteines linked by two disulfide bridges, at different positions than those from OBPs. Their signature motif is $CX_6CX_{18}CX_2C$.

The first proposed role of CSPs was based on their tissue localization. They are mainly expressed in chemosensory sensillae in antennae, legs, proboscis and other chemosensory organs and they have been attributed a role in carrying chemosensory or gustatory molecules. However, no binding to taste or odor molecules has yet been demonstrated. On the other hand, binding to pheromone compounds and fatty acids has been shown in *Mamestra brassicae* and in *Apis mellifera*. Moreover, they have a role in solubilizing cuticular hydrocarbons in ants. Thus, the role of CSPs can be extended to carrier proteins for hydrophobic ligands.

Here we report the determination of the structure of *Bombyx mori* CSP1 (BmorCSP1) by NMR. BmorCSP1 is expressed mainly in antennae and legs and was cloned from antennal cDNA. The overall fold of BmorCSP1 is globular and comprises six -helices. These helices span residues 10 14, 17 27, 35 49, 57 72, 75 85, and 92 100. The hydrophobic edges of the helices are formed mostly by leucine and isoleucine residues and therefore well suited to constitute a binding site for hydrophobic ligands.

P6

COMUTATIONAL STUDY OF RESTRICTION ENDONUCLEASE HINCII

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Restriction endonuclease HincII cleaves DNA at GTPyPuAC sequence. X-ray structure of Hinc II/DNA complex was determined with the central sequence GTCGAC in DNA 13mer [1] and in complex with $Ca2^{+}$ ions [2] which serves as an inhibitor. Magnesium is an essential cofactor for this enzyme. Cleavage leads to inversion of configuration at the scissille phosphate and results in two DNA fragments with 3'-OH and a 5'-phosphate.

We have examined the stability and/or dynamics of protein/DNA complex HincII by computational tools. Although molecular dynamics is not able to follow the reaction mechanism itself, it may serve as a good tool to describe reaction partners or intermediates. We want to describe the structure of the complex to provide detailed view of the active site and relationships in it. The stability of the ions coordination can be seen from our simulations, as well as electrostatics around them. We attempt to bring some ideas about the structure of the active site and possible role of the ions in it. We have found strong electrostatic potential around catalytic aminoacids, which is ballanced by presence of ions. It leads to the idea that B site ion is required at least to stabilize the reaction environment.

Exploring molecular tunnels brings some explanation of the ion transport between active sites and bulk solvent. Although electrostatics can strongly control this process (and maybe refuse it), this possibility of ion exchange is still there. However, negative electrostatic potential is concentrated around active sites and around DNA (due to phosphates) and the ions may get inside during the early stage of folding.

Computational tools can provide a deeper insight into the structure and dynamics of the protein/DNA complex active site and can describe experimentally unknown features of the complex. Also the role of possible mutations in DNA or protein can be usefull in evaluation of activity and prediction of complex dynamics.

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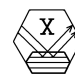

P7

COMPUTATIONAL STUDY ON RUTHENIUM(II) COMPLEXES AND THEIR INTERACTIONS WITH NA BASES

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Structures = of ruthenium(II) piano-stool complexes [(arene)Ru(II)(en)X] (arene = benzen, p-cymene; en = ethylendiammine; X = Cl, H_2O, NA basis) were computed and compared with experimental measured data reported by groups of P. J. Sadler and V. Brabec. DFT/B3LYP optimizations and MP2/6-31**G++ energy analysis were performed in both gas phase and COSMO regimes. Bonding energies of individual ligands and stabilization energy of whole complex were determined together with charge analyses. NA bases were bound in several positions and their energies were compared. Reaction profiles of hydratation reaction and water replacement by purine bases were examined in supermolecular approach. Both thermodynamical and kinetical descriptions of these processes were obtained. Changes of the average local ionization potential along the reaction coordinate were evaluated.

P8

NMR STUDY OF PROTEIN CD69

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Protein CD69 is transmembrane disulphide-linked glycoprotein of natural killer cells, which are important in the immune response to cell-surface displayed antigens. C13 and N15 labeled sample was prepared for NMR experiments. Resonance frequencies of 74% backbone atoms and 39% side chains atoms have been assigned. Titration experiments with various mono- and oligosaccharides have been performed. N15 relaxation data have been measured and used to calculate the rotational diffusion coefficient. The results showed that the studied protein was present in a form of a dimer and that it exhibited specific binding affinity towards several ligands.

P9

LYS^{735} PARITICIPATES ON THE ATP BINDING TO THE ISOLATED C-TERMINUS TRPV1

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Transient receptor potential channel vanilloid receptor 1 (TRPV1) is member of TRP ion channel family. TRPV1 is responsible for a heat and chemical – evocated pain responses and it could be activated by vide range of chemical stimuli. Its activity is modulated by intracellular ATP, by direct interaction with ATP binding domains. Despite missing experimental evidence of the tertiary structure, TRPV1 is predicted to be composed of six transmembrane domains, short hydrophobic stretch that line the pore region and two cellular terminuses.

In this study, we have tested the ability of the Walker A motif located on the isolated C-terminus of TRPV1 to bind ATP. We have identified, using steady state fluorescence experiments and molecular biology tools, single amino acid residues that play crucial role in ATP binding to Walker A domain. We have mutated following amino acids residues for alanine one at a time: P732A, D733A, G734A, K735A, D736A, and D737A. Employing TNP-ATP competition assay and FITC labelling and quenching experiments, we have confirmed the key role of the K735 residue for the binding of the nucleotide. These experimentally obtained data verify the predictions of the molecular homology model of the C-tail of TRPV1.This model has been created using a restraint-based comparative modelling approach, and the value of K_D fits within the experimental error with the value estimated previously (3.3 mM) from electrophysiological experiments [1].

