A60

APPLICATION OF QM/MM CALCULATIONS WITH NOVEL POLARIZED EMBEDDING TO DETAIL INVESTIGATION OF THE FLAVOPROTEIN WrbA

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The mechanisms of NADH:quinine oxidation reduction reaction in flavoprotein WrbA was studied by improved molecular docking based on the novel implementation of QM/MM method with polarized embedding. The molecular docking estimates the positions and relative binding energies of the substrates of the flavoprotein WrbA and thus can lead to better understanding of the mechanisms of NADH:quinine oxidation reduction reaction.

Standard molecular docking estimates the positions and binding energies of the flexible ligand to the rigid protein. The binding energies are calculated by nonpolarized molecular mechanical (MM) forcefield. This approach is appropriate for the study of the wide variety of the ligands, however it can fail in the case of the electrically charged ligands, like NADH and quinine in flavoprotein, where the charge is transferred from ligand to the protein cofactor and the polarization effects become very significant and cannot be neglected.

The polarization in molecular docking was introduced via atomic charges derived from QM/MM calculations with polarized embedding. The ligand (QM part) charges were derived from quantum mechanical (QM) electronic wave function and polarized charges of the rigid protein (MM part) were derived by the novel method of induced charges [1]. This approach is based on the method of induced dipoles, where the induced dipole on particular atom (calculated from atomic polarizability) is approximated by the set of induced charges on the neighboring atoms. This brings a considerable simplification and allows the use of standard programs for molecular docking, as they already evaluate electrostatic interactions using atomic charges.

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A61

SPECTROSCOPIC STUDY OF THE MADS BOX

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The MADS box family of transcription factors plays an essential role in the gene regulation of higher organisms. Its acronym is derived from initials of four of the originally identified members of the family: MCM1, AG, DEFA and SRF [1]. These transcription factors share a highly conserved DNA sequence of 56 amino acids, called the MADS box.

The serum response factor (SRF) plays a crucial role in the activation of genes that respond to mitogenes and also in the regulation of muscle specific genes [2]. The SRF transcriptional activity is determined by its binding as a symmetric dimer to the so called CArG box element, one of the most vastly characterized DNA binding sequences recognized by the MADS box transcription factors. Preceding studies on 20-mer oligonucleotides bearing the specific high affinity CArG box of the c-fos enhancer, disclosed an equilibrium between bend and linear conformers [3] which is partly maintained even after the protein/DNA complex formation [4]. Still little is known about the recognition process, how targeted DNAs are recognized by these transcription factors. Thus the conserved MADS box motif may serve as a basic model for study of common functional properties of DNA recognition within the whole protein family (over 200 members).

The structural and dynamic properties of the MADS box and its shorter segments were studied by advanced spectroscopy techniques: fluorescence, UV absorption and Raman scattering. Acquired data were treated by a recently developed method based on factor analysis, the singular value decomposition. Owing to this method we were able

to detect even very weak spectral changes. Vibrational spectra of the most probable conformers of shorter MADS box segments were simulated in Gaussian09 program suite in order to obtain the detailed interpretation of experimental data. Effects caused by variations of environmental conditions were also investigated. Especialy, a high sensitivity to pH (charged amino acids of MADS box i.e. tyrosine, lysine, and glutamic acid) was observed.

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A62

IMPROVEMENT OF THE PSII PURIFICATION PROCEDURE FOR CRYSTALLIZATION

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The aim of this work is to develop purification procedure for higher plant PS II providing material for its crystallization and high resolution X-ray diffraction.

We have re-designed the standard protocol and implemented novel procedures for isolation of the PS II enriched thylakoid membranes from the plant material. Handpicked leaves of the *Pisum sativum L*. were homogenised and at the first step highly active thylakoid membranes were isolated. Then, sucrose density gradient ultracentrifugation and ion exchange chromatography techniques were used for purification of the PSII complexes from the solubilised thylakoid membranes. The complexes' activity was followed throughout the isolation routine with absorption and fluorescence spectroscopy. The purity of the samples was assessed by optical spectroscopy and western blotting.

Purification procedure that included the ion exchange chromatography preceding the ultracentrifugation yielded highly pure sample. Unfortunately the quantity of the sample was not enough for crystallization trials.

We currently continue to optimize the protocol for PSII complex purification. Methods and approaches for crystallization of both membrane and soluble proteins will be implemented subsequently.

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A63

INCREASING AFFINITY BETWEEN INTERFERON GAMMA AND ITS RECEPTOR BY COMPUTER DESIGN OF RECEPTOR MUTATIONS. I. DESIGN OF THE MUTATIONS

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Rational design of molecules with function changed in desired direction is still a challenge. We intend to design interferon gamma receptor molecules that bind more strongly to their natural ligand, interferon gamma (IFNg), using simple computer models. The IFNg/receptor system is used as a model because it is important for innate and acquired immunity in vertebrates and its targeted modification can lead to practical applications. The model is also suitable because crystal structures of the IFNg/receptor complex are available.

