Posters

P1

METACENTRUM — E-INFRASTRUCTURE FOR COMPUTATIONAL AND DATA CHALLENGES

J. Kmuníček[1], V. Žáková[2], S. Licehammer[2], M. Kuba[1], I. Křenková[1], and D. Antoš[1]

[1]CESNET, z.s.p.o., Zikova 4, 160 00 Praha 6, Czech Republic
[2]Faculty of Informatics, Masaryk University, Botanicka 68a, 620 00 Brno, Czech Republic

MetaCentrum, the Czech National Grid Initiative, runs more than 1300 CPU cores in four major cities of the Czech Republic. The computation power is available for scientists in many research areas. The software installed on the infrastructure supports, among many others, biomolecular simulations, enzymatic structure engineering, quantum mechanics/molecular mechanics, studies, exploration of reaction mechanisms at atomic details.

In addition to routine computations, MetaCentrum is also active in development generic and/or specialised solutions, starting from specific application interfaces to bringing user-empowered element to the infrastructure by means of thorough virtualisation, allowing users to run their own environment and control the infrastructure.

The poster describes basic principles of MetaCentrum, available applications, running international projects, and user benefits from the infrastructure.

P2

NMR STUDY OF NRD1 AND ITS BINDING TO RNA

V. Bačíková[1], M. Zimmermann[2], R. Štefl[1]

[1]National Centre for Biomolecular Research
[2]Institute of Experimental Biology, Masaryk University, Faculty of Science, 611 37 Brno, Czech Republic
bacikova@chemi.muni.cz

The exosome - a complex of several exoribonucleolytic and RNA-binding proteins - is the central 3'-end RNA degradation and processing factor in RNA quality control apparatus. It operates with many auxiliary factors that stimulate its activity and recruit its RNA substrates in the crowded cellular environment. One of this factor is the nuclear pre-mRNA down-regulation (Nrd)1 complex, which consists of the RNA helicase Sen1, and the proteins Nrd1 and nuclear polyadenylated RNA-binding (Nab)3 that recognize specific sequence motifs on RNAs. This complex is required for transcription termination of small nuclear RNA and small nucleolar RNA genes as well as for cryptic unstable transcripts. Nrd1 protein contains RNA-recognition motif (RRM) that is known to bind its specific terminator sequence, GUA[A/G].

We will present a nuclear magnetic resonance (NMR) chemical shift perturbation and fluorescence anisotropy studies of the interaction of Nrd1 RRM with its RNA target. These studies reveal RNA-binding interface of Nrd1 and its affinity to the 5'-GUAA-3' RNA.

P3

CRYSTALLIZATION OF MUTATED ENZYMES POTENTIALLY INVOLVED IN COUPLING OF ENDONUCLEASE AND TRANSLOCASE FUNCTIONS IN EcoR124I

Tatsiana Baikova, Mikalai Lapkouski, Ivana Kutá Smatanová, Rudiger Ettrich and Eva Csefalvay

Department of Structure and Function of Proteins, Institute of Systems Biology and Ecology ASCR and Institute of Physical Biology USB, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic

Restriction and modification (R-M) systems provide bacteria with protection against infection by DNA-based bacteriophage. R-M classified on three distinct types based on their composition and cofactor requirements, the nature of their target sequence, and the position of the site of DNA cleavage with respect to the target sequence. The type I R-M enzymes are sophisticated and differ significantly from type II enzymes commonly used as molecular biology reagents. RE Type I are hetero-oligomeric enzymes composed from 5 subunits, which are responsible for specificity (HsdS), methylation activity (2*HsdM), ATP-dependent DNA translocation and endonuclease activity (2*HsdR). Structure of HsdR subunit of EcoR124I restriction enzymes (RE) has been described recently by our group. The crystal structure of the motor subunit responsible for DNA translocation and cleavage by the type I enzyme EcoR124I, resolved at 2.6 Å, shows a lysine residue on the endonuclease domain to contact N3 on the exposed edge of ATP bound at the helicase domains, potentially coupling endonuclease and translocase functions [1]. To prove this prediction the Lys220 was replaced by Arg, Glu, and Ala. Our preliminary crystallization experiments under the conditions used for WT HsdR yielded crystals of only Lys220Arg and microcrystals of Lys220Ala. The aim of our work is to find suitable crystallization conditions for functionally altered enzymes Lys220Ala and Lys220Glu. Obtained crystals will be measured on synchtron to determine their structure. Structural information will be combined with *in vivo* and *in vitro* biochemical testing of the altered enzymes, which will give us complete information about accouplement of endonuclease and translocase functions in EcoR124I and allow drawing conclusions valid for type I R-M complexes in general.

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

P4

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

L. Bednárová[1], P. Maloň[1], H. Dlouhá[1], E. Kočišová[2], K. Hofbauerová[2,3], V.Kopecký Jr.[2]

[1]Inst. of Organic Chemistry and Biochemistry AS CR, Flemingovo náměstí 2, Prague 6, 166 10, Czech Rep.
[2]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, Praha 2, 121 165, Czech Republic
[3]Institute of Microbiology AS CR, Vídeňská 1083, Prague 4, 142 20, Czech Republic
bednarova@uochb.cas.cz

Naturally occurring antimicrobial peptides (AMPs) represent one successful form of chemical defense of eukaryotic cells against bacteria, protozoa, fungi, and viruses [1]. Many of them have been already isolated, thousands of their synthetic analogs were synthesized and a broad spectrum of their antimicrobial, anticancer and antiviral activities was proven [2, 3]. In spite of large number of known AMPs and their therapeutic potential, exact mechanism of their action remains a matter of controversy. There is a consensus that these peptides selectively disrupt cell membranes and it is believed that their amphiphatic structure plays an important role in this process.

Interaction of peptides with membranes or their models leads to changes of their secondary structure, which could be detected using various spectroscopic methods. Here we present spectroscopic studies of halictines, AMP isolated from the venom of the eusocial bee Halictus sexcinctus, which exhibited potent antimicrobial activity against Gram-positive and Gram-negative bacteria but also noticeable hemolytic activity [4]. Circular dichroism, infrared and Raman spectra of HAL-1 (Gly-Met-Trp-Ser-Lys-Ile-Leu-Gly-His-Leu-Ile-Arg-NH2) in a membrane/like environment, showed ability to form a-helical structure whereas the HAL peptide exhibits random coil conformation in water. The attempts to measure HAL peptides in an interaction with liposomes with different kinds of membranes are presented as well.

1. M. Zasloff, *Nature*, **415**, (2002), 389.
2. R. E. Hancock, G. Diamond, *Trends Microbiol*., **9**, (2000), 402.

3. R. E. Hancock, M. G. Scott, Proc. Natl. Acad. Sci. U.S.A., 97, (2000), 8856.

4. L. Monincová, M. Buděšínský, J. Slaninová, O. Hovorka, J. Cvačka, Z. Voburka, V. Fučík, L. Borovičková, L. Bednárová, J. Straka,V. Čeřovský, Amino Acids, submitted.

The Grant Agency of the Czech Republic is gratefully acknowledged for support (No. 208/10/0376).

P5

HOW IS A-RNA TREATED BY DIFFERENT FORCE FIELDS AND SALT CONDITIONS?

Ivana Beššeová,[1,2] Michal Otyepka,[1,3] Kamila Réblová[1] and Jiří Šponer[1]

[1]*Inst. of Biophysics, Acad. of Sciences of the Czech Republic, Královopolská 135, 612 65, Brno, Czech Rep.*
[2]*Gilead Sciences & IOCB Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo náměstí. 2, 166 10, Prague 6, Czech Republic*
[3]*Department of Physical Chemistry and Centre for Biomolecular and Complex Molecular Systems, Palacký University, tr. Svobody 26, 771 46 Olomouc, Czech Republic*
i.besse@mail.muni.cz

An extensive molecular dynamics study (totaling 0.6 ms) of three different A-RNA duplexes is presented. We investigated the dependence of the A-RNA geometry on AMBER force fields (Parm99 [1] and Parmbsc0 [2]) and salt strength conditions (0.18 M net-neutralizing Na^+ and 0.3 M KCl).

The A-RNA duplexes were more compact when using the Parmbsc0 force field compared to the Parm99. In addition, the Parmbsc0 temporarily reduced t/t flips. Nevertheless, since the t/t sub-state occurs to a certain extent in experimental A-RNA structures, we consider both force fields as viable. The effects of the Parmbsc0 force field included visible reduction of the major groove width, increase of the base pair roll, larger helical inclination and small increases of twist. The Parmbsc0 shifted the simulated duplexes more deeply into the A-form. [3]

A narrowing of the deep major groove was observed in excess salt simulations, again accompanied by larger roll, inclination and twist. [3]

The differences between Parm99/lower-salt and Parmbsc0/higher-salt Parmbsc0 conditions were small; nevertheless their cumulation induced visible stabilization of the A-RNA helices. In addition, the effects of the force field and salt conditions were sequence-dependent. Thus, the compactness of A-RNA is sensitive to the sequence and the salt strength which may, for example, modulate the end-to-end distance of the A-RNA helix.

1. J. M. Wang, P. Cieplak and P. A. Kollman, *J. Comput. Chem.*, 21 (2000) 1049–1074.

2. A. Perez, I. Marchan, D. Svozil, J. Sponer, T. E. Cheatham, C. A. Laughton and M. Orozco, *Biophys. J.*, 92 (2007) 3817–3829.

