Posters

P1

PREPARATION OF HsdR SUBUNIT OF EcoAI ENDONUCLEASE

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Restriction enzymes (RE) are components of bacterial restriction-modification (R-M) systems that serve to protect the cells against bacteriophage infection, because the incoming foreign DNA is endonucleotically cleaved by the restriction enzyme if it contains the enzyme's recognition sequence. R-M systems can be divided into several different types according to subunit composition and cofactor requirements. RE Type I are hetero-oligomeric enzyme compose of 5 subunits, which are responsible for specificity (HsdS), methylation activity (HsdM), ATP-dependent DNA translocation and endonuclease activity (HsdR). Although RE Type I has been extensively characterized biochemically, there is not much direct structural information available about particular subunits. Structure of HsdR subunit of EcoR124I (RE TypeIC) has been described recently by our group (1). Due to differences in length and composition in primary sequence of HsdR of particular RE Type I, we decide to structurally characterize HsdR subunit of EcoAI endonuclease belonging to the type IB family. Comparison of structural information supposes to give us more complex information about catalytical domains and function of these peliculiar endonucleases. Our project described here is aimed to produce enzyme in amount and purity sufficient for crystallization.

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P2

DISULPHIDE CHROMOPHORE AND ITS OPTICAL ACTIVITY

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The use of various spectroscopic methods and their chiroptical variants, for the determination of peptide/protein conformation is relatively well established [1-2]. Although the information is of rather low resolution it can be obtained for samples in solution and therefore has a distinct advantage over more informative methods like NMR or X-ray crystallography. Electronic circular dichroism (ECD) measured in the visible and near UV spectral region carries majority of structural information via the amide group. Detailed analyses of ECD give also additional structural data about other functional groups existing in peptide/protein molecules. These involve aromatic chromophores of Phe, Tyr and Trp side chains, the not very well understood contribution of the imidazole ring of histidine and a contribution of cystine disulphide chromophore, which is sometimes detectable as the high wavelength tail of the CD spectrum. Disulphide group is the only chromophore in proteins and peptides, which by itself exhibits inherent chirality and therefore should give rise to substantial chiroptical manifestation in electronic spectra (the non-planar disulphide chromophore itself is of C_2 symmetry). In practice, it is unfortunately not the case and especially the low energy CD bands of the disulphide group with the maximum at about 260 nm are low in intensity and rather broad. If we consider, in addition, the possible overlap with CD bands of aromatic chromophores of phenylalanine, tyrosine and tryptophan residues, it is not surprising that structure oriented application of electronic CD spectroscopy to a disulphide chromophore is quite difficult.

In this contribution we scrutinize chiral disulphides by other variants of chiroptical spectroscopy, namely vibrational optical activity measured in Raman scattering [3] Raman spectroscopy is for this purpose rather promising already in its non chiral variant (it gives information on the C-S bond conformation), but one should underline that the obtained information is not complete. In that way no information about 'absolute' conformation of the disulphide bridge can be acquired. According to theoretical calculations [4] Raman optical activity could provide this very specific information using the S-S (~500 cm^{-1}) and C-S (~700 cm^{-1}) stretching vibrations.

The ECD, IR and Raman spectra, VCD and ROA spectra of model systems are presented with the aim to cast light

on this unresolved problem. The spectra are compared with theoretical predictions.

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P3

PREPARATION OF RECOMBINANT PROTEINS RhsA AND RhsB FROM *E. COLI*

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Genes rhsA and rhsB are widely distributed in the *E. coli* K12 chromosome. RhsA and RhsB proteins are products of these genes and have unknown functions and structures. There is the theory that protein RhsA is required for the maximal biosynthesis of the K5 polysaccharide [1].

The main objective of our project is to get the crystals of RhsA and RhsB proteins for synchrotron diffraction experiments. This work includes next steps: to construct the vectors containing genes rhsA and rhsB and to build them in bacterial DNA, to get RhsA and RhsB proteins, and finally to prepare proteins for crystallization experiments. The polymerase chain reaction (PCR) technique has been used to obtain rhsA and rhsB templates from bacterial DNA. Optimal conditions for PCR have been found, and structures of expression systems are under solution.

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P4

MODULATION OF ENANTIOSELECTIVITY IN HALOALKANE DEHALOGENASE DbjA BY ENGINEERING OF A SURFACE LOOP

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The conversion of structurally and chemically simple chiral molecule 2-bromopentane to 2-pentanol is catalyzed by the novel haloalkane dehalogenase DbjA [1] with high enantioselectivity (E = 145), while other two closely related enzymes, DhaA and LinB, exhibit only low enantioselectivity (E = 7 and E = 16, respectively) with this substrate. The analysis of sequence of these enzymes identified a unique surface loop as possible determinant of this high enantioselectivity. Mutant enzyme DbjA constructed by deletion of this extra loop has significantly lowered enantioselectivity with 2-bromopentane (E = 58). The high enatioselectivity was re-introduced in DbjA + H139A (E = 120), carrying additional single-point mutation in the floppy residue H139.

In this study, we employed computer modeling to study a molecular basis for modulation of 2-bromopentane enantiodiscrimination by engineering of a surface loop. Free energies of binding were calculated using linear re-

sponse analysis (LRA) [2] and the reactivities were estimated with populations of near attack configurations (NACs) for both enantiomers [3]. The calculations showed preference of *(R)*-enantiomer over *(S)*-enantiomer in all DbjA variants which is in correspondence with experimental observations. The calculated preferences arise mainly from the difference in reactivity since *(S)*-enantiomer occurs in NACs less frequently. The preference is further increased by tighter binding of *(R)*-enantiomer. Calculated data correctly reproduce observed changes in enantioselectivity in DbjA and DbjA + H139A. Deletion of the loop results in rotation of His139 towards the center of the active site pocket and reduced width of the pocket. Interactions with His139 displace *(R)*-enantiomer from its reactive position and lead to significant drop in enantioselectivity of DbjA mutant (NACs = 19.7 %, E_{calc} = 71 in DbjA *versus* NACs = 6.4 %, E_{calc} = 49 in DbjA). Additional mutation of His139Ala reconstitutes width of the active site pocket, reactivity of *(R)*-enantiomer and enantioselectivity of DbjA +H139A to its original level (NACs = 24.1 %, E_{calc} = 79).

In summary, we show that the width of the active site pocket is important for enantioselective discrimination of structurally and chemically simple molecule 2-bromopentane by DbjA, and explain why DhaA and LinB with narrow active site pockets discriminate linear brominated alkanes poorly. Our study further demonstrates that enantioselectivity of enzymes can be modulated by the surface loop engineering which may have important implications for construction of new enantioselective biocatalysts.

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P5

RECOGNITION OF PHOSPHORYLATED CTD OF RNA POL II BY NRD1

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The transcription by RNA polymerase II (Pol II) in *Saccharomyces cerevisiae* can be terminated via at least two pathways - the mRNA pathway and ncRNA pathway. Both of them require interaction of CTD domain of RNA poly II with CID domain of a specialized protein. The CTD domain of RNA polymerase II contains tandem repeats of a heptad sequence that are dynamically phosphorylated or dephosphorylated on Ser5 and Ser2 over the course of transcription. The Nrd1 protein acts in ncRNA pathway – where Nrd1 (in comlex with Nab3 and Sen1) is via its CID domain bound to CTD domain of RNA poly II. Depending on the extent of exosome degradation, this termination pathway can lead to either 3'end trimming or complete degradation. Unlike other factors of transcription termination, Nrd1 binds preferentially to CTD phosphorylated at Ser5, which has the highest levels of fosforylation in early elongation. We will present our NMR study of the Nrd1 CTD domain and its interaction with the Ser5-phosphorylated CTD of RNA Pol II.

P6

GENERATION OF BACTERIAL STRAINS FOR PRODUCTION OF THERAPEUTIC HUMAN PEPTIDE HORMONE

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Therapeutically active peptides and proteins (biopharmaceuticals or biodrugs) represent a rapidly growing proportion of marketed drugs and have an undisputed place alongside many therapies; for certain indications they even are the only effective therapy. Therapeutic peptides and proteins interact with targets that are not accessible for small chemistry-based molecules. Biopharmaceuticals cover many therapeutic areas including treatment of cancer, autoimmune diseases, diabetes, anemia, disorders as-

sociated with lack of certain proteins (e.g. human growth hormone) and others.

Parathyroid hormone (PTH) is a peptide hormone secreted by the parathyroid glands that consists of 84 amino acid residues. In human it regulates calcium and phosphate metabolism. A C-terminal truncated version consisting of the 34 first amino acids retains the biological activity.

The aim of this work was to generate a high-level expression system for production of recombinant human parathyroid hormone which eventually can be used for pharmaceutical purposes.

Because it is known, that short peptides are difficult to express in *Escherichia coli*, several different strategies were used to obtain suitable expression constructs for production of PTH in bacteria. One method relies on the use of fusion partners (glutathione S-transferase (GST), maltose binding protein (MBP) and others). By including an appropriate protease recognition sequence, the peptide can be separated from the fusion partner by proteolytic cleavage. Another method involves gene polymerization. Here, the gene of interest is expressed and purified as polymer and subsequently cleaved into monomers. A third approach is to express the target gene in a bacterial strain exhibiting low proteolytic activity what should also lead to higher yields of the produced peptide.

We have developed several bacterial expression systems, using all three approaches mentioned for yield enhancement. As an alternative to bacterial expression, we are also developing a yeast expression system, producing and secreting the desired peptide.