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P10

STRUCTURE – FUNCTION RELATIONSHIPS OF PROTEIN-DNA INTERACTIONS

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Despite extensive effort to understand structural features of protein-DNA complexes governing specific DNA-protein interactions, neither general, nor simple rules have been found to date [1, 2]. Understanding of these principles would serve as a guideline for protein-DNA recognition in various families of regulatory and other DNA-binding proteins. We have recently reported conformational variability of DNA based on the analysis of torsion angles in almost eight thousand dinucleotides from well resolved DNA x-ray structures [3]. Although double helical A and B conformations represent majority of the population, we have defined many substates that represent A-to-B, B-to-A, BI-to-BII, and other unusual conformations. Such a detailed conformational characterization allowed us, in the current work, to ascribe different DNA conformational substates to four groups of transcription regulatory proteins, three groups of DNA-binding proteins and four groups of enzymes. The majority of examined DNA-protein complexes exhibit the BI and BII conformations as the most populated substates. These conformations are observed for binding with different protein motifs such as helix-turn-helix, helix-loop-helix, leucine zipper and zinc fingers. Within the BI and BII DNA conformations, no rules governing specific recognition of DNA sequences by individual protein structural motifs were observed. This conclusion confirms the results of the previous study [1] that the recognition via certain motifs does not depend on functional characteristics of protein-DNA complexes. In contrast to these findings, here we also show that other proteins in DNA-protein complexes bind a substantial number of A type or A-to-B type of DNA conformations. Analysis of these interactions suggests that different protein domains, such as TBP-like motif, are more specific for classification of protein-DNA complexes according to their function in transcription. Identification of amino acids involved in side chain/base interaction among regulatory and DNA binding proteins distributed into distinct families is also being performed to further explore the common and unique principles responsible for protein-DNA recognition.

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P11

RAT NKRP1A AND NKRP1B PROTEINS STUDIED BY VIBRATIONAL SPECTROSCOPY AND MOLECULAR MODELING

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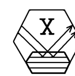
Lectin-like receptors of the C-type lectin family are important antigens at the surface of immune cells. Natural killer receptor protein 1 (NKRP1) is a lectin-like receptor of a natural killer cell, where NKRP1A is an activating and NKRP1B an inhibitory one. The activating and inhibitory signals launched by these receptors regulate effector functions of natural killer cells such as killing malignantly transformed, viral infected, or stressed cells [1]. NKRP1 proteins are unique for their high affinities for the classical ligands of C-type lectins – carbohydrates. These proteins prefer to bind linear but not branched oligosaccharides [2]. Binding of complex carbohydrates opens a possible way to medical applications.



Therefore, the aim of our study is to characterize structure of NKRP1 proteins and structural changes upon ligand binding. To initiate ligand binding studies, we have prepared soluble forms of rat NKRP1A and B proteins by recombinant expression of their extracellular domains in E. coli followed by in vitro refolding. Structure of the proteins was investigated by infrared, Raman and drop coating deposition Raman spectroscopy. Homology modeling of the proteins was provided as well. It was found that the structure of both proteins is very similar and spectral differences are caused mainly by a different amino acid composition of the peptide chains. However, significant differences exist. The influence of binding of ligands, complex branched oligosaccharides, on the secondary structure as well as on local environments was followed by infrared and Raman difference spectroscopy. Protein behaviour in solution was studied by molecular dynamics simulation. Results from theoretical calculations were critically confronted with experimental measurements, whereas good agreement was reached.

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P12

VIBRATIONAL OPTICAL ACTIVITY SPECTRA SIMULATION FROM CLASSICAL TRAJECTORIES

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Interpretation of infrared and Raman spectra are often based on ab-initio methods. These, however, are limited to relatively small molecules. Therefore, we try to develop combined QM/MM approaches based on the autocorrelation functions that can be used for more complicated systems, like hydrated proteins. At the current stage, we focus on the calibration of the methodology with respect to accurate ab-initio data. The results show that the simplified calculations can reproduce the spectra if a large number of MD trajectories are averaged. In the future, we plan to extend the simulations to larger molecules with usual force fields (Amber).

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P13

STRUCTURAL CHARACTERIZATION OF HUMAN PROTEIN KINASE CKI EPSILON

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Casein kinase I epsilon (CKI) is a human homologue of *Drosophila* Disc overgrown (*Dco*). In case of mutations in the *Dco,* mutant larvae (*Dco*^{3}) fail to arrest growth of imaginal discs when they reach their normal size and the discs grow continuously to several times the wild-type final size [1]. Moreover a remarkably high frequency of somatic mutations was found in CKI in breast cancer [2]. In this project we would like to identify proteins which form stable association with CKI under *in vivo* conditions and characterize the topology of the CKI complex. These investigations will provide a molecular basis for understanding of CKI function and for design of drugs modulating CKI activity.

This work was supported by Grant Agency of the Czech Republic (301/07/0814).

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P14

INFLUENCE OF THE MUTATION TOPOLOGY ON PROPERTIES OF HUMAN CYSTATHIONINE BETA-SYNTHASE (CBS) POLYPEPTIDE

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Cystathionine beta-synthase, a key enzyme of the methionine cycle, catalyzes condensation of toxic homocysteine and serine to cystathionine. More than 140 different mutations have been found. However only a small proportion has been characterized in more detail.

In this study we have classified topology of 27 known mutations based on the CBS active core structure and the structure of CBS domains in various proteins. Selected mutations represent disturbances in various protein domains and cover about one fifth of all mutations found in CBS patients. Further we expressed all 27 mutants in *E.coli* and Chinese hamster ovary cells expression systems. We measured the catalytic activity and monitored the degree of assembly by native western blotting. We correlate tetramer amounts and activity with topology of CBS mutations.

Our data suggest that a substantial proportion of selected mutations have an internal propensity to misfold and aggregate. The properties of the mutant enzyme is significantly affected by the location of the mutation. Mutations buried in CBS globule cause more severe disturbances of quaternary enzyme structure and activity than the mutations at the surface of the enzyme.

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P15

HUMAN $_1$-ACID GLYCOPROTEIN INVESTIGATED BY RAMAN SPECTROSCOPY AND RAMAN OPTICAL ACTIVITY

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$_1$-Acid glycoprotein (AGP), also known as orosomucoid, contains 183 amino acids with 21 possible substitutions in one polypeptide chain linked by two disulfide bonds. The content of carbohydrate moiety is slightly above 40% of the total molecular weight of 41 kDa of the protein. Five branched carbohydrate units terminated by sialic acid residues are linked to Asn residues of the peptide chain. It is known that AGP plays a role under inflammatory or other pathological conditions and is able to bind basic drugs and certain steroid hormones [1]. However, the exact role of AGP as well as its 3D structure is not entirely clear.