3. I. Besseova, M. Otyepka, K. Reblova and J. Sponer, *Phys. Chem. Chem. Phys.*, 11 (2009) 10701-10711.

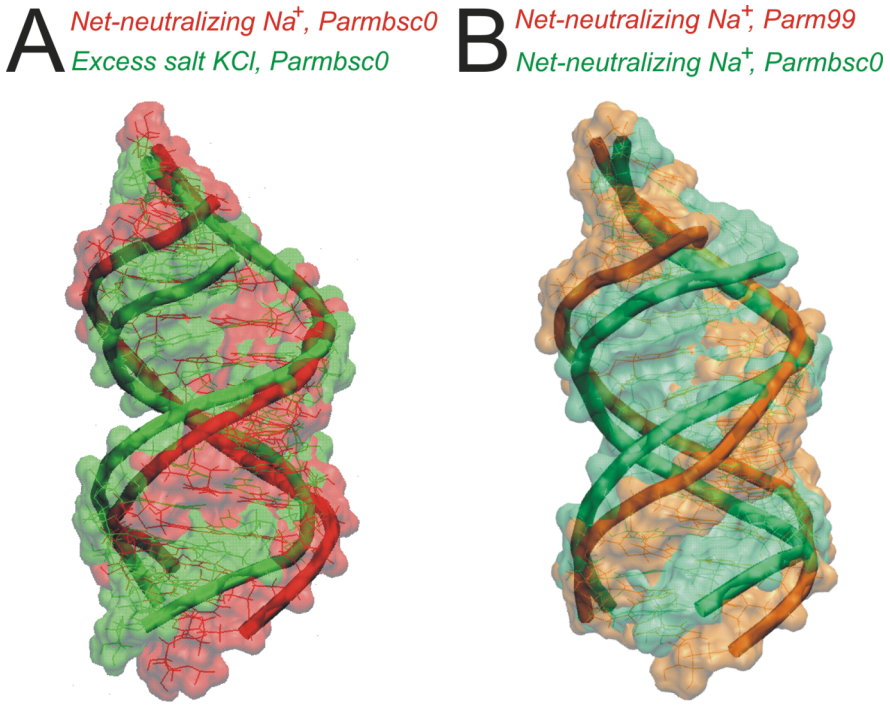

Figure 1. A-RNA simulations: ionic conditions influence the major groove width and compactness of the helix.

P6

CLONING AND EXPRESSION OF RHSA AND RHSB GENES FROM *E.coli*

Vitali Bialevich, Eva Csefalvay and Rudiger Ettrich

Department of Structure and Function of Proteins, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, and Institute of Physical Biology, University of South Bohemia in Ceske Budejovice, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic

RhsA and RhsB proteins are products of genes rhsA and rhsB accordingly. These proteins 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 RhsA and RhsB proteins in purity sufficient for biophysical 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 biophysical experiments. The polymerase chain reaction (PCR) technique has been used to obtain DNA fragments encoding for rhsA and rhsB genes from genomic DNA of *E. coli* K12. Optimal conditions for PCR have been found. Both rhsA and rhsB have been cloned into pBluescript SK+ and pET32a(+): the clones contained good expression system have been selected by restriction analysis and sequencing. Several expression *E. coli* strains have been used to test expression under several cultivation conditions.

1. C. McNulty, J. Thompson, B. Barrett, L. Lord, C. Andersen and I. S. Roberts. *M. Microbiology* (2006) 59 (3), 907–922.

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 (AVOZ 60870520).

P7

CHARACTERIZATION OF THE CALMODULIN BINDING ON THE TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL TRP M5

J. Bílý, B. Holakovská, J. Teisinger

Department of Protein Structures, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, Praha 4, 142 20

Transient receptor potential (TRP) proteins are a diverse family of proteins with structural features typical of ion channels. TRPM5, a member of the TRPM subfamily, plays an important role in taste receptors. TRPM5 is a monovalent-specific, nonselective cation channel that carries Na^+, K^+, but not Ca^{2+} ions. It is directly activated by Ca^{2+} , but activation mechanism remains controversial.

On basis of a comparison of the similarity with TRPM4 and Calmodulin target database, there have been identified several binding sites.

To detect Calmodulin binding site we used anisotropy measurements between Calmodulin and short peptides of the TRPM5 labeled with fluorescent probe. Short peptides were designed based on amino acids sequence of the TRPM5, also mutant version of peptides with replaced amino acids with neutral alanine to determine role of the residues in Calmodulin binding.

This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (GAAV IAA 600110701, GACR 303/07/0915) project (No. H148), Centre of Neurosciences No. LC554 The Ministry of Education, Youth and Sports of the Czech Republic, Research project No. AV0Z.

P8

ENGINEERING THE ACTIVITY OF HALOALKANE DEHALOGENASE WITH TOXIC SYNTHETIC SUBSTRATE USING METHODS OF FOCUSED DIRECTED EVOLUTION

P. Dvořák, M. Pavlová, M. Klvaňa, J. Brezovský, R. Chaloupková, Z. Prokop, and J. Damborský

Loschmidt laboratories, Institute of Experimental Biology and National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4, 625 00 Brno, Czech Republic
paveld@chemi.muni.cz

Haloalkane dehalogenases (HLDs; EC 3.8.1.5.) are microbial enzymes that catalyze hydrolytic conversion of haloalkanes to the corresponding alcohols and hydrogen halides [1]. Some synthetic halogenated alkanes, like 1,2-dichloroethane (DCE), are produced worldwide and are classified as persistent environmental pollutants. DCE is widely used as solvent and intermediate in chemical industry. Its toxic and carcinogenic effects are well recognized. HLDs have been investigated for possible applications in detoxification of halogenated pollutants including DCE [2,3]. It remains a challenge to provide HLDs that could degrade these substrates efficiently. Here we report construction of a mutant dehalogenase with improved catalytic activity towards DCE.

Dehalogenase DhaA from *Rhodococcus erythropolis* Y2 [4] has only minimal activity with DCE. Large cavity of DhaA active site and wide mouth of the main tunnel cannot effectively accommodate small DCE molecule and results in non-productive binding. We hypothesized that reduction of size of DhaA active site and tunnels together with elimination of non-productive binding could lead to the increased activity with DCE.

Initially, computer modeling, site-directed and saturation mutagenesis were applied to target the passage of ligands through the access tunnels [5]. Up to seven bulky substitutions (I135F, A145F, A172F, C176Y, V245F, L246I, Y273F) were introduced into the walls of DhaA tunnels and in vicinity of the active site. Closing up the tunnels influenced the substrate specificity of DhaA and improved the enzyme's activity towards DCE 8-times. This DhaA variant was used as a template for the second round of *in vitro* evolution. During this second step four residues (I132, L209, F245 and I246) were randomized with the aim to reduce the size of DhaA active site and to influence the non-productive binding of DCE above the catalytic histidine. Screening of four thousand clones from resulting mutant libraries revealed one positive DhaA variant (F245T+I246L) which showed 12-times higher specific activity with DCE compared to the wild-type DhaA.

The substitutions showing the most significant impact on DhaA activity with DCE will be inserted to another HLD to validate their effect. Understanding the structure-function relationships in constructed mutants is important to design next generation of biocatalysts for degradation of anthropogenic substrate.

1. D.B. Janssen, *Curr. Opin. Chem. Biol.* **8,** (2004), 150-159.
2. G. Stucki, M. Thuer, *Environ. Sci. Technol.* **29**, (1995), 2339-2345.
3. M. Pavlova, M. Klvana, Z. Prokop, R. Chaloupkova, P. Banas, M. Otyepka, R.C. Wade, M. Tsuda, Y. Nagata, J. Damborsky, *Nat. Chem. Biol.* **5,** (2009), 727-733.
4. A.N. Kulakova, M.J. Larkin, L.A. Kulakov, *Microbiology* 143, (1997), 109-115
5. M. Klvana, M. Pavlova, T. Koudelakova, R. Chaloupkova, P. Dvorak, A. Stsiapanava, M . Kuty, I. Kuta-Smatanova, J. Dohnalek, P. Kulhanek, R.C. Wade, J. Damborsky, *J. Mol. Biol.* **392**, (2009), 1339-1356.

P9

NMR STUDY OF *Saccharomyces cerevisiae* AIR2 PROTEIN

H. Černá, M. Sanudo, R. Štefl

National Centre for Biomolecular Research, Masaryk University, Faculty of Science, 625 00 Brno, Czech Republic

The Air2 protein from *Saccharomyces cerevisiae* is an RNA-binding subunit of the Trf4–Air2–Mtr4 polyadenylation (TRAMP) complex. This complex plays an important role in the nuclear RNA-surveillance machinery. It adds short poly(A) tails to aberrant or misfolded RNA transcripts and thus promote their degradation by the nuclear exosome. It is suggested that the Air2 protein is involved in the RNA substrate recognition, as it contains five consecutive zinc knuckles–these domains are well known to bind nucleic acids. Here we present the biochemical and nuclear magnetic resonance (NMR) studies of the five zinc-knuckles construct of Air2.

P10

TOWARDS IDENTIFICATION OF HYDROPHOBIC CORE IN GLOBULAR PROTEINS

Fačkovec Boris, Vondrášek Jiří, Hobza Pavel

Institute of Organic Chemistry AS CR, Center for Biomolecules and Complex Molecular Systems, Flemingovo nám. 2., 166 10 Praha 6
borislavujo@gmail.com

The concept of hydrophobic core is a contributory paradigm, which is successfully used when studying a protein deeper. A core has remarkable properties that might shed light on problems related to thermostability of proteins and protein folding kinetics.

However, there is not yet any exact definition of core that would integrate different physical models and would be transferable and definite.

The present study aims at integrated algorithm of searching for protein residues, that takes into account more core identification criteria and definitely determines whether residue belongs or does not belong to core set.

Analysis is theoretical based on PDB structures. Mainly empirical force field (Amber ff03) computations are employed, what makes method fast and feasible.

1. Hobza P., Müller-Dethlefs K.: Non-Covalent Interactions: Theory and Experiment, RSC Publishing, 2010.
2. Berka K., Hobza P., Vondrášek J.: Analysis of energy stabilization inside the hydrophobic core of rubredoxin. Chemphyschem. *10*, 543 (2009).

