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Conference Committee

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Thursday, March 24, Session I

L1

LIPID HELICES FORMATION IN *BACILLUS SUBTILIS* CELL MEMBRANE

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The domains of different lipid composition are present in eukaryotic and prokaryotic cell membranes. Using membrane binding fluorescent dyes, we demonstrate previously, the presence of lipid spirals extending along the long-axis of cells of the rod-shaped bacterium *B. subtilis* (1). These data indicate a higher level of membrane lipid organization than previously observed. Little is known however of the origin of these helical structures.

Principally, there are at least three main specifically localized molecular structures in the membrane or close proximity to it what can help to form or influence the formation of lipid helixes. In our work we have focused on analyzing these lipid structures in correlation with other above mentioned helical structures in the cell membrane or its close proximity. We were analyzing lipid domains by using lipid specific dyes in protoplasted cells, in Mbl, MreB and MreBH mutant strains. We have used FRAP and FRET experiments to determine dynamics of lipid domains and co-localization of lipid dyes with GFP fused proteins, respectively.

We have also studied the role of lipid helices in cell division by directing the Min system to the helices from pole to pole. We inspected cell division when *E. coli* Min-system was introduced into *B. subtilis* cells. We show that $MinD_{Ec}$ can partially substitute function of its *B. subtilis* protein counterpart (2). Additionally, we observed dynamic behavior of $MinD_{Ec}$ and MinE in *B. subtilis* when expressed together. All these findings indicate that these two Min systems resemble each other more than was thought previously.

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L2

ENGINEERING OF BIOCHEMICAL PATHWAY FOR DEGRADATION OF ANTHROPOGENIC ENVIRONMENTAL POLLUTANT 1,2,3-TRICHLOROPROPANE

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Toxic anthropogenic compound 1,2,3-trichloropropane (TCP) is typically released into the environment as a result of its manufacture and use as a solvent, extractive agent and an intermediate in the production of some other industrially relevant chemicals. It has been detected in low concentrations in surface, drinking and ground water, and is anticipated human carcinogen [1]. TCP is likely to persist for long period of time in the groundwater environment, because no living organism posses the complete biochemical pathway for its utilization [2]. The persistence problem observed with various man-made environmental pollutants, including TCP, could be possibly solved by assembling missing biochemical pathways using protein and metabolic engineering.

The aim of this project is to assemble the biochemical pathway for complete degradation of TCP *in vitro*, using

immobilized enzymes, and *in vivo,* employing suitable microbial host. We proposed pathway consisting of haloalkane dehalogenase DhaA from *Rhodococcus sp*., haloalcohol dehalogenase HheC and epoxide hydrolase EchA from *Agrobacterium radiobacter* AD1. These enzymes are expected to catalyze five subsequent steps of TCP degradation to the harmless product glycerol, which can be further utilized in nature through the microbial glycolysis. The genes *dhaA*, *hheC* and *echA* were synthesized using the sequences available in public databases. Purified enzymes were used for determination of kinetic parameters with the target substrates of proposed pathway. Degradation of TCP and its intermediates by respective enzymes was followed using gas chromatography methods. Possible pathway bottlenecks were identified according to the obtained data. Detected poor catalytic efficiency of wild-type DhaA with TCP was improved 26-times by the application of computer-assisted design and focused directed evolution [3]. Resulting mutant DhaA31 has four amino acid substitutions within the main tunnel and the slot tunnel, which connects the buried active site of enzyme with bulk solvent. Bulky hydrophobic residues introduced into the tunnels limit the access of water molecules into the active site, otherwise preventing formation of the reactive complex.

Once the problem of poor DhaA activity was solved, we have studied degradation of TCP in a multi-enzyme reaction with DhaA31, HheC and EchA in buffer solution. Almost complete conversion of 5 mM TCP to the final product glycerol was observed at 37°C, 30°C and 19°C, thus confirming the concept of TCP degradation through assembled biochemical pathway *in vitro*. In the future, we will study this metabolic pathway with immobilized enzymes and enzymes expressed in the suitable host microorganism or bacterial chassis. Comparison of *in vitro* and *in vivo* systems engineered for degradation of TCP will provide unique information on conversion of non-natural compounds by assembled biochemical pathway.

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L3

EXPRESSION AND PURIFICATION OF MYRISTOYLATED MATRIX PROTEIN OF MASON-PFIZER MONKEY VIRUS FOR STRUCTURAL STUDIES

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Matrix proteins (MA) are the N-terminal domains of structural polyproteins Gag of all retroviruses. They play multiple roles both in the early and late stages of the viral replication cycle. Most of the retroviral matrix proteins are N-terminally myristoylated which is a common post-translational modification of eukaryotic proteins. The myristoylation is necessary for transport of immature virus particles to the cytoplasmic membrane and for budding of virus particle out of the cell. Unfortunately prokaryotic expression systems, like *Escherichia coli*, are unable to produce myristoylated proteins. To overcome this problem and yet retain the advantage of strong expression in bacterial cells we co-expressed the matrix protein with yeast N-myristoyl transferase. We managed to prepare a C-terminally His-tagged matrix protein of Mason-Pfizer monkey virus in a large quantity and a high purity using a single-step purification procedure. This protein has been used for NMR and MALDI-TOF studies. The MS analysis proved a high degree of myristoylation of the MA (more than 95%). The comparison of NMR spectra of the matrix protein with and without the myristic acid revealed substantial structural changes of the myristoylated protein.

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L4

THE MOLECULAR PUZZLE OF C-TYPE LECTIN LIKE NATURAL KILLER CELL RECEPTORS

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Natural killer cells belong to the innate immune system and are able to recognize and destroy virus-infected or tumour cells [1]. Apart from immunoglobulin-type receptors these cytotoxic lymphocytes utilize a row of cell surface C-type lectin like (CTL) molecules involved either in activation or inhibition of the NK cell response which results in release of perforin and granzymes and lysis of a target cell within minutes. In mammals the number of types of CTL receptors identified for individual species differs while the overall architecture of such a receptor is relatively constant. An intracellular part responsible for signaling is connected via a transmembrane linker (helix) to an extracellular domain involved in receptor-ligand or cell-cell interactions. Several studies confirmed that the interacting partners for these receptors are again cell surface proteins of the same type (Clr – C-type lectin related in rodents or human LLT1 – Lectin-like transcript-1) [2]. Structural details underlying the functionality of these receptors and especially formation of these protein-protein complexes are still only sparse. Our recent work has been focused on mouse and rat receptors and ligands of the NKR-P1 family (CTL type). Three-dimensional structures based on x-ray diffraction analysis of mouse NKR-P1A and Clrg complemented by a high resolution structure of human CD69 together with sequence analysis of the protein family give first indications of their specific properties [3]. The surface loop of the CTL domain fold may play an important role in receptor-ligand interactions and amino acid sequence analysis leads to predictions of similar behaviour within the NKR-P1 family.

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L5

GENERATION OF ARTIFICIAL BINDERS WITH AFFINITY TO HUMAN CYTOKINES VIA COMPUTER-ASSISTED MUTAGENESIS OF A STABLE PROTEIN SCAFFOLD AND RIBOSOME DISPLAY SELECTION

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Albumine binding domain (ABD), a three-helix bundle of native *Streptococcal* G protein, represents a promising scaffold for the construction of small artificial ligands with required properties, useful as new diagnostic tools or next

generation therapeutics. To generate a set of novel binding proteins with high affinity and specificity to human cytokines, surface residues of the ABD molecule were subjected to a detailed analysis using interaction energy map, calculation of solvent accessibility and *in silico* alanine scanning, resulting in the identification of key residues for the stability and 11 randomization-accessible amino acid positions. Combinatorial DNA library with a theoretical complexity up to 10^{14} ABD variants, generated using randomized oligonucleotide primers followed by fusion with cDNA coding for TolA helical helper protein, served as a template for the reverse transcription, followed by in vitro translation and ribosome display selection. After several round of two independent screening campaigns, high affinity ligands for human interferon gamma (hIFN) were identified, with K_d ranging from 0.2 to 10 nM. Molecular modeling and docking of four best hIFN binders suggested that these ABD variants share a common binding region different from that known for the hIFNă receptor. The predicted binding model for novel ligands is supported by competition ELISA and SPR using a synthetic form of one of the ABD variants and recombinant form of hIFN receptor Novel ligands represent unique artificial probes for structure-function studies and promising tools for the construction of new biosensors. The ABD library and the established screening approach are currently being used for the generation of novel inhibitory ligands blocking the function of human interleukin-23.

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Thursday, March 24, Session II

L6

CHEMICAL CROSS-LINKING AND H/D EXCHANGE COMBINED WITH MASS SPECTROMETRY: A TOOL TO VALIDATE AND REFINE 3-D PROTEIN X–RAY MODEL

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Chemical cross-linking and Hydrogen/Deuterium exchange combined with mass spectrometry are powerful tools for elucidating protein conformation in solution. We applied both methods to study structural details of an important activating lymphocyte receptor NKR-P1A and to distinguish between structural characteristics in crystal and physiological conditions. The determination of crystal structure of NKR-P1A evokes questions about the unique flap region containing residues Pro161-Asp187 which is in crystal directed towards symmetrically related molecule. Chemical cross-linking and H/D exchange combined with high resolution mass spectrometry have been employed to investigate the flexibility and conformation of the flap region.

In order to study the flexibility and conformation of the flap region in the NKR-P1A protein, we have prepared recombinant NKR-P1A protein and its mutant NKR-P1A-NF in which the flap region containing residues Pro161-Asp187 was deleted and replaced by two alanines. While the main structure of NKR-P1A obtained by mass spectrometry techniques was consistent with the previously known crystal data, a difference was found in the flap region. In the crystal structure the flap region extends from the compact core region. On the contrary, analysis of the peptic fragments showed decreased local H/D exchange in the NKR-P1A protein in region containing residues 115-131, 138-143 and 190-206 in comparison with NKR-P1A-NF. This indicates a reorganization of the flap region and its association with the compact core region. The mass spectrometry results were further confirmed by the NMR structural analysis.

L7

STRUCTURAL DETAILS OF NKR-P1D : CLRB INTERACTION ELUCIDATED BY PROTEIN CROSS-LINKING AND MASS SPECTROMETRY

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Interaction between murine NKR-P1D and Clrb receptors was originally described as a novel type of „MHC class-I independent missing-self recognition" and was shown to confer protection from killing by natural killer cells [1]. However, further studies suggested that it is not in fact NKR-P1D but rather NKR-P1B, an allelic form expressed in different strains of mice, which binds Clrb and that NKR-P1D binds with significantly lower affinity if at all [2].

In order to address the issues arising from these conflicting results, we have recombinantly expressed the extracellular domains of both receptors in *E. coli* cells and refolded the proteins *in vitro.* The quality of refolding was confirmed both by determining the disulphide bonding pattern using FT-MS and measuring $^{1}H/^{15}N$-HSQC spectra using 600MHz NMR spectrometer. Interaction between the proteins in solution was immobilized using protein cross-linking technique. Three cross-linking reagents, EDC, DSG and DSS with spacer arm lengths of 0 Å, 7.5 Å and 11.5 Å respectively were used. The reaction mixture was separated by means of SDS-PAGE and protein bands corresponding to dimers were digested in gel. Using FT-MS we were able to find peptides from both proteins connected by the cross-linkers.

Using recently resolved structures of extracellular domains of NKR-P1A and Clrg receptors bearing 86% and 76% sequence identity with NKR-P1D and Clrb respectively, we were able to build homology models of both NKR-P1D and Clrb and a model of the interacting pair. Part of the data obtained from cross-linking experiments fitted nicely into the model of homodimers of both proteins interacting in a face-to-face fashion as would be expected; however significant portion of the observed cross-links could not be explained by this model. In order to allow for these data we suggest that these receptors do not only interact in the face-to-face fashion but also in a chain-like or cluster-like fashion with each homodimer contacting two homodimers of the second protein at the same time.

To our knowledge this would be the first interaction of this kind observed for this type of receptors.

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L8

SUPRAMOLECULAR ASSEMBLIES FORMED BY DIOLEIN AND STEARYL ALCOHOL

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Diolein (1,2-dioleoylglycerol, DOG) and stearyl alcohol (1-octadecanol, ODA) are typical amphiphiles, which can be conveniently used as glycolipid models. They have some structural similarities, namely both contain 18 carbon atoms in the chain and have a single hydroxyl group. Despite these similarities, they assemble into very different supramolecular structures. It was shown by a combination of IR spectroscopy and MD/QM simulations that the assemblies formed by DOG are characterized by low structural order and very weak hydrogen bonding, while those formed by ODA, on the contrary, exhibit highly ordered structures stabilized by strong hydrogen bonds (Figure 1).

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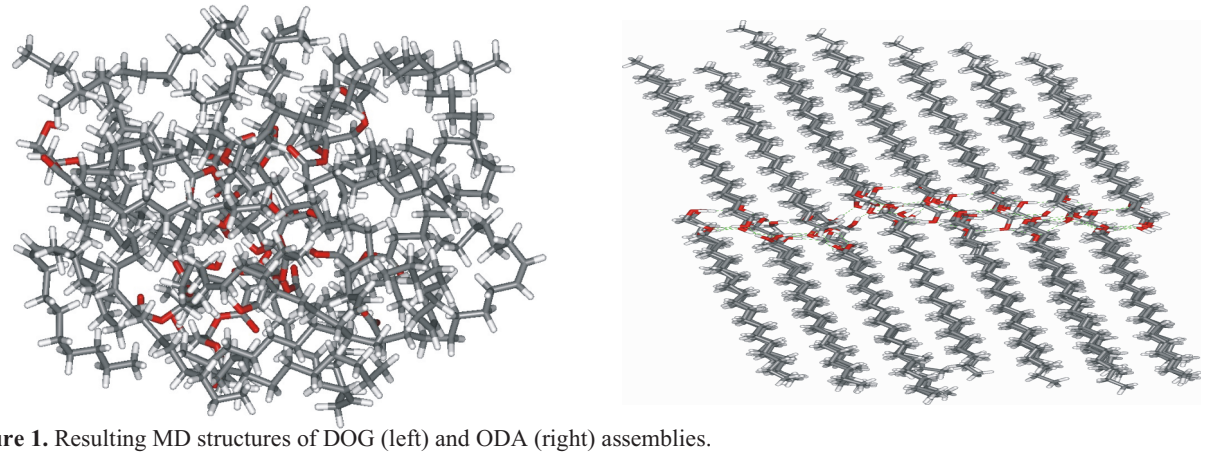

Figure 1. Resulting MD structures of DOG (left) and ODA (right) assemblies.

L9

STRUCTURE OF THE 14-3-3/RGS3 COMPLEX: NEW DETAILS ON ARCHITECTURE OF COMPLEXES FORMED BY 14-3-3 PROTEINS

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14-3-3 proteins are a family of highly conserved dimeric regulatory molecules that are expressed in all eukaryotic cells and specifically bind to the phosphoserine (or phosphothreonine)-containing motifs in a sequence-specific manner. To date more than 300 binding partners of 14-3-3 proteins have been identified. It remains largely unresolved how 14-3-3 proteins exhibit such a diverse assortment of ligands involved in so many different signaling pathways. Not all ligands show equal affinity for different 14-3-3 isoforms, despite the relatively high sequence homology that all 14-3-3s share. Despite structural knowledge of the 14-3-3 protein alone, there is only restricted information about the structure of 14-3-3 proteins in complex with their binding partners [1]. Exactly this information is of paramount importance for mechanistic understanding by which 14-3-3 proteins regulate signal transduction, metabolic pathways, cell cycle, apoptosis etc. We were particularly interested in 14-3-3 /RGS3 protein complex. RGS proteins bind specifically to the GTP-bound forms of Gá and significantly stimulate their GTPase activity by stabilizing the transition state. Upon phosphorylation the RGS protein interacts with the 14-3-3 protein. Phosphorylation-dependent binding of 14-3-3 acts as a molecular switch that controls the GAP activity keeping a substantial fraction of RGS proteins in a dormant stay [2].

To map the interaction between 14-3-3 and RGS3 protein we performed a wide range of biophysical measurements: H/D exchange and cross link experiments coupled to mass spectrometry were used to determine the interacting surface, FRET (Förster resonance energy transfer) time-resolved fluorescence experiments were used to measure distances between AEDANS-labeled RGS3 molecule and fluorescein-labeled 14-3-3 . The stoichiometry of 14-3-3 /RGS3 protein complex was elucidated using the analytical ultracentrifugation. The crystal structure of RGS domain of RGS3 at 2.3 Å resolution was solved. Time resolved tryptophan fluorescence spectroscopy was employed to characterize the conformational changes of RGS3 induced by 14-3-3 protein binding [3]. SAXS (small angle X-ray scattering) measurement was used to obtain molecular envelope of 14-3-3/RGS3 protein complex. Based on all these results we build 3D model of 14-3-3 /RGS3 protein complex.

Our model revealed new details on architecture of complex formed by 14-3-3 proteins. To date all known structure of 14-3-3 proteins complexes suggests that the ligand is docked in the central channel of 14-3-3 protein. Our results indicate that the RGS domain of RGS3 protein is located outside the central channel of 14-3-3 protein interacting with less-conserved residues of 14-3-3 . This could explain why different ligands show different affinity for different 14-3-3 isoforms.

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L10

STRUCTURAL AND FUNCTIONAL STUDY ON THE GLYCOSYLATION IN MAIZE CYTOKININ OXIDASE/DEHYDROGENASE1

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Cytokinin oxidase/dehydrogenase (CKO; EC 1.5.99.12) catalyzes the metabolic degradation of the plant hormones cytokinins. The presence of a glycosylation in maize CKO isoenzyme 1 (ZmCKO1) was originally deduced from its binding to lectin affinity columns [1]. This was further confirmed by electrophoretic migration, which provided significantly higher molecular mass estimates than was a theoretical calculation. The amino acid sequence of ZmCKO1 comprises eight potential N-glycosylation sites [2]. The published crystal structures have shown that at least six of them may be occupied by glycans attached at the residues N63, N89, N134, N294, N323 and N338 [3,4]. However, the composition and size of the carbohydrate chains have not yet been investigated as well as their contribution to the activity and stability of the enzyme. In this work, a recombinant ZmCKO1 expressed in *Yarrowia lipolytica* was treated by endoglycosidase H, which resulted in a complete deglysosylation (except for an expected preservation of one *N*-acetyl-D-glucosamine per each glycosylation site). The released N-glycans were purified by reversed phase chromatography on graphitized carbon and subjected to MALDI-TOF MS using 6-azathiothymine as a matrix. Sodium and potassium adduct peaks were observed in the positive ion reflectron mode. The sodium adduct peaks appeared in a ladder starting at *m/z* 1216, which continued gradually by adding a mass difference of 162 units at least to *m/z* 2676. By means of a simple calculation and search in a library of known structures (http://www.expasy.ch/tools/glycomod), the peaks could be assigned to high-mannose type N-glycans from $Man_6GlcNAc$ to $Man_{15}GlcNAc$. On a Q-TOF instrument with electrospray ionization (ESI), the same N-glycans were detected predominantly as doubly charged ions $[M+H+K]^{2+}$. Several structures were confirmed by collision-induced tandem mass spectrometry (CID-MS/MS). A tryptic digest of ZmCKO1 was then prepared to analyze N-glycopeptides directly by liquid chromatography coupled to mass spectrometry. The digest was separated on a reversed phase column with gradient elution and detected by Q-TOF ESI-MS. In CID-MS/MS, a double charged ion with *m/z* 1267.53 ($[M+2H]^{2+}$) yielded a peptide fragmentation pattern corresponding to the sequence 133-INVSADGR-140 binding two *N*-acetyl-D-glucosamines plus 8 mannoses. The final proof of the presence of high-mannose type N-glycans in recombinant ZmCKO1 was obtained by MALDI-TOF MS of the tryptic digest using ferulic acid as a matrix. A series of glycopeptides was observed with *m/z* values spanning the interval of 7986-12360 and differing from each other by 162 mass units. Calculations allowed assigning of the measured glycopeptides to the tryptic peptide 269-LTAPRPGGGGA SFGPMSYVEGSVFVNQSLATDLANTGFFTDADVAR -313 bearing large N-glycan chains from $Man_{20}GlcNAc_2$ to $Man_{45}GlcNAc_2$ (indicating a hyperglycosylation.) The enzyme deglycosylated under non-denaturing conditions showed decreased activity as well as thermostability indicating the physiological importance of the carbohydrate chains in ZmCKO1.