A model of 3D structure of AGP has been proposed by an approach that combines molecular modeling and vibrational spectroscopy [2]. It shows that AGP folds as a symmetrical all- protein dominated by an eight-stranded antiparallel -sheet. Binding of progesterone is described with a coincident transformation of the -helix above the binding pocket into antiparallel -sheet [2]. Here we investigated ligand binding of AGP followed by three ligands (natural and artificial) with different physico-chemical properties, i.e. progesterone, propranolol and warfarin. Raman difference spectroscopy shows the same pattern upon binding of all ligands which suggest presence of only one high-affinity binding site in AGP. The spectral changes reflect a presence of Trp^{122} in the binding site as was proposed by molecular modeling and dynamics simulations. The ligand release must be connected with a reverse structural behavior where the carbohydrate moiety in interaction with membranes could play a role. Therefore, we investigated structure of AGP with respect to carbohydrate moiety and its potential role in ligand binding/release by Raman optical activity (ROA). It was demonstrated, on the case of N-acetyl neuraminic acid, that ROA spectra of carbohydrates are very useful in interpretations of ROA bands of glycoproteins.

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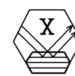

P16

TEXT MINING OF ARTICLES IN METABOLOMICS RESEARCH

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More than 80% of information is stored in textual form. A large number of papers appears every day. It is beyond power of anybody to go through even a part of the published works. A use of search machines yields excessive numbers of results and does not make a problem more simple. There are additional difficulties in biochemical research such as an abundance of terms with ambiguous nomenclature, acronyms and abbreviations. That is the reason for a boom of text mining methods in this area. Text mining methods transfer unstructured textual data into a structured data matrix, which can be evaluated with data mining methods.

We have developed a program, which takes a list of terms we are interested in and searches for occurrence of them in abstracts or articles from the internet databases or generally internet sites. The program is based on the algorithm Aho-Corasick and works as a efficient searching machine. Running articles through this algorithmus takes almost the same time for 10 or 5 000 given terms. The program goes through the articles and creates a data matrix from the found terms. At the end it sorts its results and deletes useless information.

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P17

UNRAVELING THE PROBLEMS OF PROTEIN - SACCHARIDE INTERACTIONS VIA COMPUTATIONAL CHEMISTRY

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Detailed knowledge of interactions between proteins and small molecules is important for understanding of significant processes in organisms. Saccharides and various glycoconjugates play a significant role in many host-pathogen interactions. Lectins are sugar-binding proteins of non-immunoglobulin nature that agglutinate cells or precipitate glycoconjugates. Their specificity is usually defined by the monosaccharides or oligosaccharides that are best at inhibiting the agglutination or precipitation the lectin causes. Lectins are of interest because of their wide variety of properties and potential applications (pharmacology, immunology, cancer therapy, agriculture ...).

Since host carbohydrates have been known for many years to constitute specific attachment sites for pathogen protein receptors, there is a great interest in structure-function studies of bacterial proteins enabling the pathogen attachment to host glycans. However, only a limited number of their complexes with receptors have been characterized by crystallography [1]. The molecular modeling methods can help in the study of the complexes.

The study will be focused on docking of a set of monosaccharides into two different lectins originally from bacteria *Pseudomonas aeruginosa* (PA-IIL) [2] and *Ralstonia solanacearum* (RS-20L) using the Dock v. 6.0 program [3]. The best docked structures (using standard scoring function) were scored again using AMBER scoring function with solvation energy based on implicit solvent model. The same structures were also solvated using TIP3P water model and MD simulations have been run. The binding energies were afterwards calculated from the trajectories. There will be shown the differences in calculated binding energies by using different scoring functions and also the effect of solvation energy on the binding energy.

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P18

MOLECULAR DYNAMICS: MOLECULAR DOCKING SIMULATION OF SUBSTRATES AND INHIBITORS OF BETA-N-ACETYLHEXOSAMINIDASE OF *Aspergillus Oryzae*

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Fungal beta-N-acetylhexosaminidases catalyze the hydrolysis of chitobiose into monosaccharides. It plays an important role in life cycle of the fungus for formation septa, germ tubes and fruit bodies. The interesting features of the enzyme from *Aspergillus Orizae* are its high catalytic activity and good relationship with a wide range of modified substrates [1]. Recently was examined the tolerance of beta-N-acetylhexosaminidase to C4- and C6 -modified carbohydrates [2]. Our main interest is to examine possible substrates and inhibitors of the mentioned enzyme.

Models of modified ligands were prepared in Yasara – p-nitrophenol-2-acetamino-2deoxy-6-sulfo-beta-D-gluco pyranoside (normal and reduced at sulfo-group) and p-nitrophenol-2-acetamino-2deoxy-beta-D-glucopyranosi de. Partial charges and force field parameters were calculated in Gaussian for the YAMBER2 force field. The substrates were docked into the active site of the enzyme and data were collected for 5 ns of molecular dynamics simulation. The temperature was adjusted to 298K. The experiment was carried out in TIP3 water, periodic boundary conditions were applied and pressure was kept constant to account for the changes of the structure in solution.

To predict the stability of the complex "substrate-enzyme" and to examine the possible inhibition ability of certain substrates we calculate internal and binding energies over time and analyze displacement of critical amino acids.

One of the predicted structures shows a similar binding energy as the natural substrate, with a similar small contribution of estimated solvation contribution to the binding energy. A structural analysis gives clear theoretical evidence that the substrate might work as a competitive inhibitor. These results will be used by organic chemists to synthesize this substrate and to verify our results.

The support by grants from the Ministry of Education of Czech Republic (MSM6007665808, MSM0021620835, LC06010), the Institutional Research Concept of the Academy of Science of the Czech Republic (No. AVOZ6087 0520) and the Grant Agency of Czech Republic (203/05/ 0172) is acknowledged.

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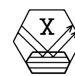

P19

STRUCTURAL STUDIES ON THE HSDR SUBUNIT OF THE ECOR124I ENDONUCLEASE FROM *E. coli*

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Many genetic processes require the action of large protein machines that act on DNA. We are currently concentrating on the Type I restriction enzymes which are complex molecular machines that protect bacteria by cutting invading viral DNA in an ATP-dependent manner [1]. The complex is composed of three distinct subunits responsible for site-specific DNA sequence recognition (HsdS), methylation (HsdM) and ATP-dependent DNA translocation and cleavage (HsdR).

We are interested in solving the structure of the 119 kDa HsdR subunit of the Type I restriction-modification enzyme EcoR124I (R.EcoR124I) from *E. coli*. Solving the structure will shed the light onto the enzymatic action and on the protein-DNA interface.