P11

STUDY OF HINCII ENDONUCLEASE BY MOLECULAR DYNAMICS METHODS

J. Fukal, P. Kulhánek, E. Fadrná, J. Koča

National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno
enigma@chemi.muni.cz

HincII is one of restriction endonucleases which are present in bacterial cells. They work in defense against alien DNAs (e.g. phages). HincII is homotetramer consisting of two functional dimers which recognize sequence GTPyPuAC. Bound oligonucleotide is double-bent and its specific bending is partially caused by intercalating glutamine 138 on each monomer. HincII enzyme needs ions for its function, Mg^{2+} or Mn^{2+} ions work like natural cofactors and Ca^{2+} inhibits the cleavage reaction.

The goals of this study are as follows: a) what is the influence of intercalated glutamine 138, b) what is the role of ions in the active site, c) describe dynamics of HincII complexes. To address these questions, molecular dynamic simulations were performed on pre-reaction complexes of restriction endonuclease HincII. The crystal structures data from work [1] and [2], deposited in PDB database, were used. The simulated systems consist of wildtype protein or Q138F mutated protein and of substrate 5'-GTCGAC-3' or 5'-GTTAAC-3'. From crystals containing Ca^{2+} ions were prepared two variations of each: original with Ca^{2+} ions and changed with Mg^{2+} ions. Force field parm99sb [3] with parmbsc0 [4] was used for all of the simulations.

The analyses show the protein is a rigid part and the DNA is flexible part of the complex. The ions' analyses demonstrate the ions with 2+ formal charge stay almost in their places but some Na^{+} ions changed their positions. The influence of intercalation of amino acids 138 is studied by anisotropic thermal diffusion method (ATD) [5]. ATD method should reveal how the thermal energy is distributed in the system, in this case, which part of system is affected by the intercalated amino acid. The molecular dynamics simulation of DNA molecule is rather problematic because DNA is sensible for imbalances in parameters. Even though the corrected force field was used, it is observed the DNA is forced to the canonical form but still better than containing artefacts. In spite of existing problems of simulations, usable results were obtained.

This work has been supported by Ministry of Education of the Czech Republic (MSM0021622413, LC06030). The access to the METACentrum supercomputing facilities provided under the research intent MSM6383917201 is highly appreciated. The research leading to these results has received funding from the European Community's Seventh Framework Programme under grant agreement No. 205872.

1. H.K. Joshi, C. Etzkorn, L. Chatwell, J. Bitinaite, N.C. Horton, *J. Biol. Chem.*, **281**, (2006), 23852-23869.
2. C. Etzkorn, N.C. Horton, *Biochemistry*, **43**, (2004), 13256-13270.
3. V. Hornak, R. Abel, A. Okur, B. Strockbine, A. Roitberg, C. Simmerling, *Proteins: Struct, Funct Bioinformatics*, **65**, (2006), 712-725.
4. A. Pérez, I. Marchán, D. Svozil, J. Sponer, T.E.C. III, C.A. Laughton, M. Orozco, *Biophys J.*, **92**, (2007), 3817–3829.
5. N. Ota, D.A. Agard, *J. Mol. Biol.*, **351**, (2005), 345-354.

P12

CALMODULIN INTERACTS WITH C-TERMINAL REGION OF TRPC6

L. Grycova, B. Holakovska, J. Teisinger

Department of Protein Structures, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, Praha 4, 142 20

The transient receptor potential channel TRPC6 is a non-selective cation channel involved in regulation of calcium levels in eukaryotic cells (including sensory receptor cells) in response to external signals. Calmodulin (CaM) is a ubiquitously expressed Ca^{2+}-binding protein that serves as an important mediator of Ca^{2+}-dependent regulation of the TRPC6 channel. One CaM binding site has been identified within the C-tail of TRPC6.

In this project we mapped in detail the CaM and inositol (1,4,5)-triphosphate receptor binding (CIRB) domain in the C-terminal region of mouse TRPC6 (801-878) capable of interacting with CaM using in vitro binding assays. Besides we used a set of positively charged amino acid residues Arg852, Lys856, Arg864 and Lys859/Arg860, and a hydrophobic residue Ile857 placed in position 1 in consensus CaM binding motif 1-5-10. We replaced these amino acids with neutral alanine and investigated role of the residues in CaM binding to TRPC6 C-terminus using fluorescence anisotropy measurement. Participation of Ile857 could indicate a strong role of this conserved CaM binding motif.

This project was supported by Grant GAAV IAA 600110701, GACR 301/10/1159, GACR P205/10/ P308, project (No. H148), Centre of Neurosciences No. LC554 MSMT CR, Research project No. AV0Z.

P13

RAMAN AND RAMAN OPTICAL ACTIVITY CONFORMATIONAL STUDY OF A CYCLIC HEXAPEPTIDE

J. Hrudíková[1,2]*, J. Kapitán[1,2], V. Baumruk[1] and P. Bouř[2]

[1]*Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, 121 16 Prague 2*

[2]*Institute of Organic Chemistry and Biochemistry, Acad. Sci., Flemingovo nám. 2, 166 10 Prague 6*

hrudikova@uochb.cas.cz

Raman optical activity (ROA) measures vibrational optical activity as a difference in the intensity of Raman scattering from chiral molecules for right and left circularly polarized light. Raman and ROA spectral intensities, band positions, and, in the extreme case, ROA signs can be significantly modified by molecular flexibility [1]. All the conformations present in the sample provide unique ROA signal due to the fast molecular response to the light.

A model cyclohexapeptide, cyclo-(Phe-D-Pro-Gly-Arg-Gly-Asp) was selected as a convenient model of -hairpin induced by the D-Pro-Gly sequence, and a short anti-parallel -sheet. Its Raman and Raman optical activity (ROA) spectra were used to investigate the structure and flexibility of the side chains, for which the IR and VCD techniques are not very sensitive [2]. This system is accessible to relatively precise computations thanks to its size and a constricted conformation of the backbone [3]. Information about peptide side chains could be extracted from the experiment via combined density functional theory (B3LYP/CPCM/ 6-31G**) and molecular dynamics (MD) simulations of spectral intensities. Spectral averaging for many MD structures provided better spectral profiles than a fixed geometry.

The results suggest that neither the side chains move freely, but oscillate around preferred conformations. According to the simulations the ROA spectrum senses local side chain conformation; in the case of the cyclohexapeptide it senses mostly about the hydrophobic phenylalanine and proline residues, and their interaction.

1. J. Kapitán, V. Baumruk, V. Kopecký Jr., P. Bouř, *J. Phys. Chem. A* **110** (2006) 4689-4696.
2. P. Bouř, J. Kim, J. Kapitán, R.P. Hammer, R. Huang, L. Wu and T. A. Keiderling, *Chirality* **20** (2008) 1104-1119.
3. J. Hilario, J. Kubelka, F. A. Syud, S. H. Gellman, T. A. Keiderling, *Biospectroscopy* **67** (2002) 233236.

Grant Agency of the Czech Republic (203/06/0420) and Grant Agency of the Academy of Sciences (A400550702) are gratefully acknowledged.

P14

A FOURIER TRANSFORM METHOD FOR GENERATION OF ANHARMONIC VIBRATIONAL MOLECULAR SPECTRA

Ivan Ivani [1*], Vladimír Baumruk[1] and Petr Bouř[2]

[1]*Faculty of Mathematics and Physics, Institute of Physics, Ke Karlovu 5, 12116, Prague, Czech Republic*
[2]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences, 166 10 Prague, Czech Republic*
ivan.ivani@centrum.cz

Accurate computations of vibrational energies and vibrational spectra of molecules require inclusion of the anharmonic forces [1]. In standard computational protocols, a large vibrational Hamiltonian matrix is diagonalized, and spectral intensities are calculated for individual transitions separately.

We propose an alternate direct generation of the spectral curves based on a temporal propagation of a trial vibrational wavefunction followed by a Fourier transformation [2]. For example, the absorption intensity is calculated as

$$I_R(\ ) \quad \frac{\sqrt{2}\ dN}{4\ ^2} |\ _R(\ )|^2, \qquad (1)$$

where

$$_R(\ ) \qquad _R(t)\exp(\ i\ \ t)dt \qquad (2)$$

is Fourier transform of a time-dependent electric dipole moment. The lack of the lengthy and computer-memory demanding diagonalization makes the method suitable for larger molecules. It is especially convenient for sparse Hamiltonians that are commonly obtained within the harmonic oscillator basis set, and the algorithm is amendable to parallelization. On a model water dimer basic convergence properties are discussed. The method is then applied to vibrational Raman intensities of the fenchone compound, were it provides spectral shapes comparable with those obtained by the classical approaches.

1. Daněček, P.; Bouř, P. *J. Comp. Chem.*2007, **28**, 1617-1624.
2. Kubelka, J.; Bouř, P. *J. Chem. Theory Comput.* 2009, **5**, 200-207.

P15

ANALYSIS OF BACKBONE MOTIONS OF DELTA SUBUNIT OF RNA POLYMERASE FROM *Bacillus subtilis*

P. Kadeřávek[1], C. Diehl[2], V. Motáčková[1], H. Šanderová[3], L. Žídek[1], L. Krásný[3], V. Sklenář[1], M. Akke[2]

[1]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic*
[2]*Center for Molecular Protein Science, Biophysical Chemistry, Lund University, 22100 Lund, Sweden*
[3]*Institute of Microbiology and Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, v.v.i., Vídeňská 1083, 14220 Praha 4-Krč, Czech Republic*
kada@ncbr.chemi.muni.cz

RNA polymerase plays a fundamental role in the process of protein synthesis because it is responsible for the DNA transcription. The RNA polymerase of Gram positive bacteria consists of seven subunits. The motions of delta subunit from *Bacillus subtilis* was investigated within this project. The previous experiments revealed the delta subunit increases the transcriptional specificity [1-6] and efficiency of RNA synthesis [4-6]. Delta subunit is a two domain protein. The N-terminal domain has a well defined structure while the C-terminal domain is disordered. The backbone dynamics of the N-terminal domain of delta subunit was studied to revealed the functionally important parts of the molecule.