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L11

ACCESSIBILITY CHANGES WITHIN DIPHTHERIA TOXIN T DOMAIN UPON MEMBRANE PENETRATION PROBED BY HYDROGEN EXCHANGE AND MASS SPECTROMETRY

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Diphtheria toxin is a protein secreted by *Corynebacterium diphtheriae* as a single polypeptide chain of 58 kDa. During cell intoxication, it is cleaved by furin into two fragments, the A chain corresponding to the catalytic (C) domain and the B chain corresponding to the translocation (T) and receptor-binding domains. The C and T domains remain covalently linked by a disulfide bond. Following binding to its cell surface receptor, diphtheria toxin is internalized through the clathrin coated pathway. The acidic pH in the endosome triggers a conformational change leading to the insertion of the toxin in the membrane. The C domain is then translocated across the endosomal membrane into the cytosol. The C domain ADP-ribosylates the elongation factor 2, blocking protein translation and leading to cell death.

At neutral pH, the T domain is a globular protein made of ten alpha-helices, named TH1 to TH9 and TH5'. The insertion of the T domain into membranes is pH-dependent and follows a two-step process. During the first step, between pH 7 and pH 6, the T domain adopts a partially folded state, the so-called molten globule, because of the tertiary structure destabilization. At that stage, the T domain binds to membrane, mainly through hydrophobic interactions, leading to the membrane-bound (MB) state. During the second step, between pH 6 and pH 4, the conformation of the T domain is reorganized, leading to a membrane-inserted (MI) state. While the tertiary structure is lost, the secondary structure, i.e. the helical content, of the T domain is preserved throughout the process. The MI state is the functional state of the T domain; it permeabilizes the membrane and enables the passage of the N-terminal region from the *cis* to the *trans* side of the membrane.

In this work we used Hydrogen/Deuterium exchange coupled to mass spectrometry to describe the individual steps of T-domain membrane insertion. Accessibility changes of T-domain were followed for T-domain at pH 7, 6 and 4 and for different states – either free T-domain in solution, or T-domain bound to the lipid membrane (mimicked by large unilamellar vesicles). Also the influence of high-ionic strength (masking of electrostatic interactions) was followed.

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Friday, March 25, Session III

L12

MECHANISM OF THE OXALIPLATIN BINDING TO THE DOUBLE-STRANDED 1,2-DS(PGPG) DINUCLEOTIDE IN AQUEOUS SOLUTION

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A mechanism of the formation of the intrastrand 1,2-crosslink between ds(pGpG) dinucleotide and hydrolyzed oxaliplatin is studied using theoretical RI-DFT-D and ONIOM/MP2/B3LYP methods. It is a two-step reaction in which the monoadduct formation is the rate-determining step with activation free energy of ~ 20 kcal/mol followed by the diadduct formation step with activation free energy of ~ 15 kcal/mol. The binding in the 5'-G 3'-G direction is kinetically preferred over the 3'-G 5'-G direction. Non-bonded interactions and steric effects influence considerably the structure of transition states affecting energetics of the reaction. The overall reaction is exergonic by more than 20 kcal/mol. The changes of the ds(pGpG) structure and charge transfer effects upon the oxaliplatin binding will be discussed.

We gratefully acknowledge support from the Grant Agency of the Czech Republic (grants 204/09/J010 and P205/10/0228) and from the Ministry of Education, Youth and Sports of the Czech Republic (grants ME09062 and ME 10149).

L13

TORSION ANGLE DEPENDENCE OF PHOSPHORUS CHEMICAL SHIFTS IN A NUCLEIC ACID BACKBONE FROM COMBINED MOLECULAR DYNAMICS AND DENSITY FUNCTIONAL CALCULATIONS

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^{31}P chemical shifts (P) in nucleic acids proved to be sensitive to the conformation of the backbone [1]. Therefore, the understanding of torsion angle-chemical shift relationships can aid nucleic acid structure determination. We present a comprehensive study of these relationships relying on density functional (DFT) calculations of P [2].

The chemical shift calculations were performed using geometries of hydrated dimethyl phosphate (DMP), the model of the phosphodiester linkage, extracted from molecular dynamics (MD) snapshots of [d(CGCGAATT CGCG)]$_2$. A thorough analysis of P obtained has shown that while P-O torsion angles and dominate P, C-O torsion angles and only modulate the chemical shift trends established by and . Moreover, the dependences of P on P-O torsion angles are described by simple functions, which can serve as alternatives to the so-called Karplus equations, typically used in NMR to determine torsion angles. We also present chemical shift maps revealing the joint effects of and .

A B-DNA backbone undergoes transitions between two conformational states referred to as B_I and B_{II}. These transitions occurring on a nanosecond time scale influence P. The chemical shift difference between the pure B_I and B_{II} states obtained from our calculations is in a very good agreement with the difference inferred from experimental data. The combined MD/DFT approach employed in the study thus proved to bring a considerable improvement over static calculations reported so far.

This work was supported by the Grants MSM0021622413 to M.M. and LC06030 to V.S. from the Ministry of Education, Youth and Sports of the Czech Republic, and by the Grant No. IAA500040903 from the Grant Agency of the Academy of Sciences of the Czech Republic to JP.

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L14

PROPOSAL OF ALTERNATIVE PATHWAY OF THE GLYCOSIDIC BOND CLEAVAGE MECHANISM OF 8-OXOGUANOSINE CATALYZED BY THE HUMAN DNA REPAIR PROTEIN HOGG1

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The enzymes repairing chemically damaged DNA molecules are crucial for maintaining integrity of genetic information. One of the frequently occurring DNA mutations is the oxidative damage of nucleic acid bases that can be initiated by reactive oxygen species, ionizing radiation, and also by free organic radicals. The product of oxidation of guanine base, the 7,8-dihydro-8-oxo-guanine (oxoG), can be mispaired with adenine what may have serious consequences in DNA replication. To eradicate the lesions such as oxoG and restore original DNA cell evolved base excision-repair pathways. The 8-oxoguanosine glycosylase (OGG1) is an enzyme designed for excision of oxoG in eukaryotes. Several possible pathways were suggested and investigated theoretically during past decade based on the structural information derived from crystals of oxoG captured in catalytic pocket of hOGG1 enzyme. We come up with theoretical model of new alternative pathway for the oxoG excision that is coherent with the structural information in crystals. In order to obtain reliable picture of the glycosidic bond cleavage we confront energetic of the new reaction pathway with those for other suggested mechanisms.

This works was supported by the Young Investigator's Grant of the Human Frontier Science Program (HFSP)..

L15

COMPLEMENTATION OF 3D STRUCTURE OF DELTA SUBUNIT OF RNA POLYMERASE FROM *BACILLUS SUBTILIS* WITH DESCRIPTION OF INTERNAL MOTIONS IN TERMS OF REDUCED SPECTRAL DENSITY MAPPING

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Full text see on page 3.

Friday, March 25, Session IV

L16

EXPERIMENTAL AND THEORETICAL STUDY OF FORMATION OF POLYPROLINE II HELIX BY MEANS OF RAMAN OPTICAL ACTIVITY

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Polyproline-II helix (PPII) represents a less common protein secondary structure motif. Its structure is rather specific due to absence of internal stabilizing hydrogen bonds. The stability of the helix is caused only by sterical reasons and the interaction with surrounding solvent molecules. Number of recently discovered evidences [1] has lead to a presupposition that the PPII helix is the dominant element of the structure of unfolded proteins.

In our experiment we measured Raman and Raman optical activity (ROA) spectra of several oligo- and poly-L--proline samples in a wide frequency range between 120 cm^{-1} and 1800 cm^{-1} and analyzed them with respect to the length of the proline chain. The relatively new technique of ROA [2,3], which is based on a different interaction of a specimen with the 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 peptide chain backbone. There is also a strong link to previous experiments [4] which were focused on the characterization of proline side chain conformation and its interaction with solvent.

The stress was laid on the interconnection between experiments and simulations. For that purpose we performed *ab initio* calculations of Raman and ROA spectral bands and their intensities for all measured samples in order to obtain more accurate interpretation of recorded spectra and observed phenomena. Computations were done in Gaussian program, whose latest implementation enables analytical calculations of optical activity tensors leading to the significant decrease of computational time.

So far, we were able to determine the characteristic spectral peaks associated with formation of stable PPII helical conformation in studied systems. The most relevant peaks were found at 405, 535 and 945 cm^{-1}. Based on our experimental data analysis we were able to determine the minimal length of $(L\text{-proline})_N$ chain necessary for formation of the stable PPII conformation as N=6 [5]. Moreover, based on the results of calculation we managed to assign specific vibrations to the most proliferating spectral peaks and discuss the influence of various peptide bond and side chain conformers in experimental spectra.

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L17

CONFORMATIONAL STUDY OF THE CYCLO(L-TRP-L-TRP)DIPEPTIDE

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Cyclic dipeptides are convenient model compounds because they have a restricted flexibility due to the ring containing two peptide bonds [1]. Some cyclic peptides are also known to possess antiviral, antibiotic, and anti-tumour activity.

The aim of our study is to understand the interaction of two aromatic tryptophan sidechains in c(Trp-Trp) on the basis of DFT calculations and experimental vibrational spectra. Previous studies suggest that the diketopiperazine ring is nonplanar [2],[3]. This was studied for two simplified models, cyclo(Gly-Gly) and cyclo(L-ala-L-Ala), the potential energy surface has been calculated in Gaussian 09 (B3LYP/6-311++G**, DMSO solvent). Two shallow minima have been found. For cyclo(L-Trp-L-Trp), equilibrium conformations were found also for the sidechains. Geometries with the lowest energies were used for calculation of Raman and ROA spectra.

Raman spectra of different conformations were similar, whereas Raman optical activity (ROA, the difference in scattering for right and left circularly polarized light) spectra were more sensitive to structural details, and could better distinguish positions of the tryptophane residues and nonplanarity of the central ring.

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L18

APPLICATION OF NEW QM/MM INTERFACE TO STUDY OF RU DRUGS INTERACTING WITH DNA

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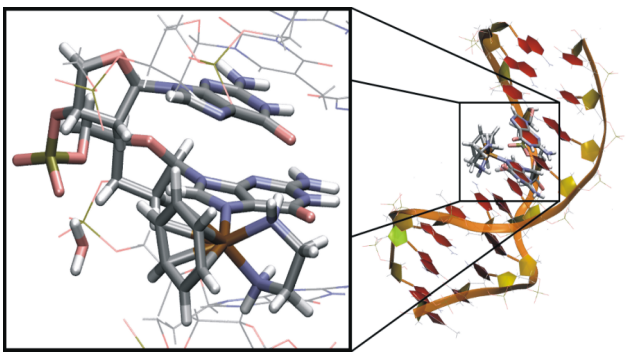
New program interface capable to inter-connect standard QM and MM software to perform hybrid QM/MM calculations on molecular systems was implemented. Subtractive QM/MM scheme with link atoms and electronic embedding is used. Several geometry optimization and molecular dynamics algorithms are coded into the interface. Advantage of this system is its flexibility to combine the most suitable programs or methods for description of all parts of computational model.

The QM/MM technique was applied on problem of studying interaction of Ru(II) piano-stool complexes with DNA. The interaction is interesting and important because of significant anti-cancer activity of these complexes caused probably by structural changes of DNA. Interaction with DNA was proven by experimental measurements but the precise reaction mechanism at molecular level is not known. We use DFT(B3LYP) for calculation of properties on Ru(II) complex and nearest nucleic bases (guanines) and Amber FF03 for DNA parametrization.

The reaction energy profile obtained from relaxed and optimized structures will be presented as well as basic properties such as geometry parameters, molecular orbitals or atomic charge analysis. Also size effect of arene ligand will be discussed.



L19

KINETIC ANALYSIS OF *E. Coli* WRBA REDOX ACTIVITY

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The flavoprotein WrbA from *E. coli* was identified as a founding member of a new family of multimeric flavodoxin-like proteins that are implicated in cell protection against oxidative stress. WrbA hereby bridges bacterial flavodoxins and eukaryotic NAD(P)H:quinone oxidoreductases with its three-dimensional structure clearly revealing a close relationship to mammalian NAD(P)H: quinone oxidoreductase, Nqo1 [1]. A closer analysis of apo and holo crystal structures, together with flavodoxin structures, rationalizes functional similarities and differences of the WrbAs relative to flavodoxins, suggesting that WrbAs are not a remote and unusual branch of the flavodoxin family as previously thought but rather a central member with unifying structural features [2].

In this study we try to elucidate the kinetic mechanism of WrbA and its range of substrates, activators, and inhibitors. Assays of WrbA NADH oxidation/quinone reduction activity were carried out. These reactions follow the decrease in NADH absorbance at 340 nm upon oxidation during reduction of ubiquinone or benzoquinone. The reactions are carried out by manual mixing under ambient (i.e., aerobic) conditions since non-enzymatic oxidation is negligible. Standard kinetic approaches to evaluate alternative mechanisms are well-established in studies with the NQO1s, and the classical work of Hosoda et al. [3] provides a model for the comparison with NQO1s. Discrimination among various mechanisms is based on the patterns of substrate and inhibitor concentration dependence of reaction rates. Thus, the concentrations of NADH, NAD, and quinone are varied separately and together to determine patterns of competition that are used to infer which compounds can occupy the active site simultaneously. Ping-pong kinetics have been generally accepted as the mechanism for NQO1s [3, 4], indicating that FAD is reduced in a first step by oxidation of NADH, which then dissociates. In a second step, quinone binds to the site vacated by NAD and is reduced by FADH2. In addition to defining the basic kinetic mechanism, we test WrbA also for a number of the characteristic mechanistic features of NQO1s.

Comparison of the high-resolution *E. coli* WrbA structure with NQO1 implies significant differences in access to the enzyme active site by the cofactor and the NADH and quinone substrates. Despite these structural differences our results clearly demonstrate the unusual two-plateau behaviour on the substrate concentration-dependence plots for NADH or benzoquinone as described for NQO1 by Hosada et al. [3]. The experiments show that WrbA activity increases upon addition of membrane-mimicking detergents, and they demonstrate the ability of the protein to inactivate reversibly by shifting temperature from 5 to 25 ^{o}C. The similarity of these properties to NQOs implies a common structural and functional basis of the kinetic mechanism, however, even for the NQOs the reported two-plateau behaviour could not be explained in structural and molecular terms to date.

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Friday, March 25, Session V

L20

RELIABILITY AND ACCURACY IN PROTEIN STRUCTURE DETERMINATION

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Protein science made an immense progress in the last decade. Combination of information obtained by advanced methods of mass spectrometry, dynamic light scattering, small angle scattering, NMR techniques, molecular modeling and protein crystallography gave already a complete insight into the function of many biological systems on molecular level.

Namely protein crystallography made a huge progress. It has become a very common technique available practically for any laboratory and produces about 8 thousand new bio-macromolecular structures per year. Hundreds of structures of the largest macromolecular complexes (as e.g. ribosomes [1]) and their components in different molecular environments have already been determined by protein crystallography and deposited into the PDB [6] at atomic resolution.

People also learned how to measure intermediate states of molecular complexes acquiring thus a complete insight into the function of "the molecular machines" responsible for correct function and regulation of processes in their native environment (e.g. pumping of Na/K ions by ATPase [2]).

Former difficulties with preparation of good quality "protein crystals" were significantly reduced. Realizing the dynamic character of protein crystals, where the protein molecules remain "floating" in an equilibrium with solution, leads to development of methods for crystallization of macromolecules in various adhesion modes. This provides opportunities for direct observation of many different adhesion modes between molecules which are often decisive for signaling, intermolecular communication, transport, and formation of intermediate molecular complexes [3].

New X-ray Free Electron Lasers (XFEL) allow structure determination of large molecular complexes from nano-crystalline material promising thus structure determination of materials resistant to classical crystallization experiments, namely the structure determination of membrane proteins (e.g. structure determination of photosystem I [4]).

However, all standing experimental and theoretical methods have still some severe limitations which should be taken into account in attempts to understand the "molecular machines" responsible for correct function and regulation of processes in living environment. Failure to realize these limitations can result in molecular models with very problematic relation to reality.

There are some global measures of reliability and accuracy (as e.g. resolution, R factor, number and quality of restraints used in refinement [5]). However no structure provided by protein crystallography is static. The protein molecules are in dynamic equilibrium with solvent and it is usually reflected in different displacement factors (B-factors reflecting motion of individual atoms) and different stability of some parts of molecular complexes. It is necessary to learn "the crystallographic dialect" to get the real multi-conformational view of the real protein complex, to get a view of the motions inside of the protein complex and to read unbiased details of water interactions with protein surface and its motion in the protein channels and clefts.

There are also some human errors introduced into the PDB by inexperienced scientists. A relative easiness of protein crystallography, and accessibility to non-specialists brought non-negligible amount of incompletely refined structures. The remediated version of the PDB [6], containing nowadays over 70 thousand experimentally determined bio-macromolecular structures, removed some global discrepancies only.

Thus a closer insight into reliability of the important structure segments is absolutely necessary before any serious structure study. When trying to use PDB deposited data one should always read comments in the first part of PDB file and observe some warning indicators along full lengths of protein chains (e.g. unusual B-factors, occupation factors, indicators of disorder, missing atoms, etc.). All warning marks or some errors which can be found in some structure depositions in the PDB [6] should be analyzed.

It is important to realize that the real result of experiment is not the structure model deposited in the PDB, but that it is the tree-dimensional electron density into which the structure model is built by crystallographer. Therefore if in any doubts, one should check the three-dimensional map of electron density on the screen of his own personal computer. It is very easy to connect to the Electron Density Server (http://eds.bmc.uu.se/eds/) [7] and of course a little more difficult to interpret the indicated problem alone.

It is also important to read the provided information on the experiment planning, status of protein during measurement and also the procedures used during structure refinement. All that can be important to get a relevant picture of the real state of the protein complex in its cellular or extracellular milieu.