We have recently crystallized the motor subunit (HsdR) of the EcoR124I enzyme and preliminary crystallization studies showing that crystals belong to the primitive monoclinic space group P2/P2(1) and with lattice parameters a = 86.0 Å, b = 125.1 Å, c = 128.8 Å, = 108.1 . Native data were collected to 2.45 Å resolution at the X12 synchrotron beamline of the EMBL Hamburg.

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P20

SINGLE-CELL FLUORESCENCE SPECTROSCOPY

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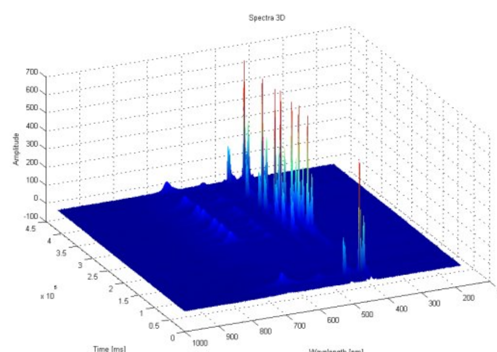
We are developing software for spectrum analysis measured in single living cell. We want to automatize analysis of measured data and to accelerate generating of results.

We measure cells of *trichodesmia.* We are measuring changes in spectra of one cell selected in the sample. Cell is irradiated by the blue light and intensity is changed according to the protocol. Spectrum is measured by spectrometer in range from 177 [nm] to 992 [nm] (from IR to UV). Output of measurement is the large table with light intensities measured on different wavelengths (rows) and time (columns) of measurement. Fluorescent parameters like F_M, F_0, F_S are calculated of this table. Due the size of this table cannot be used common available tools like MS Excel or OpenOffice Calc etc.

Therefore I am developing program in the Matlab environment, which allows to process this large data set and to automatize generating of desired results. Program was named 'Sandra'.

Illustrative picture displays table in 3D representation. This table has size 1044x1630 values. This is repeated measurement several times during the day. Manual processing of one table using MS Excel takes one day in the case that we use only of part measured spectra and required spectra is averaged. We are able to do the same and without



restriction which we mentioned in several minutes using our software Sandra.

Up to now we have solved head parts like reading data of file, visualization data in 3D and partially plotting of fluorescent parameters in depend on wavelength.

The aim of those calculations is to express fluorescent parameters like functions wavelength (F_M( ), F_0( ),F_S( ),...) of measured data. Later we will concentrate on calculation time delays between excitation light and emission light of cell, which will allow us to better understand to elementary processes in metabolism of living cell.

P21

A COST-EFFECTIVE APPROACH FOR AMINO-ACID-TYPE SELECTIVE ISOTOPE LABELING OF PROTEINS EXPRESSED IN *Leishmania tarentolae*

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We report a cost effecient approach for preparation of amino acid type selective (AATS), isotopically labeled proteins for nuclear magnetic resonance (NMR) spectroscopy using the *Leishmania tarentolae* expression system. The method is based on cultivation of the *L. tarentolae* inducible expression strain in a rich complex (BHI) medium supplemented with labeled amino acid(s). In this protocol, a labeled amino acid is deliberately diluted in the medium of undefined composition, which leads to a low level AATS isotope enrichment upon protein over-expression. Although low level isotope enrichment implies the decreased sensitivity of NMR experiment, the use of a rich complex medium leads to a two to three fold increase of the yield of the recombinant protein and, importantly, more than 10-fold reduction of the overall costs as compared to the recently established protocol for isotopic labeling using synthetic media (Niculae et al., 2006). We show that low-level enrichment does not compromise an NMR experiment and makes preparation of the recombinant proteins over-expressed in *L. tarentolae* economically viable. The method is demonstrated for selective labeling of ~27 kDa enhanced green fluorescent protein (EGFP) with ^{15}N-labeled valine.

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P22

THEORETICAL STUDY OF CISPLATIN INTERACTIONS WITH GLYCINE IN GAS-PHASE AND IMPLICIT WATER SOLUTION – COSMO

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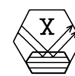
Interactions of hydrated cisplatin complexes with glycine were explored. The square-planar cis-$[Pt(NH_3)_2(H_2O)X]^+$ complexes (where X = Cl, OH and H_2O) were chosen as models for mono- and dihydrated reactants. Theoretical calculations using DFT techniques with B3LYP functional were performed. Both gas-phase and polarizable continuum model (in COSMO version) were employed for the reaction energies and bonding energies determinations in approach of isolated molecules as well as supermolecular complexes. The formations of monodentate complexes by replacing aqua-ligand with N and O atoms of the amino acid represent exothermic processes. The formation of chelate structures is an exothermic reaction for dihydrated form of reactants.



P23

NMR STUDY OF A SINGLE-STRANDED DNA BINDING TO THE C-TERMINAL DOMAIN OF RETROVIRAL PROTEASE

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Proteases play a crucial role in the retroviral viral infection but the mechanism of their regulation remains unclear. The C-terminal domain of the retroviral proteases, rich in glycines, has been proposed to bind RNA. Biochemical tests showed that the C-terminal domain binds single-stranded oligonucleotides (both RNA and DNA) without inhibiting the proteolytic activity. The N-15 labeled C-terminal domain of mouse intracisternal A-type particles endogenous retrovirus (CT) was investigated using NMR. CT was titrated with a single-stranded DNA 20-mer. The titration was monitored in 2D HN-HSQC and 1D proton spectra. 3D TOCSY-HSQC, NOESY-HSQC, and HNHB spectra were recorded on the over-titrated sample. The protocols of the overexpression on minimal media have been optimized and C-13, N-15 labeled C-terminal domain and C-13, N-15, H-2 labeled full-length protease samples were prepared. A set of triple resonance experiments have been measured. The assignment of the C-terminal region is presented.