We performed mutation analysis at the models of IFNg/receptor interface determined from crystal structures using empirical force filed as implemented in web-based tool FoldX (http://foldx.crg.es/). The analysis lead to predictions of receptor mutants that should strengthen the interaction. Only residues with lower than 50% conservation were allowed to mutate; the final set of suggested mutations contained nine single mutants, four double and one triple mutant. To check non-random nature of alleged improvement of the binding affinity we also selected four single mutants that should lower the affinity. All eighteen receptor mutants were successfully expressed and their affinities determined, results are shown in a presentation by Mikulecký et al.

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A64

HOW CAN STALLING OF TRANSLOCATION ACTIVATE THE ENDONUCLEASE IN THE TYPE I RESTRICTION-MODIFICATION SYSTEM ECOR124I

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Restriction–modification enzymes provide a bacterial defence mechanism against foreign DNA. Hemi-methylated host DNA is fully methylated at specific sequences by a methyltransferase, thus protecting its DNA from restriction by the accompanying endonuclease. Foreign DNA is unmethylated at these sites and is cleaved [1, 2].

Type I restriction endonucleases consist of three different subunits: methylase (M), restriction (R) and specificity (S) encoded by the hsdM, -R and -S genes, where hsd=Host Specificity for DNA [3]. Together, they form an intriguing, multifunctional complex which can either restrict or modify DNA. Here, the mode of action of the complex is dictated by the methylation state of the recognition sequence [4]. A fully methylated site results in no action being taken and in enzyme dissociation; hemi-methylated target sequences direct the enzyme into a protective methylation mode producing fully methylated DNA, while unmethylated DNA shifts the enzyme into a destructive (and protective) restriction mode. It is in this protective mode that type I enzymes restrict foreign DNA and thereby maintain the integrity of the host genome [3].

Molecular mechanism of dsDNA translocation, cleavage and ATP hydrolysis has not been jet structurally investigated. Crystal structure of the 120 kDa motor HsdR protein of the Type I EcoR124I nuclease in complex with ATP was recently reported by our group, and it is the first know structure of the motor subunit throughout group of Type I restriction enzymes. This structure characterization, among others, disclose unexpected endonuclease domain contributing to ATP binding by Lys220 residue. This contact could potentially couple endonuclease and translocase functions [5].

To more clearly understand correlation of ATPase and restriction activities of the type I amino acid residues from sequence-variable region around Lys220 have been chosen which could be potentially engaged in conformational changes that occur once translocation is stalled and a signal is transmitted to the endonuclease. Those residues have been replaced by site-direct mutagenesis and the influence of the replacement is monitored using *in vitro* ATPase activity assays of the whole pentameric enzyme complex and is tested by restriction assay *in vitro* and *in vivo*.

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A65

IN SILICO CHARACTERIZATION OF THE MOTORSUBUNIT OF THE *E.COLI.* RESTRICTION-MODIFICATION SYSTEM ECOR1241

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Type1 restriction modification system are intriguing multifunctional multisubunit molecular motors that can catalyze both restriction and modification activity .The type 1 RM enzymes binds to its target sequence and its activity as an endonuclease or methyltransferase is determined by the methylation state of the target sequence. If the target sequence is unmodified, the enzyme while bound to its target site is believed to translocate or pull the DNA towards itself simultaneously in both directions in an ATP dependent manner.

The crystal structure of the motor subunit R has been determined by our group but the molecular mechanism by which these enzymes translocate and cleave the DNA is not fully understood.

Our current research effort focuses on full-length three-dimensional structures of the R-subunit, utilizing computational and bioinformatics methods. Optimization of intersubunit contacts is performed by energy minimization followed by molecular dynamics simulations in solution at 300K. The dynamic behavior of WT and mutant holo and apo systems is explored by molecular dynamics simulation in GROMACS using the AMBER99SB force field. Conformational changes connected to coupling of translocation and endonuclease activity are observed and QM/MM methods are applied to calculate binding energies.

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A66

STRUCTURAL STUDIES OF NK RECEPTORS AND LIGANDS

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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 Clr-g [2], a target of this structural study, is a part of immune system of mouse. It is a ligand of NK receptor NKR-P1F. Clr-g oc-

curs in dendritic cells and macrophages. The extracellular part of Clr-g was expressed, purified and crystallized. Structure was solved at 1.95 Å resolution using X-ray diffraction data measured at Bessy II of the Helmholtz Zentrum Berlin.

The overall fold of mouse Clr-g is the fold typical of C-type lectin like proteins. Mouse Clr-g forms dimers and is most similar to human CD69 [3].

An interesting crystal contact was found in the crystal structure: N-terminus of the extracellular part of Clr-g binds to neighbour dimer in the crystal, and thus shows feasibility of peptide binding into the central pocket of the dimer.

Mutual orientation of monomers in the dimer is slightly different than in the CD69 structure. However, moderate variability in orientations of monomers was found also among single structures of CD69. It seems that this difference gives testimony about flexibility in dimer formation and not about differences between mouse Clr-g and human CD69.

Electrostatic potential was computed for several proteins structurally and functionally related to mouse Clr-g and surprisingly big differences were found.