A neglected inspection of PDB file is the most frequent source of errors and misinterpretations in the comparative studies and the structure-function analyses.

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L21

CRYSTAL STRUCTURES OF TWO PROTEASE INHIBITORS FROM TICK SALIVA

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The saliva of ticks is a rich source of various protease inhibitors which help the blood-feeding parasite to overcome the defense of host immune system. Two proteins (named IRS-2 and OmC2) belonging to distinct families of protease inhibitors were identified in the saliva of two tick species and their roles in parasite-host interaction were investigated as well as their crystal structures.

Protein IRS-2 belongs to the serpin family of protease inhibitors. It is produced in the salivary glands of the hard tick *Ixodes ricinus* and exhibits an activity in suppressing the adaptive host immune response [1]. Recombinant protein was prepared by heterologous expression in bacterial system and the crystal structure was determined at resolution of 1.8 Å [2]. IRS-2 adopt a typical serpin fold composed of 3 large -sheets and 9 -helixes (Figure 1A). Interestingly, the structure represents a relaxed state of serpin, in which the loop responsible for interaction with target protease (so called reactive-centre loop, RCL) is cleaved and inserted into the central -sheet.

Protein OmC-2 belonging to cystatin family of protease inhibitors was identified in the salive of the soft tick *Ornithodorus moubata* by proteomic approach. The inhibitory activity of OmC2 toward broad-range of human peptidases was confirmed as well as its immunomodulatory activity *in vivo*. OmC2 represents a promising target for the development of anti-tick vaccine. Crystal structure of recombinant OmC2 determined at resolution of 2.45 Å was used to explain the structure-inhibitory activity relationship [3]. OmC2 adopts a typical cystatin fold characterized by five-stranded twisted antiparallel -sheet wrapping around a central helix (Figure 1B).

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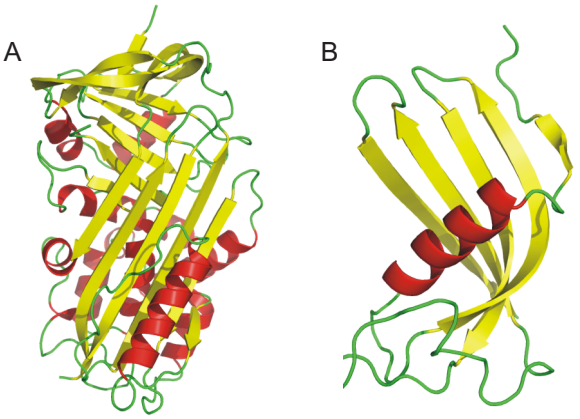

Figure 1. A. Crystal structure of serpin IRS-2 from hard tick *Ixodes ricinus* (PDB code 3NDA). B. Crystal structure of cystatin OmC2 from soft tick *Ornithodorus moubata* (PDB code 3L0R).

L22

THREE-DIMENSIONAL STRUCTURE OF BINUNCTIONAL ANHYDROLASE

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Organophosphorus acid anhydrolases (OPAA; E.C. 3.1.8.2) belong to a class of enzymes that are able to hydrolyze a variety of toxic organophosphorus compounds such as pesticides and also some types of chemical nerve agents which are acetylcholinesterase inhibitors. Due to their significant proteolytic activity against various Xaa-Pro dipeptides (Xaa stands for any amino acids), OPAA enzymes also belong to prolidases.

Enzymatic biodegradation is safer and more economical than detoxification via purely chemical means or incineration, so recombinant OPAA can be applied as an enzymatic tool for detoxification of widespread pesticide waste and for deactivation of chemical warfare agents.

Three X-ray structures of recombinant wild type enzyme OPAA from a marine bacterium *Alteromonas macleodii* are presented here. The data were collected at the beam line BL14.1 of the source of synchrotron radiation Bessy II (Helmholtz-Zentrum, Berlin). In two cases, the crystals belong to space group *C*2 with unit cell parameters a = 134.3 Å, b = 49.1 Å, c = 97.2 Å and = 125.0°. Data were collected at resolutions 1.8 Å and 1.9 Å, respectively. In other case, the crystal belongs to space group $P2_12_12_1$ with unit cell parameters a = 75.6 Å, b = 111.2 Å, c = 138.1 Å and data were collected to the resolution 2.2 Å.

The data were processed using *HKL*2000 and the structures were refined by Refmac5. All the 442 amino acids of the recombinant protein were located in electron density. The protein fold is mainly -helical. The binuclear metal center is located within the pita bread domain in the active site. Manganese ions, which are required for protein activity, were observed in full occupancy in the active sites. The enzyme form dimers. Existence of dimers was confirmed both in crystal and also in solution by dynamic light-scattering. The enzyme shares the so-called "pita bread" fold of the C-terminal domain with other enzymes with prolidase activity.

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L23

STRUCTURAL ANALYSIS OF A RECOMBINANT PLANT BIFUNCTIONAL NUCLEASE TBN1

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Bifunctional nuclease TBN1 (sequence accession no. AM238701) from *Solanum lycopersicum* (red tomato) is a Zn^{2+}- dependent plant glycoprotein composed of 277 amino acids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated). TBN1 belongs to plant nuclease I group and plays a considerable role in specific apoptotic functions, vascular system development, stress response and tissue differentiation in plants [1]. In addition, TBN1 exhibits anticancerogenic properties [2]. Therefore, a detailed structural study of this enzyme can contribute to development of new drugs for cancer, bacterial and viral disease treatment. Nuclease P1 from *Penicillium citrinum* with 24% sequence identity, the structure of which is known (PDB ID 1ak0) [3], is probably the closest structural homologue of TBN1.

Heterologous expression of TBN1 in tobacco leaves yields amounts and quality of the enzyme suitable for structural studies. Crystals with sufficient quality for X-ray diffraction analysis can be obtained. The first diffraction experiments were performed using an in house Gemini Enhanced Ultra diffractometer with the Atlas CCD detector (Oxford Diffraction) and three different crystal morphologies were identified (orthorhombic, rhombohedral and trigonal). Datasets for structural analysis were collected at the synchrotron radiation source BESSY II (Helmholtz-Zentrum Berlin), beam line MX-14-1, with a MARmosaic CCD detector. Presence of zinc in the protein was confirmed by X-ray fluorescence and an absorption edge scan and two MAD datasets (for a rhombohedral and a trigonal crystal) were collected. The phase problem was solved using the SHELXC, D and E program suite [4]. The TBN1 structure resembles some features of P1 nuclease with differences near the active site and in the glycosylation pattern.

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L24

STRUCTURAL BASIS FOR SUBSTRATE RECOGNITION BY GH30 GLUCURONOXYLANASE FROM *Erwina Chrysanthemi*

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The important industrial enzymes, endo- -1,4-xylanases (EC 3.2.1.8), have been classified into several glycoside hydrolase (GH) families based on hydrophobic cluster analysis, three dimensional structure and mode of action [1]. Best known enzymes belong to GH families 10 and 11. These enzymes do not seem to be specialized for hydrolysis of a particular xylan. Unique xylanases were assigned to GH family 30 [2]. Some of bacterial GH30 xylanases are specialized for hydrolysis of xylans containing D-glucuronosyl or 4-O-methyl D-glucuronosyl side residues. With enzyme species from *Bacillus subtilis* (Xyn C) and *Erwinia chrysanthemi* (XynA) it was clearly demonstrated that the cleavage of xylan main chain is dependent on the presence of uronic acid side residues [3-7].

Recently, the structure of xylanase A from the phytopathogenic bacterium *E. chrysanthemi* with free active site was reported [7]. Here we report the crystal structure of xylanase A in the complex with aldotetraouronic acid $MeGlcA^2Xyl_3$, an analogue of the product of enzymatic reaction. The crystal structure of the enzyme-ligand complex was solved at 1.39 Å resolution [8]. The ligand xylotriosyl moiety occupies three earlier recognized subsites -1, -2 and -3, while the MeGlcA residue attached to the middle xylopyranosyl residue of xylotriose is bound to the enzyme through hydrogen bonds to five amino acids and by the ionic interaction of the negatively charged uronic acid carboxylate with positively charged guanidinium group of Arg293. The interaction of the enzyme with MeGlcA residue appears to be indispensable for proper distortion of the xylan chain and its effective hydrolysis. Such a distortion does not occur with linear -1,4-xylooligosaccharides which are hydrolyzed by the enzyme at a negligible rate. In the close proximity to the catalytic amino-acids Glu165 and Glu253 an electron density has been found into which a molecule of imidazole was modeled. Imidazole is a part of the crystallization buffer. It is suggested that imidazole occupies +1 subsite binding xylose or xylosyl residues of the enzyme-cleaved substrates. The structure of the protein-$MeGlcA^2Xyl_3$ complex was used for calculation of the binding energy for the ligand itself and for its analogues.

Structural analysis, energy calculations and experimentally measured specific activity of the enzyme on various substrates are used to answer the question why the enzyme does not attack efficiently linear -1,4-linked xylooligosaccharides.

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Friday, March 25, Session VI

L25

CRYSTAL STRUCTURE OF THE MOUSE GALECTIN-4 N-TERMINAL CARBOHYDRATE RECOGNITION DOMAIN

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Galectin-4, a member of the tandem repeat subfamily of galectins, participates in cell membrane interactions and plays an important role in cell adhesion, and modulation of immunity and malignity. Previously, we have reported on the oligosaccharide specificity of the mouse galectin-4 carbohydrate recognition domains (CRD). In this work, we further investigated the structure and binding properties of the N-terminal domain CRD1 and determined the crystal structure of CRD1 in complex with lactose at 2.1 Å resolution. We characterized lactose binding affinity by fluorescence measurements and identified two lactose binding sites: a high affinity site with the K_d value in micromolar range (K_{d1} = 600 ± 70 μM) and a low affinity site with K_{d2} of 28 ± 10 mM.

This study was supported by grants from the Grant Agency of the Czech Republic, reg. no. 203/09/0820 and 304/03/0090, by the grant 1M0505 from the Czech Ministry of Education, and by the institutional research projects AV0Z 40550506, AV0Z50520514, and AV0Z50520701 awarded by the Academy of Sciences of the Czech Republic.

L26

BIOLOGICAL APPLICATIONS OF QM/MM CALCULATIONS WITH MM POLARIZATION

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Hybrid quantum mechanical/molecular mechanics (QM/MM) calculation can be widely used for the study of the biological systems. The effect of polarization on the charge distribution is usually included in the QM part only and MM part is often neglected due to difficulties of the implementation. The MM polarization can however play an important role. Furthermore, there is inconsistence since the one part of the system (QM part) is polarized and the other (MM part) is not. Therefore we have introduced simple approach to treat the polarization of the MM part of the model. The approach is based on the treatment of polarization by induced atomic charges instead of induced dipoles. In this method, the induced dipoles (calculated from atomic polarizabilities and electrostatic potential arising from QM part) are represented by induced charges on the MM atom itself plus its neighboring atomic sites [1]. The main advantage of this approach is the easy implementation to the existing MM programs, since the induced charges are simply added to the permanent MM atomic charges and there is no need to implement the evaluation of charge-dipole interactions. We have implemented the method in various QM/MM programs.

We have applied the method for the study of the enzymatic reaction of chorismate to prephenate within the chorismate mutase. The polarization has stabilizing effect on the transition state and we have observed the decrease of the activation energy by 5-7 kcal/mol.

We have also applied the MM polarized QM/MM method in the attempt to improve the results of the ligand docking in the case of the fragment based drug design. Since the smaller fragments bind more weakly to the protein than the bigger drug like molecules, the more accurate docking is required. The standard docking by program Glide was performed on the series of the fragments with known crystal structure [2]. The docking results were then significantly improved by rescoring of the poses using QM/MM calculations and by re-docking the ligands using the polarized charges of both the ligand and the protein. We have successfully predicted 11 out of 12 structures when using our approach, compare to just 5 out of 12 structures when the standard docking by Glide was used.

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L27

STRUCTURE AND CONFORMATIONAL BEHAVIOR OF THE DNA OLIGONUCLEOTIDE ATTACHED TO THE CHARGED SURFACE: A MOLECULAR DYNAMICS STUDY

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Although surface plays an important role in microarray hybridization, many software tools available for the probe design are still based on standard hybridization conditions, i.e. nucleic acids in solution, not being attached to a surface. First steps to estimate some aspects concerning the influence of the surface have already been made, based both on experiments and on theoretical considerations, but the underlying phenomena remain poorly understood.

In our theoretical study we focused on the role of the electric field on structure and conformational behavior of DNA anchored to the surface. The surface is represented by a graphene monolayer with partial charges on carbon atoms varying from -0.1 to +0.1e in the simulations. It corresponds to the typical values of charge densities in similar experiments. Both single strand and double strand oligonucleotides were studied to mimic conditions before and after hybridization. The single stranded decanucleotide 5'-CCACTAGTGG-3' was attached to the graphene layer via a C6 aliphatic linker.

Two types of interactions of DNA with surface are crucial for the stabilization of the DNA – surface system. Whereas for surface with zero or low positive charge density the dispersion forces between the closest base/base pair and the surface dominate, higher charge densities on the surface lead to strong electrostatic interaction between the phosphate groups of DNA and the surface and ions.

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L28

COMPETING REACTIONS OF DISULPHIDES AND HYDROXYL ANION – QM STUDY

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Disulphide moiety is found in the majority of proteins and attack by the hydroxyl anion can be expected in the water environment. The studied competing reaction mechanisms encompass (i) nucleophilic substitution on sulphur with two different angles of attack, (ii) nucleophilic substitution on -carbon, (iii) eliminatinon from or (iv) -carbon and (v) internal elimination reaction without participation of OH^- nucleophile. The minimal energy pathways do not allow for unequivocal distiction of the preffered mechanism.

Saturday, March 26, Session VII

L29

IMPACT OF POINT MUTATIONS ON PROTEIN FUNCTIONS: EXAMPLE CASE OF RAS GTPASES

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Proteins point mutations belong to the biological variations that are indispensable driving force of evolution. They play a significant role in the adaptation of organisms to the changing environment or in the micro-evolution of immune system. However, they are in the focus of interest predominately because of their harmful potency. Occurring during the life can lead to various diseases and cancer with possible lethal consequences. Inherited via the germline can be also lethal or cause live-long handicaps. Although many cases are known, mechanism by which the point mutations influence the functions of proteins is vastly unknown. Moreover, general question arises: "Is there some general mechanism at all?"

In several studies we have investigated the impact of single or multiple point mutations on protein functions, most recently on the function of key GTPase Ras. Nevertheless either at molecular or cellular level, under in-vivo or in-vitro conditions, with natural or artificial mutations – results indicate that the issue is much harder then anticipated.

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L30

STRUCTURE-BASED DRUG DESIGN OF SELECTIVE 5'-NUCLEOTIDASES INHIBITORS

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The monophosphate 5'-nucleotidases, including 5'(3')-deoxyribonucleotidase, belong to a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates. The ribonucleotides and deoxyribonucleotides can be synthesized *de novo* from low-molecular-weight precursors or by salvage from nucleosides or nucleobases produced in catabolism of nucleic acids [1]. In this salvage pathway, ribonucleotides and deoxyribonucleotides are phosphorylated by nucleoside and nucleotide kinases to maintain sufficient pools of dNTP's and NTP's for synthesis of DNA and RNA, respectively. The phosphorylation by cellular nucleoside kinases is opposed by 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates [2,3,4]. Besides their role in the regulation of physiological dNTP pools, substrate cycles between ribonucleotidases and kinases may affect the therapeutic action of pyrimidine nucleoside analogs used as anticancer and antiviral agents. Such compounds require the nucleoside kinases activity for phosphorylation to their active forms. Results of clinical and *in vitro* studies propose that an increase in nucleotidase activity can interfere with nucleoside analogue activation resulting in drug resistance [5].

The main goal of this project is the search for potent and selective inhibitors of mammalian 5'-nucleotidases based on nucleoside phosphonic acids and their derivatives and comparison of sensitivity of 5'-nucleotidases isolated from various sources toward individual inhibitors.

We have prepared 2 types of human 5'-nucleotidase: cytosolic and mitochondrial by recombinant expression in *E. coli*. Two strategies of finding potential inhibitors are used. First, a random series of nucleoside phosphonic acids derivatives are tested. Second, testing of rationally designed compounds based on a published structure of known inhibitor-enzyme complex [6]. In this structure-based design we have already found 100x better inhibitor

of mitochondrial nucleotidase and some other promising compounds.

In general, compounds of strong and selective inhibitory potency are of high medicinal interest as antimetabolites for anticancer and antiviral therapy.

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L31

CERIT-SC CENTRE: LOOKING FOR SYNERGIES IN SCIENTIFIC COMPUTING

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Recently we presented the intent to form the CERIT-SC (CERIT—-Scientific Cloud) data and computing centre at the Institute of Computer Science of Masaryk University. Since then a project proposal was submitted, it passed evaluation, and currently the project is in its negotiation phase. Here we describe the main principles and goals of the emerging CERIT-SC centre, and we expect to start discussions on future collaboration with the conference attendees.

Considering the startup of ESFRI and CZ OP "Research and Development for Innovations" projects, deluge of experimental data is expected in near future. The unprecedented amounts of data, together with the development of the computing equipment (increasing number of CPU cores in particular), will enforce changes of both data storage and processing paradigms. Many currently deployed methods will become unusable when applied on data larger by several orders of magnitude.

New methods will have to be developed to work with the emerging equipment and with the expected data sizes efficiently. This can be achieved only by close collaboration of experts in both the application area and IT. The main sustaining goal of the CERIT-SC centre is provision of this IT expertise, besides computing and storage resources of competitive quantity. Following its 15 years computing centre tradition at the Institute, the CERIT-SC centre will build an expert team of researchers, administrators, and developers, including undergraduate and Ph.D. students. The team members will, together with the centre users, work on improvement of current tools and programs, as well as development of new computational methods appropriate for emerging scientific challenges. This joint effort is expected to yield efficient and extensive use of the computing resources, and consequently novel scientific results to beachieved.

Provision of the physical resources will follow the modern "cloud" paradigms and they will be provided to the scientific community free of charge. The practical outcome is a very flexible resource provision the users, once registered with the centre, will not be bothered repeatedly to pass any formal process to be granted resources; those will be available on-demand (as opposed to standard supercomputing centres). On the other hand, the centre capacity is still limited, therefore the amount of resources actually allocated to the users will be controlled, and rather complex policies will be applied. Once the user/group achieves recognized scientific results, it will get prioritized appropriately on top of always available entry-level share. However, such priority will decay over time if it is not "refreshed" by further results. In this way, successful communities are prioritized while new users, students, etc. are not prevented to use the resources.

With the exception of assessment of scientific results (which will follow generally accepted criteria) all the priority assignments and resulting resource allocation will be achieved by technical means, combining advanced resource scheduling, virtualization, and the cloud paradigm; no administrative process will be involved. We have several years experience with development and deployment of such system, and we will also leverage the R&D potential of the Institute and the collocated Faculty of Informatics.