P24

EXCITONIC INTERACTIONS OF PHOTOSYSTEM II REACTION CENTER STUDIED BY MOLECULAR MODELING TECHNIQUES

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Temperature dependent changes in excitonic interactions between pigments of photosystem II reaction center (PSII RC) from Thermosynechococcus elongatus upon reduction of primary acceptor pheophytin (D1-Pheo) have been studied by molecular modeling techniques. Six molecular dynamics (MD) simulations of so-called dark-adapted and light-adapted equilibrated PSII RC model in overall duration of ~20ns provided valuable information about structural changes in pigment and protein part of PSII RC complex at atomic level. In order to follow time and spatial distribution of structural changes in protein environment of D1-Pheo four protein layers in different distances from D1-Pheo were selected. Results from conformational analysis of all protein layers and eight core PSII RC pigments are presented and discussed. The major structural changes were detected for protein layers of D1 protein and for PSII RC pigments of active D1-branch. Absorption spectra calculated on equilibrated dynamic structures of PSII RC complex developed by performed MD simulations at 298 K and 77 K were in good accordance with experimental PSII RC absorption spectra [1, 2].

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P25

BIOCOMPUTATIONAL PREDICTION OF SMALL NON-CODING RNAS IN *Streptomyces coelicolor*

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A prediction of small non-coding RNAs (ncRNAs, sRNAs) in gram-positive differentiating bacteria of *Streptomyces* is presented. The prediction is based on sequence conservation within intergenic regions (IGRs) of *S. coelicolor* and *S. avermitilis* genomes, and predicted termination factors.

58 novel *Streptomyces* sRNAs were predicted and tested for expression. 15 of them have been shown experimentally to be expressed during cell development at standard conditions.

Among the predicted *Streptomyces* sRNAs, m1-like, 4.5S-like and spot42-like RNA candidates were identified computationally using sequence and / or structural similarity with known sRNAs of different bacteria. The already known *Streptomyces* tmRNA was also identified as a positive control.

As termination factors for computational identification of the sRNAs, either predicted Rho-independent terminators or new putative transcription terminators were used. The new termination factor was proposed as it most likely terminates m1-like sRNA and other two sRNAs that were computationally identified and experimentally validated with high reliability.

P26

HOW TO DEAL WITH THE MULTI-SCALE DYNAMICAL SYSTEMS: A CASE STUDY – MODEL FOR PHOTOSYNTHESIS, PHOTOINHIBITION AND PHOTOACCLIMATION

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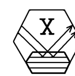
The multi-scale modelling and computation became essential in solution process of multi-scale systems frequently arising in chemistry and biology. The systems with multiple time-scales are characterised by the wide separation between the *O*( ) time-scale, and the *O*( ), or even *O*( ). In this paper we describe three different ways to solve the problem of microalgal photosynthesis under periodic intermittent light. The lumped parameter model for photosynthesis, photoinhibition and photoacclimation in microalgae represents the so-called stiff system, i.e. the dynamical system with multiple time-scales. The separation of time-scales in the system is modeled by a small parameter . From a mathematical point of view, the problem in hand is a system of two ordinary non-linear differential equations (ODE), and we have assumed that the phase-space can be decomposed into the slow phase, x_3, and the fast one, x_2. Ordinarily, the ODE system is not amenable to analytical treatments and requires an efficient computational tool for its solution. However, in our case, i.e. for the piecewise constant input (light intensity u), is the analytical solution reachable because we deal with the linear ODEs in both subintervals of light-dark cycles period: h_a - light-off interval, and h_b - light-on interval. Consequently, we are able to evaluate the average value of the fast phase x_2 (which is reflecting the rate of photosynthesis in microalgal culture) depending on the period of light-dark cycles $h = h_a + h_b$. Next, we can analytically compute the solution of the same ODE system applying two common approaches for solving the stiff systems: (i) neglecting the fast system dynamics, and (ii) neglecting the slow dynamics. Once having the exact analytical solution, we can observe the limits of validity of both approximated solutions. The presented simulation results based on published data demonstrate the perfect concordance between the theoretical assumptions and the numerical results and could serve as the motivation example for applying the multi-scale method, and at the same time, to eliminate some frequent errors in treatment of stiff systems.



P27

DETERMINATION OF STRUCTURE OF DOUBLE MUTANT T41I/T78I OF M-PMV MATRIX PROTEIN

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The Mason-Pfizer`s Monkey Virus (M-PMV) is a prototype of D type retroviruses. In type B and D retroviruses inmature virus particles pre-assemble in cytoplasma, whereas in type C retroviruses (HIV) Gag is targeted to the plasma membrane, where the particle formation occurs. The N-terminal domain of Gag, the matrix protein (MA), plays a critical role in determining this morphogenic difference. Several single- or multi-point mutations of MA have been described that alter various stages of the M-PMV life cycle. Our goal is to characterise the influence of mutant proteins in M-PMV on the molecular level by studying their structures. The three- dimensional structures of the wild-type and R55F mutant, a mutant which changes capsid assembly from D type to C type, are known so far.
In this work we focus on the determination of the three-dimensional structure of the double-mutant T41I/T78I, which doesn`t affect assembly and transport of immature virus particles. However, these particles are unable to bud through the cytoplasmatic membrane and rather accumulate on it. We determined the three-dimensional structure of the MA mutant using heteronuclear NMR spectroscopy. For calculation of the structure we used distance restraints obtained from N and C edited NOESY spectra and residual dipolar couplings measured in polyakrylamide gel. Comparison of the calculated structure and the structure of the wild-type proteins shows that the mutation altered angles between protein helices. In contrast to wild-type proteins, mutated isoleucines 41 and 78 are oriented inside the protein core where they may interact with a myristic acid which is linked to the N-terminus. This finding supports a hypothesis that the phenotypic change of the mutant is caused by enhanced interaction of the myristic acid with the protein core, which prevents association of immature viral particles with the cytoplasmatic membrane.

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P29

HIGH-COVERAGE PROTEOMIC IDENTIFICATION OF POST-TRANSLATIONALLY MODIFIED PROTEINS

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Proteomics is systematic analysis of proteins for their identity, quantity and function. It is a very important tool for study of life processes in cells, tissues or organisms. In contrast to a cell's static genome, the proteome is both complex and dynamic. Proteome analysis is most commonly accomplished by the combination of a proper separation technique, mass spectrometry and bioinformatics.

Both top-down (combining protein separation with MS analysis of intact proteins) and bottom-up (MS analysis of digested proteins) proteomic approaches were used for a detailed characterization of various proteins with the aim to obtain the high-coverage of the primary structure including post-translational modifications. The influence of different proteomic protocols (differing in separation technique, enzyme and digestion procedure) on the extent of coverage of protein primary structure was studied. The most successful protocols were in-gel digestion of the alkylated protein with trypsin and in-solution digestions of the nonalkylated protein with trypsin or trypsin/chymotrypsin mixture.