The dynamics of the system was investigated by NMR techniques. The experiments were based on the measurement of relaxation rates of backbone ^{15}N-^{1}H spin pair. Both the motions at fast (ps-ns) and slow (s-ms) timescales were studied. In order to explore subnanoseconds motions, the standard set of NMR experiments including R_1, R_2 and NOE were performed at two magnetic fields (500 MHz, 600 MHz) and the obtained data were interpreted within the Model-Free approach [7][8]. The CPMG [9][10] and T_1 [9] experiments were utilized to inspect the slow motions. The experiments and dynamic analyses were carried out at two temperatures 300 K and 280 K to obtain the temperature dependence of the dynamical parameters.

It was revealed that the parts of the molecule which exhibit extensive motions at the s-ms timescale correlates with the conserved residues in the sequence, expected to form an interaction surface with other subunits. On the other hand the residues which are the most flexible on the ps-ns timescale are located in another part of the protein.

1. Achberger E.C., Whiteley H.R., Journal of Biological Chemistry, 256, 7424-7432,1981.
2. Achberger E.C., Tahara M., Whiteley H.R., Journal of Bacteriology, 150, 977-980, 1982.
3. Dobinson K.F., Spiegelman G.B., Biochemistry, 26, 8206-8213, 1987.
4. Juang Y.L., Helmann J.D., Journal of Molecular Biology, 239, 1-14, 1994.
5. Juang Y.L., Helmann J.D., Biochemistry, 34, 14270-14270, 1995.
6. Juang Y.L., Helmann J.D., Biochemistry, 34, 8465-8473, 1995.
7. Lipari G., Szabo A., Journal of American Chemical Society, 104, 4546-4559, 1982.
8. Lipari G., Szabo A., Journal of American Chemical Society, 104, 4559-4570, 1982.
9. Palmer A.G., Kroenke C.D., Loria J.P., Methods in Enzymology, 339, 204-238, 2001.
10. Long D., Liu M.L., Yang D.W., Journal of American Chemical Society, 130, 2432-2433, 2008.

This work was supported by the Swedish Research Council and by the Grants MSM0021622413 and LC06030 from the Ministry of Education, Youth and Physical Culture of the Czech Republic, by the Grant 204/09/0583 from Czech Science Foundation, FRVŠ 1851/2010, and by the Grant NK/9138-3 from the Ministry of Health..

P16

ORGANIC SOLVENT EFFECTS ON PROTEIN TERTIARY STRUCTURE AND ENZYME STABILITY: A COMPUTATIONAL STUDY

Morteza Khabiri[1], Babak Minofar[1], Veronika Štěpánková[2], Radka Chaloupková[2], Jan Brezovský[2], Jiří Damborský[2], and Rüdiger Ettrich[1]

[1]Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zámek 136, 373 33 Nové Hrady
[2]Loschmidt Laboratories, Institute of Experimental Biology, Masaryk University, Kamenice 5A/4, 625 00 Brno

Enzymes never exist *in vacuum*, but either in crystalline, glass-transition, or solvated state, with the solvated state being the "functional" one and the primary solvent being a water/salt mixture. However, already in the 1970s the stabilizing effect of various organic solvents on a protein was described experimentally (1). Nevertheless, to date computational simulations focused mainly on modeling enzyme reactivity in water. Little computational work has been done with the aim to study the molecular reasons for the stabilizing or destabilizing effect of organic solvents and gain a fundamental understanding of its influence on protein structure-function relationships. In this study we have chosen two enzymes, DhaA from *Rhodococcus rhodochrous* NCIMB13064 and DbjA from *Bradyrhizobium japonicum* USDA110, belonging to the haloalkane dehalogenase family that are able to cleave carbonhalogen bonds (2). As the enzymes come from bacteria that use halogenated organic compounds as their growth substrate, their interaction with organic solvents is not an artifical *in vitro* situation, but physiologically relevant. These two enzymes are structural and functional homologs with a primary sequence identity of 50%, and a homology of 63%. Despite this fact, they exhibit a very different behavior in various organic solvents. While an isopropanol/water mixture dampens vibrational and rotational modes in the DbjA structure, the structure of DhaA gets destabilized. For formamide/water mixtures the result is opposite. Therefore a detailed analysis of the molecular interactions on the protein surface and in the solvent shell around the protein leads to a fundamental understanding and a generalization of the organic solvent effects on protein structure-dynamics-function.

The access to METACentrum supercomputing facilities provided under the research intent MSM6383917201 is acknowledged. We gratefully acknowledge support from the Ministry of Education, Youth and Sports of the Czech Republic (LC06010), Academy of Sciences of the Czech Republic (AVOZ60870520), Grant Agency of the Czech Republic (203/08/0114 to R.E and J.D.).

1. Asakura, T., Adachi, K. and Schwartz, E., Stabilizing effect of various organic solvents on protein.J. Biol. Chem.1978 253: 6423-6425.
2. Damborský, J., Chaloupková, R., Pavlová, M., Chovancová, E., Brezovský, J., 2009: Structurefunction relationships and engineering of haloalkane dehalogenases. *In.* Timmis, K.N. (Ed.), *Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds*, Springer, Heidelberg.

P17

X-RAY DIFFRACTION STUDIES OF RED TOMATO NUCLEASE TBN1

T. Kovaľ[1,2], J. Dohnálek[1,3], P. Lipovová[4], T. Podzimek[4], J. Matoušek[5], J. Dušková[3], T. Skálová[3], A. Štěpánková[3], P. Kolenko[3] and J. Hašek[3]

[1]Institute of Physics, Academy of Sciences of the Czech Republic, Cukrovarnická 10, Praha 6, Czech Republic,
[2]Fac. Of Mathematics and Physics, Charles University in Prague, Ke Karlovu 3, Praha 2, Czech Republic,
[3]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského nám. 2, Praha6, Czech Republic
[4]Institute of Chemical Technology, Technická 5, Praha 6, Czech Republic
[5]Institute of Plant Molecular Biology, Biology Centre, Academy of Sciences of the Czech Republic, Branišovská 31, České Budějovice, Czech Republic
koval@fzu.cz

Bifunctional nuclease TBN1 (accession no. AM23701) from *Solanum lycopersicum* - red tomato is a Zn^{2+}- dependent plant glycoprotein composed of 277 amino acids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated). TBN1 belongs to plant nuclease I group and plays a considerable role in specific apoptotic functions and in plant tissue differentiation, vascular system development and stress response [1]. Therefore detailed structural study of this enzyme can provide knowledge applicable in research leading to new ways of cancer, bacterial and viral disease treatment. Nuclease P1 from *Penicillium citrinum* with 24% sequence identity is probably the closest structural homologue of TBN1, the structure of which is known (PDB ID 1ak0) [2].

Heterologous expression in tobacco leaves yields amounts and quality of the enzyme suitable for structural studies. Crystallization leads to crystals with sufficient quality for X-ray diffraction analysis. First diffraction experiments were performed using in house Gemini Enhanced Ultra diffractometer with the Atlas CCD detector and two different crystal morphologies were identified (orthorhombic and rhombohedral). Datasets for structural analysis were collected at the synchrotron source BESSY, beam line BL14.1, detector MARmosaic CCD 225 and Rigaku rotating anode FR-E^+ SuperBright with dual wavelength anode at copper wavelength, CCD detector Saturn A200. Presence of zinc in the protein was confirmed by an absorption edge scan at the synchrotron. A partial MAD dataset was collected at BESSY (Helmholtz-Zentrum Berlin) and attempts to solve the phase problem were performed using the SHELXC, D and E program suite [3].

1. Matousek J., Kozlova P., Orctova L., Schmitz A., Pesina K., Bannach O., Diermann N., Steger G., Wiesner D. (2007). Accumulation of viroid-specific small RNAs and increase in nucleolytic activities linked to viroid-caused pathogenesis. *Biol. CHem*., **388**, 1–13.
2. Romier C., Dominguez, R., Lahm, A., Dahl, O., Suck, D. (1998). Recognition of single-stranded DNA by nuclease P1: high resolution crystal structures of complexes with substrate analogs. *Proteins* **32**, 414–424.
3. Sheldrick, G.M. (2008). "A short history of SHELX", *Acta Crystallog*r. **D64**, 112-122.

The work on this project was supported by the Czech Science Foundation, projects no. 310/09/1407, 202/06/0757 and.521/09/1214 and by the European Commission under ELISA grant agreement number 226716 (synchrotron access funding, projects 09.1.81077 and 09.2.90262). We also gratefully acknowledge support from Praemium Academiae of AS CR and Institution research plan AVOZ 10100521 of the Institute of Physics AS CR. The authors wish to thank Dr. Uwe Müller of the Helmholtz-Zentrum Berlin, Albert-Einstein-Str. 15 for support at the beam line BL14.1 of Bessy II.

P18

CONFORMATIONAL BEHAVIOR OF BETA AMYLOIDS AND THEIR INTERACTIONS WITH CHOLESTEROL DERIVATIVES

Zdeněk Kříž[1], Zdena Krištofíková[2], Jaroslav Koča[1]

[1]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic, zdenek@chemi.muni.cz
[2]Prague Psychiatric Centre, Ústavní 91, 181 03 Praha 8 – Bohnice, Czech Republic

Proteins play a critical role in most cellular processes, from signal transduction to enzyme catalysis. Folding to the correct three-dimensional native state is crucial to their function. Under pathological conditions, proteins can misfold, typically to structures in which the hydrophobic residues, which form the hydrophobic core of the folded protein, are exposed to the solvent. These misfolded proteins can self-assemble into a variety of aggregate structures, including large, insoluble fibrillar entities known as amyloids. A number of diseases, including Alzheimer´s disease (AD)

and type II diabetes, are associated with the presence of amyloid [1]. Extracellular proteic plaques found in the brains of patients affected by Alzheimer´s disease contain fibrils composed of -amyloid (A) peptides. These range in length from 39 to 43 amino acids, the most abundant form being A -(1-42). The A -(1-42) peptide nucleates and aggregates more rapidly than shorter A peptides [2,3]. It indicates that the C-terminus sequence is critical for the nucleation of amyloid formation and suggesting that production of A -(1-42) may be pathogenic.