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P7

CRYSTALLIZATION OF CARBOHYDRATE OXIDASE FROM *MICRODOCHIUM NIVALE*

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Carbohydrate oxidase from *Microdochium nivale* containing 475 amino acids belongs to a family of flavin- or Cu-containing enzymes whose function consists in catalytic oxidation of the primary alcohol in various oligosacharides. The product of the reaction is the corresponding saccharide acid and the process is accompanied by reduction of molecular oxygen to hydrogen peroxide, which has large potential industrial utilization.

Hampton Research Crystal Screens and Index solutions were used for the initial screening and crystallization experiments were performed using vapour diffusion and micro-batch methods at 290 K and 298 K. Morphology of the crystals varied depending on crystallization conditions and crystals featured varying stability.

A hexagon-shaped crystal grown with Jeffamine ED2001 as precipitant was measured at beamline BM14, ESRF in Grenoble using the MARMosaic 225 detector. The data were collected up to 2.7 Å resolution, however, a rapid intensity fall-off occurred beyond 3.5 Å. The space group was assigned as $P6_222$, with unit cell parameters $a = b = 55.7$Å, $c = 610.4$ Å. The crystallographic symmetry was verified with program Pointless [1]. Analysis of the intensity statistics clearly showed the presence of merohedral twinning. This was confirmed by the web-based service for analysis of twinning http://nihserver.mbi.ucla.edu/. Twinning which indicated merohedral twinning with a twin factor of 43% [2].

Extensive molecular replacement trials with a model of 41% sequence identity (PDB code 1zr6) [3] were performed in *MOLREP* [4] and *AMORE* [5], however, without satisfactory results. Further experiments to produce well diffracting crystals are necessary.

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P8

DETAILED CHARACTERIZATION OF THE CALMODULIN BINDING DOMAIN ON THE C TERMINUS OF TRPV1 RECEPTOR

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Transient receptor potential vanilloid 1 (TRPV1) is a transmembrane ion channel that participates in physical and chemical pain evoked signal transduction. This nonselective cation channel allows monovalent and some divalent cations (Ca^{2+}, Mg^{2+}) to move into the cell. Many TRP channels possess multiple CaM-binding domains (CaM-BDs) located at both termini and which could show varying behaviors towards calcium. Despite the fact that CaM-BDs have no sequence homology, they share key structural features such as interspersed basic and bulky hydrophobic amino acid residues. The C-terminal region of TRPV1 (TRPV1-CT) does not possess any classical previously defined CaM-binding motif. Several possible binding motifs for CaM have been recently identified on the TRPV1-CT by sequence homology and structural analyses.

In this work we explored this unusual TRPV1 CaM-binding motif in detail. Based on our three dimensional computer homology model, we generated nine point mutant proteins of TRPV1-CT containing the putative binding site for CaM (V769A, R771A, L777A, R778A, R781A, V782A, R785A, K788A, and R797A) and evaluated their effects on TRPV1-CT interaction with CaM. The part of the rat TRPV1-CT DNA subcloned into the expression vector pET32b was used as a template for the introduction of point mutations. Recombinant fusion proteins were used for steady state anisotropy measurements and the dissociation constants for CaM on all constructions were estimated. We have found that among the basic residues the arginine R785 is the most essential residue for TRPV1-CT binding to CaM. In addition, the analysis of hydrophobic valines located at the first and the last position of a tentative ‘‘1-8-14” conserved motif revealed that the residue at the first position plays a more important role in TRPV1-CT’s binding to CaM.

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P9

SURFACE MAPPING OF CYSTATHIONE BETA SYNTHASE: INSIGTH INTO ENZYME AUTOINHIBITION USING MASS SPECTROMETRY

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Cystathionine -synthase (CBS) is a tetrameric enzyme containing 551 amino acids, which catalyzes condensation of serine with homocysteine. Sequence of CBS can be divided to three regions: N-terminal part (1-39), active core (40-413) and C-terminal part (414-551); interaction of active core with C-termain domain causes enzyme autoinhibition.

The 3-D structure was determined only for the truncated CBS lacking C-terminal part (amino acids 1-413, trCBS) since full-length CBS protein (wtCBS) could not be succesfully crystallized. The aim of this work is to describe molecular mechanism of the autoinhibition using the chemical modification of surface exposed amino acid residues followed by mass spectrometric detection.

Initially, we tested eight labelling compounds and six of them were suitable since they have not altered the quarternary structure and activity of CBS, namely 4 –hydroxyphenylglyoxal (HPG), N-ethylmaleinimide (NEM), diethylpyrocarbonate (DEP), N- hydroxysulfosuccinimide acetate (NHS), N-brom-succinimide (NBS) and N-acetylimidazole (NAI).

In our ongoing study, we have analysed reactivity of four agents (NEM, DEP, NBS, NAI) with trCBS and wtCBS. Cluster of three tryptophane residues (Trp408-Trp410) was differentially reactive with NBS, modified in trCBS but not in wtCBS, indicating that the cluster is sterically hindered in wtCBS.

Contradictory, cysteine (reacted with NEM), histidine (DEP) and tyrosine (NAI) modification sites were identically localized in both forms of CBS. These data shows subtle differences in surface of trCBS and wtCBS and the modular character of the enzyme. Futhermore, this dataset provides the restrains for computation modelling which would have explained the molecular mechanism of CBS activity regulation.

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P10

STRUCTURE AND RNA BINDING OF Nab3

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Besides mRNA, RNA polymerase II also transcribes a subset of small nuclear and small nucleoar RNAs, and a class of intergenic and anti-sense RNAs. Termination of these transcripts requires the nuclear pre-mRNA down- regulation (Nrd)1 and the nuclear polyadenylated RNA-binding (Nab)3 proteins, and the RNA helicase Sen1. In this so-called nonpoly(A) termination pathway, the RNA-binding proteins, Nrd1 and Nab3, recognize Nrd1- and Nab3-binding sites which is an initial step in the termination and subsequent processing or degradation of these transcripts.

Recent studies identified sequence motives for Nrd1 and Nab3, as the signals that direct termination and exosome-TRAMP trimming/degradation of nonpolyadenylated transcripts. It was shown that the RNA-recognition motif (RRM) of Nrd1 and Nab3 bind to the GUA[A/G] and UCUU sequences, respectively. In addition, it was demonstrated that Nrd1 and Nab3 form a stable heterodimer and bind to snoRNA terminators that contain multiple Nrd1- and Nab3- binding sequences.

To fully understand the structural basis behind the RNA recognition by the Nrd1-Nab3 complex, we use multidimensional NMR spectroscopy to determine the three- dimensional structures of the individual RRMs of Nrd1 and Nab3 and the minimal Nrd1- Nab3 heterodimer alone as well as in complex with their RNA substrates.

P11

THE PURIFICATION PROCESS OF TRPC6 C-TAIL AND CALMODULIN- OPTIMIZATION FOR CRYSTALLIZATION

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TRP (transient receptor potential) channels represent multifunctional sensors perceiving wide spectrum of environmental cues in form of physical and chemical stimuli. They are widely expressed in the CNS and peripheral cell types. They are involved in numerous fundamental cell functions. Recently, many pathological conditions have been linked to TRP dysfunctions. However many aspects of physiology and regulation of TRPs are still elusive.

It has been proved that TRPC6 plays an important role in vascular and pulmonary smooth muscle cells and it has been proposed to play a critical role in the intravascular pressure-induced depolarization and constriction of small arteries and arterioles.

Unveiling the structure of complex of TRPC6 C-tail with calmodulin could contribute to elucidation of mutual structural adjustment of these two molecules in space, their mutual interactions on molecular level. Detailed studies of the structure of this complex could help with prediction of therapeutically significant substances applicable in clinical practice for treatment of diseases caused by TRP dysfunctions.

Purification process of recombinant TRPC6 C-tail and calmodulin was suggested. Both proteins were expressed in E. coli expression system. The cells were resuspended, digested with lysozyme and sonicated. The TRPC6 C-tail peptide was obtained by successive affinity chromatography on HisPur resin column and gel filtration. Calmodulin was purified on the column of CL4B-Sepharose and gel filtration. The proteins were mixed together and refined to a high purity by gel filtration. The sample was further used for crystallization experiments using the method of "hanging drop ".

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P12

COMPUTATIONAL STUDY OF THE NH-TAUTOMERISM IN CALIX[4]PHYRINS OBSERVED BY NMR SPECTROSCOPY

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Calix[4]phyrins are novel compounds derived from porphyrins. They can be used as excellent agents in complexes and macromolecular chemistry [1]. For some derivatives the inner hydrogen movement can bee seen in NMR spectra, where the NH-tautomerism can be followed by measurement of the coalescent temperatures. The transition energy barrier for the phenomenon can be obtained from the NMR data. Because of the importance of the hydrogen transfer processes we studied the effect in detail, by simulating the transition path and two-dimensional potential energy surfaces. The PM3, HF/3-21G and DFT BPW91/6-31G** and B3LYP/6-31G** methods were used for the ab initio computations. The Qgrad program [2, 3] using normal modes coordinates was adapted for suitable coordinate definition corresponding to the hydrogen movement. Calculated barriers and equilibrium geometries well correspond to the observed data.