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P30

EXPRESSION, MITOCHONDRIAL LOCALISATION AND ISOLATION OF HUMAN CELL INDUCED DEATH EFFECTOR - A (CIDEA)

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CIDEa, CIDEb proteins are related to both N terminals of the heterodimeric DNA fragmentation factor DFF, consisting of the 40-kDa caspase-3-activated nuclease (DFF40 or CAD), & its 45-kDa inhibitor (DFF45 or ICAD) [1]. The DFF45&DFF40 complex is cleaved by caspase-3 and released nuclease then causes apoptotic DNA fragmentation. CIDE-induced apoptosis is not sensitive to caspase inhibitors but is inhibited by DFF45. The N-domain of CIDEa binds to the homologous domain on DFF45 opposing its inhibitory effect on DFF40. However, mitochondrial localization and CIDEb(a) dimerization is likely required for induction of apoptosis [2].

In this work we have confirmed mitochondrial localization of recombinant human CIDEa in W303 and JB516 yeast strains of *S. cerevisiae* and of human CIDEa or CIDEa-fused with a red fluorescent protein (RFP-CIDEa) in selected culture cells, such as embryonic kidney 293T cells, hepatocellular carcinoma HEPG2 cells, and insulinoma INS1-E cells. The CIDEa import into the inner membrane was proven by immunodetection of fractionated mitochondria and its identity was verified by Western blotting and by MALDI-TOF-assisted peptide mapping of the trypsinized samples of isolated yeast mitochondria. RFP-CIDEa transfected to cultured cells was co-localized with mitotrackers and with mitochondrially-targeted green fluorescent protein. Further we transformed Rosetta Competent cells with His-tag CIDEa and isolated protein on Ni –NTA agarose column. Pure protein was used for reconstitution and protein-protein interaction studies.

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P31

OPTICAL SPECTROSCOPIC STUDIES OF L-ALANYL-L-ALANINE

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Many spectroscopic methods used in analyses of biomolecules make use of their chirality. For example, the electronic circular dichroism (ECD) spectroscopy and Raman optical activity (ROA) are often used for structural and dynamical studies of peptides and proteins. The interpretation of the spectra is based on *ab initio* computations, which cannot be applied directly for bigger molecules. Therefore, we study the role of geometry fluctuations and the polar solvent (water) in formation of the spectral shapes of usual peptide structures on a simple model dipeptide – L-Alanyl-L-Alanine (Ala-Ala). The solvent is modeled explicitly as well as by a polarizable continuum dielectric model (PCM).

Both ECD and ROA spectra of Ala-Ala dipeptide in all the three ionic forms were simulated using combined quantum mechanics and molecular mechanics (QM/MM) procedures. Cluster geometries were obtained from MD simulations (TINKER software, AMBER forcefield).

In the case of ECD, calculated spectral frequencies and intensities reasonably well correspond to the experiment, but only when the explicit solvent is used. Also the amide geometry dispersion has to be included for faithful modeling. Preliminary ROA simulations indicate that the Raman spectroscopy probes the peptide structure more locally, while the ECD spectra are more sensitive to the solvent.

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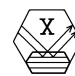

P32

CONFORMATIONAL CHANGES OF FoxO4-DNA BINDING DOMAIN UPON BINDING TO 14-3-3 PROTEIN

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14-3-3 proteins are abundant regulatory molecules expressed among all eukaryotes. Their function is represented by protien-protein interactions and thus regulation of the function of the binding partners. Seven isoforms of 14-3-3 proteins have been found in human and over 200 target proteins is currently known to interact with some of these isoforms (e.g. Cdk2, p21^{CIP1}, p27^{Kip1}, p53, MDM2, BAD, A20, TERT, ExoS, Raf-1) [1,2]. 14-3-3 proteins preferentially bind the conserved motif RXY/FXpSXP in phosphorylation dependent manner [5]. Phosphorylation-independent binding has been observed to be important in several cellular processes [3]. Versatile 14-3-3 proteins action is involved in cell signaling events, enzymatic or binding activity of bound ligands and localization within the cell. Despite a large number of the X-ray structures of 14-3-3 proteins and their shorted ligands the exact mechanisms of 14-3-3 action remains unclear. It seems to have distinct ways of action such as hindrance of binding site for other component of the cell followed by e.g. sequestration, inhibition of phosphorylation or degradation [4]. On the other hand 14-3-3 binding of the ligand causes significant conformation changes that leads to altered function of the ligand. 14-3-3 proteins are preferentially homodimeric and thus contain two binding. It is thought that rigid character of 14-3-3 protein's structure may facilitate conformation change and was proposed that 14-3-3 proteins act like molecular anvil when bind ligand by both binding sites. The molecular anvil theory says that by the binding of two sites of ligand by one molecule of 14-3-3 may induce greater changes of the ligand structure and modify its function (e.g. enzymatic or binding affinity) [4].

Protein FoxO4 (Afx) is member of the family of forkhead transcription factors regulating life span and apoptosis. DNA binding domain (DBD) is responsible for interaction between FoxO4 and DNA. PKB-induced phosphorylation of FoxO4 at threonine and serine residues (28T, 198S) generates two 14-3-3 protein binding sites. 14-3-3/FoxO4 protein complex is subsequently exported from the nucleus via 14-3-3 hindrance of the nuclear localization sequence (NLS) [6]. It is also known than phosphorylation and further 14-3-3 binding to FoxO4 disrupt FoxO4/DNA complex [7].

To illustrate the molecular anvil theory of 14-3-3 proteins we have investigated conformational changes of FoxO4-DBD caused by 14-3-3 protein binding to one or both binding sites. We have constructed mutants of 14-3-3 protein without any tryptofan residue and mutants of FoxO4 containing two tryptofan residues within the DBD. Fluorescence anisotropy decays of single and double phosphorylated FoxO4 mutants complexed with 14-3-3 protein were measured to reveal differences of mobility DBD.