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A67

NON-ROBOTIC HIGH-THROUGHPUT SETUP YIELDING DIFFRACTION-QUALITY CRYSTALS IN NANOLITER DROPS MANUALLY ASSEMBLED BY MOTORIZED PIPETTE

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Nanoliter-sized drops of protein solution and precipitant are characteristic of high-throughput protein crystallizations screening. Present-day crystallization practice requires almost inevitably the robotized setup for reliable nanoliter crystallization drop pipetting. In this work, a protocol for reproducible manual assembly of protein crystallization screening experiments adopting nanoliter-sized drops in 96-well format has been designed and developed. The protocol exploits the repetitive-pipetting mode of commonly available motorized single and multichannel pipette, together with simple tools available in ordinary laboratory. For assembly of one crystallization screen hawing 96 crystallization conditions with 10 mg/ml of protein in equilibrated drop, less than 350 µg of protein was used. The protocol approach was verified on the finding crystallization conditions for antibody Fab fragments and their complexes with tau protein antigens. Diffraction-quality crystals were grown directly from the screen conditions on 96-well plates and yielded complete diffraction data sets on synchrotron source. The results proved that successful crystallization in nanoliter high-throughput format is affordable even for a small-sized crystallography laboratory, without the need of expensive instrumentation.

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A68

THYLAKOID MEMBRANE STUDIED BY THE MEANS OF MOLECULAR DYNAMIC

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Thylakoid membranes are the most abundant membranes in nature. They are found in all phototrophic organisms, where they make a matrix for proteins participating in photosynthetic reactions. Contrary to the well-characterized animal phosphoglycolipidal membranes, thylakoid membranes are composed from more than 90 % (depending on organism) of galactoglycolipids. In the cyanobacterium *Synechocystis PCC6803* two electrically neutral components – monogalactosyl diacylglycerol (MGDG), that makes 37% of the membrane, and digalactosyl diacylglycerol (DGDG, 20 %) – and negatively charged sulfoquinovosyl diacylglycerol (SQDG, 29 %) are the major components of the membrane. Only 14 % of the membrane is made of a phospholipid phosphatidyl glycerol [1]. Other phosphoglycolipids can be found in traces only.

As there is only a little experimental data available on the above mentioned galactolipids and their mixtures, molecular dynamic simulations are the theoretical tool that enable us to examine the systems on the atomic detail level. The coarse-grained MARTINI [2] model of the thylakoid lipids was constructed by the method described by Hinner *et al* [3]. This model is based on atomistic simulations using the Gromos 45A4 force field [4] with some yet-unpublished adjustments for the tail part of the lipid molecules. The gained MARTINI parameters provide the most important characteristics of the membranes (such as membrane thickness, area per lipid) in very good agreement with atomistic simulations what enables us claim that the coarse-grained simulations reflect the real behavior of the system.

A model of a thylakoid membrane from cyanobacterium *Synechocystis PCC6803* will be presented on atomistic and coarse-grained level and the properties of the membrane will be characterized.

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A69

STRUCTURAL BIOINFORMATICS OF CBS DOMAIN

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CBS (cystathionine -synthase) domain pair, also known as Bateman domain, is an ancient scaffold present in numerous organisms form Archaea to mammals. It regulates the function of many enzymes such as cystathionine -synthase, AMP-activated protein kinase or inosine monophosphate dehydrogenase, as well as voltage gated chloride channels and other transporters. It can bind different nucleotide phosphates, *S*-adenosylmethionine, ions and other ligands. During our attempt aimed at refinement of a model of the CBS domain pair from human cystathionine -synthase we collected and structurally aligned ~140 CBS domain pairs from ~70 experimental 3D structures of proteins containing this structure. This scaffold forms a pseudo-symmetric pair of two subdomains and this pseudo-symmetry causes that a ligand can bind either in the binding site A or B or both sites can be occupied by ligands, where sides A and B are associated by the (pseudo-)symmetry. Here we show a comparison of CBS domains in terms of their overall structure, binding sites and amino acid sequence in order allow better prediction of binding site details, prediction of the ligand, its binding mode and conformational changes associated with biological function.

A70

PLASMA-MEMBRANE PERMEABILITY OF *S. CEREVISIAE* BY PGSE NMR

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The plasma membrane is an essential component of all living cells. It is involved in cell integrity and volume maintenance, metabolism, signal regulation and transport. It is fully permeable for small uncharged molecules like water.

The principle of monitoring of the plasma-membrane fluidity relies on the distinction between extra- and intracellular water – which in fact requires some "labeling" of the molecules. The widely used and well established NMR method for measurement of exchange over plasma membrane utilizes doping of the extracellular compartment by paramagnetic agents which enhance relaxation of extracellular molecules [1, 2]. Alternatively, the difference in apparent translational diffusion coefficients can be used as a "labeling" method to distinguish extra- and intracellular water NMR signal. Such method was proposed and applied to monitor the temperature dependent plasma-membrane fluidity of bakers yeast [3]. The Filter Exchange spectroscopy (FEXSY) experiment comprises two building blocks formed by pulsed gradients of magnetic field. The amplitude and spacing of gradient pulses in first block are set to a fixed value to effectively suppress the NMR signal originating from extracellular water molecules. After short delay (10 – 400 ms) called mixing time, during which the water molecules exchange over plasma membrane, the second block is applied. Here the amplitude of gradient pulses is gradually incremented in sixteen steps in order to perform a standard NMR experiment for measurement of translational diffusion. The information about plasma-membrane fluidity is encoded in the water NMR signal intensity recorded for four values of mixing time. The most interesting parameter – the intracellular water life-time – is then obtained by fitting the theoretical equations (Eq. 2-12 in [3]) to experimental signal intensities.