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P13

2'OH ACTIVATION IN RNA CATALYSIS: METAL-ION / NUCLEOBASE MECHANISMS IN SELF-CLEAVING RIBOZYMES

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The reaction catalyzed by a small catalytically active self-cleaving ribozymes is a phosphate ester hydrolysis that is thought to proceed via activation of the 2'OH group of the ribose moiety at the cleavage site. In this work the mechanism of the 2'OH activation is studied theoretically by DFT and MP2 methods. The effect of bulk solvent is treated with continuum solvation CPCM model. Mg^{2+} ion and the G-12 guanine nucleobase are considered as the catalysts of the proton transfer to activate 2'OH. Calculated free energy barriers of 2'OH activation are similar for Mg^{2+} ion and G-12. However negative charge on $2'O^-$ is not stabilized enough by the bare G-12 nucleobase and further stabilization by other species (e.g. by metal ions) is necessary and/or the 2'-OH activation is concomitant with the nucleophilic attack on the phosphorus followed by an immediate cleavage reaction. Mg^{2+} ion stabilizes $2'O^-$ by a direct coordination and kinetics of 2'OH activation by the Mg^{2+} ion is determined by the equilibrium between hexa- and penta-coordinated Mg^{2+} structures. The metal ion coordination to 2'OH lowers the pK_a value of the 2'OH group by more than three log units.

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P14

STRUCTURAL ANALYSIS OF THE EXTRINSIC PsbP PROTEIN OF PSII FROM *SPINACEA OLERACEA* BY X-RAY CRYSTALLOGRAPHY, RAMAN SPECTROSCOPY AND BIOINFORMATICS

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Introduction

Photosynthesis is the process by which the light energy is converted into the chemical energy. It takes place in the thylakoid membrane of higher plants, algae and cyanobacteria, where membrane-embedded, pigment-protein PSII complex performs light-driven oxidation of water with concomitant reduction of the plastoquinone pool. Water splitting is performed in a cluster of four Mn^{2+} ions located on the lumenal side of photosystem II (PSII) and Ca^{2+} and Cl^- ions are required for optimal activity of this water–oxidase complex. In higher plants the function of Ca^{2+} and Cl^- is modulated by the presence of four extrinsic proteins [1], PsbP, PsbQ [2], PsbO, and PsbR, at the lumenal surface, the so called oxygen-evolving complex. To understand the molecular mechanisms of the oxygen–evolving reaction, an essential prerequisite is the structural knowledge of these proteins and their relative interactions.

Results and Discussion

The structure of recombinant protein PsbP of the oxygen-evolving complex from *Spinacia oleracea* was determined at a 1.98 Å resolution by X-ray crystallography [3] and the unresolved parts further refined by Raman and FTIR spectroscopy. Spectroscopy gives a unique opportunity to study protein samples in different phases, we report the spectra of PsbP in crystal, solution and as DCDR deposit. For protein preparation the overexpression system *Escherichia coli* (*BL21DE3)* transformed by plasmid DNA with gene PsbP (recombinant HisPsbP) was used to produce protein stable in bisTRIS buffer (pH=6,00) in a concentration of 15 mg/ml. A more clear picture about the location of extrinsic protein in higher plants is emerging from interaction studies between PsbP and PsbQ proteins from spinach, approached in both, theoretical (MD analysis) and experimental level (affinity chromatography).

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P15

CHARACTERIZATION, CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDIES OF TOMATO NUCLEASE I

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Tomato Nuclease I is a Zn^{2+} dependent plant endonuclease, which cleaves both RNA and DNA in ss and ds form. This enzyme belongs to plant nuclease I group, is relatively small glycoprotein and shows considerable activity in cell apoptosis. Therefore detailed structural study of this enzyme can lead to new ways of cancer and bacterial disease treatment. Heterelogous expression yields amounts and quality of the enzyme suitable for structural studies. Biochemical characterisation of tomato nuclease I focused on 3D structure determination using crystallographic methods and also crystallization and preliminary X-ray diffraction experiments are reviewed.

P16

EXPLORING THE 3D STRUCTURE OF THE N-ACETYLGLUCOSAMINYLTRANSFERASE V (GNT-V)

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Glycosyltransferases (GT's) are indispensable to cellular life in eukaryotes by producing glycan linkages with a unique contribution to the development and function of physiological systems in the context of living organisms. Also connections between GT's and mammalian disease processes are being made recently. In this group of glycosyltransferases is -1,6-N-acetylglucosaminyltransferase V (GnT-V) also included. Genetic experiments on mice with blocked GnT-V production (Mgat5-/- knockout) clearly show reduction in cancer metastasis [1]. Due to the clear evidence of the involvement GnT-V in the cancer metastasis is only little information on the structure and reaction mechanism.

In the present study, we have attempted to build model of the GnT-V 3D-structure. Unfortunately, it is not clear to which GT family GnT-V belongs, therefore, we used one structure from the GT-A family and one structure from the GT-B one as structure templates. Selected structures had the best primary sequence alignment with GnT-V minimal catalytic domain [2]. We have chosen structure of Core2 transferase (2GAM) [3] as the representative of the GT-A family and MurG transferase (1NLM) [4] as the representative of the GT-B family, respectively. We made several alignments employing different algorithms, namely with Blosum62, 120PAM or 3D-PSSM. We obtain altogether 10 models of the GnT-V structure. On the basis of the 3D-structure analysis we have chosen five models for the molecular dynamic equilibration in water. All structure models have been equilibrated at 300 K during 4ns. The molecular dynamic simulation show, that more reliable model might be the model based on the MurG template. Due to these result we did multiple sequence alignment with another seven members of this family, including MurG, to observe more precise alignment. In addition we also took one glycosyltransferase, which family hasn't been specified yet, -1,6-fucosyltransferase FUT8 (2DE0) [5].

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P17

EXPERTOMICA HELA CELLS 3.03 RC

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Expertomica Hela Cells (EHC) is a program that creates computer description of the behavior of a cell culture photographed with Time-Lapse microscopy. It is being developed especially for the description of HeLa cells with an experiment range up to 1500 analyzed images with 30-50 cells per image. The EHC can be used for larger experiments or for other cell types as well with minor limitations only.

EHC consists of two logical blocks, the data control block and GUI. However, the user can see only GUI, which he uses to control the whole program. In the program he creates a project into which he loads the directory with analyzed pictures and then enters the position of the cells and their neighbors with a mouse click. The internal cell features, shape in our case, are entered after the cell was highlighted. When the cell divides itself, moves or merges with another cell, a new cell has to be created with a click and its parents and neighbors have to be marked. The whole process goes on this way until a complete computer description is finished.

The topological representation of the complete computer description can be exported into a *.dot file or just a part of it relating to a single cell can be viewed in the Graph panel.

The above mentioned operations are analogous to those performed by biologists decades ago with just one difference. They drew the topological graph by hand on millimeter paper and were able to analyze the culture up to approximately 15 cell cycles. The benefit of the computer description, apart from paper saving and facile storage, is the fact that we can easily compute any parameters of the cell culture (e.g. time between cell division in a single line or within the whole culture), get exact growth speed of the colony, etc.

EHC is written in the Java language version 6 because of its almost unlimited transferability between PC platforms and OS. Another advantage is the small program size.

The following EHC version is to eliminate the above mentioned limitations by adding semi-automatic analysis and extending program usage to other cell types. After that, the user will solve only disputable analytic cases.

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P18

GLYCOSIDE HYDROLASE FAMILY 31 PROTEINS IN *CAENORHABDITIS ELEGANS*

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Human acid alpha-glucosidase (GAA, EC 3.2.1.20) catalyses lysosomal glycogen degradation and belongs to glycoside hydrolase family 31 (GH31). The objective of our study was to assess GH31 proteins in *Caenorhabditis elegans* (*C. elegans*), to identify the acid active GAA ortholog and to evaluate *C. elegans* as a model organism for glycogen storage disease type II (acid alpha-glucosidase deficiency).

C. elegans genome contains four acid alpha-glucosidase related (aagr-1-4) genes. These predicted ORFs were amplified from mixed stage N2 culture's cDNA and sequenced. Multiple protein sequence alignment demonstrated common evolutionary origin of AAGR-1-4 and other selected GH31 members. We further performed protein structure homology modeling of all four AAGR proteins on the basis of available GH31 templates (YicI, MalA and Nt-MGA) as well as molecular docking of the specific inhibitor of non-alpha-glucosidase GH31 enzymes - acarbose. All these bioinformatic analyses suggested clustering of AAGR-1 and 2 with acid-active, and AAGR-3 and 4 with neutral pH optimum GH31 enzymes. The expression of AAGR-1 and 2, evaluated by

transcriptional GFP tagging, was in both cases limited to intestinal cells and six coelomocytes. RNA interference (RNAi) of each of the four nematode's genes did not reveal any changes in morphological phenotype. Additionaly, RNAi efficacy was assessed by glucosidase activity measurements at two pH values (4.0 and 6.5) with and without addition of acarbose (pH 4.0), a clinically used alpha-glucosidase inhibitor. We observed predominant neutral or acidic glucosidase activities associated with individual AAGRs. Nevertheless, AAGR-1 was found to possess acidic glucosidase activity with relatively pronounced acarbose resistance, a result further replicated in aagr-1 deletion mutant.

To conclude, four *C. elegans* (AAGR-1-4) orthologs of human GAA were evaluated by combination of bioinformatic, cellular and biochemical approaches. It was determined that AAGR-2 has predominating acid and AAGR-3 neutral glucosidase activity, though AAGR-1 was the least acarbose sensitive acidic AAGR and therefore could represent the most probable ortholog of human GAA.

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P19

Structure-function relationships of maize -glucosidase Zm.p60.1

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The basic structure of the vast majority of -glucosidases is the ( )$_8$ barrel fold which is a fundamental structural element in many proteins. Proteins containing the ( )$_8$ barrel fold occur in five of the six general classes of catalytic activities according to the Enzyme Commission classification scheme.