The centre will offer computational clusters of two types "High Density" (many nodes of 8 16 CPU cores and up to 32 GB RAM) and "Symmetric Multi-Processing" (fewer "fat" nodes of more than 64 CPU cores and over 128 GB RAM), and a hierarchy of storage capacities the fastest disks to be used during computation, standard disks for active data, and Hierarchical Mass Storage for long term data archiving. Together with an appropriate network interconnect (40 Gbps Infiniband typically), these resources cover the entire spectrum of possible user requirements, with the exception of the most demanding tasks which require specialized supercomputers.

The centre initial phase is expected to be funded by the mentioned OP Research and Development for Innovations project. According to the current plans, the first SMP clus-

ter of approx. 500 CPU cores and 250 TB of disk space will be purchased in Q3/2011. Another 500-core SMP cluster and a 500-core HD cluster will follow in 2012. Later in 2012, the HSM storage of 3 PB will be purchased. In 2013, a 2000-core HD cluster and additional 350 TB of disk space is planned. With this installation the planned sustained size of resources will be reached. Further on, the equipment will be renewed periodically, keeping it at least at this level.

The plans of the CERIT-SC centre were clarified over the last year. Despite the project funding has not been formally confirmed at the time of writing this contribution, our expectations are positive. Our goal is to prepare both users for new infrastructure possibilities and the infrastructure for users' needs. Close cooperation of the Centre and the users, from mere ad-hoc consultations to common projects, will be beneficial for both sides. As such, it is one of the cornerstones of the Centre long-term strategy.

L32

METACENTRUM IN E-INFRASTRUCTURE ERA

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In recent years, various infrastructure projects are being prepared on the European level (ESFRI projects) on national level (Research and Development for Innovations program) in different scientific areas. In IT area, three major infrastructure projects are emerging in Czech Republic (eIGeR, CERIT-SC and IT4Innovations) with common goal — support IT-related requirements of projects from various scientific areasand create common e-Infrastructure, which will will encompass advance networks, computing and storage capacitiesand higher-level services. In this presentation, position of MetaCentrum will be presented, together with our actual and future plans, and services already provided or planned.

Under umbrella of CESNET e-Infrastructure project eIGeR, where grid computing is one of four major directions of development (together with national optical network, storage services and collaborative tools), MetaCentrum project is transforming itself to National Grid Infrastructure (NGI), which will cover not only provisioning of computational resources to users, but also interoperability with international grid infrastructures (namely EGI) and access coordination to these services with remaining IT providers.

In following years, moderate expansion of computational clusters already provided by MetaCentrum is expected, with first acquisition planned in summer 2011 (additional 500 CPU cores, 100TB of disk space) and similar extensions are in next years too. MetaCentrum resources will encompass cluster installations in several cities (Pilsen, Prague, Brno according current plans) with hardware architecture following currently deployed clusters (x86 architecture, mixture of high-density and larger shared-memory (SMP) nodes, accompanied with large shared filesystem). However, this resources should be perceived as a "glue", which should allow integration of larger resources, provided by other IT projects but also integration of clusters directly purchased by scientific projects from different application areas. To support integration of such more independent clusters, we have prepared major change in job management and scheduling tools, which includes transition from central scheduling system to decentralized pool of cooperating scheduling systems and further development in area of virtualization. We will present first results of this development, together with practical impact to users and owners of clusters already connected to MetaCentrum. We will also present first features inspired by cloud evolution, which allows easier adoption of this computing model in science.

National grid infrastructure must be directly connected to similar projects abroad. NGI will follow up with established cooperation with EGI, European project which build corresponding infrastructure across Europe. Native integration of national resources, or even resources (clusters) owned by research groups, into worldwide collaborative environment will support easier and seamless cooperation with scientific partners across the world. Transformation of MetaCentrum to more stable and dependable NGI gave us possibility to expand our services to areas, which are either induced from empowered coordination role or traditionally provided by standard high-performance centers. NGI will provide unified access to compute resources provided by various centers and will be, in cooperation with national and European partners, working on development, deployment and enforcement of these standards across all IT providers.

New possible area of cooperation is deployment of various application software tools; NGI itself will have a budget, which will allow broader set of development and application tools to be deployed in NGI, but we will also offer cooperation in software purchase process, again not only with other IT projects, but also with large scientific groups, which are also planning acquisition of new software tools and licenses in their projects.

Based of long experience with management of grid systems and with development of such environment, including non-trivial experience with evolving cloud paradigm, NGI will continue to provide modern computing environment not only for users, but also for scientific groups, which will own their own computational resources and will be willing to integrate them to larger infrastructure. NGI will also provide assistance to users and provide IT expertise, required for successful adoption of cluster or grid programming and optimization of computational code to such environments.

Saturday, March 26, Session VII

L33

QSPR MODELING – ALGORITHMS, CHALLENGES AND IT SOLUTIONS

O. Skřehota, R. Svobodová Vařeková, S. Geidl, M. Kudera, D. Sehnal, C. M. Ionescu, T. Bouchal, and J. Koča

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Nowadays, a large amount of experimental and predicted data about the 3D structure of organic molecules and biomolecules is available. Advanced computational methods and high performance computers allow us to process these data and calculate descriptors – numerical values, which encode the structural characteristics of molecules. Hundreds if not thousands of molecular descriptors have been designed for various goals. One very useful application is to employ descriptors in Quantitative Structure-Property Relationship (QSPR) models for physicochemical properties (e.g. dissociation constants, partition coefficients, solubility, lipophilicity, etc.) prediction.

QSPR modeling has become very popular in chemical, biological and pharmaceutical research. However, the design of QSPR models for predicting many important physicochemical properties is still a topic of research. This is caused, among other things, by the fact that the process of QSPR model design and evaluation is relatively complicated. At the beginning of this process, one only has a rough suggestion of descriptors. One uses these first ideas and implements algorithms for processing molecular structures and for calculating the descriptors. Afterwards, an equation expressing the relation between the descriptors and the property in question must be formulated, i.e., the QSPR model has to be parameterized. And finally, one evaluates how accurately the model correlates with reference (mainly experimental) data. The results of this evaluation extend our knowledge and help us to correct the model (e.g. add or remove some descriptors). The procedure of improvement of a model can be repeated many times.

OSPR modeling covers many different areas of interest. Therefore currently available software packages (e.g. OCHEM [1] or Arguslab [2]) can read descriptors from input and create the QSPR model, but they can not calculate descriptors, and they are able to evaluate models only one by one, along with other limitations.

For this reason, we have developed a modular and easily extensible program, called QSPR Designer [3], which can read or calculate structural properties of atoms and bonds, employ them as QSPR descriptors, and evaluate relationships between the descriptors and the examined physicochemical property of the molecules in question. Furthermore, the software allows us to design and parameterize QSPR models, calculate physicochemical properties via the models, test the quality of the models, and provide graphs and tables summarizing the results.

The performance of the software is demonstrated by a case study on the prediction of pK_a, which is one of the most challenging properties to calculate [4]. Using the QSPR Designer, we have successfully designed, evaluated, and compared a lot of different QSPR models for the prediction of pK_a from charges.

1. S. Novotarskyi, I. Sushko, R. Krner, A. P. Kumar, M. Rupp, V. V. Prokopenko, I. Tetko: OCHEM - on-line CHEmical database & modeling environment. Journal of Chemoinformatics, 2 (2010), P5.
2. M. A. Thompson: ArgusLab 4.0, Planaria Software LLC (2008). Available from: http://www.arguslab.com.
3. O Skřehota, R. Svobodová Vařeková, S. Geidl, M. Kudera, D. Sehnal, C.-M. Ionescu, J. Koča: QSPR Designer – a program to design and evaluate QSPR models. Case study on pK_a prediction. 6th German Conference on Chemoinformatics, (2010).
4. A. C. Lee, G. M. Crippen: Predicting pK_a. J. Chem. Inf. Model., 49 (2009), 2013 -2033.

L34

Aleuria aurantia LECTIN FAMILY – STRUCTURAL INSIGHT INTO DIFFERENCES OF CARBOHYDRATE BINDING AMONG RELATED PROTEINS

Josef Houser[1], Jan Komárek[2], Nikola Kostlánová[1], Gianluca Cioci[3], Anne Imberty[4], Michaela Wimmerová[1,2]

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Aleuria aurantia lectin (AAL) has been studied for many years. Its structure was solved in 2003 [1] revealing 6-beta propeller fold with five binding sites formed in between blades. The existence of several different binding sites leads to the difficulties in determination of their binding properties, although several studies were undertaken [2]. Interestingly, over last decade several lectins from AAL family have been identified. They all posses the same fold as AAL, but some important structural differences make them an ideal targets for structural-functional relationship study.

AAL homologue RSL from *Ralstonia solanacearum* is one of the strongest monosaccharide binders known. It was found out that it is able to bind ligands with subnanomolar affinity with no ions involved in the interaction [3]. Another lectin from *Aspergillus fumigatus* (AFL) with six different binding sites per monomer has even wider variability in carbohydrate binding than AAL itself. All these proteins are studied in our group intensively on molecular level. Thanks to the combination of crystallographic data and advanced functional studies (ITC, SPR,…) we build up a complex picture of lectin-saccharide interaction with a great potency for future medicinal and biotechnological applications.

The research has been supported by Ministry of Education of the Czech Republic (MSM0021622413, LC06030, ME 08008), Grant Agency of the Czech Republic (303/ 09/ 1168) and the European Community's Seventh Framework Program under grant agreement n° 205872.

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2. J. Olausson, L. Tibell, B.H. Jonsson, *Glycoconjugate J.* **25**(8) (2008), 753-762.
3. N. Kostlanova, E.P. Mitchell, H.Lortat-Jacob, S. Oscarson, M. Lahmann, N. Gilboa-Garber, G. Chambat, M. Wimmerova, A. Imberty, J. Biol. Chem **280**(30) (2005), 27839-27849.

L35

FUNCTIONAL COUPLING OF DNA DUPLEX TRANSLOCATION TO ITS CLEAVAGE IN A TYPE I RESTRICTION ENZYME

R. Ettrich[1, 2], M. Lapkouski[1], A. Guzanova[3], L. Csefalvay[1], T. Baikova[1, 2], E. Dellapenna[4], M. Khabiri[1,2], I. Shevelev[4], P. Janscak[5,6], S. Panjikar[7], J. Carey [1,7], Marie Weiserova[3], I. Kuta Smatanova[1, 2], E. Csefalvay [1, 2]

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The type I restriction-modification enzymes differ significantly from the type II enzymes commonly used as molecular biology reagents. On hemi-methylated DNAs type I enzymes act as conventional adenine methylases at their specific target sequences, but unmethylated targets induce them to pull thousands of basepairs through the enzyme before cleaving distant sites nonspecifically. Biochemical, biophysical, and molecular biological studies of their translocation and cleavage mechanisms offer a wealth of detail that has lacked a structural framework. The crystal structure of the motor subunit responsible for DNA translocation and cleavage by the type I enzyme EcoR124I, resolved at 2.6 Å [1], shows a lysine residue on the endonuclease domain to contact N3 on the exposed edge of ATP bound at the helicase domains, potentially coupling endonuclease and translocase functions. Protein crystallography, point-mutations, *in vivo* and *in vitro* testing, in combination with computational modelling are used as tools to explain the coupling of endonuclease and translocase functions in Type I restriction-modification complexes in general.

1. Lapkouski M, Panjikar S, Janscak P, Kuta Smatanova I, Carey J, Ettrich R, Csefalvay E *Nat Struct Mol Biol* **16**: 1. (2009) 94-95.

We gratefully acknowledge support from the Ministry of Education, Youth and Sports of the Czech Republic (MSM6007665808, LC06010), the Academy of Sciences of the Czech Republic (AVOZ60870520), the Grant Agency of the Czech Republic (Nos. P207/10/1934), and joint Czech - US National Science Foundation International Research Cooperation (ME09016 and INT03-09049), Additionally, T.B. and M.K. were supported by the University of South Bohemia, grant GAJU 170/2010/P.

PROGRAMME

Thursday, March 24

12:00 13:15 Registration

13:30 13:45 Opening

13:45 15:45 **Session I** chairperson: *Vladimír Sklenář*

L1 13
I. Barák
Lipid helices formation in *Bacillus subtilis* cell membrane * (*30 min*)

L2 13
P. Dvořák
Engineering of biochemical pathway for degradation of Anthropogenic environmental pollutant 1,2,3-trichloropropane

L3 14
J. Prchal
Expression and purification of myristoylated matrix protein of Mason-Pfizer monkey virus for Structural Studies

L4 15
J. Dohnálek
The molecular puzzle of C-type lectin like natural killer cell receptors

L5 15
P. Malý
Generation of artifical binders with affinity to human cytokines via computer-assisted mutagenesis of a stable protein scaffold and ribosome display selection *

15:45 16:00 *Coffee break*

13:45 15:45 **Session II** chairperson: *Petr Novák*

L6 16
D. Rozbeský
Chemical cross-linking and H/D exchange combined with mass spectrometry: A tool to validate and refine 3-D protein X–ray model

L7 17
P. Hanč
Structural details of NKR-P1D: Clrb interaction elucidated by protein cross-linking and mass spectrometry

L8 17
V. Andrushchenko
Supramolecular Assemblies Formed by Diolein and Stearyl Alcohol

L9 18
L. Řežábková
Structure of the 14-3-3/RGS3 complex: New details on architecture of complexes formed by 14-3-3 proteins

L10 19
M. Šebela
Structural and functional study on the glycosylation in maize cytokinin oxidase/dehydrogenase1

L11 20
P. Man
Accessibility changes within diphtheria toxin T domain upon membrane penetration probed by hydrogen exchange and mass spectrometry

18:30 20:00 Opening dinner at the Novy Zamek

20:00 22:00 **Poster Session I**, at the Novy Zamek

Friday, March 25

7:30 8:30 Breakfast

9:00 10:20 **Session III** chairperson: *Bohdan Schneider*

L12 21
Yoshiuki Tanaka
NMR spectroscopic approaches to the mechanism of action of hammerhead ribozyme*

L13 21
J. Přecechtělová
Torsion angle dependence of phosphorus chemcial shifts in a nucleic acid backbone from combined molecular dynamics and density functional calculations*

L14 22
V. Sychrovský
Proposal of alternative pathway of the glycosidic bond cleavage mechanism of 8-oxoguanosine catalyzed by the human DNA repair protein hOGG1

10:20 10:40 *Coffee break*

10:40 12:20 **Session IV** chairperson: *Vladimír Baumruk*

L15 23
V. Profant
Experimental and theoretical study of formation of polyproline II helix by means of Raman optical activity

L16 24
J. Hudecová
Conformational study of the cyclo(L-Trp-L-Trp)dipeptide

L17 24
Z. Futera
Application of New QM/MM Interface to Study of Ru Drugs Interacting with DNA

L18 25
O. Kroutil
Structure and Conformatial Behavior of the DNA Oligonucleotide Attached to the Charged Surface: A Molecular Dynamics Study

L19 25
I. Kishko
Kinetic analysis of *E. coli* WrbA redox activity

12:30 13:45 Lunch

14:00 16:00 **Session V** chairperson: *Petr Malý*

L20 26
J. Hašek
Reliability and Accuracy of Structure Determination in Diffraction Methods
L21 27
P. Řezáčová
Crystal structures of two protease inhibitors from tick saliva*
L22 28
A. Štepánková
Three-dimensional structure of binunctional anhydrolase
L23 29
T. Koval
Structural analysis of a recombinant plant bifunctional nuclease TBN1
L24 30
L. Urbániková
Structural basis for substrate recognition by GH30 glucuronoxylanase from *Erwinia Chrysanthemi**

16:00 16:15 *Coffee break*

16:15 17:45 **Session VI** chairperson: *Jiří Vondrášek*

L25 31
J. Brynda
Crystal structure of the mouse galectin-4 N-terminal carbohydrate recognition domain *
L26 31
D. Řeha
Biological Applications of QM/MM Calculations with Explicit MM Polarization
L27 32
Z. Chval
Mechanism of the Oxaliplatin Binding to the Double-Stranded 1,2-ds(pGpG) Dinucleotide in Aqueous Solution
L28 32
M. Krupička
Competing reactions of disulphides and hydroxyl anion - QM study

18:00 19:15 Dinner

19:30 20:30
First General Assembly of the Czech Society for Structural Biology, at the Novy Zamek. Meeting will be held in the Czech and/or Slovak languages.

20:30 23:00 **Poster Session I**I, at the Novy Zamek

Saturday, March 26

7:00 9:00 Breakfast

9:15 10:45 **Session VII** chairperson: *Pavlína Řezáčová*

L29 33
R. Dvorský
Impact of Point Mutations on Protein Functions: Example Case of Ras GTPases*
L30 33
P. Pachl
Structure-based drug design of selective 5'-nucleotidases inhibitors
L31 34
M. Ruda
MetaCentrum in e-Infrastructure era
L32 35
A. Křenek
CERIT-SC Centre: Looking for Synergies in Scientific Computing

10:45 11:00 *Coffee break*

11:00 12:00 **SessionVIII** chairperson: *Richard Hrabal*

L33 36
O. Skřehota
QSPR modeling - algorithms, challenges and IT solutions
L34 37
J. Houser
Aleuria aurantia lectin family - structural insight into differences of carbohydrate binding among related proteins
L35 38
R. Ettrich
Functional coupling of DNA duplex translocation t

12:15 14:00 *Lunch*

Unless stated otherwise, all events are held at the Rezidence hotel.

Thirty minute talks are labeled by an asterix, (*), other talks have a limit of 20 minutes.

All times include discussion and will be strictly observed.

POSTERS

Posters

P1

CRYSTALLIZATION AND STRUCTURE-FUNCTIONAL ANALYSIS OF MUTATED MOTOR SUBUNITS OF TYPE I RESTRICTION ENZYME ECOR124I

Tatsiana Baikova[1,2], Mikalai Lapkouski[1], Santosh Panjikar[3], Ivana Kuta Smatanova[1,2], Rudiger Ettrich[1,2] and Eva Csefalvay[1,2]

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Invasion of foreign DNA into microorganisms can be prevented by restriction–modification (R-M) systems. Though Type I R-M enzymes are not site-specific endonucleases they remain an important area of research mainly because of their unusual biochemical properties and their unique mechanisms of DNA translocation and restriction. The crystal structure of the motor subunit, responsible for translocation and restriction, of the type I enzyme EcoR124I , resolved at 2.6 Å, shows a square-planar arrangement of globular domains with a prominent cleft that accommodates DNA extending from the two canonical helicase domains to the endonuclease active site [1]. Unexpectedly, ATP bound in the helicase cleft is also engaged by Lys220 of the nuclease domain, potentially coupling the nuclease and translocation activities. To shed light on the coupling mechanism, mutants were designed that should alter the coupling at this position by influencing DNA cleavage but leaving the ATPase activity of EcoR124I unaltered. Three-dimensional structural information is essential to complement experimental data in a way, that lets us draw conclusions about the molecular mechanism. We discuss mutations of Lys220 to Ala, Arg and Glu, and the successfully solved crystals structures of the Arg220 and Glu220 mutants, that we determined to 2.74 A (PDB 2w74) and 2.99A resolution (PDB 2y3t), respectively.