Recently we have performed series of MD simulations on rat and human amyloid beta. The AMBER molecular modeling package was used for MD simulations and analyses of results. The NMR solved structure of human A -(1-42) peptide from the PDB database (pdb code 1z0q) was used as starting point of the simulations. The rat A -(1-42) peptide have been prepared using the Triton and Modeller software from human A -(1-42) peptide by in silico mutation method. Each molecule was immersed into octahedral simulating box with TIP3P water molecules (minimal thickness of water layer was 12 A) and counter ions were added to neutralize electrostatic charge of the molecule. We prepared three different simulation conditions with concetration of NaCl 0.00, 0.15 and 0.30 mol/l. The AMBER force field ff03 was used for all simulations. The snapshots from the trajectories were used for docking studies of interactions with 24S-hydroxycholesterol – cerebrosterol. The docking studies have been performed by the DOCK 6.3 software[4].

The results show the differences in conformational behavior of peptides in solvent with different ionic strength and different ability to interact with cholesterol derivatives.

This work has been supported by the Czech Science Foundation (305/09/0457) and by the Ministry of Education of the Czech Republic (MSM0021622413, LC06030). The access to the METACentrum supercomputing facilities provided under the research intent MSM6383917201 is highly appreciated.

1. M. G. Krone, L. Hua, P. Soto, R. Zhou, B. J. Berne, J.-E. Shea, *J. Am. Chem. Soc.,* **130**, (2008), 11066.
2. L. L. Iversen, R. J. Mortishire-Smith, S. J. Pollack, M. S. Sherman, *Biochem. J.*, **311**, (1995), 1.
3. S. Tomaselli, V. Esposito, P. Vangone, N. A. van Nuland, A. M. J. Bonvin, R. Guerrini, T. Tancredi, P.A. Temussi, D. Picone, *ChemBioChem*, **7**, (2006), 257.
4. P. T. Lang, S. R. Brozell, S. Mukherjee, E. T. Pettersen, E. C. Meng, V. Thomas, R. C. Rizzo, D. A. Case, T. L. James, I. D. Kuntz, *RNA,* **15,** (2009) 1219.

P19

RAMAN OPTICAL ACTIVITY AND CONFORMATION OF STRUCTURALLY IMPORTANT GROUPS IN PEPTIDES AND PROTEINS

M. Kubáňová[1], L. Bednárová[2], P. Maloň[2], V. Baumruk[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, Prague 2, CZ-121 16, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo nám 2, 166 10, Prague, Czech Republic
kubanova@karlov.mff.cuni.cz

Raman optical activity (ROA) represents a modern spectroscopic technique that can be applied to a wide range of chiral molecules starting from small organic molecules up to complex biomolecular systems [1]. Among other things ROA provides information about solution structure of peptides and proteins [2]. The aim of this work was to determine the relationship between three-dimensional structure and Raman optical activity of disulphide and amide groups in peptides. Characteristic band patterns of the polyproline II conformation (left-handed 3_1-helix) were found in the ROA spectra of poly(Pro-Gly-Pro), oxytocin and hinge peptide linked to the antigen sequence. ROA signal in the S-S and C-S stretching region was observed in ROA spectra of model cyclodextrin compounds connected with disulphide bonds [3]. Positive ROA band in the S-S stretching region was found in the ROA spectrum of oxytocin (the peptide with one S-S bridge). According to the theoretical studies of model disulphides, positive ROA signal in this region indicates positive dihedral angle C -S-S-C . This result is in agreement with the crystal structure. We have also worked on extension of ROA measurements to the hydrogen stretching region (2500–3200 cm^{-1}).

1. L. D. Barron, L. Hecht, I. H. McColl, E. W. Blanch, *Mol. Phys.* **102** (2004), 731-744.
2. L. D. Barron, E. W. Blanch, L. Hecht, *Adv. Protein Chem.* **62** (2002), 51-90.
3. P. Maloň, L. Bednárová, M. Straka, L. Krejčí, L. Kumprecht, T. Kraus, M. Kubáňová, V. Baumruk, *Chirality* (2010) accepted 2010.

The Grant Agency of the Czech Republic is acknowledged for the support (projects 203/07/1335 and P205/10/1276).

P20

2-DEOXY-2-FLUOROHEXOSES AS INHIBITORS OF GLYCOSYL HYDROLASES

M. Krupička, I. Tvaroška

Slovak Academy of Sciences, Institute of Chemistry, Dubravska cesta 9, Bratislava, Slovak Republic
chemmakr@savba.sk

The 2-deoxy-2-fluorohexoses are known to act as inhibitors for glycosyl hydrolases and are widely used to obtain the crystal structure of enzyme with substrate. The question has been raised, whether the stabilization of reaction intermediate is result of enzyme-substrate interaction, or the substrate itself is stabilized and does not undergo further reaction steps.

The reaction was modeled as acid catalyzed hydrolysis, where the protonated intermediate and oxocarbenium ion were considered. The relative stability of these intermediates and the differences in charge distribution are discussed. The properties of intermediates are calculated at B3LYP/6-311++G(d,p), the enzymatic environment is omitted.

It was found that both reaction intermediates are less stable for the 2-deoxy-2-fluoro substrates. The energy difference is in order 6-8 kcal/mol, and this suggests two modes of action for the inhibitor, (i) higher barrier towards the protonation and (ii) electron-withdrawing properties of the fluorine atom destabilizing the oxocarbenium intermediate.

This work has been supported by MRTN-CT-2006-035866 (REVCAT) and VEGA 2/0176/09.

P21

THE -GALACTOSIDASE TYPE A GENE FROM *Aspergillus niger* ENCODES A FULLY FUNCTIONAL -N-ACETYLGALACTOSAMINIDASE

Natallia Kulik[a,b], Lenka Weignerová[c], Tomáš Filipi[c], Petr Pompach[d], Petr Novák[d], Hynek Mrázek[e], Karel Bezouška[d,e], Vladimír Křen[c], and Rüdiger Ettrich[a,b]

[a]Center for Biotransformation and Biocatalysis, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zámek 136, 373 33 Nové Hrady
[b]Institute of Physical Biology, University of South Bohemia, Zámek 136, 373 33 Nové Hrady
[c]Center for Biotransformation and Biocatalysis, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4
[d]Institute of Microbiology, Laboratory of Molecular structure characterization, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4
[e]Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, CZ-12840 Prague 2

Two genes in the genome of from *Aspergillus niger*, *aglA* and *aglB,* are up to now assigned to encode for two -galactosidases, an A and B variant. However, a careful structural analysis based on structural models of these two enzymes revealed significant differences of the active centers, which contributes to the understanding of the specificity of the hydrolyzed carbohydrates.

To confront this unexpected result with experimental data, purified protein from *Aspergillus niger* CCIM K2, that exhibited both -galactosidase and -*N*-acetylgalactosaminidase activity, was sequenced and could be assigned to the enzyme encoded by the gene *aglA*. In accordance with enzyme activity measurements, substrate docking clearly demonstrates the preference of the identified enzyme for -D-*N*-acetylgalactosamine over galactose and enzyme activity measurements in the presence of thiazoline((3aR,5R,6R,7R,7aR)-(6,7-dihydroxy-5-(hydroxymethyl- 2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole) suggests for this -*N*-acetylgalactosaminidase an analogous mechanism as homologous -galactosidase.

Thus, we provide evidence that the -galactosidase type A gene *aglA* from *Aspergillus niger* encodes in fact a fully functional -*N*-acetylgalactosaminidase.

This work supported by the Academy of Sciences (AVOZ50200510, AVOZ60870520), the Ministry of Education of the Czech Republic (LC 06010, LC 7017, MSM6007665808, MSM_21620808) and by the Grant Agency of the Czech Republic (204/06/0771).

P22

CRYSTALLIZATION AND CRYSTALLOGRAPHY ANALYSIS OF HALOALKANE DEHALOGENASE MUTANT DHAA12 FROM *Rhodococcus rhodochrous*

Maryna Lahoda[a], Tana Koudelakova[b], Jiri Damborsky[b] and Ivana Kuta Smatanova[a,c]

[a]*Institute of Physical Biology University of South Bohemia Ceske Budejovice, Zamek 136, 373 33 Nove Hrady, Czech Republic*
[b]*Loschmidt Laboratories, Faculty of Science, Masaryk University, Kamenice 5/A4, 62500 Brno, Czech Republic*
[c]*Institute of Systems Biology and Ecology Academy of Science of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic*

Haloalkane dehalogenases comprise a group of enzymes that hydrolyse carbon-halogen bounds in a wide range of haloalkanes [1]. DhaA12 mutant contains several of amino acid residues typical for haloalkane dehalogenase DbjA that were introduce into DhaA. DbjA shows significant enantioselectivity with selected substrates in contrast to DhaA that is showing no enantioselectivity [2]. Enantioselectivity plays an important role in cleaning substratum in the production of pharmaceuticals and agrochemicals. The aim of creating the DhaA12 enzyme mutant was to enhance the enantioselectivity of DhaA to the extent of the DbjA enantioselectivity. The goal of this project is to solve the protein structure to understand changes in their enantioselectivity and possibly to design new mutants.

DhaA12 protein was crystallized using sitting-drop vapor-diffusion technique [3]. Crystals of DhaA12 grew at room temperature in the crystallization solution containing 20% PEG 4000, 100mM MES Sodium salt, pH 6.1. Crystallization experiments have been performed in Combi Clover Crystallization Plates (EBS plate, Emerald BioStructures, WA, USA). Diffraction data for DhaA12 was collected at the beamline 14.1 of BESSY (Berlin) to the resolution of 1.68 Å. Crystals of mutant belong to the orthorhombic space group $P2_12_12_1$. The known structure of the haloalkane dehalogenase from *Rhodococcus sp*. [4] was used as a template for molecular replacement.The process of the DhaA12 structure refinement is in progress.