We studied three *S. cerevisiae* deletion mutant strains (erg2, erg4 and erg6) which lack genes involved in the synthesis of ergosterol, the most important steroid compound present in the plasma membrane. Blocking of different steps of ergosterol synthesis leads to accumulation of various intermediates within the cell. Therefore the composition of plasma membrane differs among selected strains. We found the intracellular water life-time to be very similar for wild type *S. cerevisiae* and for erg2 mutant strain, while erg6 and erg4 mutants show significantly faster water exchange over plasma membrane.

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A71

THE CLUSTER OF ACIDIC RESIDUES IN THE C TERMINUS OF TRPA1 CONTRIBUTES TO CALCIUM SENSING

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The ankyrin transient receptor potential channel TRPA1 is a Ca^{2+}-permeable, voltage-sensing cation channel. It is predominantly expressed in a subpopulation of nociceptive neurons. There it mainly acts as a polymodal sensor, activated by pungent compounds. From the cytoplasmic side, TRPA1 is critically regulated by Ca^{2+} ions and this mechanism represents a self-modulating feedback loop that either augments or inhibits the initial activation. It has been suggested that an EF-hand like domain in the N-terminus is responsible for Ca^{2+} dependent activation, but these results have been questioned. Here, we suggested for such role the cluster of acidic residues in the distal C-terminus. We investigated its contribution to Ca^{2+} sensing using mutagenesis and whole-cell electrophysiology. We found that neutralization of four conserved residues, namely Glu1077 and Asp1080-Asp1082 in human TRPA1, had strong effects on Ca^{2+}- and voltage-dependent potentiation and/or inactivation of agonist-induced responses. In addition, the

surprising finding of this study was that truncation of the C-terminus by only 20 residues selectively slowed down the Ca^{2+}-dependent inactivation by 2.9-fold without affecting other functional parameters. Our findings identify the conserved acidic motif in the C-terminus that is actively involved in TRPA1 channel modulation by Ca^{2+} and may represent its long-sought Ca^{2+}-sensing domain.

A72

CRYSTAL STRUCTURE OF THE IRON-REGULATED OUTER MEMBRANE LIPOPROTEIN FRPD (NMB0584) FROM *NEISSERIA MENINGITIDIS*

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

The biological function of FrpD appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity (K_d = 0.2 nM) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of any structural information on these proteins. 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.

We found out that 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 $P6_4$, 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. The calculated structure was used as a search model in molecular replacement to determine the structure of native $FrpD_{43\text{-}271}$ protein. Here we present the crystal structures of the native and Se-Met derivative of the $FrpD_{43\text{-}271}$ (Se-Met $FrpD_{43\text{-}271}$) protein that were solved using diffraction data to 2.25 Å and 1.40 Å resolution, respectively.

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A73

METALOPHILLIC ATTRACTION IN THE CONSECUTIVE T-HGII-T DNA BASE PAIRS

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The metal atoms like AuI, PtII, AgI or HgII attract each other owing to the so called metalophilic attraction. The DNA containing mismatched Thymine-Thymine base pairs represents an ideal matrix for HgII atoms that allows formation of the T-HgII-T metal-mediated DNA base pairs. Such binding of mercury in the mismatches substitutes structurally the hydrogen bonding in cannonical DNA base-pairs. The non-covalent interactions in the consecutive T-HgII-T base-pairs include usuall base-base stacking and in addition also metal-base and metal-metal interaction. We found that the HgII…HgII metalophillic attraction contributes by ca 10% to the overall stabilization [1]. The Raman spectra of T-HgII-T base-pairs were recently used for the anlysis of mercury binding in the metallo base-pairs [2].

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A74

QUANTUM CHEMISTRY STUDY OF REPAIRING FUNCTION OF HOGG1 ENZYME

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Low-energy pathway for excision of 8-oxoguanine catalysed with the human 8-oxoguanine glycosylase 1 (hOGG1) is proposed by means of theoretical calculations. Initiation of the reaction proceeds via attacked glycosidic nitrogen of 8-oxo-2'-deoxyguanosine (oxoG) with N -ammonium group of Lysine 249 (Lys249). Notably pyramidal geometry of the glycosidic nitrogen accompanied by loss of the five-membered ring aromaticity are key markers of the reaction with significantly lower activation energy as compared to activation energies for S_N2 cleavage mechanisms employing attack of the anomeric carbon C1' or simple dissociation of oxoG base.

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A75

REACTION MECHANISM OF MUTH ENZYME – QUANTUM MECHANICS/MOLECULAR MECHANICS STUDY

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Enzymes are catalysts of many crucial reactions in living organisms. Therefore, the knowledge of their reaction mechanisms can be helpful in many fields such as biology, medicine or pharmacy. In our study, we are focused on MutH enzyme, which is an integral part of Methyl-directed Mismatch Repair process, together with MutL and MutS enzymes. A mismatch introduced during DNA replication is recognized by MutS enzyme, information about the mis-

match is transferred through MutL to MutH enzyme. MutH specifically recognizes the GATC sequence on daughter DNA strand and cleaves this strand next to the G base. After wrong paired base is removed, the missing part of DNA is to be re-synthesized by the DNA polymerase and the correct base pairing is reestablished [1].