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MODELLING CONFORMATIONAL CHANGES USING METADYNAMICS IN ESSENTIAL COORDINATES

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Accurate modelling of conformational changes in bio-macromolecules is fundamental for understanding the role of protein motions in catalysis, allosteric effect, induced fit, molecular motors, folding, unfolding, misfolding and many other processes. However, conformational motions are often not accessible by means of standard molecular dynamics simulation because of time-consuming nature of this method. Moreover, standard molecular dynamics simulation does not provide any quantitative information about free energy changes. The recently introduced method of metadynamics [1] makes possible to explore a free energy surface of a molecular system in the space of collective variables. These collective variables are parameters that determine the progress along the modelled conformational (or other chemical) change. Parameters such as distances between two atoms or dihedral angles are often used as collective variables. The choice of these collective variables is usually intuitive and a matter of experience. In modelling conformational changes it is often difficult to find suitable collective variables. Here we present metadynamics in space of essential coordinates obtained by essential dynamics analysis [2] of molecular dynamics trajectory. Essential dynamics (principle component analysis) allows tracing major collective motions in the dynamics of a bio-macromolecule. The results of metadynamics in space of essential coordinates applied to the model molecule (alanine dipeptide, Ace-Ala-Nme) are presented. These results demonstrate that a combination of these two methods (essential dynamics and metadynamics) has great potential in modelling of conformational changes.

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P35

CRYSTALLIZATION STUDY OF THREE MUTANT HALOALKANE DEHALOGENASES DERIVED FROM DEHALOGENASE DHAA OF *Rhodococcus rhodochrous* NCIMB 13064

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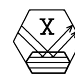
Haloalkane dehalogenases (EC 3.8.1.5) are enzymes that belong to the -hydrolase fold family. These microbial enzymes catalyze hydrolytic conversion of halogenated hydrocarbons to corresponding alcohols [1]. Dehalogenation is a key step in aerobic mineralization pathways of many halogenated compounds that occur as environmental pollutants [2]. Haloalkane dehalogenases are potentially important biocatalysts with both industrial and bioremediation applications that could be used for industrial biocatalysis or as active compounds of biosensors, respectively [3, 4].

Wild-type DhaA was isolated from bacterium *Rhodococcus rhodochrous* NCIMB 13064 [5]. Derived mutant enzymes DhaA04, DhaA14 and DhaA15 were constructed to reveal importance of product transporting pathways (tunnels) in DhaA for its enzymatic activity. Our project is aimed to produce crystals of haloalkane dehalogenases DhaA04, DhaA14 and DhaA15 purified mutants (Fig. 1) in efficient quality for diffraction experiments and finally compare results with known structure of wild-type DhaA [3].

Standard vapor diffusion technique has been used for searching and optimization of crystallization conditions. Crystallization experiments have been performed in Hampton Research Linbro and Cryschem plates (Hampton Research, CA, USA) as well as in Emerald BioStructures CombiClover Crystallization Plate (EBS plate, Emerald BioStructures, WA, USA) using commercial crystalliza-



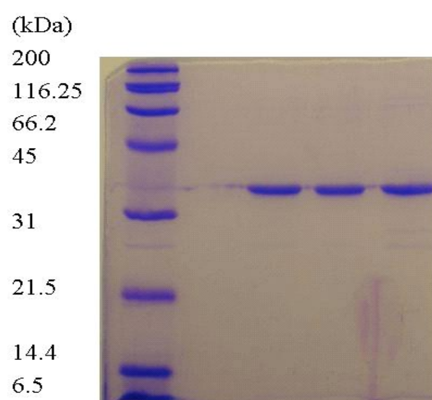


Fig. 1 SDS-PAGE of the Dehalogenases
Lane 1: Marker:
Lane 2: DhaA04
Lane 3: DhaA14
Lane 4: DhaA15

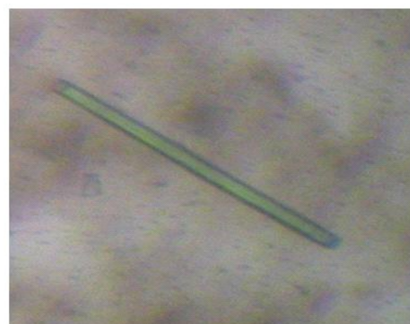


Fig. 2 Crystals of DhaA15
(70 x 5 x 5 um)

tion kits as Crystal Screen Lite and Crystal Screen of Hampton Research, and Clear Strategy Screen 1 of Molecular Dimensions Limited (MDL, Suffolk, UK). The first microcrystals of DhaA15 were obtained from PCT, reagent B2 of Hampton Research (Fig. 2).

Crystallization experiments with all enzymes are in the progress.

This work is supported by the Ministry of Education of the Czech Republic (MSM6007665808 and LC06010) and by the Academy of Sciences of the Czech Republic (AVOZ608 70520).

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P36

PHYSICAL AND MATHEMATICAL CHALLENGES IN MOLECULAR AND CELL BIOLOGY – USERS POINT OF VIEW

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In the paper will be discussed the origins of physical differences between the test-tube biochemistry and "living cell as test tube" and the subsequent necessities of new kind of mathematics [1]. It will be discussed:

(1) Ratchets in protein-based systems (namely information ratchets) [2, 3], the principle that allows the protein to act as Maxwell´s demon. Machines based on this principle, seemingly being the perpetuum mobile of second kind, are widespread in the world of proteins.

(2) Stochastic resonance, the non-trivial principle that may lead to synchronised switching of noisy systems invoked only by low level trigger signal [3, 4] and acting generally as one of the switching mechanism of Brownian motors.

(3) The role of intracellular noise in systems of low number of particles that leads to the differences in reaction rates and courses [5] and may cause the bifurcations, oscillations and other types of complex behaviour [6], i.e. the cases not predicted by classical chemical kinetics or Langevin equation [7].

(4) Macromolecular crowding caused by high concentrations of large molecules in the cell (5-30% volume occupancy). This principle, exemplified by two extreme cases of crowding (influence of macromolecules) and confinement (trapping of large particles in small compartments) [8], leads to large deviation from ideal behaviour in protein-protein reactions and may be the leading principle in chaperone function [9].

(5) Effects of nanoscales, referred in protein word as non-equilibrium thermodynamics of small systems [10, 11]. At these scales the state of the system is not described by quantities such as temperature, pressure or chemical potential, therefore we have to define the so-called control parameters [12]. In effects, we may observe work fluctuations, transient violations of the second law of thermodynamics and free-energy recovery which are the elements leading to miraculous efficiency of proteins as molecular nanomachines.

Popular reviews summarizing the above mentioned principles may be found in [13, 14, 15 and 16].

We set aside the questions of modularity, evolution and population dynamics although they are not fully separable from questions discussed in this paper. Interested learners are invited to courses of Physical Biology organized annually as a part of the lifelong education by the Institute of Physical Biology, University of South Bohemia.