1. Martina Pavlova, Martin Klvana, Zbynek Prokop, Radka Chaloupkova, Pavel Banas, Michal Otyepka, Rebecca C Wade, Masataka Tsuda, Yuji Nagata, Jiri Damborsky Redesigning dehalogenase access tunnels as a strategy for degrading an anthropogenic substrate Nature Chemical Biology 5, 727 - 733 (2009).
2. Sato, Y., Monincova, M., Chaloupkova, R., Prokop, Z., Ohtsubo, Y., Minamisawa, K., Tsuda, M., Damborsky, J., Nagata, Y., 2005: Applied and Environmental Microbiology 71: 4372-4379.
3. Hiroaki Adachi, Kazufumi Takano, Masaaki Morikawa, Shigenori Kanaya, MasashiYoshimura, Yusuke Mori, Takatomo Sasaki Application of a two-liquid system to sitting-drop vapor-diffusion protein crystallization Biological Crystallography ISSN 0907-4449.
4. Newman, J., Peat, T. S., Richard, R., Kan, L., Swanson, P. E., Affholter, J. A.,Holmes, I. H., Schindler, J. F., Unkefer, C. J. & Terwilliger, T. C. (1999).Biochemistry, 38, 16105–16114.

This work is supported by the Ministry of Education of the Czech Republic (MSM6007665808, LC06010) and the Academy of Sciences of the Czech Republic (AV0Z 60870520).

P23

CHIROPTICAL PROPERTIES OF THE DISULFIDE GROUP

P. Maloň,[1] L. Bednárová,[1] M. Kubáňová[1], M. Urbanová[2] and V. Baumruk[3]

[1]*Institute of Organic Chemistry and Biochemistry, 166 10 Prague 6,*
[2]*Institute of Chemical Technology, 166 28 Prague 6*
[3]*Faculty of Mathematics and Physics, Charles University, 121 16 Prague 2*
malon@uochb.cas.cz

Disulfide group is an important structural constituent of peptides and proteins. It is a part of definition of protein primary structure, although it can be created /broken much easier than the general peptide chain forming linkage – the amide. Despite its evident importance for the peptide /protein structure the relation of its stereochemistry and spectroscopic properties is still not known at full detail.

We present here a comparison of our calculations on simple model disulfides [1] with experimental data obtained by spectroscopic investigation (especially chiroptical) of several rigid and semi-rigid model compounds. These include disulfide-bridged cyclodextrins [2] and neurohypophyseal hormone analogs [3]. We have attempted electronic spectroscopy and vibrational spectroscopy including their chiroptical variants – circular dichroism (ECD, VCD) and Raman optical activity (ROA).

1. L. Bednárová, P. Bouř and P. Maloň, *Chirality*, accepted 2009.
2. P. Maloň, L. Bednárová, M. Straka, L. Krejčí, L. Kumprecht, T. Kraus, M. Kubáňová, V. Baumruk, *Chirality*, accepted 2010.
3. P. Maloň, L. Bednárová, H. Dlouhá, M. Flegel, J. Hlaváček, V. Setnička, M. Urbanová, S. Hynie, V. Klenerová, M. Kubáňová, V. Baumruk, *Coll.Czech.Chem.Commun.*, submitted 2010.

This work was supported by Grant Agency of the Czech Republic (no. 203/07/1335 and P205/10/1276).

P24

CRYSTALLIZATION OF SPOIISA TOXIN AND SPOIISB ANTITOXIN FROM DIFFERENT *Bacilli* SPECIES

Patrik Florek[1], Jana Melničáková[1], Anthony J. Wilkinson[2], S. Rešetárová[1] and Imrich Barák[1]

[1]*Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava*
[2]*Department of Chemistry, University of York, York, UK*

SpoIISA-SpoIISB killer-antikiller system was originally found in *B. subtilis* and its homologues were identified also in *B. cereus* and several other relative *Bacilli.* In absence of SpoIISB protein, activity of SpoIISA toxin blocks *B. subtilis* sporulation process in early stages and its forced expression during vegetative growth leads to cell lysis both in *B. subtilis* and *E. coli* [1].

SpoIISA protein (248 aa) was proposed to comprise two domains – N-terminal, which is formed by three membrane spanning helices and globular C-terminal part, which contitutes negatively charged cytosolic domain (C-SpoIISA). SpoIISB (56 aa), on the other hand, is basic, hydrophilic protein, which was shown to bind SpoIISA through its predicted cytosolic domain [2].

In our previous work we prepared crystals and solved molecular structure of C-SpoIISA–SpoIISB complex, where we observed binding of two SpoIISB monomers to opposite sites of C-SpoIISA dimer [unpublished data].

Here, to further understand mechanism of regulation of SpoIISA toxicity, we purified and crystallized cytosolic domain of SpoIISA protein without its cognate antitoxin partner. For now, these crystals were successfully tested for diffraction, but the further optimization of the crystallization conditions and search for better cryoprotectants is still required. We also determined expression level of SpoIISA protein during life cycle of *B. subtilis*. Moreover, we focused on SpoIISA-SpoIISB killer-antikiller system in *B. cereus* and analyzed SpoIISA toxic activity in *E. coli*. We purified protein complex of cytosolic domain SpoIISA and SpoIISB for further crystallization experiments.

1. E. Adler, I. Barák, P. Stragier. *J. Bacteriol.*, **183** (2001) 3574-3581.
2. P. Florek, K. Muchová, P. Pavelčíková, I. Barák. FEMS Microbiol. Lett. **278** (2008) 177-184.

This work was supported by the grant APVT-51-027804, No. ESF-EC-0106, LPP-0218-06 and VEGA grant 2/7007 /27 from the Slovak Academy of Sciences and The Wellcome Trust Grant 082829/Z/07/Z.

P25

IN SILICO MUTAGENESIS AND DOCKING STUDY OF RSL LECTIN

Sushil K. Mishra[1], J. Adam[1], M. Wimmerová[1], R. Abagyan[2] and J. Koča[1]

[1]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
[2]The Skaggs School of Pharmacy, 9500 Gilman Drive, La Jolla, CA 92093 University of California San Diego, CA, USA

Lectins are proteins showing highly specific and selective binding towards mono or oligosaccharides. This large family of the proteins encompasses many biological functions that involve the deciphering of the sugar code, like cell–cell signalization or host recognition in pathogen infection. Since host carbohydrates are responsible for specific attachment sites for pathogen proteins, it is of great interest to perform the computational study of the binding and nature of interactions in lectin-saccharides complexes.

The gram-negative bacterium *Ralstonia solanacearum* is a pathogen causing lethal wilt in many agricultural crops and it produces a potent L-fucose binding lectin (RSL). This lectin is a trimer of 90 amino acids long chain monomer units and attains six-bladed -propeller structure after oligomerization. RSL lectin shows very strong affinity towards fucose and fucosylated oligosaccharides and in contrary to RS-IIL lectin from *R. solanacearum*, do not have ions in the binding sites but is still able to create stable enough complexes with carbohydrates[1].

This work will be focused on *in silico* mutagenesis, docking of fucose and fucosylated oligosaccharides in RSL lectin. Binding energy pattern of various saccharides was investigated by a series of docking simulations. Docking experiments were performed using the software AutoDock 3, AutoDock Vina, DOCK v.6.2 and ICM Dock [2-5]. Performance of various docking programs will be discussed with respect to binding energy of those saccharides measured from SPR experiment. *In silico* mutants of RSL lectin was prepared using in house developed program TRITON and docking of methyl- -L-fucoside was done to identify the important residues around the binding site and define their contributions[6]. Merits and disadvantages of the docking programs in modeling lectin-saccharide interactions will be discussed in detail. Energetically favorable mutations obtained from the study will be investigated further by computational methods like thermodynamic integration or free energy perturbation and eventually will be determined experimentally.

1. Kostlánová N., Mitchell E., Lortat-Jacob H., Oscarson S., Lahmann M., Gilboa–Garber N., Chambat G., Wimmerová M., Imberty A. *J Biol Chem*. (2005) 280,27839-49.
2. Morris GM., Goodsell DS., Halliday RS., Huey R., Hart WE., Belew RK., Olson AJ. *J. Comput. Chem*., (1998), 19, 1639-1662.
3. Troot O., Olson A.J. *J Comput. Chem*. (2009), 31(2):455-461.
4. Lang P., Moustakas D., Brozell S., Carrascal N., Mukherjee S., Pegg S., Raha K., Shivakumar D., Rizzo R., Case D., Shoichet B., Kuntz I. DOCK, Version 6.2. (2008). University of California, San Francisco.
5. Totrov M., Abagyan R. *Proteins* (1997) 29(0):215-220.
6. Prokop M., Adam J., Kříž Z., Wimmerová M., Koča J. *Bioinformatics* (2008) 24, 1955-6.

This work has been supported by Ministry of Education of the Czech Republic (MSM0021622413, LC06030, ME08008) and the Grant Agency of the Czech Republic (301/09/H004). The research leading to these results has received funding from the European Community's Seventh Framework Programme under grant agreement n° 205872.

P26

DRUG DESIGN OF SELECTIVE 5'-NUCLEOTIDASES INHIBITORS

Petr Pachl[1], Jiří Brynda[1,2], Ivan Rosenberg [2], Milan Fábry[1], Pavlína Řezáčová[1,2]

[1]Institute of Molecular Genetics, Flemingovo nam. 2, Prague 6, Prague, 16610, Czech Republic,
[2]Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nam. 2, Prague 6, Prague, 16610, Czech Republic

The monophosphate 5'-nucleotidases, including 5'(3')-deoxyribonucleotidase, belong to a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates. The ribonucleotides and deoxyribonucleotides could be synthesized *de novo* from low-molecular-weight precursors or by salvage from nucleosides or nucleobases coming from catabolism of nucleic acids[1]. In this salvage pathway, ribonucleotides and deoxyribonucleotides are phosphorylated by nucleoside and nucleotide kinases to maintain sufficient pools of dNTP's and NTP's for synthesis of DNA and RNA. The phosphorylation by cellular nucleoside kinases is opposed by 5'-nucleotidases that dephosphorylate ribo- and deoxyribonucleoside monophosphates[2,3,4]. Besides their role in the regulation of physiological dNTP pools, substrate cycles between ribonucleotidases and kinases may affect the therapeutic

action of pyrimidine nucleoside analogs used as anticancer and antiviral agents. Such compounds require the nucleoside kinases activity for phosphorylation to their active forms. Results of clinical and *in vitro* studies propose that an increase in nucleotidase activity can interfere with nucleoside analogue activation resulting in drug resistance[5].