Data collection of Glu220 crystal was performed on beamline X11 at EMBL/DESY (Hamburg). Data from 451 images were integrated and scaled with XDS and XSCALE. The motor subunit Glu220 crystals belong to space group P21 and unit cell dimensions are a =127.11 Å, b =123.11 Å, c =160.11 Å, and = 111.48°. The structure was solved by molecular replacement using Auto-Rickshaw19 and MOLREP programs, and using our previously solved wild type structure (PDB 2w00) as the search model.

1. Lapkouski M., Panjikar S., Janscak P., Kuta Smatanova I., Carey J., Ettrich R., Csefalvay E. *Nat. Struct. & Mol.Biol*, 2009, 16, 94.

We gratefully acknowledge support from the Ministry of Education, Youth and Sports of the Czech Republic (MSM6007665808, LC06010), the Academy of Sciences of the Czech Republic (AVOZ60870520), the Grant Agency of the Czech Republic (Nos. P207/10/1934), and joint Czech - US National Science Foundation International Research Cooperation (ME09016 and INT03-09049), Additionally, T.B. was supported by the University of South Bohemia, grant GAJU 170/2010/P.

P2

HOW COULD FLEXIBILITY OF RNA THREE WAY JUNCTION FROM THE GTP-ASE ASSOCIATED CENTER OF 50S ASSIST TO THE ACCOMMODATION OF THE TRNA INTO THE RIBOSOME?

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[2] Gilead Sciences&IOCB Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo namesti 2, 166 10, Prague 6

We studied RNA three-way junction (3WJ) formed by Helices 42-44 (H42-H44) organizing the GTP-ase associated center (GAC) of 23S rRNA by using explicit solvent molecular dynamics (MD) simulations. The GAC 3WJ is the RNA component of the L7/L12 stalk forming a side protuberance of the large ribosomal subunit, which interacts with the EF-G and with EF-Tu+GTP+tRNA complex, see the Figure. We have included ribosomal protein L10 in our study while the L7/L12 proteins could not be included do to absence of sufficiently complete experimental data.

The aim of the study was to analyze the intrinsic flexibility of the H42-H44 rRNA and to consider it in the ribosomal context in available crystal structures of the ribosome including tRNAs, elongation factors, etc. The flexibility of the RNA is evaluated based on preferred stochastic thermal fluctuations sampled in unrestrained simulations. The simulations thus identify the intrinsic

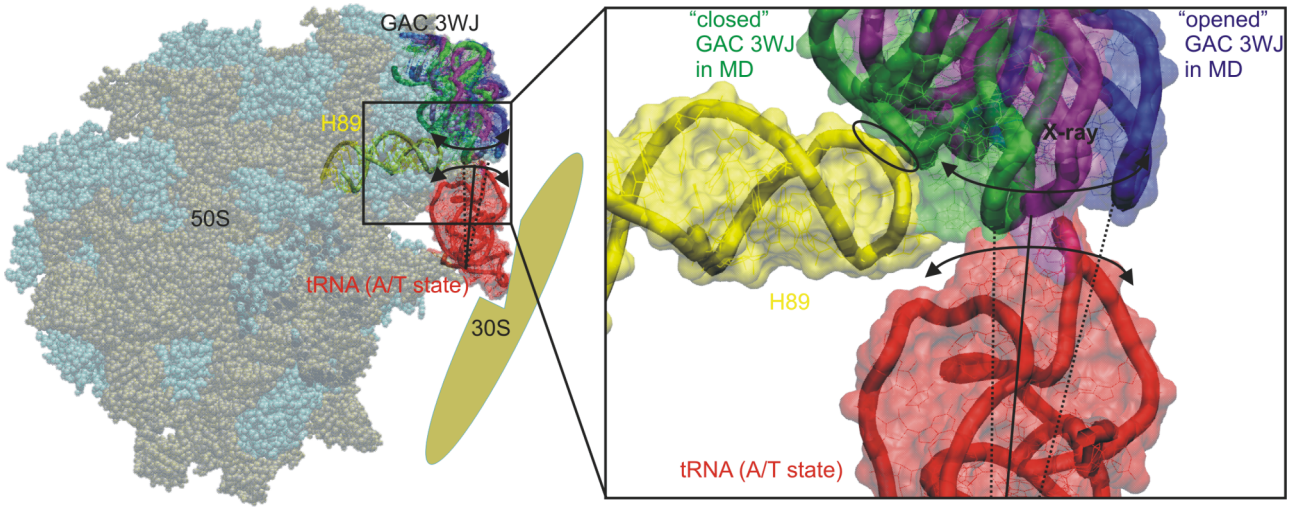

Figure 1: Left - Large ribosomal subunit (RNA in tan, proteins in cyan, pdb code 2WRO [2]) from *Thermus termophilus* including tRNA in the A/T state (in red, 2WRN [2]). The GAC 3WJ in the X-ray data is in purple, opened and closed geometry of the junction occurred in the MD simulations is shown in green and blue, respectively. Right - Detailed view on coordinated hypothetical movement of the tRNA and GAC 3WJ. The closed MD geometry of the GAC 3WJ forms contact with H89 (marked by ellipse), which might be a key point, where GAC 3WJ deliver tRNA to H89 (in yellow).

low-energy deformation modes of the molecules that can co-operate with the surrounding ribosomal elements to achieve the functional dynamics.

MD reveals that GAC 3WJ possesses significant anisotropic hinge-like flexibility [1]. Projecting observed movement into the ribosome, H43/H44 is flexible in direction towards and away (closing-opening geometry path of the GAC 3WJ) of the large ribosomal subunit, see the Figure. When the H42-H44 domain is in the overall "closed" conformation, the tip of the hairpin loop of H89 can fit into the groove defined by the docking of the hairpin loops of H43/44. However, such contact is only seen in the 2AW4 crystal structure of vacant *E.coli* ribosome [2]. In other crystal structures the distance between the H89 and GAC RNA varies widely (it even exceeds in some cases 10 Å) [3, 4]. The experimental structures show a wide range of positions sampling a set of more inward and more outward structures with respect to the A-site of the large subunit and H89. The range of observed positions coincides well with the anisotropic flexibility direction predicted by MD.

The simulations show that flexibility of the GAC RNA stems from the 3WJ and includes also the H42 stem region below the 3WJ and above the conserved H42-H97 tertiary interaction. The GAC rRNA could undergo large-scale rapid thermal fluctuations or structural adaptations to facilitate gliding of the tRNA to H89, which lead it into its functional destinations (A/A state) [5], see the Figure 1.

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2. B. S. Schuwirth, M. A. Borovinskaya, C. W. Hau, W. Zhang, A. Villa-Sanjurjo, J. M. Molton & J. H. D. Cate, *Science*, 310 (2005) 827-834.
3. T. M. Schmeing, R. M. Voorhees, A. C. Kelley, Y. G. Gao, F. V. Murphy, J. R. Weir & V. Ramakrishnan, *Science*, 326 (2009) 688-694.
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5. K. Y. Sanbonmatsu, S. Joseph & C. S. Tung, *Proc. Natl. Acad. Sci. U. S. A.*, 102 (2005) 15854-15859.

P3

HELICAL-HELICASE 2 DOMAINS INTERACTIONS IN HSDR SUBUNIT OF ECOR124I RESTRICTION-MODIFICATION COMPLEX

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Procaryotic type I Restriction-Modification systems effectively recognize and destroy phage DNA by cooperative recruitment of endonuclease, ATPase and DNA translocase and protect the host genome from being self-restricted by DNA methyltransferase action [1].

Type I R-M systems are multi-subunit enzymes. The complex is composed of two HsdR (**R**estriction and translocation) subunits, two HsdM (**M**ethylation) subunits, and one HsdS (**S**pecifity) subunit. HsdR subunit is the biggest part of the complex and responsible for DNA cleavage. Additionally it acts as an ATP-dependent DNA translocase [1]. HsdR is organized into four approximately globular structural domains in nearly square-planar arrangement: the N-terminal endonuclease domain, the recA-like helicase domains 1 and 2 and the C-terminal helical domain. The near-planar arrangement of globular domains creates prominent grooves between each domain pair. The two helicase-like domains form a canonical helicase cleft in which double-stranded B-form DNA can be accommodated without steric clash. A positively charged surface groove proceeds from the helicase cleft and continues between the helical and endonuclease domains where it passes over the cleavage site recessed slightly from the surface [2]. The C-terminal helical domain resembles the fold of HsdM and has strong interactions with helicase 2 domain. During translocation large-scale rotation of helicase domain 2 relative to the helicase domain 1 is expected, which would significantly alter the helical-helicase domain interface. Salt-bridges or hydrogen bonds over this interface are the major energetic contribution to the binding energy between both domain, and must play a key role in accommodating the different rotational stages of helicase domain 2 during the translocation cycle. We map residues that are essential for helical-helicase 2 domains interactions by using a combination of site-directed mutagensis and *in vivo* and *in vitro* activity assays and put the results into the broader context of DNA translocation and following restriction.

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2. Lapkouski M., Panjikar S., Janscak P., Kuta Smatanova I., Carey J., Ettrich R., Csefalvay E. *Nat. Struct. & Mol.Biol*, 2009, 16, 94.

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P4

INVESTIGATION OF LIGAND PASSAGE IN PROTEINS

Lada Biedermannová[1], Zbyněk Prokop[2], Radka Chaloupková[2], Pavel Dvořák[2], Jiří Damborský[2] and Rebecca Wade[1]

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The function of many globular proteins depends on their interaction with small organic molecules - substrates, products, cofactors, or inhibitors - as well as the solvent. The interaction sites, however, are often not located on the surface of the protein, but rather deeply buried in the structure. In order to understand the function of such proteins, we need to understand the process in which ligands pass through the tunnels leading from the bulk solvent to the binding site. Here, we investigate ligand passage in haloalkane dehalogenase enzyme LinB [1], and the effect of mutation on it. The Random Acceleration Molecular Dynamics (RAMD) method [2], developed in our lab, is employed to find the pathways available for the products of the LinB catalyzed reaction. The free energy profiles of the product unbinding process are investigated using the Adaptive Biasing Force (ABF) method [3]. In order to understand the molecular details of the process, we focus on analyzing the dynamics of solvent molecules during the ligand passage. The hydration patterns turn out to play an important role in facilitating the passage of ions.

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P5

LOCALIZATION OF CALMODULIN AND PIP2 BINDING SITES ON THE C-TERMINI OF THE TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL TRPM5

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Transient receptor potential (TRP) channels are involved in the perception of a wide range of physical and chemical stimuli, including temperature, pain, taste, light, osmolarity changes and pheromones. Recent studies have indicated that members of the TRP family of ion channels can function as calcium influx channels both in excitable and non-excitable tissues. The channel subunits have six transmembrane domains that most probably assemble into tetramers to form non-selective cationic channels, which allow for the influx of calcium ions into cells. On the basis of structural information the TRP family is subdivided in three main subfamilies: the TRPC (canonical) group, the TRPV (vanilloid) group and the TRPM (melastatin) group.

TRPM5 channel is member of the TRPM ion channel family. Members of TRPM family are divided into 4 groups - TRPM1+3, TRPM2+8, TRPM4+5, TRPM6+7. TRPM4+5 are closely related cation channels that are ubiquitously expressed. TRPM5 is found mainly in the intestine, taste buds, pancreas, stomach, lung, testis and brain. Channels TRPM4+5 are impermeable to $Ca2^{+}$, but they however play roles in $Ca2^{+}$ modulation. TRPM4 channel is activated by $Ca2^{+}$ is often via complex signaling cascades including $Ca2^{+}$-Calmodulin binding. Characterization of binding site has not been determined in case of TRPM5. The series of different length of highly purified fusion proteins of C-terminus of TRPM5 was performed to get sufficient amount of soluble proteins. To study the location of Calmodulin binding site on the C-terminal segments of TRPM5 was used the fluorescence spectroscopy measurements. Similarly was identified PIP_2 binding site in C-termini of TRPM5 using side directed mutagenesis. It could be deduced that both sites (CAM and PIP_2) were overlapped in C-terminus of TRPM5. This project can help us understand the regulation of TRPM5 ion channel.

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P6

REDUCTION MECHANISM OF THE PT(IV) SATRAPLATIN DERIVATES BY GUANOSINE MONOPHOSPHATE; QUANTUM MECHANICAL STUDY

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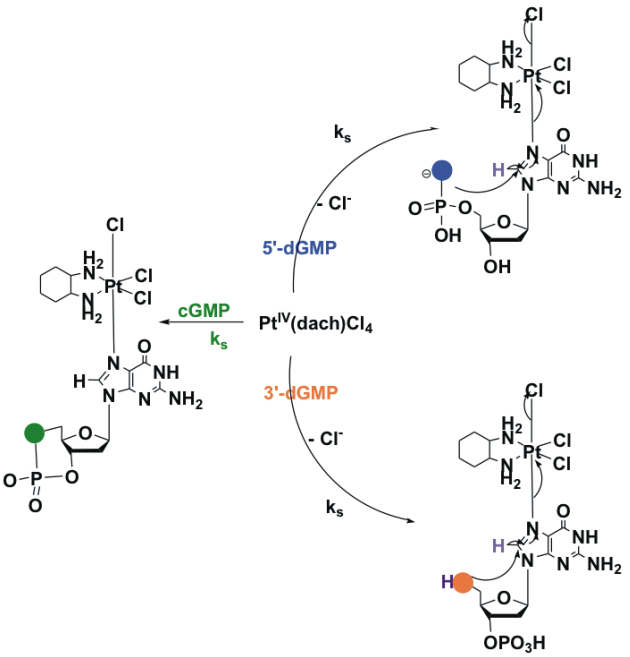
The platinum IV compound Satraplatin (JM216) was selected for clinical development because of: a) high cytotoxic activity *in vitro* against several solid tumor cell lines, including cisplatin resistant ones; b) *in vivo* oral antitumor activity against a variety tumor models; c) a relatively mild toxicity profile and oral bioavailability. In Phase 2 clinical trials, satraplatin showed activity against several different cancers, including prostate, ovarian, and small cell lung cancers [1].

When PtIV(dach)Cl_4 reacts with dGMP or GMP the pH drops form 8.3 to 3.4, and yellow crystals are formed. The crystals were identified as PtII(dach)Cl_2 by IR analysis (3267, 3190, 3066, 2936, 2863, and 1564 cm-1). This indicates that both dGMP and GMP can reduced Pt(IV) to Pt(II) [2].

Quantum chemical tool was used to confirm the suggested reactions and explore the detailed reduction mechanism. Both thermodynamic and kinetic characteristics were determined at several computational levels. The AIM, NBO and frequency analyses were used for examination of all the individual complexes.



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P7

THERMODYNAMICAL ANALYSIS OF THE MUTANT CV-IIL LECTINS FROM *CHROMOBACTERIUM VIOLACEUM*

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Lectins are proteins or glycoproteins of non-immune origin capable agglutinate cells. They are able to specifically recognize saccharides with high affinity. However, the major function of lectins appears to be in the cell recognition process, they generally recognize diverse sugar structures and mediate a variety of biological processes. Bacterial lectins play a crucial role in recognition of sugar moieties on the host cell surface and consequently can cause bacterial adhesion. An attractive approach is the use of agents that interfere with the ability of the bacteria to adhere to the host cell surface, since such adhesion is one of the initial stages of the infectious process. However, the process of ligand binding is very complex and complicated, deeper understanding of the thermodynamics of lectin-saccharide interaction can be useful for the molecular design of potent anti-adhesion therapeutics.

Chromobacterium violaceum is an opportunistic pathogen that commonly occurs in water and soil in tropical and subtropical regions. Infection caused by this bacterium can be fatal for immunocompromised people and children. Bacterial unusual resistance to antibiotics is the reason of high mortality.

The lectin from *C. violaceum* named CV-IIL is a tetramer (the structural functional unit is a dimer), each monomer is composed of 113 amino acids. This lectin shows ability to bind L-fucose (6-deoxy-L-galactose) and D-mannose with high affinity. Each monomer contains two calcium ions in the carbohydrate binding site, which mediate binding of the saccharides. The crystal structure of CV-IIL demonstrates that there is also one water molecule, which plays a special role by bridging sugar with backbone nitrogen atom and also with side chain of the amino acid threonine in position 97. (Fig.1) [1] Therefore, several representatives from different groups of amino acids were chosen for the mutagenesis in this position 97.

The complete thermodynamical analysis was performed using isothermal titration microcalorimetry method (ITC 200, Microcal). From the single measurement we can obtain and consequently calculate important thermodynamical values (enthalpy, entropy, Gibbs free energy) as well as binding affinity and stoichiometry of the lectin-saccharide interaction. The interaction with high affinity ligand -Me-fucoside was measured in case of each prepared mutant lectin at different temperatures in the range from 10 to 40°C at constant pressure. These detail measurements allow the determination of the heat capacity C_p - the slope of the enthalpy dependence on temperature. Accurate values of C_p for individual mutant allow partial

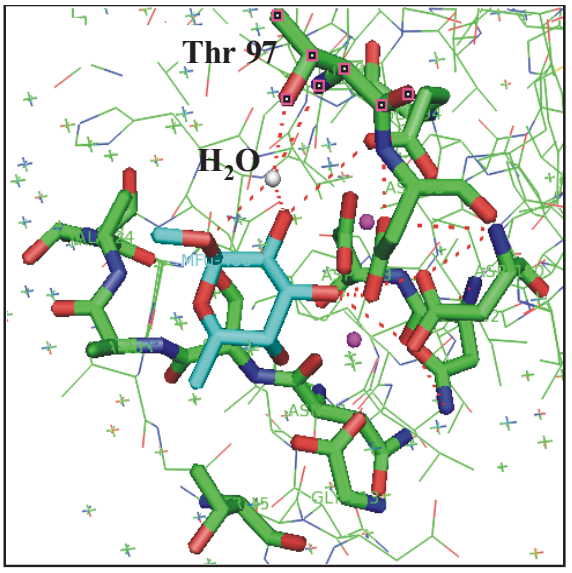

Fig.1 CV-IIL carbohydrate binding site with a -Me-fucoside

discrimination of the entropy change associated with the solvent release and the loss of configurational entropy. The total entropy of binding can be devided into terms for changes in solvation and losses in configurational, rotational and translational entropy.

$$S° = S_{solv} + S_{config} + S_{rot} + S_{transl} \quad [2]$$

The two major contributions to the binding entropy are the change in conformational and the solvation entropy, which will be discussed. However, the values of the Gibbs free energy are very similar, the difference in enthalpy and entropy contributions were marked in some cases.

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P8

PREPARATION OF AHP PROTEINS FOR STRUCTURAL ANALYSIS

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A histidine-containing phosphotransmitters (HPts) from *A. thaliana* (AHP1-5) mediate signal transduction downstream from receptor histidine kinases (HK) to subsequent phospho-accepting response regulators (RR) via so called multistep phosphorelay (MSP). AHP proteins are involved in and potentially integrate various MSP signalling pathways (e.g. cytokinin, ethylene, osmosensing). In this study, we cloned genes for AHP proteins into pRSET B expression vector enabling production of AHPs in fusion with N-terminal His tag. Expression levels were tested at different cultivation conditions in BL21(DE3)pLysS *E.coli* host strain. Proteins were extracted under denaturing and native conditions to estimate the proportion of produced AHPs in the soluble and insoluble form, respectively. Both forms of respective protein were identified by Western blotting. Surprisingly, quantification of bands from Western blot analysis showed significant differences in expression levels of AHP proteins. The first purification step (affinity chromatography) was optimized using different buffers and gradient shape. Following purification step (gel filtration or anion exchange chromatography) allowed obtaining of homogenous proteins under denaturing condition. Purified AHP2 was used for crystallization screening.