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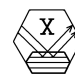

P38

GENE SYNTENIES CONSERVED ON VERY LARGE GENETIC DISTANCES

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Conserved synteny genes are homologous genes that map to the same chromosome in two or more genomes. We set up to characterize the syntenies conserved on very large genetic distances (billions of years). We used orthologous sets of genes from human, fruit fly *Drosophila melanogaster*, worm *C. elegans* and yeast *S. cerevisiae* and computed conserved syntenies for all pairs of organisms. As controls, we generated random genomes and counted conserved syntenies in the same way as for the real sets. We then identified combinations of chromosomes enriched for and depleted of conserved syntenies.

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P39

AUTOMATIC ANALYSIS OF NOSTOC SP. GROWTH

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Objectives: Metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind. Specifically, the study of their small-molecule metabolite profiles. The small-molecule profiles can be monitored by high-sensitive chemical analysing machines. But the amount of collected data is huge and hard to analyse by human sense.

Methods: High-Performance Liguid Chromatography followed by Mass spectrometry was used as a measuring tool. In Matlab and C++ enviroment automatic analysing tools for metabolomic measurements were developed. Random and Systematic noise filters are presented. A peak detector based on certainty factor evaluation is introduced as well. Metabolomic content of measuremt set can be comparised

Conclusion: Metabolite history of 11-day growth of cyanobacteria Nostoc sp. was automatically extracted from measurements and a dendrogram was evaluated.

P40

CO-CRYSTALLIZATION OF WrbA PROTEIN WITH ITS FLAVIN COFACTOR LEADS TO 3D-STRUCTURE DETERMINATION

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The WrbA protein from *Escherichia coli* belongs to a new family of multimeric flavoproteins [1,2], that contain flavin mononucleotide (FMN) as a physiological cofactor. The proper function of WrbA had been unknown until recently. According to the latest observations the *E. coli* WrbA and its homologues exhibit the NAD(P)H:quinone oxidoreductase activity, which is proposed to be implicated in protection mechanisms against oxidative stress [3]. Determination of crystal structure of the *E. coli* WrbA is aimed at proper characterization of the new protein family with respect to its recently revealed oxidoreductase activity.

Recombinant WrbA protein was expressed in *E. coli* CY15071(DE3) cells and purified as described previously [2]. Due to the loss of FMN cofactor during purification, the WrbA apoprotein was crystallized first. Single crystals for diffraction analysis were obtained after optimization of crystallization by using additives (Cd-chloride and Li-citrate) [4], but due to inconvenient diffraction pa-

rameters the structure couldn't be solved. Crystallizability of the WrbA protein improved significantly after binding of its cofactor, FMN. Yellow, well-formed crystals of WrbA protein in complex with its flavin cofactor were obtained even from several crystallization conditions. Yielding of diffraction-quality WrbA holoprotein crystals without additional optimzation steps shows the positive influence of FMN cofactor on crystallization of WrbA protein. This effect reflects FMN as a potential stabilizer of the protein structure. In correspondence to this finding, FMN binding was shown to stabilize WrbA protein providing it with increased resistance against proteolytic digestion (data not shown) and thermal denaturation [5]. Data collected for the WrbA holoprotein crystals by using synchrotron radiation were used for 3D-structure determination. The structure of WrbA in complex with FMN was refined to 2 Å.

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P41

LIPID TRANSFER PROTEINS OF BARLEY

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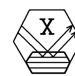
Nonspecific lipid transfer protein 1 (LTP1) was isolated from barley seeds and malt. MALDI-TOF mass spectrometry in combination with in-solution tryptic digestion revealed that Asp 7 of LTP1 was modified by cis-14-hydroxy-10,13-dioxo-7-heptadecenoic ester in both samples. However, no modification was observed when the protein was digested by the standard proteomic protocol using SDS-PAGE. It was found that the ester was hydrolyzed by the harsh conditions of protein denaturation. In addition to the lipid modification, glycations of the protein were identified in the intact LTP1 samples.

A large-scale purification protocol was optimized for isolation of LTP1 from barley flour, yielding approximately 20 mg of pure LTP1. The sample was studied by proton NMR spectroscopy at 600 MHz. In addition to NOESY and TOCSY spectra recorded at 310 K, a series of NOESY spectra was measured up to 350 K. Inspection of the spectra showed that the protein was not denatured in the temperature range tested. The obtained spectra differed from the results reported for the non-modified LTP1 in literature. Assignment of the resonance frequencies is in progress.

Nonspecific lipid transfer protein 2 (LTP2) was identified in barley by MALDI-TOF/TOF MS-MS. No modification was observed. Therefore, a synthetic gene was obtained, in order to clone it into a overexpression vector. This approach should provide an access to large quantities of isotope-labeled LTP1 for NMR studies.



P42

THE LENGTH OF ESTERIFYING ALCOHOL DETERMINES THE AGGREGATION PROPERTIES OF CHLOROSOMAL BACTERIOCHLOROPHYLLS

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Chlorosomes, the main light harvesting complexes of green photosynthetic bacteria, contain bacteriochlorophyll (BChl) molecules in the form of self-assembling aggregates. To study the role of esterifying alcohols in BChl aggregation we have prepared a series of bacteriochlorophyllide *c* (BChlide *c*) derivatives differing in the length of the esterifying alcohol (C1, C4, C8 and C12). Their aggregation behaviour was studied both in polar (aqueous buffer) and non-polar (hexane) environments and the esterifying alcohols were found to play an essential role. In aqueous buffer, hydrophobic interactions among esterifying alcohols drive BChlide *c* derivatives with longer chains into the formation of dimers, while this interaction is weak for BChlides with shorter esterifying alcohols and they remain mainly as monomers. All studied BChlide *c* derivatives form aggregates in hexane, but the process slows down with longer esterifying alcohol due to competing hydrophobic interactions with hexane molecules. In addition, the effect of the length of the solvent molecules (*n*-alkanes) was explored for BChl *c* aggregation. With an increasing length of *n*-alkane molecule, the hydrophobic interaction with the farnesyl chain becomes stronger, leading to a slower aggregation rate. The results show that the hydrophobic interaction is the driving force for the aggregation in aqueous environment, while in non-polar solvents it is the hydrophilic interaction. The data also suggest that BChl dimers are the building blocks of BChl *c* aggregates.