Main goal of our project is the understanding of the reaction mechanism of MutH enzyme. We present the Quantum Mechanics / Molecular Mechanics (QM/MM) study of the MutH enzyme reactivity based on models prepared from the available crystal structures of protein / DNA complex [2].

The cleavage mechanism is studied on ab initio level using CPMD [3] implementation of Density Functional Theory and on semi-empirical level using PM3 Hamiltonian [4] in Amber package [5]. We are considering two possible nucleophiles. We are also comparing two different models of the protein / DNA complexes with both Ca^{2+} ions (inhibitor) and Mg^{2+} ions co-factor) of the cleavage reaction. We evaluate the free energy profiles of various processes in the active site including cleavage itself.

This work was realized in CEITEC - Central European Institute of Technology with research infrastructure supported by the project CZ.1.05/1.1.00/02.0068 financed from European Regional Development Fund. The access to the MetaCentrum computing facilities provided under the program "Projects of Large Infrastructure for Research, Development, and Innovations" LM2010005 funded by the Ministry of Education, Youth, and Sports of the Czech Republic is acknowledged. The work has been supported by the Grant Agency of Czech Republic (GD301/09/H0040).

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A76

MOLECULAR MECHANISMS OF ACTIVATION AND INHIBITION OF HALOALKANE DEHALOGENASES BY ORGANIC CO-SOLVENTS

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Enzymatic transformations in organic co-solvents are being increasingly used for many applications, for example in organic synthesis of chiral compounds [1]. However, the presence of organic co-solvents in the reaction mixture generally leads to enzyme inactivation by denaturation, conformational rigidity or inhibition [2]. Detailed understanding of the effects of solvent molecules on enzyme functionality can be useful for selection of appropriate type and concentration of solvent for particular enzymatic reaction.

In the present study, the structure and function of haloalkane dehalogenases DbjA from *Bradyrhizobium japonicum* USDA110, DhaA from *Rhodococcus rhodochrous* NCIMB13064 and LinB from *Sphingobium japonicum* UT26, were investigated in the presence of three representative organic co-solvents: formamide 5% (v/v), acetone 20% (v/v) and isopropanol 10% (v/v). Systematic activity screening, structural analysis, molecular dynamics simulations and steady-state kinetic measurements were employed to get an insight into the mechanisms controlling the enzyme-solvent interactions at the molecular level. The results demonstrated that enzyme inactivation at high co-solvents concentrations is attributed to the conformation changes, while at lower concentrations both inhibition and activation took place. All three studied solvents were found to enter the enzymes' access tunnels and active sites, but did not act as competitive inhibitors. At low concentrations, the co-solvents either enhanced catalysis by lowering $K_{0.5}$ or increasing k_{cat}, or caused enzyme inactivation by promoting substrate inhibition. A computational method, involving molecular dynamics simulations and quantitative analysis of the active sites and access tunnels occupancies by the solvent molecules, was developed. This tool can be used for prediction the effect of organic co-solvents on enzymatic activity, due to identified

correlation between the portion of the active site cavity occupied by the solvent and enzyme activation or inhibition.

Our study contributes to understanding of protein-solvent interactions and demonstrates that rational selection of an optimal protein-solvent pair and effective co-solvent concentration is possible.

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A77

ISOTOPE-ASSISTED STRUCTURAL CHEMICAL ANALYSIS OF NUCLEIC ACIDS

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In the mechanistic/physicochemical studies of biological molecules, it is important to probe catalytic sites and molecular interfaces. In order to probe a specific site in a biological macromolecule, site-specific isotope labeling is a straight-forward way. By using the site-specifically labeled RNA and DNA molecules, we have studied their structures and physicochemical properties. To date, with the site-specific labeling technique, we have studied 1) a weak hydrogen bond of a C-U mismatched base-pair in an RNA duplex, 2) metal cation-binding to conserved sequence motif of hammerhead ribozyme and 3) a chemical structure determination of Hg^{II}-mediated T-T base-pair (T-Hg^{II}-T base-pair) in a DNA duplex. In all systems, a single atom or a single residue was site-specifically labeled with ^{15}N. For the detection of hydrogen-bonding and metal ion-binding, the traces of ^{15}N chemical shifts against temperature (hydrogen bond) and metal cation-concentration (metal ion-binding) was efficient. For the determination of the chemical structure of the T-Hg^{II}-T base-pair, we detected the 2-bond ^{15}N-^{15}N *J*-coupling through the N-Hg^{II}-N bond ($^{2}J_{NN}$). In addition, the direction of chemical shift perturbations can be qualitatively predictable for imine-type nitrogen, based on the theory of the chemical shift and the bonding state of the nitrogen atoms. Thus, NMR spectroscopic parameters of ^{15}N nuclei are sensitive probe for micro-environment around the corresponding nitrogen atoms. Lastly, site-specific labeling technique for "long" RNA molecules will be presented.