Current knowledge as represented by more than 150 hits in the CaZy (carbohydrate active enzymes) database shows that the -glucosidase (-glucoside hydrolase, EC 3.2.1.21) class comprises extended "families" of enzymes hydrolyzing a broad variety of aryl- and alkyl- b -D-glucosides as well as glucosides made up only of carbohydrate moieties [1]. Widespread interest in -glucosidase research reflects their essential functions in a variety of basic biological processes ranging from developmental regulation to chemical defense against pathogen attack, and in a number of industrial applications such as biomass conversion.

Our model enzyme, the maize -glucosidase Zm-p60.1, is important for the regulation of plant development due to its role in the targeted release of free cytokinins from their inactive storage forms, cytokinin-O-glucosides [2,3].

Our research is focused on attempting to modulate enzyme specificity and understanding the functional relationships between key amino acid residues that form the entrance to the active site. We are interested in plant -glucosidases and Zm-p60.1 is the best described among them. This group of proteins is highly diverse in terms of homologous enzymes, which enables bioinformatics as well as the elucidation of the biological significance of -glucosidases.

In our work we have mainly used methods of rational design in protein engineering. that approach, however, has several limitations. Therefore we have adopted a strategy of random site directed mutagenesis followed by directed evolution to investigate the functional relationships between amino acid residues. Generally, this work will shed more light on the complex evolution of enzyme substrate interaction at the active site. Simultaneously, the ability to modulate specificity of -glucosidases holds considerable promise in terms of biotechnological applications.

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P20

BUILDING A STRUCTURAL MODEL FOR TRANSIENT RECEPTOR POTENTIAL CHANNELS: POSSIBILITIES AND LIMITATIONS

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Transient receptor potential (TRP) channels are a large superfamily of nonselective cation channels that play an important role in many sensory functions. In the absence of high resolution TRP channel structural data, structural prediction and computational modeling is the only possibility to help with the interpretation of experimental data on an atomic and structural level. TRP channels are characterized by six transmembrane domains and a short, pore-forming hydrophobic stretch between the fifth and sixth transmembrane domain. Many TRP channels have a long amino terminus containing several ankyrin-repeat domains and a C-terminal domain on the cytoplasmic side of the membrane. We demonstrate on two examples, the transient receptor potential vanilloid receptor-1, which is a cation channel present in sensory nerve endings, and the transient receptor potential TRPA1 as a candidate for a mechanically gated transduction channel, the possibilities and limitations of computational modeling. Starting with secondary structure prediction and prediction of transmembrane regions, over fold prediction, threading and homology modeling up to molecular dynamics in an explicit phoshatidyl oleoyl phosphatidylcholine membrane and ligand docking calculations, a wide variety of attempts are made to gain structural insides. Their reliability and confrontation with experimentally gained data is discussed.

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P21

NMR STRUCTURAL STUDY OF N-TERMINAL DOMAIN OF RNA POLYMERASE DELTA SUBUNIT FROM *BACILLUS SUBTILIS*

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Contrary to the well studied RNA polymerases of gram negative bacteria, RNA polymerase of *Bacillus subtilis*, a gram positive bacterium, contains two additional subunits, referred to as delta and omega1. A well-structured N-terminal domain of subunit delta has been overexpressed in an *E. coli* expression system, labeled with stable isotopes C-13 and N-15, and investigated by nuclear magnetic resonance.

A standard set of triple resonance NMR experiments was measured and almost all resonances of the protein backbone were assigned. Resonance frequencies of the side-chains were assigned using 3D TOCSY- and NOESY-type spectra. Three-bond coupling constant $J(H^NH)$ were obtained from 3D HNHA experiments. Chemical shifts of backbone nuclei, medium range NOEs, and the three-bond coupling constants were analyzed and secondary structure was predicted. Internuclear distance restraints were extracted from NOESY spectra and used in structure calculation.

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P22

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF NOVEL HALOALKANE DEHALOGENASE DBEA FROM *BRADYRHIZOBIUM ELKANI* USDA94

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Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are bacterial enzymes that catalyze the hydrolytic conversion of halogenated aliphatic compounds to their corresponding alcohols. These enzymes play a key role in aerobic mineralization of many halogenated compounds that occur as environmental pollutants. HLDs are applicable for bioremediation, decontamination and industrial biocatalysis [1]. Isolation of a new family members is important for evolutionary and mechanistic studies as well as for biotechnological applications.

Three subfamilies, denoted HLD-I, HLD-II and HLD-III, can be recognized within this family [2]. A novel enzyme DbeA belonging to the subfamily HLD-II was isolated from *Bradyrhizobium elkani* USDA94. This new enzyme is closely related to DbjA from *Bradyrhizobium japonicum* USDA110 [3], which exhibits a unique insertion in the N-terminus of the cap domain absent in other HLDs. DbeA has 71% identity to DbjA [3], 47% identity to DhaA [4], 41% identity to LinB [5] and 39% identity to DmbA [6].

Proper folding of DbeA was assessed by measurement of CD spectra in far-UV and near-UV spectral regions. Thermal stability of DbeA was evaluated by determination of the melting temperature ($T_m = 48.82 \pm 0.16$°C), which is in the same range as observed with other family members. Gel filtration and native polyacrylamide gel electrophoresis were used for determination of oligomerization state of DbeA. Determined molecular weight of DbeA confirmed dimeric state of DbeA under native conditions. Activity data of HLDs were measured with a set of 30 various substrates. The principal component analysis of the specific activities showed that DbeA is less active than DbjA and LinB, while it has similar activity as DhaA. DbeA posses a unique substrate specificity, the enzyme has the highest activity towards brominated and iodinated compounds from all tested HLDs. DbeA showed high enantioselective conversion of 2-bromopentane, 2-bromohexane and brominated ester of propionic and butyric acid into chiral alcohols. The temperature and pH profiles of DbeA were detected by activity measurement with 1-iodohexane as a substrate. The highest activity of the enzyme was detected at the temperature range 45-55 °C, which is in good agreement with the temperature profiles of other HLDs. Surprisingly, DbeA showed more than one pH optimum with the maximal activity detected at pH conditions 6.0 and 8.5-9.5. Similar pH profile was described only for DmbA, while other HLDs exhibited single pH optimum. Crystallographic analysis of DbeA was initiated to understand the structure-function relationships and evolution of this interesting enzyme.

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P23

PREDICTION OF ^{13}C CHEMICAL SHIFTS IN NUCLEIC ACIDS

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Chemical shifts are sensitive probes of biomolecular structure [1]. The structural information contained in chemical shifts is, however, very different from the distance and dihedral angle constraints provided by NOE intensities and scalar coupling constants, respectively. The value of chemical shift of a specific nucleus is a sum of many contributions whose sources are not readily identifiable. Therefore, a necessary first step in the studies of chemical shifts in relation to the biomolecular structure is establishing a reliable procedure of calculating the chemical shifts for known structural elements.

As quantum chemical approaches at sufficiently high level remain beyond the applicable limit for molecules of biological interest, the chemical shift calculations rely mostly on classical or semiclassical approaches considering long-range effects arising from the magnetic susceptibilities, electric field effects and close contact effect (mainly hydrogen bonds) as well as the effect of local geometry. In interpreting macromolecular chemical shifts it is usually assumed that the individual contributions are approximately independent and additive. Since hydrogen atoms are bound to only one other atom, the local geometric effect tend to be reasonably constant for a particular type of nucleus and proton chemical shifts can be adequately characterized considering the long-range effects only. On the other hand, the local geometries need to be generally taken into account for ^{13}C shifts.

For proteins, a number of programs exists to relate the proton and heteronuclear chemical shifts to structural features. Much less is known about the chemical shift – structure relationships in nucleic acids. It has been shown that ^{1}H shifts in both DNA [2] and RNA [3] can be adequately represented by just three contributions, namely by ring current, magnetic anisotropy and electric field effects, with the electric field effect playing a relatively small role. Carbon and nitrogen chemical shifts for different sugar puckers and base orientations were studied by DFT on the level of DNA and RNA dinucleotides [4].

The systematic studies of ^{13}C chemical shifts of nucleic acids are hindered by inadequate database of available shifts and structures in BioMagResBank. In this situation, we decided to concentrate on a few molecules for which reliable ^{13}C chemical shift data and accurate structures are available, namely Dickerson-Drew dodecamer (PDB code 1NAJ), d(GCGAAGC) hairpin (PDB code 1PQT), DNA duplex (part of protein-DNA complex PDB code 1AHD).

Chemical shifts were calculated for ^{13}C in nucleic acid bases. The structural dependent part of the ^{13}C chemical shifts was calculated as a sum of the following contributions: ring current shift and magnetic anisotropy (calculated by program NUCHEMICS), and electric field effect (calculated by program MOLMOL). The calculated shifts were added to the experimental shift values of free dNTP which are assumed to be devoid of the effects of stacking and base-pairing interactions.

The resulting chemical shifts were found to be rather sensitive to even slight structural changes. This confirms that ^{13}C shifts are sensitive monitors of structural features, on the other hand, it makes obtaining good agreement with experimental shift values difficult. Tight correlation with the experimental shift data were observed for well defined regions of the molecules, especially for double helices. Larger errors in less regular regions can be ascribed partially to inaccurate structural information and in part to deficiencies in the method used to calculate the chemical shifts.

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P24

THE MOLECULAR DYNAMICS STUDY OF THE RNA-BINDING DOMAIN OF ADAR2 BOUND TO dsRNA

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Like RNA splicing, RNA editing alters the sequence of an RNA from that encoded in the DNA. Typically, a single RNA splicing reaction removes a large block of contiguous sequence, whereas each RNA editing reaction changes only one or two nucleotides. Therefore splicing is a cut-and-paste mechanism whereas editing is one of fine-tuning.