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P9

PREDICTING PK_A VALUES OF SUBSTITUTED PHENOLS FROM ATOMIC CHARGES

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The acid dissociation constant pK_a is one of the fundamental properties of organic molecules determining the degree of dissociation at a given pH. Dissociation constants are of interest in chemical, biological, environmental and pharmaceutical research, because the important physicochemical properties like lipophilicity, solubility, and permeability are all pK_a dependent. For these reasons, there is a strong interest in the development of reliable methods for pK_a prediction.

Numerous methods based on different approaches were developed [1] – the Linear Free Energy Relationships (LFER) method, database methods, decision tree methods, quantum mechanical simulations, QSPR models etc.. Unfortunately, pK_a values remain one of the most challenging physicochemical properties to predict.

Partial atomic charges have proven to serve as very successful descriptors for the prediction of pK_a using QSPR models [2]. The utilization of charges has been, until recently, limited by the high computational cost of their quantum mechanical calculation. Nowadays, much more powerful computers exist than ever before. It makes charges much better accessible and, thus, very attractive for pK_a prediction.

Partial atomic charges can be calculated using a variety of quantum mechanical methods (AM1, PM3, HF, MP2, functionals, etc.), population analyses (Mulliken, ESP, NPA, etc.) and basis sets. Consequently, the way of charge calculation strongly influences their correlation with pK_a [3, 4]. We have evaluated different computational strategies and models to predict the pK_a values of substituted phenols using partial atomic charges. Partial atomic charges for 143 phenol molecules were calculated using more than 70 combinations comprising of theory levels, basis sets and population analyses. The correlations between pK_a and various atomic charge descriptors were examined and the best descriptors were selected for designing the QSPR models. Then, the accuracy of all these models was analyzed an influence of theory level, basis set and population analysis on the quality of the model was evaluated.

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P10

COMPARISON OF C-TERMINAL SEGMENTS OF TRPV ION CHANNEL FAMILY MEMBERS AND THEIR INTERACTIONS WITH CALMODULIN

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TRPV ion channels belong to vanilloid subfamily of transient receptor potential channels (TRPs). These channels are ubiquitously expressed in all eukaryotic cells and are involved in many cellular processes like transduction of sensory signals and regulation of Ca^{2+} and Mg^{2+} homeostasis.

TRPV1 and TRPV2 fall within the so called thermo-TRPs. TRPV5 is a rather distinct member of TRPV subfamily and features quite different properties then the rest of this subfamily. It is strictly Ca^{2+} selective and involved in renal Ca^{2+} absorption/reabsorption.

It is known that TRP channels are regulated by calmodulin (CaM) in calcium dependent manner via binding to their intracellular termini.

In our project we identified calmodulin binding sites on the C-termini of TRPV1 (686–837), TRPV2 (654-683) and TRPV5 (587-616). These sites correspond to the consensus CaM binding motifs with conserved hydrophobic residues in positions 1-8-14 in TRPV1 and 1-5-10 in TRPV2 and TRPV5. Interestingly, the middle hydrophobic residue in the motif is exchanged for a charged residue in TRPV1 and TRPV2, indicating that these binding sites may be extraordinary. Site directed mutagenesis experiments further revealed that basic residues within these sites may play a crucial role in TRPV channels binding to CaM.

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P11

THE EFFECT OF *ESCHERICHIA COLI* MIN PROTEINS ON *BACILLUS SUBTILIS*

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In Gram-positive *Bacillus subtilis* and in Gram-negative *E. coli*, min proteins are involved in the regulation of division septa positioning. In both systems the concentration gradient of FtsZ inhibitor MinCD is formed from poles to cell centre. In *E. coli* MinE protein drives the oscillation of MinCD from pole to pole by binding to MinD and ATP hydrolysis. In this way, the lowest concentration of MinCD inhibitors at the cell centre is forming. In *B. subtilis* MinCD complex is targeted to the cell poles by MinJ and DivIVA protein. They retain MinCD at the cell poles. Such concentration gradient from poles to cell centre allows FtsZ-ring formation at the cell centre and at the same time prevents asymmetric division to take place at the cell poles. Heterologous expression of $MinC_{Ec}$ and $MinD_{Ec}$ has an effect on *B. subtilis* cell length. Moreover $MinD_{Ec}$ partially substitutes for the function of its *B. subtilis* counterpart and localizes similarly on helices along the cell axis as *B. subtilis* MinD.

Here using yeast two hybrid system we show direct interactions between MinD *E. coli* and MinC *B. subtilis.* Additionally, we observed dynamic behavior of $MinD_{Ec}$ and MinE in *B. subtilis* when expressed together. All these findings indicate that these two Min systems resemble each other more than it was thought previously.

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P12

NOVEL LIGANDS FOR IFNG DERIVED FROM STREPTOCOCCAL PROTEIN G SCAFFOLD

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Recombinant ligands derived from small protein scaffolds show promise as robust research and diagnostic reagents and as next generation protein therapeutics. Here we describe high-affinity binders for human interferon gamma (hIFN) that were derived from the three helix bundle scaffold of the albumin-binding domain (ABD) of protein G from Streptococcus G148. On the basis of computational interaction energy mapping, solvent accessibility assessment and in silico alanine scanning analysis, 11 residues from the albumin-binding surface of ABD were selected for randomization, thus generating a combinatorial scaffold library of a theoretical complexity of 1014. Upon two independent campaigns of in vitro ribosome display selection of such scaffolds, high affinity recombinant ligands were obtained that exhibited Kd values for hIFN from 0.2 to 10 nM. Molecular modeling, computational docking onto hIFN and in vitro competition for hIFN binding between pairs of individual selected ligands or with the human IFN receptor 1 (hIFN R1), revealed that four of the best ABD-derived ligands shared a common binding surface on hIFN , which was different from the site of hIFN R1 binding. The novel hIFN ligands thus represent a proof of concept for development of novel diagnostic reagents derived from the ABD scaffold.

P13

STRUCTURAL STUDIES OF 14-3-3/PHOSDUCIN COMPLEX

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Phosducin (Pd) is an important protein, which is involved in eyesight sense biochemistry. It regulates perception of sensitivity of light. Pd is expressed in photoreceptors, especially in rods, but it is found in pineal gland and other tissues as well. It is involved in G-protein signaling that allows sight [1, 2].

Dephosphorylation of Pd is induced by light and causes its binding to G complex. The transport of the resulting Pd/G complex from the outer rod disk membrane then induces the decrease in G-protein signaling. Therefore, the dephosphorylation of Pd protects the signaling pathway against saturation in the presence of very intense light. In the dark-adapted retina Pd is predominantly fosforylated at Ser54 and Ser73 [3]. Phosforylated Pd doesn´t bind G but binds multifunctional 14-3-3 protein. This interaction increases the G-protein signaling and thus the sensitivity towards the light [4].

14-3-3 proteins are family of acidic regulatory proteins that are expressed in all eukaryotic cells. They bind to other proteins in a phosphorylation-dependent manner and affects the structure of their binding partners [5]. Interaction between 14-3-3 and phosphorylated Pd probably enables sequestering Pd from G subunit or protecting phosphorylated Pd from degradation [2].

To elucidate the mechanism of 14-3-3-dependent regulation of Pd function, we performed several biophysical studies of Pd/14-3-3 complex. We have prepared Pd/14-3-3 complex *in vitro*. Nondenaturating electrophoresis was used to verify that the formation of Pd/14-3-3 complex is phosphorylation dependent. Analytical ultracentrifugation was used to estimate the complex stoichiometry. Conformational changes of Pd, induced by 14-3-3 protein binding, were studied using fluorescence spectroscopy techniques.

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P14

COMPLEMENTATION OF 3D STRUCTURE OF DELTA SUBUNIT OF RNA POLYMERASE FROM *BACILLUS SUBTILIS* WITH DESCRIPTION OF INTERNAL MOTIONS IN TERMS OF REDUCED SPECTRAL DENSITY MAPPING

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Full text see on page 3.

P15

ISOLATION OF THE EXTRINSIC PROTEINS FROM THE OXYGEN-EVOLVING COMPLEX OF HIGHER PLANTS

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In oxygenic photosynthesis sunlight is converted into chemical energy and the oxygen (essential for life on earth) is released to the atmosphere. Water splitting, giving the rise to molecular oxygen, is performed on a cluster of four Mn^{2+} ions located on the lumenal side of photosystem II (PSII). For the optimal activity of this oxygen-evolving complex are required Ca^{2+} and Cl^- ions, which is modulated in higher plants by the presence of three extrinsic proteins named PsbO, PsbP and PsbQ and create the correct ionic environment during water oxidation. These proteins are located at the lumenal surface of membrane [1]. Additionally, the protein PsbR has been described to play important role in water oxidation in plant PSII [2,3].

The isolation of the protein complex consisting of proteins PsbO, PsbP, PsbQ and the extrinsic protein PsbR from *Pisum sativum* L. for other structural analysis was optimized. Western blotting was used for detection of extrinsic proteins and preliminary crystallization experiments were performed. It was shown that complex PsbO, PsbP, PsbQ is stable in the Tris buffer, pH 8.6 while for protein PsbR is sufficient Tris buffer pH 7.00 containing the detergent octyl-thioglucopyranoside.

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P16

INFLUENCE OF RNA BINDING ON THE STRUCTURE AND FUNCTIONALITY OF BORNA DISEASE VIRUS MATRIX PROTEIN

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Borna disease virus (BDV) is the causative agent for Borna disease, a non-cytolytic, persistent infection of the central nervous system detected originally among horses in Borna, Germany. BDV has been used as a model system to investigate and understand persistent viral infections of the brain. Known hosts of BDV range from rodents to non-human primates [1] and recently it was shown that BDV is responsible for the first endogenized non-retroviral virus-derived elements in mammalian genomes [2]. BDV is the only member of the family of *Bornaviridae* that belongs to the order *Mononegavirales*, which includes among others the viruses Marburg, Ebola and Rabies.

BDV has the smallest genome among all known negative stranded non-segmented RNA viruses, with a size of 8.9 kb, encoding for six proteins [3]. The matrix protein of BDV (BDVM), a 16.2 kDa protein that forms either a stable homotetramer or –octamer [4], is associated with virus assembly and budding and may also be associated with the regulation of the viral ribonucleoprotein activity [5]. We have recently shown that BDVM binds single stranded RNA [6], as does the matrix protein VP40 of the Ebola virus [7].

To further investigate the structural and functional influence of RNA binding on BDVM, we mutated the specific RNA binding site, creating the variant BDVM H112W. This variant shows a previously unknown dodecameric oligomerization state and altered RNA binding abilities. More variants of BDVM were created to anticipate the new oligomerization state of BDVM H112W, giving a more detailed insight concerning the oligomerization state and RNA binding abilities of BDVM. An additional cell-based investigation of recombinant viruses harbouring these mutations showed potent growth attenuation and an atypical cytoplasmic accumulation in Vero cells.

Taken together the structural and functional data indicates that RNA binding and appropriate oligomerization of BDVM is required for proper viral growth.

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P17

PREPARATION OF REGULATORY DOMAIN OF TYROSINE HYDROXYLASE FOR NMR STUDIES

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Tyrosine hydroxylase belongs to the group of hydroxylases of aromatic acids, class oxydoreductases and subgroup oxygenases. This enzyme catalyses key step in the biosynthesis of catecholamine neurotransmitters – the conversion of the tyrosine to the 3,4-dihydroxyfenylalanine. We can found it mainly in cells of the adrenal gland in the heart, in the liver, in gonads and in the central nervous system [1, 2].

The tyrosine hydroxylase has the homotetrameric structure and contains three diverse structural domains: N-terminal regulatory domain, catalytic domain and C-terminal tetramerization domain [3]. The activity of tyrosine hydroxylase is regulated by phosphorylation and through the regulation of its expression. All phosphorylation sites (Ser-8, Ser-19, Ser-31 and Ser-40) are located within the regulatory domain [2]. Phosphorylation at Ser-40 by cyclic AMP-dependent protein kinase (PKA) induces the most potent activation of tyrosine hydroxylase. It has been proposed that phosphorylation of Ser-40 alters the conformation of the regulatory domain and its interaction with the catalytic domain. Phosphorylation at Ser-19 induces binding of the 14-3-3 protein, which affects the structure of the regulatory domain and protects it against dephosphorylation (at phosphorylated Ser-40) and its degradation [4, 5].

Since the structure of the regulatory domain is still unknown we decided to perform its structural characterization using NMR techniques. The regulatory domain of tyrosine hydroxylase was expressed as six-His-tag fusion protein by IPTG induction for 12 h at 20 °C and purified from *Escherichia coli* BL21(DE3). Its purification consists of two steps: the chelating chromatography and the size-exclusion chromatography on Superdex 200 column. The dynamic light scattering and the ^{1}H nuclear magnetic resonance were used to verify that the recombinant protein is not aggregated and can be used for further experiments.

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P18

BIOCHEMICAL CHARACTERIZATION OF A RECOMBINANT PLANT ALDEHYDE DEHYDROGENASE 7 FROM *Pisum sativum* (PSALDH7)

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[1]-Piperideine-6-carboxylate dehydrogenase (P6C DH), also named -aminoadipic semialdehyde dehydrogenase, antiquitin or simply aldehyde dehydrogenase 7 (ALDH7) represents a member of the ALDH7 family within the ALDH protein superfamily. Antiquitin's name (in Latin antiquusmeans old) is derived from the supposed high age of the respective coding gene. Human and plant ALDH7 proteins show roughly 60% identity in amino acids despite the evident evolutionary distance occurring between both organisms. Such a high degree of sequence similarity between species often indicates an essential and functionally conserved role within the cell. Human antiquitin was found to metabolize a wide range of aliphatic aldehydes, aromatic aldehydes and betaine aldehyde but it is mainly connected with lysine metabolism and mediates the conversion of -aminoadipic semialdehyde (-AASA) to -aminoadipate. Mutation in human ALDH7A1 gene results in the accumulation of P6C in plasma that inactivates pyridoxal 5´-phosphate, an essential cofactor for many enzymatic reactions. The physiological function of plant antiquitin is believed to be related to a general stress response but no detailed data exist on the enzyme structure and substrate specificity. The gene coding for pea antiquitin (*PsALDH7*, GenBank accession number X54359) was cloned into a pCDFDuet vector and expressed in T7 *E. coli* cells. The final protein of 522 amino acids (55 kDa) carried an N-terminal 6xHis-tag and was purified on HIS-Select cobalt affinity gel. Protein identity was verified by MALDI-TOF peptide mass fingerprinting. The enzyme utilizes NAD^+ but not $NADP^+$ as a coenzyme and prefers -AASA to other aliphatic and aromatic aldehydes.

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P19

CARBOHYDRATE CH/ INTERACTIONS – THEORETICAL INVESTIGATION OF THE FAVOURABLE REGIONS IN THE CARBOHYDRATE SURROUNDINGS

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Molecular recognition of carbohydrates by proteins plays a key role in many biological processes including immune response, pathogen entry into a cell, cell-cell adhesion and so forth [1-5]. The list of proteins that recognise carbohydrates via aromatic residues include lectins, carbohydrate-binding modules, glycosidases, glycosyl transferases, "greasy slides" of carbohydrate transporters and complimentary sugar-binding sites ("sugar tongs"). Carbohydrates typically interact with aromatic systems in a parallel orientation.

In this study we present the first systematic computational three-dimensional scan of carbohydrate hydrophobic patches for the ability to interact via CH/ dispersion interactions. The carbohydrates -D-glucopyranose, -D-mannopyranose and -L-fucopyranose were studied in a complex with a benzene molecule, which served as a model of the CH/ interaction in carbohydrate/protein complexes.

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P20

STRUCTURAL ASSEMBLY OF -*N*-ACETYLHEXOSAMINIDASE COMPLEX

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-*N*-acetylhexosaminidase (EC 3.2.1.52) belongs to exoglycosidase and is one of the most abundant enzymes found in organism from bacteria to human. The fungal -*N*-acetylhexosaminidase from *Aspergillus oryzae* is composed of propeptide and catalytical domain. The propeptide is a 10kDa large peptide noncovalently associated with the catalytical domain of the enzyme. Propeptide is essential for the enzyme activity. Although the structure of the catalytical domain was revealed by homology modeling, the structure of the propeptide has not been solved. In this study we uncover the position where the propeptide is associated with the catalytical domain.

-*N*-acetylhexosaminidase was purified from the medium of the producing organism. For EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) experiment the enzyme was transferred by gel filtration to 50mM Pyridine pH 5.5, 150mM NaCl and for DSG (disuccinimidyl glutarate) experiment to 50mM Triethylamine carbonate pH 7.5. After the cross-linking reaction was over, the products of enzyme were separated by SDS electrophoresis. In gel digestion was performed and the resulting peptides were analyzed by LC-ESI FT MS (Agilent 1200, APEX-Ultra)

Combinating chemical cross-linking and high resolution mass spectrometry we revealed that the structural changes of the catalytical domain depend on the presence/absence of the propeptide molecule. These results nicely correlated with the previously described homology model of the catalytical domain. Several cross-linked peptides between the propeptide and catalytical domain disclosed the position of the propeptide within the enzyme.

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P21

PMFLIB – A TOOLKIT FOR FREE ENERGY CALCULATIONS

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The majority of chemical and biochemical processes can be described by underlying free energy behavior. This is the case, for instance, in assembly of supramolecular complexes, chemical reactivity, protein folding and protein-ligand binding. It is evident that the calculation of free energies by computer simulations can be very helpful in better understanding of these processes. One of the most commonly used simulation technique providing the free energy is the calculation of potential of mean force (PMF). PMF represents the free energy along a prescribed reaction coordinate and thus provides information about kinetics and thermodynamics of the studied (bio)chemical events.

We have implemented five well established PMF methods into a single suite of programs, which we call PMFLib. Implemented methods are as follows: adaptive biasing force (ABF) [1], constrained dynamics (CD) [2], metadynamics (MTD) [3], umbrella sampling (US) [4], and string method (SM) [5]. The PMFLib is a set of utilities and libraries written in Fortran90 and C/C++ programming languages providing PMF calculations in transparent and uniform way. PMFLib can be easily plugged into any existing molecular dynamics code and thus to extend its features towards reliable free energy calculations. Currently, interfaces to AMBER, CPMD, and XdynBP molecular dynamics codes are available. PMFLib also offers multiple-walkers (MW) [6] extension of MTD and ABF methods. Another implemented method improving sampling is replica-exchange molecular dynamics (REMD). Both MW and REMD [7] extensions are implemented as weakly bound client-server. This allows utilization of PMFLib in calculations employing massive parallel simulations in heterogeneous computer environments, for example in computational grids.