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A78

CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF NEW PSYCHROPHILIC HALOALKANE DEHALOGENASES DMXA AND DPCA AND COMPLEX OF DPCA WITH 1-BROMOHEXANE

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Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are microbial enzymes with catalytic activity for the hydrolytic conversion of xenobiotic and highly toxic halogenated aliphatic compounds to the corresponding alcohols [1]. The active site is buried inside the protein and lined with

hydrophobic residues. The reaction proceeds via a covalent substrate-enzyme complex [2].

To date, HLD activity has been experimentally confirmed in only about a dozen different proteins [3]. A novel HLD were found in Gram negative psychrophilic bacteria: DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marinobacter* sp. ELB 17.

Both haloalkane dehalogenase DpcA and DmxA were crystallized in optimal conditions and diffraction data were collected using in-house source (IOCB, Prague) as well as the synchrotron beamline (BESSY, Berlin). To understand the changes in the active site of the enzyme DpcA, the native enzyme was co-crystallized with substrate 1-Bromohexane. Structure determination is currently in progress now.

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A79

QUANTUM-CHEMICAL INSIGHT INTO THE REACTION MECHANISM OF POLYPEPTIDE UDP-GALNAC TRANSFERASE 2, A RETAINING GLYCOSYLTRANSFERASE

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Protein glycosylation is thought to be main means of cell recognition. Misregulation of the cascade of glycosyltransferases is related to many diseases with the most prominent example being cancer [1]. There is thus significant scientific interest in the reaction mechanisms of glycosyltransferases because knowledge of transition state structures would enable targeted design of selective inhibitors usable as potential drugs. However, reaction mechanism of the configuration-retaining group of glycosyl-tr ansferases hasn't been explained yet [2].

For his reason we have chosen a retaining glycosyltransferase – polypeptide UDP-GalNAc transferase (ppGal NAcT) – as the subject of our quantum-chemical study. This enzyme catalyses the transfer of N-acetylgalactosamine moiety onto protein serine or threonine hydroxyls, forming the first bond of the so-called O-linked glycosylation pathway. Increased activity of this enzyme has been found to enable metastasis of breast and colorectal cancer [3]. These enzymes form a large family with twenty isoforms described in humans to date. Even though all ppGal NAc transferases exhibit strong mutual structural similarity, there are significant differences in their preference for protein substrates and glycosylation site location [2].

Thanks to the availability of high-resolution X-ray structures of three members of the ppGalNAcT family (human transferases 2 and 10, murine transferase 1) we have been able to successfully mount a quantum chemistry study of the human ppGalNAcT2, leveraging information on substrate positioning in active site from the ppGalNAcT10. We are using a hybrid quantum mechanics/molecular mechanics approach using density functional theory on the BP86/TZP level for the important part of the active site. Structures in reactant and product energy minima have been successfully obtained, enabling a potential energy surface scan to find the locations of transition state candidates. Results clearly show that proton transfer between the acceptor hydroxyl moiety and the donor phosphate plays a crucial role in enabling the reaction to take place. The 2D energy map suggests that the reaction proceeds via a two-step mechanism with formation of a carbocation intermediate and its subsequent nucleophilic trapping by the acceptor oxygen. However, exact location of the transition states is yet necessary to prove this conclusively.

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A80

STRUCTURE-FUNCTION STUDY ON MAIZE NUCLEOSIDE N-RIBOHYDROLASE FAMILY

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Nucleoside hydrolases also called nucleoside N-ribohydrolases (NRHs; E.C. 3.2.2.-) are glycosidases that catalyze the excision of the N-glycosidic bond in nucleosides to allow recycling of the nitrogenous bases and ribose. The recycling of nucleosides and nucleobases is also known as the salvage pathway and together with *de novo* synthesis and catabolism of purines and pyrimidines appears in the overall nucleotide metabolism. NRHs belong to metalloproteins. All enzymes characterized so far impose a strict specificity for the ribose moiety, but they exhibit variability in their preferences for the nucleobase. NRHs were initially identified and characterized in parasitic protozoa such as *Trypanosoma, Crithidia* and *Leishmania.* These organisms rely on the import and salvage of nucleotide derivatives as they do not possess enzymes for nucleotide *de novo* synthesis. In plants, only *NRH* gene family in *Arabidopsis thaliana* was deeply analyzed so far. In maize (*Zea mays*), five related genes can be found. Two paralog genes of *ZmNRH1a* and *ZmNRH1b* are localized to chromosome 8 and 3. Other two paralogs, genes of *ZmNRH2a* and *ZmNRH2b*, lie at chromosome 4 and 1.The *ZmNRH3* gene is localized to chromosome 2. All five genes from *Zea mays* were cloned and expressed in *E. coli*. The substrate specificity of the recombinant enzymes was analyzed and significant differences were observed. While both ZmNRH2a and ZmNRH2b preferentially catalyze the conversion of pyrimidine nucleosides, ZmNRH3 hydrolyzes purine nucleosides including inosine and xanthosine. The ZmRNH3 was crystallized and then diffraction data were collected at 2.5 Å resolution on Proxima 1 beamline at SOLEIL synchrotron (Saclay, France). The crystals belong to the orthorhombic space group $P2_12_12_1$ and contain one dimer per asymmetric unit. The enzyme comprises four Asp residues in a conserved sequence motif DXDXXXDD at the N terminus. The first aspartate residue is involved in catalysis while the second and fourth aspartate are involved in the coordination of a calcium ion at the active site. Finally, ZmNRHs also cleave cytokinin ribosides to the corresponding bases indicating that the enzyme could contribute to cytokinin homeostasis. Cytokinins (N^6-substituted adenine or adenosine derivatives) are the plant hormones regulating cell division as well as a large number of developmental events, such as shoot and root branching, leaf development, and chloroplast ripening.