RNA editing by adenosine deamination is catalyzed by members of an enzyme family known as adenosine deaminases that act on RNA (ADARs). ADARs are RNA editing enzymes that target double-stranded regions of nuclear-encoded RNA. ADARs are also interesting in regard to the remarkable double-stranded structures of their substrates and how enzyme specificity is achieved with little regard to sequence.

ADARs from all organisms have a common domain structure that includes variable numbers of double-stranded RNA (dsRNA) binding motifs (dsRBMs) followed by a highly conserved C-terminal catalytic domain. We focused on the N-terminal non-catalytic domain ADAR2, which recognizes the dsRNA with A-C mismatches. Using MD simulations, we study the role of mismatches and their flexibility for the formation of dsRBM-RNA complexes.

P25

HOTSPOT WIZARD: WEB SERVER FOR IDENTIFICATION OF MUTAGENESIS HOT SPOTS IN ENZYME STRUCTURES

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HotSpot Wizard is a web server for identification of hot spots for rational engineering of substrate specificity, activity and enantioselectivity of enzymes. Rational design usually requires considerable knowledge of structure-function relationships of a target protein. Mutations targeting the residues located in the active sites or lining the access tunnels, have a better chance to produce enzyme variants with novel catalytic properties, than mutations localized in other parts of the protein structure. On the other hand, replacement of the amino acid residues that are highly conserved through evolution due to their essential role for the structural stability or functionality, may lead to the loss of enzymatic activity. Mutagenesis of variable sites, which contributes to the substrate binding, transition state stabilization or product release, represents a useful concept in protein engineering [1]. To follow this concept, the users need to have experiences with analyses of protein sequences and structures. Furthermore, these analyses are time-consuming even for experienced users.

HotSpot Wizard automates and speeds-up the analysis by integration of structural and evolutionary information obtained from selected bioinformatics databases and tools. HotSpot Wizard requires a structure of the query protein in PDB format and an e-mail address as the only obligatory inputs. In the first step, Catalytic Site Atlas [2] and UniProt [3] databases are used to determine the residues indispensable for enzyme function. HotSpot Wizard then searches for potentially important residues by CASTp [4], identifying the active site pocket, and by CAVER [5, 6], calculating tunnels connecting buried cavities with the outside solvent. Finally, evolutionary conservation of individual positions in the query structure is estimated by ConSurf [7] from the multiple sequence alignment of closely related proteins. In the output, HotSpot Wizard lists residues ordered by predicted suitability for mutagenesis together with information on their conservation level, potential structural and functional importance, available mutagenesis data and existing sequence variants. Results are mapped on the enzyme structure and can be visualized directly in a web browser using Jmol [8] or downloaded to the local computer as input for PyMOL [9] or text files.

The primary application of HotSpot Wizard is in the rational design of the hot spots for site directed mutagenesis or focused directed evolution. Alternatively, HotSpot Wizard can serve as a structure annotation tool. Altogether, using HotSpot Wizard one can perform several structural and evolutionary analyses at once with minimal demands on a user, making this server potentially useful for experimentalists with no prior knowledge of structural and bioinformatics analyses.

HotSpot Wizard is freely available at
http://loschmidt.chemi.muni.cz/hotspotwizard/.

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P26

INTERACTION OF THE N-TERMINAL MYRISTIC ACID WITH MATRIX PROTEIN FROM MASON-PFIZER MONKEY VIRUS

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Polyprotein Gag as a precursor of structural proteins plays a key role in formation and budding of retroviral particles. The N-terminal domain of Gag, the matrix protein (MA) interacts with the cytoplasmic membrane of infected cell through the bipartite signal that involves a cluster of basic residues and myristic acid which is covalently attached to the amino-terminal glycine.

In this work we focus on the determination of molecular basis of the phenotypic changes of M-PMV double-mutants T41I/T78I and Y28F/Y67F, which are unable to bud through the cytoplasmic membrane and rather accumulate on it. In contrast, they do not affect assembly and transport of immature virus particles. We determined the three-dimensional structures of unmyristoylated species of both mutants using isotopically aided NMR spectroscopy. Comparison of their structures with the structure of the wild type MA shows that the mutation caused only marginal changes of the structural motif. In both cases it increased the size and hydrophobicity of the protein interior. Isoleucines 41 and 78 in T41I/T78I and phenylalanines 28 and 67 are oriented to the protein core in contrast to the original amino acid residues and they influence the interaction of the matrix protein interior with the myristic acid. This finding supports a hypothesis that the phenotypic change of the mutant is caused by an enhanced interaction of the myristic acid with more hydrophobic protein core, which prevents its exposure and finally abrogates the interaction of immature viral particles with the cytoplasmic membrane. To prove the hypothesis we focus on the determination of structure of myristoylated MA proteins with the emphasis to the interaction of the myristoyl with protein core.

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P27

MOLECULAR DYNAMICS SIMULATIONS OF WATER AND IONS INTERACTING WITH RUTILE SURFACES

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In the last 8 years we have been studying by molecular dynamics (MD) the dynamic and structural properties of aqueous solutions in contact with solid metal-oxide surfaces, rutile and cassiterite. During this period we obtained numerous results on structure of water and adsorbed ions, which were successfully linked with experimental results using X-ray techniques [1-3]. We gathered data on the adsorption of Rb^{+}, Na^{+}, Sr^{2+}, Ca^{2+}, and Zn^{2+} on neutral, negatively, and positively charged hydroxylated or nonhydroxylated 110 rutile surfaces.

In this communication, we summarize our results and outline the range of properties, which were studied on this system, including inhomogeneous diffusivity and viscosity [4] in the inhomogeneous interfacial region. We also de-

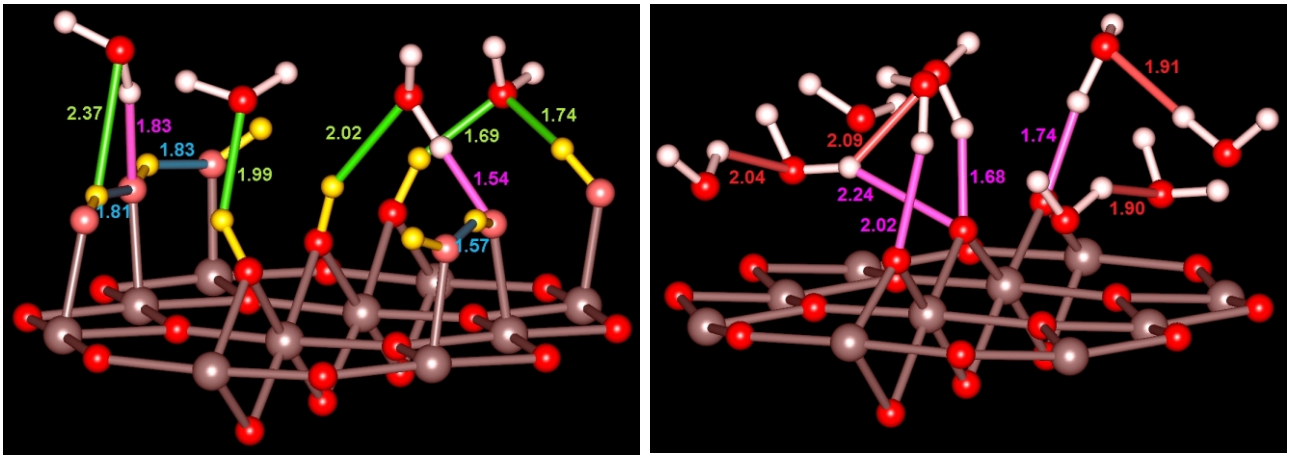

Figure 1. Hydrogen bonding at the neutral hydroxylated surface (left) and neutral nonhydroxylated rutile (110) surface (right) as extracted from a large periodic surface MD structures. Intramolecular O-H bonds in water (white) and surface hydroxyls (yellow), surface oxygen-water H-bonds (violet), surface hydrogen-water H-bonds (green). For the nonhydroxylated surface, water-water H-bonds are in red. Bond lengths are in Ĺ.

scribe the ab initio derived method to simulate charged surfaces at surface charge densities linked to solution pH values via surface titration experiments. Discussions with computational biophysicists about transferability of this study to e.g. interfaces with lipid membranes etc. and modeling of interactions of biomolecules with mineral surface are very welcome.

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P28

14-3-3 PROTEIN INTERACTS WITH AND AFFECTS THE STRUCTURE OF RGS DOMAIN OF REGULATOR OF G PROTEIN SIGNALING 3 (RGS3)

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Regulator of G protein signaling (RGS) proteins share a highly conserved 125-amino-acid large domain that was first identified by its ability to negatively regulate GPCR signaling [1]. Some RGS proteins consist of little more that the RGS domain while others posses long N-terminal or C-terminal extensions (e.g. RGS3) that usually contain additional protein-protein interaction motifs and domains [2]. RGS proteins function as GTPase-activating proteins (GAP) for the G subunit of heterotrimeric G proteins. They bind specifically to the GTP-bound forms of G and significantly stimulate GTP hydrolysis by stabilizing the transition state.

The activity of RGS proteins is tightly regulated through various mechanisms including phosphorylation and interactions with other proteins. Several RGS proteins, e.g. RGS3, RGS4 and RGS7, have been found to interact with 14-3-3 proteins. 14-3-3 proteins are a family of acidic regulatory proteins that function as molecular scaffolds by modulating the structure of their binding partners. Results published by several groups suggested that the 14-3-3 protein binding to selected RGS proteins decreases their inhibitory effect on G protein signaling presumably by blocking the interaction between RGS and G subunit [3,4].