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

We have prepared 2 types of human 5'-nucleotidase: cytosolic and mitochondrial by recombinant expression in *E. coli*. The inhibitory properties of a series of nucleoside phosphonic acids derivatives are tested and for the most promising compounds the enzyme-inhibitor structure will be determined to serve as a lead for structure-based drug design efforts.

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

1. P. Reichard: Interactions between deoxyribonucleotide and DNA synthesis. *Annu Rev Biochem.,* 57 (1988), 349-74.
2. S.A. Hunsucker, B.S. Mitchell, J. Spychala: The 5'-nucleotidases as regulators of nucleotide and drug metabolism. *Pharmacol Ther*., 107 (2005), 1-30.
3. V. Bianchi, E. Pontis, P. Reichard: Interrelations between substrate cycles and de novo synthesis of pyrimidine deoxyribonucleoside triphosphates in 3T6 cells. *Proc Natl Acad Sci U S A,* 83 (1986), 986-90.
4. P. Bianchi, E. Fermo, F. Alfinito, C. Vercellati, M. Baserga, F. Ferraro, I. Guzzo, B. Rotoli, A. Zanella: Molecular characterization of six unrelated Italian patients affected by pyrimidine 5'-nucleotidase deficiency. *Br J Haematol,* 122 (2003), 847-51.
5. C. Mazzon, C. Rampazzo, M.C. Scaini, L. Gallinaro, A. Karlsson, C. Meier, J. Balzarini, P. Reichard, V. Bianchi: Cytosolic and mitochondrial deoxyribonucleotidases: activity with substrate analogs, inhibitors and implications for therapy. *Biochem Pharmacol,* 66 (2003), 471-9.

P27

THE MOLECULAR DYNAMICS STUDY OF THE DOUBLE-STRANDED RNA-BINDING MOTIVE OF ADAR2 BOUND TO dsRNA

J. Pašulka, J. Koča and R. Štefl

National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlarska 2, Brno, 611 37, Czech Republic

The members of an enzyme family known as ADARs (adenosine deaminases that act on RNA) play an important role in the RNA editing process in higher eukaryotes. ADARs can convert adenosines to inosines within double-stranded structures of their RNA substrates in a site-specific fashion. The mechanism for such site-specific editing remains unclear, albeit several studies have suggested that the specificity could be imposed by the presence of non-canonical elements in the dsRNA structure (e.g. mismatches, bulges, and loops). We study the second double-stranded RNA binding motif of ADAR2 bound to dsRNA with non-canonical elements using molecular dynamics (MD) simulations. Our goal in this work is to explain the role of non-canonical elements in dsRNA and their flexibility for the ADAR2-dsRNA complex.

P28

TWO-DIMENSIONAL RAMAN AND RAMAN OPTICAL ACTIVITY CORRELATION AND FACTOR ANALYSIS OF LYSOZYME FIBRILLATION

T. Pazderka[1], V. Kopecký Jr.[1], K. Hofbauerová[1,2], V. Baumruk[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, Prague 2, CZ-121 16, Czech Republic
[2]Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague 4, CZ-142 20, Czech Republic
pazderka@karlov.mff.cuni.cz

Understanding of processes of amyloid fibrils formation is one of the key tasks in searching for proteins structural origin of human neurodegenerative diseases. Therefore, hen egg white lysozyme (HEWL) can serve as a good model of amyloid fibril formation. Furthermore, this protein is homologous to human lysozyme, which is one of the proteins that cause amyloid diseases [1]. Despite the intense scientific research, studying mechanisms in homologous proteins to lysozyme, the detailed mechanism of fibril formation is still far from complete understanding. Nevertheless, Raman optical activity (ROA) and Raman spectroscopy are very powerful techniques for studies of unfolded proteins. Promising experiments on lysozyme has already been done [2].

Here we present Raman and ROA study using 2D correlation spectroscopy (2DCoS) [3]. Firstly, we model changes of ROA band shapes and positions and investigate characteristic patterns in 2DCoS because the origin of 2D patterns for spectra with positive and negative bands has not been investigated yet. Subsequently, temporal and thermal (from 20 °C to 60 °C) spectral changes in ROA and Raman spectra of HEWL were analyzed by means of factor analysis and 2DCoS. It gave us an opportunity to study delicate details of HEWL fibrillation and denaturation, i.e. sequence of the secondary structure changes upon fibrils formation. Moreover, application of heterospectral 2DCoS enabled to transfer band assignment from Raman spectroscopy to ROA and spectral changes in conformation of S-S bridges were identified in protein ROA spectra for the first time.

1. D. R. Booth, M. Sunde, V. Bellotti et al., *Nature*, **385**, (1997), 787.
2. E. W. Blanch, L. A. Morozova-Roche, D. A. E. Cochran et al., *J. Mol. Biol*., **301**, (2000), 553.
3. I. Noda & Y. Ozaki, Two dimensional correlation spectroscopy: applications in vibrational and optical spectroscopy, (Chichester: Wiley), 2004.

The Grant Agency of the Czech Republic is acknowledged for the support (projects 305/09/0457 and P205/10/1276).

P29

STRUCTURAL STUDY OF LEDGF CELLULAR BINDING PARTNERS

K. Procházková [1], M. Hořejší [2], K. Čermáková [1], P. Řezáčová [1,2]

[1]Institute of Organic Chemistry and BiochemistryAS CR, Flemingovo nam. 2, 166 10, Prague 6, Czech Republic
[2]Institute of Molecular Genetics AS CR, Flemingovo nam. 2, 166 10, Prague 6, Czech Republic
katerina.prochazkova@uochb.cas.cz, rezacova@img.cas.cz

Lens epithelium-derived growth factor p75 (LEDGF/p75) is a prominent interaction partner of human immunodeficiency virus type 1 (HIV) integrase and co-factor of HIV integration. LEDGF/p75 tethers the preintegration complex to the host chromosome and this process is crucial for HIV replication. HIV integrase interacts with the C-terminal part of LEDGF/p75, region designated integrase-binding domain (IBD, amino acids residues 347 - 429). Structural information on interaction between HIV integrase and LEDGF/p75 become an attractive target for design of small molecule inhibitors blocking this interaction [1].

While the role of LEDGF/p75 in HIV integration is well characterized, very little is known about its physiological function. As a transcriptional co-activator, LEDGF/p75 is implicated not only in HIV replication, but also in human cancer and autoimmunity. The LEDGF/p75 was shown to interact through its IBD with several cellular proteins and recent evidence implies that LEDGF/p75 is a general adaptor protein tethering various factors to chromatin [2].

In this work, we set to prepare two LEDGF/p75 physiological binding partners JPO2 [2] and pogo transposable element (pogZ) [3]. The IBD, interaction domain of LEDGF/p75 was cloned and expressed in *E. coli*. The protein was isolated from inclusion bodies and purified with yields sufficient for binding studies. The JPO2 was cloned as a full-length protein, expressed in *E. coli* and optimization of purification procedure is in progress now. From pogZ, the DDE domain responsible for interaction with

LEDGF/p75 was cloned, expressed in *E. coli* and purified from the cytosol. The DDE domain of pogZ (residues 1117-1323) is a putative catalytic domain comprising Asp and Glu residues in the catalytic site. Pre-crystallization analyses and crystallization trials were initiated.

The aim of our study is to obtain structural information on the LEDGF/p75 interaction with its physiological binding partners JPO2 and pogZ, respectively. Such structural information is essential for understanding the LEDGF/p75 biological role and might help in design of inhibitors selectively blocking interaction with HIV integrase while not interfering with the LEDGF/p75 biological function.

1. P. Cherepanov, A. L. Ambrosio, S. Rahman, T. Ellenberger, A. Engelman, *Proc. Natl. Acad. Sci. U.S.A.,* **102**, (2005), 17308.
2. G. N. Maertens, P. Cherepanov, A. Engelman, *J Cell Sci* **119,** (2006), 2563.
3. K. Bartholomeeusen, F. Christ, J. Hendrix, J. C. Rain, S. Emiliani, R. Benarous, Z. Debyser, R. Gijsbers, J. De Rijck*, J. Biol. Chem.,* **284**, (2009), 11467.

This work was supported by grant from the FP7 framework of the European Union (THINC, HEALTH-2007-2.3.2-1) and in part by projects nos. AV0Z40550506 and AV0Z50520514 awarded by the Academy of Sciences of the Czech Republic.

P30

RAMAN OPTICAL ACTIVITY STUDY OF POLY-L-PROLINE CHAINS OF VARIOUS LENGTHS

V. Profant[1]*, M. Šafařík[2], P. Bouř[2], V. Baumruk[1]

[1]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, 121 16, Prague, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo nám 2, 166 10, Prague, Czech Republic
profant@karlov.mff.cuni.cz

Raman scattering and Raman optical activity (ROA) [1, 2] techniques were used for study of oligo- and poly-L-proline samples. Specifically, formation of polyproline II (PPII) helical conformation was studied in dependence on the increasing chain length N of the (L-proline)N sample. Although PPII conformation is believed to be the main content of so-called random coil structure [3], it remains not to be completely characterized so far and problematic of its creation has not been thoroughly studied yet.

The whole study was strongly related to previous experiments [4], which were focused on the characterization of proline side chain conformation and its interaction with solvent.