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A81

COMPUTATIONAL STUDY OF RETRO TRP-CAGE

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Prediction of the three-dimensional structure of a protein from sequences unrelated to any previously known structure belongs to the most complicated tasks in computational structure biology. The generally accepted fact that sequence determines protein fold can be further elaborated in search for its ground or more general rules for sequence - structure relationship. We examined the case of reverse sequence of a known protein which keeps the sequential distance between all amino acids as in the original protein but their order is reversed. We hypothesized that such arrangement may be still potent in preserving of native inter-residual contacts between amino acids and thus retain the same fold as the original sequence.

We used reversed Trp-cage miniprotein sequence (retro Trp-cage) in testing of proposed hypothesis. The three-dimensional structures of the retro Trp-cage sequence were

obtained by the current most successful in silico predictions methods - Robetta and PEP-FOLD. Folding simulations using molecular dynamics were also used for structural characterization of the reverse Trp-cage sequence and its folding and dynamical stability. NMR study of the synthetic miniprotein provided that there is no stable 3D structure in pure water, however it could be induced by addition of trifluorethanol (TFE). The experimental NMR structure of the retro Trp-cage in TFE resembled the general Trp-cage fold but showed distinct packing of the protein core and different inter-residual contacts which were not identical with the original Trp-cage protein neither with any predicted model. The presence of TFE promoted formation of helix involving the same amino acids as in the original Trp-cage molecule.

Our results revealed that reversed sequence of Trp-cage miniprotein is not able to maintain all native Trp-cage contacts and folds differently than the original structure. Although the helical preferences in the reverse sequence was preserved (confirmed by both theoretical as well as experimental studies) it can be only manifested in suitable environment. All modeling methods and simulations predicted the occurrence of helix but overestimated its stability and did not provide the correct model of the miniprotein. Obviously, structural prediction of proteins and peptides on the edge of stability or in non-water environments remains challenging themes in molecular modeling.

A82

IMPORTANCE OF NMR DATA VALIDATION PRIOR TO STRUCTURE CALCULATION

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Quality of structures calculated using NMR restraints always reflects the quality of the experimental data. In this poster, impact of the experimantal error of residual dipolar couplings (RDCs) on calculated structures was analysed by evaluating several parameters describing structure quality and the course of the calculation. The analysis showed that the use of RDCs containing experimental errors results in considerable deterioration of evaluated parameters instead of improving calculated structures compared to calculations without using of RDCs. RDCs, from which the evidently incorrectly measured data were removed, do not deteriorate calculated structures so considerably and in the case of RMSD, better results are achieved than without RDCs, but their impact is still more or less negative. Only RDCs that passed the tests developed in our laboratory, i.e., data with a minimal presence of experimantal errors, provide in some cases better results of evaluated parameters then calculations without the use of RDCs. Our results document that validation of measured RDCs before calculating protein structure is necessary in order to obtain structures of the highest possible quality.

A83

STATE TRAJECTORY OF THE BELOUSOV-ZHABOTINSKY REACTION

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All biological systems ranging from organized herds to colonies of individual cells represent the self-organizing (SO) system. Studying and modeling of these systems will allow us to predict their future behavior and interaction with other systems. The aim of our investigation was to develop a method of analysis for SO systems. As a basic model of intracellular (or intra-organelles) pattern formation was chosen Belousov-Zhabotinsky reaction.

Data were received by non-invasive methods (photographing the surface of the reaction). For maximization information gain all images were processed by the method developed on our institute which is called information entropy. It is based on Rényi entropy equation

$$H_{\alpha}(X) = \frac{1}{1-\alpha} \log \sum_{i=1}^{n} p_i^{\alpha},$$

where α is called Rényi entropy coefficient. The colour channels and different Rényi entropy coefficients may be combined to best discriminate individual states.

For the further processing of data we used principal component analysis (PCA) provided by the Unscrambler X 10.1. We are using results of PCA for construction of state trajectory of reaction. For dividing obtained data into several clusters we use cluster analysis provides by Unscrambler. The cluster analysis is based on k-mean clustering method.

Each cluster of the trajectory represents an event or subset of the states of the reaction. Size and position of

clusters depends on the trajectory and on number of clusters. We proposed seven clusters now as a first estimation, satisfied by the results: clusters are well separated; images in transitions between clusters show some changes. The loadings of the first third principal components (PC-1, PC-2 and PC-3) on the all experiments data were compared, which indicates that the using method well describes the main characteristic of the system parameters. This allows us to assume that the method can be applied to the analysis of more complex systems such as cells.