To elucidate the mechanism of 14-3-3 protein-dependent regulation of RGS function, we performed biophysical characterization of interactions between RGS3 and 14-3-3 protein. The main goal of our work was to investigate whether the 14-3-3 protein binding affects the conformation of the RGS domain of RGS3 protein. Site-directed mutagenesis was used to generate two single-tryptophan mutants of human RGS3 with the Trp residue located either at the N-terminus close to the phosphorylation site Ser264

(Trp295) or within the RGS domain at the C-terminus of RGS3 molecule (Trp424). Time-resolved tryptophan fluorescence measurements revealed that phosphorylation of Ser264 itself induces significant structural changes in the region surrounding nearby located Trp295 but not Trp424 located within the remote RGS domain. The interaction between the 14-3-3 protein and phosphorylated RGS3 induces significant structural changes in the vicinity of both tested tryptophan residues. Moreover, experiments with isolated RGS domain suggest that this domain can, to some extent, interact with 14-3-3 protein in a phosphorylation-independent manner. We also solved a crystal structure of RGS domain of RGS3 at 2.3 Å resolution. This structure suggests that 14-3-3-induced conformational change affects the region within the G –interacting portion of the RGS domain. This can explain the inhibitory effect of 14-3-3 on GAP activity of RGS3.

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P29

CRYSTALLIZATION OF PHOTOSYSTEM II CORE COMPLEX OF *PISUM SATIVUM*

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Introduction:

Membrane proteins often play important roles in fundamental processes of life. Various methods such as electron microscopy, biochemical and biophysical spectroscopy methods, or electron diffraction have been used to study membrane proteins, yet to determine their structure, in most of the cases, remains problematic [1].

Photosystem II (PSII) is a multisubunit membrane protein complex performing light-induced electron transfer and water-splitting reactions, leading to the formation of molecular oxygen [2].

The goal of our work is to find suitable crystallization conditions, grow protein crystals and solve a protein structure using X-ray diffraction technique.

Material and Methods:

OEC PSII was isolated from green pea (*Pisum sativum*) and purified according to Ghanotakis *et al*. [3]. The purification procedure was later improved similarly to Kern *et al*. [4]. The SDS-PAGE electrophoresis in a buffer system of Laemmli [5] using 12% acrylamide solution gel was used for analysis of isolated complexes. The protein solution was concentrated to 2–3 mg/ml of chlorophyll *a* (~1.7–2.7 mM Chla) and supplemented with 1 mM $MnCl_2$ prior to crystallization trails.

Results:

Protein has been crystallized using A) vapor diffusion methods as well as B) counter-diffusion [8]. Initial crystallization screening has been performed in sitting drops [6-7] for method A) and in single capillaries [9] for B). Various commercial screens (MembFac™ and ‘‘Crystal Screen™ of Hampton Research (Aliso Viejo, CA, USA) and JBScreen Crystal Screening Kits of JenaBioscience (Jena, Germany)) were tested in individual crystallization trials. Crystallization experiments are in the progress.

This work is supported by the Ministry of Education of the Czech Republic (MSM6007665808 and LC06010) and by the Academy of Sciences of the Czech Republic (AVOZ60870520).

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P30

CONFORMATIONAL FREE ENERGY MODELLING USING METADYNAMICS

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Molecular dynamics simulation is very important tool of structural biology. Unfortunately, many interesting processes, such as conformational transitions, protein and nucleic acid folding, chemical reactions and others are too slow to be efficiently modelled using conventional simulation techniques. It is also of great interest to predict macroscopic parameters of the system (namely a free energy surface) from microscopic simulations.

Metadynamics methods [1] add an artificial history-dependent bias potential to the studied system to energetically disfavour its previously visited states. This helps to efficiently explore all possible states of the studied system. Moreover, the bias potential accumulated over the metadynamics run, approximates the free energy surface of the studied system.

Here we present an application of metadynamics in modelling of conformational equilibria of different system. Metadynamics was applied in interpretation of gas-phase spectra of a tripeptide Gly-Phe-Ala [2]. In the field of glycochemistry it was applied in modelling of ring puckering and in modelling of primary hydroxyl group conformations. Conformational equilibria of ubiquitin was studied to compare efficiency of metadynamics with the efficiency of conventional simulations and with the experiment.

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P31

CRYSTALLIZATION AND PRELIMINARY STRUCTURE ANALYSIS OF SEVERAL DHAA MUTANTS FROM *RHODOCOCCUS RHODOCHROUS*

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Introduction:

Haloalkane dehalogenases (EC 3.8.1.5) are members of the -hydrolase fold family and catalyze hydrolytic conversion of a broad spectrum of hydrocarbons to corresponding alcohols [1]. These enzymes are potentially important biocatalysts for industrial and bioremediation applications. Besides a wide range of haloalkanes, DhaA can slowly convert serious industrial pollutant 1,2,3-trichloropropane (TCP) [2]. Three mutants marked as DhaA04, DhaA14 and DhaA15 were designed and constructed to study the relevance of the tunnels connecting the buried active site with the surrounding solvent for the enzymatic activity.

Results and discussion:

The three mutants of DhaA were crystallized using the sitting-drop vapor-diffusion technique [3]. Crystal growth conditions were optimized [4] and crystals were used for synchrotron diffraction measurements at the beamline X11 of the DORIS storage ring at the EMBL Hamburg Outstation. X-ray intensities data for DhaA04, DhaA14 and DhaA15 mutants were collected to a resolutions limit of 1.23 Å, 0.95 Å and 1.15 Å, respectively. Crystals of DhaA04 belong to the orthorhombic space group $P2_12_12_1$ while crystals of DhaA14 and DhaA15 mutants belong to the triclinic space group $P1$. The known structure of the haloalkane dehalogenase from *Rhodococcus* species (PDB code 1bn6) [5] was used as a template for the molecular replacement. Analyses of crystal structures of mutants allow determine of electron densities observed for the ligands. In the case of DhaA04 the ligand is benzoic acid. DhaA15 protein contains isopropanol in the active site cavity. The refinement for DhaA14 mutant model and the initial interpretation of the structural implication in protein activities is currently in progress.

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P32

CRYSTALLIZATION STUDY OF *RHODOCOCCUS RHODOCHROUS* MUTANT PROTEIN DHAA31

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DhaA31 is haloalkane dehalogenase isolated from bacterium *Rhodococcus rhodochrous* NCIMB 13064 [1]. Haloalkane dehalogenases are enzymes that catalyze the cleavage of the carbon-halogen bond by a hydrolytic mechanism [2]. These proteins are key enzymes for the degradation of many halogenated aliphatic pollutants [3]. So haloalkane dehalogenases are potentially important biocatalysts with both industrial and bioremediation applications that could be used for industrial biocatalysis and as active compounds of biosensors, respectively [4].

The goal in our project is produce good crystals of DhaA31 for diffraction experiments. Structure study of DhaA31 allows us to determine importance of protein mutations on enzyme functions.

Standard vapor diffusion technique (sitting drop) has been used for searching and optimization of crystallization conditions [5]. In crystallization experiments have been used Hampton Research Linbro and Cryschem plates (Hampton Research, CA, USA), Emerald BioStructures CombiClover Crystallization Plate (EBS plate, Emerald BioStructures, WA, USA). Microcrystals have been obtained by sparse matrix screening using commercial crystallization kits as Crystal Screen of Hampton Research, Emerald BioSystem and Jena BioScience. Crystallization experiments are in the progress.

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P33

CRYSTALLIZATION STUDY OF THE IRON-REGULATED OUTER MEMBRANE LIPOPROTEIN (FRPD) FROM *NEISSERIA MENINGITIDIS*

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Introduction:

Neisseria meningitidis is a Gram-negative bacterium colonizing the nasopharynx of about 10% of healthy humans. Occasionally the meningococci can traverse the mucosal epithelia to reach the bloodstream, eventually cross the blood-brain barrier, and cause rapidly progressing septicemia and/or meningitis. The molecular basis of meningococcal virulence remains difficult to analyze, because human colonization and invasive disease are not adequately reproduced in current animal models. Several traits potentially required for virulence of meningococci have, however, been identified, including production of a capsule conferring resistance to serum, secretion of an IgA protease, the high antigenic variability of pili and non-fimbrial adhesins, and the presence of several iron acquisition systems.

Under conditions of limited iron availability, *N. meningitidis* produces **F**e-**r**egulated **p**roteins, FrpD and FrpC, which both are encoded consecutively in an iron-regulated *frpDC* operon controlled by a ferric uptake regulator (Fur). FrpC belongs to a family of type I-secreted RTX (**R**epeat in **t**o**x**ins) proteins and it may be involved in the pathogenesis of meningococcal infection due to the presence of high titers of anti-FrpC antibodies in convalescent-phase sera of a number of patients after invasive meningococcal disease. FrpD is synthesized with a type II signal peptide for export across the cytoplasmic membrane. It is posttranslationaly modified by a lipid molecule and is targeted to the outer bacterial membrane. FrpD is highly conserved in meningococcal strains and its primary amino acid sequence does not exhibit any similarity to any known protein sequences of other organisms. Furthermore, FrpD binds the N-terminal portion of FrpC (first 300 residues) with very high affinity (apparent K_d = 0.2 nM) and probably serves as an accessory lipoprotein involved in anchoring of the secreted RTX protein to the outer bacterial membrane.