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P22

THYLAKOID MEMBRANE AND PHOTOSYSTEM II, STRUCTURE AND MOLECULAR DYNAMICS SIMULATIONS

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Photosynthetical membrane is the most abundant membrane in the nature and differs from other membranes by the foremost glycolipid composition. The mebrane lipid composition is dependent on the organism species and its living conditions. In our previous computational study of the PSII Reaction Centre pigment-protein complex, the thylakoid membrane as the protein natural environment was replaced by assembly of octane molecules due to the lack of electron density for lipid components and computationally demanding geometry optimization of lipid-protein complexes.

Using the most common force field (FF) for membrane modelling, OPLS (Optimized Potential for Liquid Simulations) and GROMOS, the monolipid mebrane model of size 8x8 glycolipids was relaxed in the presence of water and then pressed to fit „area per lipid" and bilayer diameter to correspond with the experimental data and than again relaxed to be in equilibrium.

The complex model of PSII RC embedded into the natural environment (glycolipid membrane) will be finally involved in theoretical study of photosynthetic processes, like excitonic interaction and charge separation in PSII RC and behavior of some external parts of membrane protein subunits.

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P23

CRYSTALLIZATION AND STRUCTURAL ANALYSIS OF DHAA31 PROTEIN FROM *RHODOCOCCUS RHODOCHROUS*

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Haloalkane dehalogenase DhaA is a bacterial enzyme isolated from bacterium *Rhodococcus rhodochrous* NCIMB 13064 [1]. The enzyme catalyzes hydrolytic dehalogenation of various halogenated aliphatic hydrocarbons [2]. The mutant DhaA31 was constructed to degrade anthropogenic compound 1,2,3-trichloropropane (TCP). TCP has been detected in low concentrations in surface water, drinking water and groundwater. TCP shows strict resistant to biological degradation. To increase the efficiency of this reaction, focused directed evolution was used to construct the mutant DhaA31 with up to 32-fold higher catalytic activity and 26-fold higher catalytic efficiency, than parent wild type enzyme DhaA [3]. The main goal of this project is to solve structure of DhaA31. The structure of DhaA31 will help to understand the structure-function relationships of improved dehalogenation of TCP.

The mutant protein DhaA31 was crystallized by sitting drop vapor diffusion technique and crystals of DhaA31 in a complex with TCP were obtained using soaking experiment. Both crystals belong to the triclinic space group *P1*. Diffraction data were collected to the high resolution of 1.31 Å for DhaA31 and 1.26 Å for DhaA31 with TCP. This diffraction data were collected at the beamline X12 (DESY, Hamburg). The structures of DhaA31 and DhaA31 in a complex with TCP were solved by molecular-replacement techniques using haloalkane dehalogenase DhaA04 mutant as a template. The refinement has been done in *SHELXL* program [4].

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P24

STUDY OF STRUCTURAL DIFFERENCES IN H8-H9 LOOP AMONG 14-3-3 PROTEIN ISOFORMS

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The 14-3-3 proteins are highly conserved dimeric signaling proteins expressed in all eukaryotic cells [1]. Each monomer consists of nine antiparallel helices. It has been speculated that the loop between -helixes H8 and H9 affects the binding properties of 14-3-3 protein isoforms. In this work we focused on barley isoform 14-3-3A and human isoform 14-3-3 . It has been observed that the isoform 14-3-3A binds its ligands with significantly lower affinity compared to other barley isoforms (14-3-3B and 14-3-3C) [2]. These isoforms differ in the sequence of the loop between -helixes H8 and H9. We measured the binding affinity of 14-3-3A WT and its mutant, which contained the same H8-H9 loop as isoforms B and C. For comparison, we also measured the binding affinity of human isoform 14-3-3 and its mutant, where the serine residue within the H8-H9 loop was replaced by glycine. The binding affinity of 14-3-3 protein isoforms was studied using two different fluorescence spectroscopy techniques (the fluorescence correlated spectroscopy and the quenching of the steady-state fluorescence intensity). Our results show that the mutation of H8-H9 loop decreased the binding affinity of both tested isoforms, even though their mutations were antagonistic (Gly/Ser in the case of 14-3-3A, Ser/Gly in the case of 14-3-3). In conclusion, the H8-H9 loop plays an important role in the regulation of the 14-3-3 binding affinity in an isoform-specific manner.

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P25

FUNCTIONAL AND BIOCHEMICAL ANALYSIS OF TRUNCATED SPOIISA TOXIN IN *BACILLUS SUBTILIS*

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Toxin-antitoxin were found to be encoded on vast number of bacterial plasmids and genomes and are thought to be associated with the maintenance of extrachromosomal DNA, bacteriostasis and possibly altruistic cell death. Chromosomally encoded SpoIISA-SpoIISB system appears to define a new family of toxin-antitoxin modules that is conserved among Bacilli.

SpoIISA proteic toxin from *B. subtilis* was shown to affect cell mebrane of either mother cell during sporulation process or, when artificially over-expressed in vegetatively growing cells. This 248-amino residue protein was predicted to be composed of two domains – a membrane spanning domain consisting of three helices and a negatively charged cytosolic domain (CSpoIISA), which binds small, positively charged SpoIISB protein [1].

Recently solved crystal structure of $CSpoIISA_2$: $SpoIISB_2$ heterotetrameric complex [2] revealed that the toxin portion of the complex constitutes so called GAF (c**G**MP phospho- diesterase, **a**denylate cyclase, **F**hlA) domain, a fold which is widespread in proteins of diverse function including cell signalling proteins, transcription factors and proteins involved in light detection in bacteria, fungi and plants [3]. The GAF domain has three tiers, a basal layer of two or more -helices, a middle tier of four or more strands that form a mixed -pleated sheet and a distal layer of more variable structure made up of segments connecting the strands of the -sheet. Many GAF domains are dimers formed by the packing of -helices from the basal layer to form a four helix bundle. In case of SpoIISA, the two protomers of its cytosolic domain form extensive hy-

drophobic interactions through an intermolecular 4-helix bundle formed by the first and the last CSpoIISA alpha helices [2].

Since alternative prediction of the SpoIISA topology suggested that the last CSpoIISA -helix might swap from intermolecular interface and become incorporated into cytoplasmic membrane, in this work we assessed the toxicity of the C-terminally truncated SpoIISA lacking the last alpha helix and the ability of SpoIISB antitoxin to neutralize the eventual lethal activity in *B. subtilis* cells. Moreover, we examined the effect of this C-terminal SpoIISA deletion on the CSpoIISA dimerization and the CSpoIISA-SpoIISB complex formation *in vitro*.

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P26

VIBRATIONAL AND ELECTRONIC OPTICAL ACTIVITY OF AMIDE AND DISULFIDE GROUPS IN NEUROHYPOPHYSEAL HORMONES AND THEIR MODELS

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Vibrational and electronic circular dichroism spectra of neurohypophyseal hormones oxytocin, lysine-vasopressin, arginine-vasopressin, several of their analogs and structural models were measured in order to isolate chiroptical manifestation of their peptide backbone from the signals of aromatic chains of tyrosine and phenylalanine chromophores. We have also observed electronic disulfide-related ECD in the long wavelength spectral region and identified features in the spectra of Raman optical activity (ROA) which are associated with the S-S and C-S stretching vibrations. These allow us to infer the sense of disulfide twist in the particular compounds when signs of ROA features are compared to ROA calculations on simple model disulfides [1] and verified by a comparison with spectra of disulfide-bridged cyclodextrins [2].

The results indicate that (a) there is a remarkable similarity and consistent spectral behavior of the whole family of neurohypophyseal hormones; (b) vibrational optical activity allows to observe chiroptical manifestation of neurohypophyseal hormone backbone without the harmful interference of aromatic groups; (c) vibrational optical activity indicates that the prevailing solution conformation of neurohypophyseal hormones is a short segment of left handed helix. This is in accord with analogous ECD result, however interference with the bands due to side chains of Tyr and Phe makes this observation less discernible;(d) the ROA signals in the S-S and C-S stretching vibration regions are observable also with neurohypophyseal

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P27

THE STATE TRAJECTORY OF CELL

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Cells are living unrepeatable objects. If we want to know how they are living and evolving in time we cannot do it through invasive techniques, which modify or kill the cell. In non-invasive techniques, the content of the data is given and we have to maximize information gain. We extract from the dataset by information entropy approach. A general image, acquired by any generic microscopic techniques, is subjected to transformation which evaluates the information contribution of each point in the image. Resulting values may also be used for construction of the cell or, differently said, to objective assessment of individual cell state.

As model example we used the Belousov – Zhabotinsky reaction. The 3D plot of red vs. green vs. blue channel shows that individual attractors may be discriminated and that many state trajectories may be constructed using different entropies. The color channels and different Rényi entropy coefficients may be combined to best discriminate individual states. The same approach we use for state analysis of cells. S. cerevisiae cell was observed using video enhanced microscopy in brightfield regime. Resulting images are grayscale. We segmented the histogram and to each part we assigned different colors. By this transformation individual features in the cell were significantly better visible then in original and state trajectory may be plotted. We used this approach also for analysis of coloured, phase contrast image of HeLa and MG63 cells. Here, more channels with significantly different information content are obtained and for the construction of the state trajectory are more possibilities. The question remains whether discriminateable parts of the cell – organelles etc. - are subsystems of the cell and should be assigned their own state trajectories or whether the cell is the elementary system. To prove this idea, we separated only one organelle from the image of whole cell and try to plot the state trajectory. The question could not be unanimously answered at state of the art of the method.

There are mainly technical limits to the identification of the state trajectory given by instrument time and dynamical resolution, information channel properties etc. So we are able to construct state trajectories only for simple objects and simple cells identified manually. The progress in developing methods of automated cell and cell feature extraction is reported.

P28

STUDY OF RNA POLYMERASE DELTA SUBUNIT UNIQUE FOR GRAM-POSITIVE BACTERIA

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RNA polymerase (RNAP) from gram-positive bacteria such as *Bacillus subtilis* differs from well-studied RNA polymerase from gram-negative bacteria in the presence of two additional subunits interacting with the core enzyme, delta and omega1. Recent results indicated that the presence of delta subunit increases the transcription specificity and the efficiency of RNA synthesis. Moreover, the absence of delta subunit is proposed to decrease a virulence of some pathogens. As crystallization at structure genomics centers failed, we focused on NMR studies of the delta subunit to reveal its structure and related dynamics. As the previous study showed (López de Saro et al., JMB, 1995), the C-terminal domain of the delta subunit is unstructured and its sequence is highly repetitive. Therefore, we started a systematic investigation of the protein with a shorten construct, corresponding to the well-structured N-terminal part. The construct constitution was confirmed using mass spectrometry and the secondary structure content as well as the protein thermostability were determined from circular dichroism spectra. So far, we have published its high-quality structure and the work on the analysis of internal motions is almost finished.

The more challenging part of the protein, the C-terminal domain, was not initially studied because the NMR methodology for disordered, flexible proteins was not sufficiently developed at the beginning of the project. Fortunately, during last few years many new approaches for study of biologically interesting *intrinsically disordered* proteins appeared. In contrast to X-ray crystallography, NMR can provide valuable information on residual secondary structure, possible long-range contacts, and internal dynamics of the disordered polypeptide chain.

The full-length delta protein was prepared using a standard protocol of overexpression in the *E.coli* system to produce a $^{15}N,^{13}C$-uniformly labeled sample. The basic spectra, including a standard set of triple resonance NMR experiments, 3D TOCSY, and 3D NOESY, were measured on a 600MHz spectrometer. However, the analysis of the spectra was almost impossible due to a very small differences in chemical shifts. Therefore, a new methodology coming from Wiktor Koźmiński lab was used to improve the spectra resolution and the full-size protein was then completely assigned. It was a major step for further analysis including secondary structure prediction, study of internal motions or measurement of dipolar couplings.

The interactions between the delta subunit and the RNAP was studied by NMR titration and gel-shift assay to indicate which subunits are essential for binding of the protein to the core enzyme. The experience retrieved in the presented study will be used for the innovation of the seminar C9531 taught at Masaryk University.

This work was supported by the Grants MSM0021622413, LC06030, and 2B06065 from the Ministry of Education, Youth and Physical Culture of the Czech Republic, by the FRVŠ grant 2066/2011, by the Grants 204/09/0583 and 301/09/H004 from Czech Science Foundation, and 7FP of the EC (Contract 228461, EAST-NMR).

P29

BIOLOGICAL SYSTEMS STATE VARIABLE IDENTIFICATION USING JOINT ANALYSIS OF BIOLOGICAL AND TECHNICAL HYPOTHESIS IN THE TIME-LAPSE TISSUE CULTURE DEVELOPMENT EXPERIMENT

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The time-lapse microscopy experiment is the best representation of biological cell dynamics. Cell fate is best described by the chaotic attractor and that the observable cell states represent individual basins of attraction. It is, however, not quite clear in which relation the experimentally observable macroscopic parameters are to the state variables of the attractor space. In other words it may be said that the experiment cuts the state space by a fraction of space, possibly, of lower dimension and of unknown shape with respect to system coordinates.

The only realistic approach towards objective analysis of the experiment is to determine evolution in time of information fluxes defined by the experiment. Here we present two essential representations of the system: (1) representation of information content of each timestep portrait-image in values of information entropy (this we assume to be in relation to dynamic biological system state in time of measurement) and (2) map of information content fluxes which we assume to be in relation to image of local Lyapunov exponents or eigenvalues of Jacobian matrix in actual point in state space. 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.

P30

RAMAN AND INFRARED SPECTROSCOPY OF AMINO ACIDS AND ITS IMPLICATION FOR IMPROVEMENT OF THE PROTEIN SECONDARY STRUCTURE DETERMINATION

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Determination of the protein structure represents one of the key tasks of present molecular biology. Raman and infrared (FTIR) spectroscopy can provide good wealth of information about protein structure in comparison to other methods of optical spectroscopy. Therefore, development and improvement in such spectroscopic techniques that can be used for characterization of proteins is becoming increasingly important in the rapidly expanding field of proteomics.

In this study Raman and FTIR spectra of all amino acids (AA) presented in proteins have been measured with a view to possible protein sequence dependent subtraction of vibration bands of AA side chains in the regions of amide I, II and III. The measurements were especially focused on new method of non-enhanced Raman spectroscopy – the drop coating deposition Raman (DCDR) spectroscopy [1] – based on a coffee ring effect, which enables measurements of solutions with concentration of biomolecules down to ca. 0.01 mg/mL. Even if the DCDR measurements are a bit of problematic [2] good quality spectra of molecules in glass phase can be obtained [3]. The Raman measurements were performed on extremely diluted amino acid samples (0.005 mg/mL) – where coffee ring effect doesn't play a role – to avoid formation of microcrystals. The method of AA side chains spectral subtraction was tested on short peptide samples with known primary sequence. The influence of the subtraction on improvement of the secondary structure determination is discussed as well.

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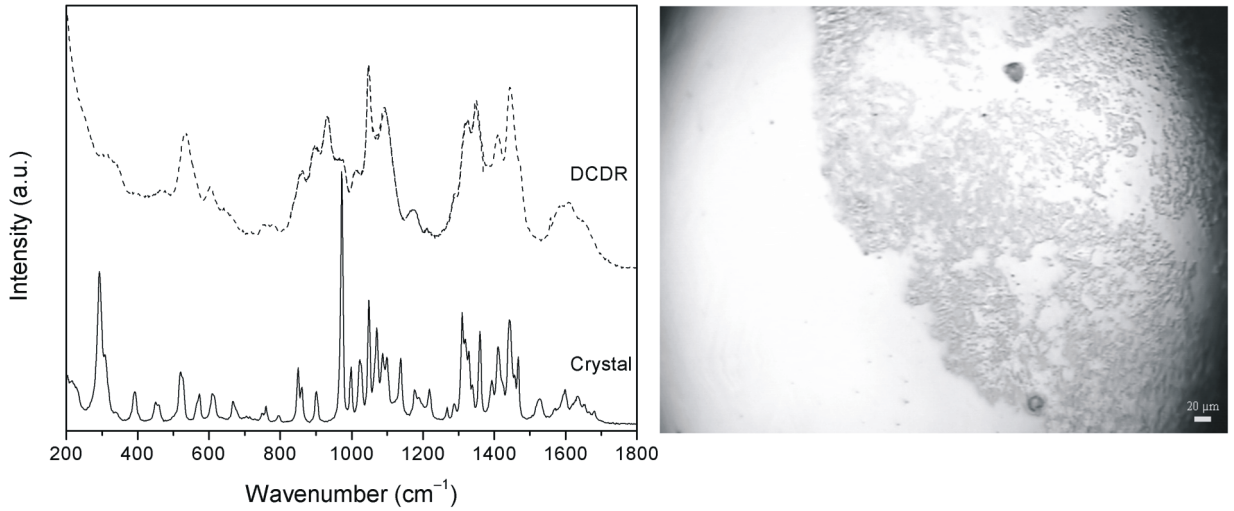

Figure 1. *Left*: Raman spectra of L-arginine measured from the polycrystalline sample and as DCDR deposit and (*right*) the detail view of DCDR deposited sample, whereas the white bar in the photography represents 20 µm.

P31

ANTIBACTERIAL PEPTIDES IN INTERACTION WITH MODEL MEMBRANES STUDIED BY VARIOUS SPECTROSCOPIC METHODS

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Naturally occurring antimicrobial peptides (AMPs) represent one successful form of chemical defense of eukaryotic cells against bacteria, protozoa, fungi, and viruses [1]. Many of them have been already isolated, thousands of their synthetic analogs were synthesized and a broad spectrum of their antimicrobial, anticancer and antiviral activities was proven [2, 3]. In spite of large number of known AMPs and their therapeutic potential, exact mechanism of their action remains a matter of controversy.

There is a consensus that these peptides selectively disrupt cell membranes and it is believed that their amphiphatic structure plays an important role in this process. Using various spectroscopic methods it was shown that interaction of AMPs with membranes or with their models lead to changes of their secondary structure. Our aim is to study these peptides in interaction with model phospholipids membranes using circular dichroism, infrared and Raman spectroscopy, and in that way contribute to clarification of these differences.

Here we present spectroscopic studies of peptide Hal-1 (Gly-Met-Trp-Ser-Lys-Ile-Leu-Gly-His-Leu-Ile-Arg-NH_2) AMP isolated from the venom of the eusocial bee *Halictus sexcinctus*, which exhibited potent antimicrobial activity against Gram-positive and Gram-negative bacteria but also noticeable hemolytic activity [4]. Circular dichroism, infrared and Raman spectra of chosen AMP and its analogs in a membrane-mimicking environment showed ability to form -helical structure whereas the HAL peptide exhibits random coil conformation in water. The attempts to measure HAL peptides in an interaction with liposomes with different kinds of membranes are presented as well.

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P32

STRUCTURE AND BINDING SPECIFICITY OF THE RECEIVER DOMAIN OF SENSOR HISTIDINE KINASE CKI1 FROM *ARABIDOPSIS THALIANA*

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Multistep phosphorelay (MSP) pathway mediate sensing of wide spectrum of signals in plants, including hormonal (cytokinin, ethylene, abscisic acid) and abiotic stress regulation. In *Arabidopsis* MSP, the signal is transferred from sensor histidine kinase (HK) via histidine phosphotransfer proteins (AHP1-5) to nuclear response regulators. In contrast to bacteria, MSP protein interactions in plants are supposed to be rather non-specific.