A84

COMPUTATIONAL MODELING OF TRPA1 C-TERMINUS

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The ankyrin transient receptor potential channel TRPA1 belongs to a roup of membrane receptors with a great importance in transfer of sensoric information. It is a non-selective cation channel assembling a tetramer of subunits with large cytoplasmic domains. Nevertheless, TRPA1 crystals with atomic resolution are still missing.

Here, we present TRPA1 C-terminus structures obtained using homology modeling and refined by molecular dynamics simulations. Several chimeras were constructed using known structures of the cytoplasmic domain of the BK channel. A site with ability to bind calcium ions was located in the C terminus of TRPA1.

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A85

THE ROLE OF WEAK NON-COVALENT INTERACTIONS IN FOXO4 BINDING TO THE TARGET DNA

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Forkhead transcription factors are structurally similar molecules containing approximately 110-amino-acid-long DNA-binding domain known as a forkhead domain. Protein FOXO4 is a member of subgroup "O" of forkhead transcription factors. Members of this subgroup play a key role in many biologically important processes [1]. For example, FOXO factors participate in metabolism control, cell-cycle control, apoptosis and oxidative stress resistance. The forkhead domain (DNA-binding domain) consists of three -helices (H1, H2 and H3), three -strands (S1, S2 and S3) and two flexible loops (called wings W1 and W2). The role of the wing W2 in FOXO binding to the target DNA is still elusive. Wing W2 probably interacts with the DNA in the region upstream of the core motif. It has been speculated that the FOXO DNA-binding affinity depends on A-T content (number of A-T pairs) in the region upstream of the core motif. In order to investigate this hypothesis, the DNA-binding domain of the FOXO4 protein was expressed and purified and its binding affinity for two dsDNA containing different number of A-T pairs in the region upstream of the core motif was determined using the steady-state fluorescence anisotropy-based method.

The crystal structure of the FOXO4:DNA complex suggested that both direct water–DNA base contacts and the unique water-network interactions contribute to FOXO-DBD binding to the DNA in a sequence specific manner [2]. To assess the importance of these interactions in the stability of FOXO4:DNA complex, we prepared a series of FOXO4 mutants and studied their DNA binding affinities using surface plasmon resonance.

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A86

STRUCTURAL BIOLOGY WITHOUT STRUCTURE - DELTA SUBUNIT OF RNA POLYMERASE FROM *BACILLUS SUBTILIS*

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Intrinsically disordered proteins are currently attracting an increased interest of biochemists and structural biologists. A (partial) structural disorder has been observed in a considerable portion of human proteome, including proteins related to serious diseases. Unstructured proteins present a particular challenge for structural biology, as the standard techniques utilizing X-ray diffraction or NMR distance data fail to describe the disordered portions of protein molecules at atomic level. Yet, various methods can be applied to describe conformational behaviour of the unstructured polypeptide chains. Several NMR techniques were used to characterize the unstructured C-terminal domain of the delta subunit of *Bacillus subtilis* RNA polymerase. Non-standard data acquisition and processing methods have been developed to overcome very poor resolution of the NMR spectra of the delta subunit. The possibility to distinguish all signals in the spectra allowed us to map the local conformation reflected by the bias of resonance frequencies from their random-coil values. Flexibility of the disordered polypeptide was described in detail by NMR relaxation measurements. Paramagnetic labels were introduced to reveal transient contacts, beyond the limit of the nuclear Overhauser effect. The average overall shape of the molecule was studied by analytical ultracentrifugation. Combination of the obtained data provided a very detailed description of the C-terminal region, complementing the high-resolution structure of the well-ordered N-terminal domain.

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COMMERCIAL LECTURES

THE BIOSAXS-1000 AND A NEW APPROACH TO COMBINING VISIBLE AND UV LIGHT IMAGING - THE LATEST DEVELOPMENTS FROM RIGAKU

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X-ray crystallography is a fundamental technique for obtaining atomic level structural information for macromolecules. However, the requirement for diffraction quality crystals often limits its efficacy for studying protein complexes or systems with inherent structural disorder. In such cases where crystallographic methods have failed, small angle X-ray scattering (SAXS) provides a complementary tool for extracting structural information from biological systems. In particular, SAXS proves ideal for studying partially disordered macromolecules, for monitoring structural changes in response to environmental perturbations, and for monitoring conformation changes due to ligand binding.

Here we introduce the first commercial system specifically designed for high-throughput solution scattering experiments with macromolecules - the BioSAXS-1000. Comprised of specially designed focusing optics and a Kratky block, the BioSAXS-1000 system eliminates smearing issues common to traditional Kratky cameras. The system includes a photon counting hybrid pixel array detector and intuitive data collection software that includes full automation of the Kratky alignment hardware. Together, the many features of the BioSAXS-1000 system allow for synchrotron-quality SAXS data from a home laboratory source.

Crystallization of proteins is a critical bottleneck in structural biology today. Prior to finding conditions that produce diffraction quality crystals, many hundreds of crystallization conditions must be examined.

The new Minstrel HT UV takes a novel approach to automated crystal imaging. Developed by Rigaku for high throughput imaging of crystallization plates, this instrument allows imaging of multiple plate types at a given temperature under a variety of imaging conditions, including black-and-white, color, polarized and UV.