Results and discussion:

The aim of this project is to produce crystals of FrpD protein for X-ray diffraction experiments and to solve the structural features of FrpD protein. The recombinant, truncated version of the FrpD protein lacking the first 21 amino acid residues ($FrpD_{250}$) with the C-terminal polyhistidine tag, was expressed in *E. coli* BL21 DE3, and purified using a combination of metal affinity and anion-exchange column chromatography. The crystals were obtained using a sitting drop vapour diffusion method. Diffraction data were collected at the beamline MX BL14.1 of synchrotron BESSY (Berlin, Germany) at 100 K to the resolution of 2.27 Å. Crystals of FrpD belong to the hexagonal space group *P 6 2*, with unit-cell parameters $a = b = 115.33$ Å, $c =$ 38.79 Å and = = 90° and = 120°. To determine the structure of the FrpD protein, phase problem has to be solved using single/multiple anomalous diffraction (SAD/MAD) experiment hence the preparation of selenomethionine labeling and/or heavy atom derivatives are currently in progress.

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P34

STRUCTURAL INTERPRETATION OF J-COUPLING CONSTANTS CALCULATED IN GUANOSINE AND DEOXY-GUANOSINE: INFLUENCE OF SUGAR-PHOSPHATE-LIKE BACKBONE CONFORMATION, SUGAR PUCKER AND SOLVENT ENVIRONMENT

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The relationship between the the glycosidic torsion angle , the three-bond couplings ^{3}J(C8-H1') and ^{3}J(C4-H1'), and the four one-bond couplings ^{1}J(C8-H8), ^{1}J(C1'-H1'), ^{1}J(C2'-H2') and ^{1}J(C2'-H2'2) in deoxyguanosine and the three one-bond couplings ^{1}J(C8-H8), ^{1}J(C1'-H1') and ^{1}J(C2'-H2') for guanosine has been analyzed using density functional theory - B3LYP /6-31G**. The influence of the backbone conformation, sugar composition and the sugar pucker, and molecular environment including water solvation has been also considered.

This work was supported by the Grant Agency of the Czech Academy of (grants no. IAA400550701), by the Grant Agency of Charles University (grant GAUK nr. 58708), by the Netherlands Organization for Scientific Research (NWO-CW), and by the National Research School Combination - Catalysis (NRSC-C).

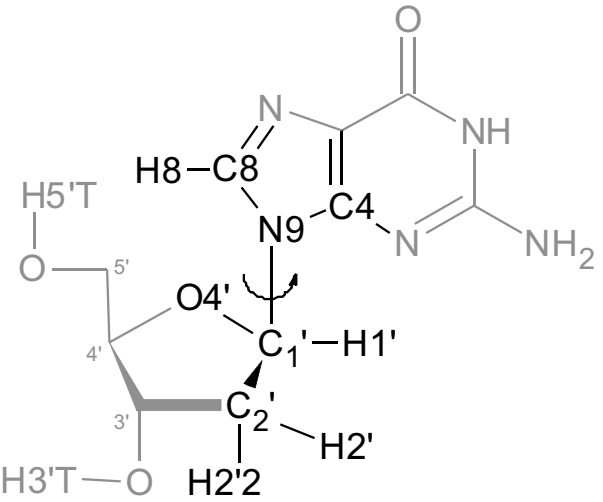

Figure 1: Numbering of atoms and definition of torsion angles in deoxy-Guanosine.

P35

IMPLEMENTATION OF PARALLEL ANALYZES OF MOLECULAR DYNAMICS SIMULATION TRAJECTORIES

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Molecular dynamics is widely used method of computational chemistry. With growing size of simulated systems and length of simulations, sequential analyzes of resulting trajectories become time demanding. Thus there is a need for tools that speed up trajectory processing and analyzes.

Recently, we have designed, implemented and tested tools for fast analyzes of long molecular simulation trajectories. Our implementation uses parallel processing of trajectories with arbitrary number of processors focused on binding free energy calculations. Moreover this approach can be easily extended to other analyzes, where analyzes of snapshots are independent on each other, e.g. radius of gyration, solute/solvent contacts, etc.

Developed tools were applied on the calculation of binding free energies using MM/PBSA method. Two biomolecular complexes were selected as a test case: a) LgtC galactosyltransferase from *Niesseria meningitidis* complexed with UDPGal and deoxylactose and b) lectin PA-IIL from *Pseudomonas aeruginosa* complexed with fucose. The 28-times acceleration on 32 processors was achieved, which shows 90 % parallel efficiency proving suitability of used approach.

P36

EFFETCS OF MAGNESIUM BINDING AND BERYLLOFLUORIDATION ON A COMPONENT OF THE CYTOKININ SIGNALING PATHWAY STUDIED BY NUCLEAR MAGNETIC RESONANCE

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Cytokinins are important regulators of intrinsic developmental programs leading to de- and re-differentiation of plant cells. The cytokinin signal transduction seems to be mediated via multistep phospohorylation, similar to the action of bacterial two-component signalling systems. A soluble receiver domain of CKI1 receptor histidine kinase of *Arabidopsis thaliana* has been investigated by nuclear magnetic resonance (NMR) in this study. The domain was expressed in *Escherichia coli* and labeled with stable isotopes (C-13, N-15). Resonance frequencies have been assigned using standard strategy and conformational changes were monitored by running 2D ^{1}H-^{15}N HSQC spectra. Effect of Mg(2+) has been studied in a series of titration experiments and the most significantly affected residues were identified using secondary chemical shift mapping. Activation of the protein, which cannot be studied in real time due to a short life-time of the activated form with phosphorylated aspartate, was investigated in an artificial system with phosphate replaced by beryllofluoride. In both cases, the observed chemical shift changes were mapped on a recently solved X-ray structure of the non-phosphorylated protein. In addition to the structural studies, molecular motions were investigated by N-15 NMR relaxation experiments. Series of relaxation spectra were obtained for free, Mg(2+)-bound, and beryllofluorinated protein and interpreted in terms of the Lipari-Szabo model-free approach. The observed changes are discussed in context of the X-ray structure of the free protein.

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P37

LIGUID CHROMATOGRAPHY – MASS SPECTROMETERY EVALUATION BASED ON PROBALISTIC THEORY

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Mass spectrometers are sophisticated, fine instruments which are essential in many applications. However, their results are usually interpreted in a rather primitive way, without knowing the errors of the results we get. We divide the output of the LC-MS into three parts: (a) useful output, (b) random noise (c) systemic noise of the instrument related to the particular experiment. The characteristics of the systemic noise change in time and depend on the analyzed substance. This allows us to quantify the probability of error and, at the same time, retrieve some peaks which get lost in the noise when using the existing methods. There are no user-defined parameters. Our software tool automatically evaluates the given instrument, detects compounds and calculates the probability of individual peaks.

The software automatically evaluates the given instrument, detects peaks, and calculates the probability of error for individual peaks. There are no artificial, user-defined parameters. The program not only quantifies the accuracy of the interpretation, but it also detects many peaks which, using the existing methods, are not distinguished from the noise.

P38

WHAT IS THE FUNCTION OF POLYQ STRETCHES IN THE C-TERMINAL TAILS OF YEAST 14-3-3 ISOFORMS?

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The polyglutamine (polyQ) sequences of certain proteins are known to induce protein aggregation that underlies the cytotoxicity of these molecules. It has been shown that peptides containing polyQ stretches adopt -structure and can form -sheets (both antiparallel and parallel), -hairpins, and also highly compact random coil [1,2].

14-3-3 proteins are abundant binding proteins involved in many biologically important processes. 14-3-3 proteins bind to other proteins in a phosphorylation-dependent manner and function as scaffold molecules modulating the activity of their binding partners. While lower eukaryotes, e.g. yeast, contain only two 14-3-3 genes, higher eukaryotes possess up to 15 14-3-3 genes. For example, in mammals seven isoforms have been identified to date. The presence of multiple 14-3-3 isoforms over the wide range of species suggests that individual isoforms can interact with different targets. Maximal isoform sequence variability occurs within the C-terminal stretch, a region that is believed to be flexible. The structure of this part of 14-3-3 molecule is unknown because it cannot be seen in any of the available 14-3-3 X-ray structures presumably due to disorder. It has been shown that 14-3-3 C-terminal stretch is involved in the regulation of ligand binding and in the absence of the ligand, the C-terminal stretch occupies the ligand binding groove of 14-3-3 molecule [3].

Yeast 14-3-3 isoforms (BMH1 and BMH2) compared to mammalian and plant isoforms, posses significantly longer C-terminal tails that contain polyQ sequences with unknown function. In this work, we used methods of fluorescence spectroscopy and studied conformational properties of these segments. Site-directed mutagenesis was used to generate single-tryptophan mutants of BMH proteins with the Trp residue located at both sides of polyQ stretches. Time-resolved fluorescence measurements revealed that ligand (phosphopeptide) binding does not affect the conformation of C-terminal segments of BMH proteins. It seems, therefore, that the C-terminal tails of BMH proteins do not function as autoinhibitors which are ejected from the ligand binding groove during the ligand binding (as has been observed for mammalian isoforms). In addition, fluorescence anisotropy measurements revealed that BMH proteins form oligomers bigger than expected dimmers. The presence of these oligomers was also confirmed using DLS. Therefore we speculate that polyQ stretches located within the C-terminal tails of BMH proteins induce their oligomerization.