A C-terminal receiver domain of histidine kinase CYTOKININ-INDEPENDENT 1 ($CKI1_{RD}$) has been investigated in this study. Using both *in vivo* and *in vitro* assays we have found that $CKI1_{RD}$ is responsible for recognition of CKI1 downstream signaling partners and specifically interacts with AHP2, 3 and 5 with different affinities. Effects of Mg^{2+}, the cofactor necessary for signal transduction, and phosphorylation-mimicking BeF_3^- on $CKI1_{RD}$ in solution were studied by NMR. Finally, crystal

structures of free $CKI1_{RD}$ and $CKI1_{RD}$ in a complex with Mg^{2+} were determined. Magnesium binding induces rearrangement of some residues around the active site of $CKI1_{RD}$ which affects its activity and specificity.

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P33

PREPARATION, CHARACTERIZATION AND CRYSTALLIZATION OF RECOMBINANT FRAGMENT OF ANTI-CD3 ANTIBODY MEM-57

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Monoclonal antibody MEM-57 recognizes CD3 surface glycoprotein, which associates with T-cell receptor and mediates activation signal transduction. CD3 antigen is expressed on peripheral blood T-lymphocytes [1]. Antibody MEM-57 can, therefore, be used as a part of the „Bispecific T-cell Engager" (BiTE) antibodies in the cancer therapy [2].

Single-chain variable fragment of MEM-57 (scFv MEM-57) was produced in *E. coli* BL21(DE3) from a pET22b vector; the product, targeted into the periplasmic space by the pelB leader sequence, contained c-myc and His_5 tag at the C-terminus. The purification consisted in two steps, nickel chelation affinity chromatography and ion-exchange chromatography. The antigen binding activity was confirmed by flow cytometry.

Crystallographic studies were initiated, as the structural information on scFv MEM-57 would be useful for humanization of the antibody. This poster focuses mainly on experiments aiming at pre-crystallization analysis and the improvement of protein crystallizability. Size-exclusion chromatography, dynamic light scattering and differential scanning fluorimetry (DSF) [3] analyses were performed to seek for conditions optimal for protein crystallization. DSF was used to select composition of the starting buffer, optimal for protein stability and homogeneity, which highly affects protein crystallizability, as confirmed by the results of our initial crystallization trials.

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P34

THE BINDING PROPERTIES OF THE H5N1 NEURAMINIDASE - MOLECULAR DYNAMICS STUDY

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The avian influenza H5N1 virus has emerged as an important pathogen, causing severe disease in humans and posing a pandemic threat. Substrate specificity is crucial for the virus to obtain the ability to spread from avian to human. Therefore, an investigation of the binding properties of ligands at the molecular level is important for understanding the catalytic mechanism of the avian influenza virus neuraminidase and for designing novel and specific inhibitors of H5N1 neuraminidase. We have performed extended molecular dynamics studies to clarify the role of the loops surrounding the active site of the H5N1 neuraminidase in the binding of the two natural substrates, namely the 3'sialyllactoside and 6'sialyllactoside. Our results suggest different binding of the two substrates to the adjanced loops. Furthermore, the MD simulations have revealed decreased mobility of the catalytic aspartate in the case of 6'sialyllactoside complex, which can explain the experimentally observed preference of the H5N1 neuraminidase for the 3'sialyllactoside.

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P35

DOCKING STUDY OF MATRIX METALLOPROTEINASE INHIBITORS

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Matrix metalloproteinases (MMPs) are a family of zinc-containing, calcium-dependent enzymes responsible for the remodelling and degradation of almost all components of the extracellular matrix [1]. They are known to be involved in a number of physiological and pathological cellular processes, such as wound healing, tumor growth and metastasis. MMP inhibitors have been explored as potential anticancer, anti-inflammatory and antiviral agents [2, 3].

With ongoing increase of computing power, *in silico* methods have become a key component of the rational drug design process. Molecular docking is one of the common computational techniques to predict preferred orientation of a small molecule in complex with a protein and to quantify its binding energy. Given the fact that MMPs are promising pharmaceutical targets, it is important to have a reliable docking method, which is able to rank the binding strength of ligands when also interactions established with ions presented in the system are taken into consideration. This is because one of the challenges in ligand-MMP docking is the presence of a zinc ion in the binding site. It has been shown that determining the correct orientation of the zinc-binding group is crucial to obtain preferred binding mode [4].

In this study we compare the docking accuracy of several contemporary molecular docking programs on several members of MMP family. The test set consists of 38 MMP-ligand complexes taken from the RCSB Protein Data Bank. Docking of the geometry optimized ligands was performed using several commonly used docking software. The docking results were correlated with experimental data. Performance of docking programs will be discussed in terms of their binding orientation and binding affinity prediction accuracy.

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P36

IDENTIFICATION OF AMINO ACID RESIDUES IMPORTANT FOR COUPLING ATPASE AND RESTRICTION ACTIVITY IN ECOR124I ENDONUCLEASE

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Restriction-modification enzymes (R-M) protect bacteria from infections by viruses, and it is commonly accepted as being their major role in nature. Type I R-M enzymes are large, multifunctional macromolecular complexes composed of three different subunits: HsdS, HsdM and HsdR [1]. The activity of the complex endonuclease is translocation DNA through enzyme complex tightly binds in specific sequence on DNA molecule. When translocation is stopped, the DNA molecule is cleaved nonspecifically outside of recognition site [2]. The first crystal structure of the 120 kDa motor HsdR protein of the Type I EcoR124I nuclease in complex with ATP was recently reported [3]. This structure discloses an unexpected contact of endonuclease domain with ATP by Lys220. Engagement of the endonuclease domain in ATP binding seems to be involved in the communication of the ATP-dependent translocase with the endonuclease, potentially coupling dsDNA translocation and cleavage.

To investigate the underpinning molecular mechanism of coupling ATP-dependent DNA translocation and DNA cleavage and the communication pathway through the motor subunit, we selected residues from the sequence variable region nearby Lys220, which could be potentially engaged in conformational changes that occur once translocation is stalled and a signal is transmitted to the endonuclease. The influence of the replacement of these selected residues to either neutral Ala or eventually to charged Arg on translocation is monitored using in vitro ATPase activity assays of the whole pentameric enzyme complex. We demonstrate that although in close proximity to the ATP binding pocket, none of the selected residues significantly alters ATPase activity, thus leaving the ATPase fully intact. Alteration of endonuclease activity of these selected residues is reported and discussed in molecular terms.

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P37

HIGHER PLANT PHOTOSYSTEM II

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The aim of this work carried out in collaboration with Laboratorio de Estudios Cristalográficos, Granada, Spain and Institute of Molecular Genetics of the Academy of Science of the Czech Republic, Prague is to crystallize higher plant PS II for high resolution X-ray diffraction and to resolve the structure of the supercomplex.

A combination of centrifugation (sucrose density) and chromatography (ion exchange, gel filtration) techniques was used for extraction and purification of the PSII complexes from solubilised thylakoid membranes of the *Pisum sativum L*. chloroplasts. The complexes' activity was followed throughout the isolation routine with optical spectroscopy and polarographic measurements of oxygen evolution rates.

A new protocol for hydroponic plant growth under controlled conditions has already been established as well as re-designed and implemented for isolation of the PS II enriched thylakoid membranes. Broad screening for most suitable buffers and detergents that sustain maximum PSII activity throughout the isolation routine has been already done. Nowadays the protocol for PSII purification is developing. Upcoming methods and approaches for crystallisation of both membrane and soluble proteins will be tested subsequently.

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P38

STRUCTURE OF MOUSE CLRG – A LIGAND OF NK CELL RECEPTOR

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Natural killer cells (NK cells) belong to lymphocytes, besides more familiar B and T-lymphocytes. They were discovered in 1970s [1]. They comprise 5-10% of lymphocytes in blood and their role in the immune system is to discover and kill cells with cancer and cells infected by viruses. NK cells have a number of receptors on their surface, which are used for contact with other cells and for initiation of the cytotoxic response.

Protein Clrg [2], a target of this structural study, is a part of immune system of mouse. It is a ligand of NK receptor NKRP1F. Clrg occurs in dendritic cells and macrophages.

The extracellular part of Clrg was expressed, purified and crystallized. Diffraction data were collected at the synchrotron radiation source Bessy II of the Helmholtz Zentrum Berlin, beamline PX14.1 at temperature 100 K using a MAR Mosaic 225 CCD detector. Data were processed by HKL2000 with resolution 1.95 Å in space group $P2_12_12$ and with unit-cell parameters 118.3, 61.4 and 32.4 Å. Molecular replacement was solved by BALBES in *P*2 using structure of the human CD69 [3]. The solution was translated to a higher space group after the structure-building phase. The structure was refined in REFMAC to final *R*-factor 18.5 % and *R*-free 26.8 %.

The overall fold of mouse Clrg is very similar to that of human CD69. There are interesting crystal contacts which may indicate inter-molecular interactions between NK receptors and their ligands.

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P39

MOLECULAR SIMULATION WITH ELASTIC NETWORK MODEL POTENTIAL IN GROMACS

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Elastic network model and its follow-ups (Gaussian and anisotropic network models), despite their simplicity, have been successfully applied in prediction of flexibility and coupled motions in proteins. Here we present an application of molecular dynamics simulation in Gromacs package with potential energy calculated by the elastic network model. It allows for microsecond simulations per day on a single commodity personal computer. The method was applied on the structure of ubiquitin and the resulting trajectory was analyzed in terms of residue flexibility and coupled molecular motions. The results were compared with atomistic molecular dynamics simulation in explicit solvent and with published experimental data. We show that simulation of elastic network models of proteins is useful in modelling of biomolecular systems where mechanical properties become more important than atomistic details.

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P40

PROTEIN OLIGOMERIZATION AND BACKBONE FLEXIBILITY

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Oligomerization capacity of the retroviral matrix protein is an important feature that affects assembly of immature virions and their interaction with cellular membrane. A combination of NMR relaxation measurements and advanced analysis of molecular dynamics simulation trajectory provided an unprecedentedly detailed insight into internal mobility of matrix proteins of the Mason-Pfizer monkey virus. Strong evidences have been obtained that the oligomerization capacity of the wild type matrix protein is closely related to the enhanced dynamics of several parts of its backbone on ns timescale. Increased flexibility has been observed for two regions: the loop between -helices 2 and 3 and the C-terminal half of -helix 3 which accommodate amino acid residues that form the oligomerization interface. On the other hand, matrix mutant R55F that has changed structure and does not exhibit any specific oligomerization in solution was found considerably more rigid. Our results document that conformational selection mechanism together with induced fit and favorable structural pre-organization play an important role in the control of the oligomerization process.

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P41

COMPUTATIONAL STUDY OF THE DIMETHYLPHOSPHATE HYDROLYSIS AS THE REFERENCE SYSTEM FOR THE UNDERSTANDING OF THE RESTRICTION ENDONUCLEASES MECHANISM

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Understanding enzymatic mechanisms is essential knowledge for further medicine and biotechnology development. Our long-standing effort is aimed at the explanation of the reaction mechanism of endonucleases (enzymes cleaving DNA chain(s)) using computational approaches. According to the published experimental and theoretical results, the suggested mechanism is S_N2 hydrolysis of the phosphate backbone [1].

Presented work is focused on the study of simplified model of the enzymatic reaction. This model consists of dimethylphosphate and the nucleophile (water, hydroxyl ion) attacking the phosphodiester bond. The hydrolysis was simulated using Car Parrinello Molecular Dynamics (CPMD) [2] in vacuum and in water. The reaction free energy profiles were evaluated using metadynamics [3] and obtained results were compared to the experimental data [4]. The model serves as a reference system for the upcoming simulation of the enzymatic reactions.

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CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF *RHODOCOCCUS RHODOCHROUS* WILD-TYPE DHAA PROTEIN AND ITS VARIANT DHAA13 COMPLEXED WITH DIFFERENT LIGANDS

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The haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064 can slowly detoxify the industrial pollutant 1,2,3-trichloropropane (TCP) [1,2]. Structural analysis of this enzyme complexed with TCP was conducted to obtain detailed information about the structural limitations of its catalytic properties. The wild-type DhaA was also complexed with two different concentrations of 2-propanol to investigate ability of this ligand to access into the enzyme active site and the effect on enzyme structure. The variant DhaA13 was constructed to terminate the catalytic cycle of enzyme at the stage of the alkyl-enzyme intermediate. The complex with coumarin dye, in which the dye is specifically located in the tunnel mouth of the DhaA13, was used in time-resolved fluorescence spectroscopy to monitor hydration, accessibility and mobility of the dye and its microenvironment in the protein [3]. In our work we aimed to obtain the structures of DhaA13 in complex with coumarin dye and TCP.

All crystallization experiments were performed using the sitting-drop vapour-diffusion method. Diffraction data for wild-type DhaA crystal grown from the solution containing 6% (*v/v*) 2-propanol was measured at a home source (Institute of Molecular Genetics, Prague). This crystal diffracted to the resolution of 1.75 Å and belonged to the triclinic space group *P*1. Diffraction data for wild-type DhaA crystal grown from the solution with 11% (*v/v*) 2-propanol, for DhaA13 crystal soaked with TCP for three hours and for DhaA13 in complex with dye coumarin were collected at the EMBL/DESY in Hamburg. The crystals diffracted to a maximal resolution of 1.26 Å, 1.60 Å and 1.33 Å, respectively. The crystals of wild-type DhaA and variant DhaA13 in complex with dye coumarin belonged to the triclinic space group *P*1 while the crystal of DhaA13 complexed with TCP belonged to the orthorhombic space group $P2_12_12_1$. Data collections for wild-type DhaA crystal grown in the presence of TCP in the crystallization solution and for DhaA13 crystal soaked with TCP for 40 hours were carried at BESSY II in Berlin. The crystals diffracted to maximal resolutions of 1.04 Å and 0.97 Å, respectively. Both crystals belonged to the triclinic space group *P*1. The structures of wild-type DhaA and variant DhaA13 were solved by molecular replacement using the coordinates from *R. rhodochrous* haloalkane dehalogenase (PDB code 3FBW) as a search model. Structure refinement of wild-type DhaA and DhaA13 proteins and their complexes are currently in the progress.

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Neisseria Meningitidis IRON-REGULATED PROTEIN FRPD: CRYSTALLIZATION AND CRYSTALLOGRAPHIC CHARACTERIZATION

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FrpD is a highly conserved lipoprotein of *Neisseria meningitidis* anchored to the bacterial outer membrane. The *frpD* gene sequence contains two translation initiation sites, which give rise to production of the full-length FrpD protein ($FrpD_{271}$) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein ($FrpD_{250}$) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported $FrpD_{271}$ precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys_{25} residue, and sorted to the outer bacterial membrane [1].

The biological function of FrpD appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity ($K_d = 2 \cdot 10^{-10}$ M) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of any structural information on these proteins. Moreover, the primary amino acid sequence of FrpD does not exhibit any similarity to known protein sequences of other organisms, and therefore, a new type of protein fold could be expected.

This project is aimed to determine the structure of FrpD protein. Our preliminary results showed the full version of $FrpD_{250}$ protein couldn't be crystallized. Therefore, we performed a specific truncation of 21 amino acid residues from N-terminus of $FrpD_{250}$ protein. The native and Se-Met substituted variants of recombinant, truncated version (lacking the first 21 amino acid residues from N-terminus) $FrpD_{43\text{-}271}$ protein were prepared and crystallized using the sitting-drop vapour-diffusion method. The crystals of native $FrpD_{43\text{-}271}$ protein belong to the hexagonal space group $P6_2$, while the crystals of Se-Met substituted $FrpD_{43\text{-}271}$ protein belong to the primitive orthorhombic space group $P2_12_12_1$ [2]. Crystal structure of Se-Met substituted $FrpD_{43\text{-}271}$ was determined using the single anomalous diffraction (SAD) method. Structure refinement of the Se-Met $FrpD_{43\text{-}271}$ protein is currently in progress. The calculated structure will be further used as a search model in molecular replacement to determine the structure of native $FrpD_{43\text{-}271}$ protein.

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DECOMPOSITION OF CELLULAR SYSTEM VIA CAUSAL RELATIONS

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Monolayer of living cells time development is the closest approximation to organ development and function. It inspired the computational approach of cellular automata and agent-based modeling. Yet, for living cell description this approach is seldom utilized. In this paper we address reasons why biochemical /molecular biology approach is so much more popular. We present the formal structure of stochastic systems theory for formal description of the cell culture experiment. We define phenomenological attributes of cell monolayer system as cells states assigned by the operator. As system variables we consider levels of metabolic fluxes in cell compartments and between them and states of intracellular signals. For the behavior of system variables we consider that of stable orbits in the state space which arise from movement in the confined intracellular space combined with chemical reactions. Recent theoretical studies indicate also that formation and maintenance of cell shapes may arise by similar mechanism. Bio-inspired computing has been a holy grail of computational theory since its eve. Recent developments in biological systems description open a question what is really meant by this term, how much the neural networks are related to neuron and cellular automata to cells. We address in this paper also the issue of reality of bio-inspired computing in production of adequate models and/or integration of living cell elements in the computational process.

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Full text see on p. 6.

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THE ACTIVITY OF NTH1 ENZYME IS MEDIATED BY YEAST 14-3-3 ISOFORMS

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Trehalase (EC 3.2.1.28) is an intrinsic glycoprotein of the small intestine and renal brush-border membranes that hydrolyzes , -trehalose (1- -D-glucopyranosyl -D-glucopyranoside) to two glucose molecules in animals [1]. Three trehalases have been identified in *Saccharomyces cerevisiae* so far: neutral trehalase 1 (NTH1), neutral trehalase 2 (NTH2) and acidic trehalase 1 (ATH1). NTH1 is responsible for trehalose degradation, which is accumulated after stress [3]. The activity of the NTH1 enzyme was just recently found to be mediated by BMH1 and BMH2 binding in yeast. Yeast BMH1 and BMH2 proteins (yeast 14-3-3 isoforms) form a complex with neutral trehalase after its phosphorylation by PKA. Either one of the two 14-3-3 yeast isoforms are required for complete activation of neutral trehalase (NTH1) [2]. However details concerning the mechanism of BMH-dependent activation of NTH1 remain still unknown.

We showed that PKA phosphorylates NTH1 *in vitro* on three Ser residues: 20, 21 and 83. To find out which site or sites are essential for the 14-3-3 binding we produced NTH1 WT (both phosphorylated and non-phosphorylated), three NTH1 mutants containing single phosphorylation site, one double phosphorylated NTH1 mutant (at Ser20 and 21) and a mutant containing none of these studied phosphorylation sites as well. The interaction between BMH1 and BMH2 protein with enzyme NTH1 was monitored using native electrophoresis and sedimentation velocity measurements. The sedimentation equilibrium analysis was used to define the stoichiometry of NTH1/BMH complexes. Finally, we used enzyme kinetic measurements to monitor the BMH-dependent activation of NTH1.

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