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P39

MAPPING THE INTERACTIONS BETWEEN 14-3-3 PROTEIN AND FORKHEAD TRANSCRIPTION FACTOR FOXO4

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The forkhead family of transcription factors shares a highly conserved 100-amino-acid large DNA binding (DBD) or FOX (Forkhead box) domain. The FOX proteins display large functional diversity and play a wide range of roles in a number of physiological and pathological processes [1]. Among the forkhead family, the FOXO class consists of four members (FOXO1, FOXO3, FOXO4 and FOXO6) that play a central role in cell-cycle control, differentiation, metabolism control, stress response and apoptosis. Transcriptional activity of FOXO proteins is regulated through insulin–phosphatidylinositol 3-kinase–AKT/protein kinase B (PI3K–AKT/PKB) signaling pathway. The AKT/PKB-mediated phosphorylation triggers phosphorylation of additional sites by other kinases and induces FOXO binding to 14-3-3 protein. This in turn both promotes the nuclear export of the resulting complex and inhibits the nuclear import of FOXO likely by interfering with the function of its nuclear localization signal (NLS) [2]. In addition to phosphorylation, the function of FOXO proteins is further controlled by other types of posttranslational modifications including acetylation and ubiquitination [3].

The role of 14-3-3 proteins in the regulation of FOXO forkhead transcription factors is at least twofold. First, the 14-3-3 binding inhibits the interaction between the FOXO and the target DNA. Second, the 14-3-3 proteins prevent nuclear reimport of FOXO factors by masking their NLS. The exact mechanisms of these processes are still unclear, mainly due to the lack of structural data. In this work, we have used fluorescence spectroscopy techniques to investigate the mechanism of 14-3-3 protein-dependent inhibition of FOXO4 DNAbinding properties. We have labeled four different sites within the forkhead domain of FOXO4 (N-terminal region, C-terminal wing W2, and both sides of helix H3) with the extrinsic fluorophore 1,5-IAEDANS and used methods of time-resolved fluorescence spectroscopy to study interaction between FOXO4 and 14-3-3 protein. Our results suggest that 14-3-3 protein physically interacts with all four tested regions of forkhead domain that represent important parts of its DNA-binding interface. Such interactions likely mask the DNA-binding interface thus blocking the FOXO4 binding to the target DNA. In addition, time-resolved tryptophan fluorescence measurements indicate no significant 14-3-3 protein binding-induced conformational change within the forkhead domain of FOXO4. Thus the 14-3-3 protein functions as a "molecular hood" that covers the DNA-binding interface of FOXO4 and blocks its interaction with the target DNA.

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P40

BACILLUS SUBTILIS AS A PRODUCTION HOST FOR MEMBRANE PROTEIN SPOIISA

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Gram-positive bacterium *Bacillus subtilis* has great potential as the host for production of cytoplasmic proteins, protein complexes and membrane proteins assigned for structural and functional analysis.

As a model for studying membrane proteins we have chosen *B. subtilis* protein complex SpoIISA – SpoIISB. These proteins belong to bacterial programmed-cell death system, forming a toxin-antitoxin couple. SpoIISA as a toxin has three putative membrane-spanning helices. The remaining two-thirds of the protein are predicted to be localized in the cytoplasm. SpoIISB antitoxin is a small cytosolic protein and it was found, that SpoIISB makes complex with cytosolic part of SpoIISA.

In order to choose the most productive expression system for production of SpoIISA as a membrane protein we have developed its expression in *B. subtilis*. We were testing different possible expression systems, namely xylose inducible, IPTG inducible and subtilin-regulated system, respectively. We have compared the strength and leakiness of all three systems. Western blot analysis revealed that the highest level of expression was obtained in SURE system (~ 10 - 100 g protein/100 ml).

To further study localization of SpoIIS system in *B. subtilis* cells we have prepared fusion of transmembrane domain of SpoIISA, under control of its own promoter, with GFP.

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P41

CHARACTERIZATION OF RECOMBINANT CYSTEINE SYNTHASE IN *CAENORHABDITIS ELEGANS*

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Nematode *Caenorhabditis elegans* could be a suitable model to study metabolic and cellular consequences of homocystinuria due to cystathionine -synthase (CBS) deficiency. However, metabolism of sulfur amino acids in *C. elegans* is as yet unknown, namely the steps in cysteine biosynthetic pathways. Cysteine can be synthesized either via the transsulfuration pathway which utilizes homocysteine by CBS or via the assimilation pathway which uses sulfide by cysteine synthase (CS).

In silico analysis of *C. elegans* database identified four homologs of human CBS, namely *ZC373.1*, *C17G1.7*, *K10H10.2* and *R08E5.2*. The aim of this study was to express the gene *C17G1.7* (predicted CS) in prokaryotic system, to purify and further characterize this recombinant protein. Molecular weight of polypeptide chain was determined to be 37,2 kDa by MALDI-TOF MS. Blue Native electrophoresis revealed a molecular weight of 70 kDa suggesting that recombinant CS is a dimer. Purified protein contains pyridoxal 5'-phosphate (PLP) as determined by UV/VIS absorption spectrometry; circular dichroism showed characteristic PLP maximum confirming its localization in a centre of organized globular protein. We determined that purified enzyme has very specific enzymic activity for CS reaction; other possible activities were not detected. Recombinant CS exhibited K_m values for O-acetyl-L-serine and sulfide of 5.54 and 4.23 mM, respectively, and a turnover number of 139 and 134 s^{-1}, respectively.

These data show that *C17G1.7* could play an important role in cysteine biosynthesis since *C. elegans* genome contains also a CBS gene, we hypothesize that nematode utilizes both cysteine biosythesis pathways - sulfur assimilation and transsulfuration pathway.

This work was supported by Wellcome trust International senior research fellowship in biomedical science in Central Europe.

P42

STRUCTURE AND DYNAMICS OF THE APA, APC, CPA AND CPC RNA DINUCLEOSIDE MONOPHOSPHATES RESOLVED WITH NMR SCALAR SPIN-SPIN COUPLINGS

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Functional diversity of nucleic acids (NAs) makes their study crucial for deeper understanding of many essential biological processes. Basic structural parameters of NAs are the , backbone torsion angles and the glycosidic torsion angle (Figure 1).

In our previous study we correlated the calculated NMR indirect spin-spin coupling constants (J-couplings) with the backbone torsion angles of ribosomal RNA [1]. Each RNA structural pattern was shown to be in principle detectable with specific magnitudes of specific J-couplings assigned to the backbone torsions [2].

In the present study we correlated the J-couplings measured in the ApA, ApC, CpA and CpC RNA dinucleoside monophosphates with the calculated ones. Since the four RNA molecules are higly flexible we used the molecular dynamics (MD) for modeling their dynamical behavior, particularly to sample the motions for the torsion angles (Figure 1). The calculated J-couplings were obtained as the dynamically averaged Karplus equations with distributions of the torsion angles modelled with MD. Other structural features, like the rate of base stacking, evolution and mutual coupling of torsional motions were also analyzed including their dependence on sequence of the four RNA molecules.

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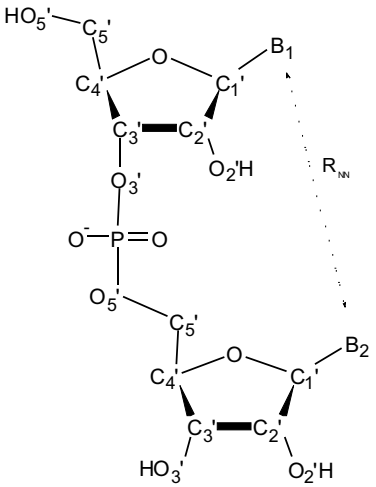

Figure 1: Backbone torsions of RNA dinucleoside monophosphate

P43

THERMAL DENATURATION OF BARLEY LIPID TRANSFER PROTEIN 1 STUDIED BY NUCLEAR MAGNETIC RESONANCE AND DIFFERENTIAL SCANNING CALORIMETRY

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Lipid transfer protein 1 (LTP1) is a ubiquitous plant protein able to transfer lipids between membranes *in vitro.* Barley LTP1 consists of 91 amino acids and adopts a typical structure comprised of four -helices and a C-terminal tail both stabilized by four disulfide bridges.

LTP1 isolated from barley grain contains a lipid-like molecule of 294 Da as a postranslational modification. Recently, this lipid-like adduct has been identified as a reactive oxylipin (-ketol of 9-hydroxy-10-oxo-12-*Z*-octadecenoic acid). LTP1 is extremely heat and protease resistant, survives all procedure of making beer, and is therefore present in beer and has effect on foaming.

In spite of the number of thermal stability studies reported in the literature, we felt that two issues still remained to be addressed in order to understand the impact of the thermal denaturation of LTP1 on the quality of beer and other barley products. First, the LTP1 denaturation was so-far systematically studied up to 100 °C, which is not enough to observe unfolding of the native protein. The second and more important issue is the irreversibility of the thermal denaturation of LTP1. While reversible denaturation curves can be interpreted in terms of van't Hoff enthalpy and of melting temperature directly related to the entropy, such approach is not relevant for irreversible denaturation.

In our study, process of thermal denaturation of LTP1 covalently modified at Asp 7 was monitored by nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) up to 120 °C. While DSC provided a complete picture of heat capacity changes, NMR selectively described the structural changes during protein unfolding. The denaturation was found to be irreversible and highly cooperative. A method of numerical quantitative analysis allowing to fit the NMR data to a transition-state model without further simplification was developed. Based on the obtained values of transition state enthalpy and entropy, rate of denaturation was calculated as a simple measure of protein stability at various temperatures. Effects of reducing agents were studied and discussed in the context of quality control of barley products during storage and processing.

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