For the analysis, five oligopeptide of different lengths (N=2,3,4,6,12) and three polypeptide samples of different mean molecular weight were used for preparation of highly concentrated water solution. Raman scattering and ROA spectra were measured in a wide frequency range between 120 cm^{-1} and 1800 cm^{-1} and analysed with respect to the main peptide chain conformation.

Due to high sensitivity of the ROA technique to the conformational stability and rigidity of peptide chain we were able to determine the characteristic spectral peaks associated with formation of stable PPII helical conformation in studied systems. The most relevant peaks are located at 405, 535 and 945 cm^{-1}. Additionally, we were able to determine the minimal length of (L-proline) N chain necessary for creation of the stable PPII conformation as N=6.

1. P.W. Atkins, L.D. Barron, *Mol. Phys*. **16**, (1969), 453-466.
2. L.D. Barron, M.P. Boggard, A.D. Buckingham, *Nature* **241**, (1973), 113-114.
3. Z. Shi, R.W. Woody, N.R. Kallenbach, *Adv. Prot. Chem.* **62**, (2002) 163-240.
4. J. Kapitán, V. Baumruk, P. Bouř, *JACS* **128**, (2006), 2438-2443.

The work was supported by the Grant Agency of the Czech Republic (grant No. 202/07/0732) and by the Ministry of Education of the Czech Republic (MSM 0021620835).

P31

CRYSTALLISATION AND STRUCTURE-FUNCTIONAL ANALYSIS OF A NOVEL HALOALKANE DEHALOGENASE DbeA FROM *Bradyrhizobium elkani* USDA94

Tatyana Prudnikova[a], Tomas Mozga[c], Pavlina Rezacova[e,f], Radka Chaloupkova[c], Yukari Sato[g], Michal Kuty[a,b], Tana Koudelakova[c], Yuji Nagata[g], Jiri Damborsky[c,d], and Ivana Kuta Smatanova[a,b]

[a]Institute of Physical Biology University of South Bohemia Ceske Budejovice, Zamek 136, 373 33 Nove Hrady, Czech Republic
[b]Institute of Systems Biology and Ecology Academy of Science of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic
[c]Loschmidt Laboratories, Institute of Experimental Biology and National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4, 625 00 Brno, Czech Republic
[d]Loschmidt Laboratories, Fac. of Science, Masaryk University, Kamenice 5/A4, 62500 Brno, Czech Rep.
[e]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 37 Prague, Czech Republic
[f]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 37 Prague, Czech Republic
[g]Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Sendai 980-8577, Japan

A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkani* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. To understand the altered activity and specificity of DbeA enzyme, its mutant variant DbeA1, carrying the unique fragment of DbjA, was also constructed. Both the wild type DbeA and the DbeA1 were crystallised using the sitting-drop vapour-diffusion method. The crystals of DbeA belong to the primitive orthorhombic space group $P2_12_12_1$, while the crystals of the mutant DbeA1 belong to the monoclinic space group C2. Crystal structure of a DbeA and DbeA1 has been solved and refined to 2.2 Å resolution. The enzymatic molecular structure of DbeA was compared with those of known haloalkane dehalogenases already deposited in Brookhaven Protein Data Bank.

Grants MECR (LC06010, MSM6007665808) and ASCR (AV0260870520) are acknowledged.

P32

BIOPHYSICAL CHARACTERIZATION OF PHOSDUCIN/14-3-3 PROTEIN COMPLEX

L. Rezabkova[1,2], M. Kacirova[1,2], P. Herman[3], J. Vecer[3], M. Sulc[4], T. Obsil[1,2]

[1]Faculty of Science, Charles University in Prague, 12843 Prague, Czech Republic
[2]Institute of Physiology, Academy of Sciences of the Czech Republic, 14220 Prague 4, Czech Republic
[3]Fac. of Mathematics and Physics, Inst. Physics, Charles University in Prague; 12116 Prague, Czech Rep.
[4]Institute of Microbiology, Academy of Sciences of the Czech Republic; 14220 Prague, Czech Republic
lenka.rezabkova@seznam.cz

Phosducin (Pd) is a regulator of G-protein-mediated signaling that is especially abundant in photoreceptors and pineal gland but expressed in other tissues as well [1]. In photorreceptors, Pd is phosphorylated in dark-adapted retina and undergoes dephosphorylation in response to light. Dephosphorylated Pd binds G , sequestering and translocating it away from disk membrane, which blocks the interaction between G and effectors or reasociation with G subunit [2, 3]. When Pd is phosphorylated at Ser54 and Ser73 it binds the multifunctional 14-3-3 protein. 14-3-3 proteins are a family of acidic regulatory proteins that function as molecular scaffolds by modulating the structure of their binding partners. The biological meaning of the interaction between 14-3-3 and Pd could be to sequester Pd from G subunit or to protect phosporylated Pd from degradation [4, 5].

To elucidate the mechanism of 14-3-3 protein-dependent regulation of phosducin function, we performed biophysical characterization of Pd and Pd/14-3-3 protein complex. We have prepared Pd/14-3-3 complex *in vitro*. Nondenaturating electrophoresis was used to verify that the formation of Pd/14-3-3 complex is phosporyalation dependent. Analytical ultracentrifugation was used to study the oligomerization state of phosducin and the stochiometry of Pd/14-3-3 complex. Various methods of fluorescence spectroscopy have been employed to charac-

terize conformational changes of Pd induced by 14-3-3 protein binding.

1. S. Banner, M. J. Lohse, *Proc Natl Acad Sci USA*, 93 (1996) 10145-50.
2. J. Xu, D. Wu, V. Z. Slepak and M. I. Simon, *Proc Natl Acad Sci U S A*, 92 (1995) 2086-90
3. R. Gaudet, J. R. Savage, J. N. McLaughlin, B. M. Willardson and P. B. Sigler, *Mol Cell*, 3 (1999) 649-60.
4. C. D. Thulin, J. R. Savage, J. N. McLaughlin, S. M. Truscott, W. M. Old, N. G. Ahn, K. A. Resing, H. E. Hamm, M. W. Bitensky and B. M. Willardson, *J Biol Chem*, 276 (2001) 23805-15.
5. K. Nakano, J. Chen, G. E. Tarr, T. Yoshida, J. M. Flynn and M. W. Bitensky, *Proc Natl Acad Sci USA*, 98 (2001) 4693-8.

This work was funded by Grant IAA501110801 of the Grant Agency of the Academy of Sciences of the Czech Republic, by Research Project MSM0021620857 and Centre of Neurosciences LC554 of the Ministry of Education, Youth, and Sports of the Czech Republic, and by Research Project AV0Z50110509 of the Academy of Sciences of the Czech Republic.

P33

DNA DUPLEX MELTING MONITORED VIA LINESHAPE ANALYSIS OF ^{1}H NMR SPECTRA

Václav Římal[1], Helena Štěpánková[1], Josef Štěpánek[2]

Charles University in Prague, Faculty of Mathematics and Physics,
[1]Department of Low-Temperature Physics, V Holešovičkách 2, 180 00 Prague 8, Czech Republic
[2]Institute of Physics, Ke Karlovu 5, 121 16 Prague 2, Czech Republic
stepanko@mbox.troja.mff.cuni.cz

Structural stability of DNA double-helix plays an important role in many biological processes. Nuclear magnetic resonance is capable to provide a detailed insight into the temperature induced base-pair breaking and corruption of regular geometry at particular location of DNA chain. This application requires (in the case of ^{1}H spectra and stationary magnetic field > 7 T) the set of temperature dependent NMR spectra to be analyzed in respect to effect of chemical exchange between the duplex and single-strand states on NMR lineshape. By solving set of Bloch-McConnell differential equations for the case of free induction decay, we have found an analytical formula for the spectral lineshape in the case of chemical exchange between unequally populated states with different relaxation rates. This allows the experimental spectra to be straightly fitted by this expression without long-term numerical calculations. This approach was applied to NMR spectra of aromatic protons in self-complementary DNA octamers, d(GATGCATC) and d(CTTGCAAG), measured in a broad temperature range covering the duplex melting. For every nucleobase we obtained independently temperature profiles of the equilibrium constant and the kinetic association constant. These characteristics were found as sensitive indicators of local deviations from the overall duplex properties.

P34

CLONING AND EXPRESSION OF PUTATIVE HELIKASE LHR FROM *E.coli* K12

Katsiaryna Shamayeva, Vitali Bialevich, Eva Csefalvay and Rudiger Ettrich

Department of Structure and Function of Proteins, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, and Institute of Physical Biology, University of South Bohemia in Ceske Budejovice, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic

Gene lhr is widely distributed in the *E .coli* chromosomes, and encoding for of 1,538 amino acids, making it the longest known protein in *E. coli*. Absence of this gene is not essential for *E.coli* growth under various laboratory conditions. The gene (tentatively termed *lhr* for long helicase related) contains the seven conserved motifs of the DNA and RNA helicase superfamily II, which seems like Lhr protein is probable very large helicase [1].

The polymerase chain reaction (PCR) technique has been used to obtain lhr gene from *E. coli* K12 genomic DNA. Optimal conditions for PCR have been found. Lhr gene has been cloned into 2 types of commercial plasmids (*pBluescript* and *pET32a*) to obtain expression system able to produce Lhr protein. Expression of Lhr has been tested in several *E.coli* expression strains.

The aim of our work is to produce and prepare Lhr protein in the amount sufficient for structural studies.

1. Nina Bacher Reuven, Eugene V. Koonin, Kenneth E. Rudd, and Murray P. Deutscher (1995), The Gene for the Longest Known Escherichia coli Protein is a Member of Helicase Superfamily II, Journal of Bacteriology, Oct. 1995, p. 5393–5400.

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 (AVOZ 60870520).

P35

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

Z. Střelcová, P. Kulhánek and J. Koča

National Centre for Biomolecular Research, Masaryk University, Kotlářská 2, CZ-61137 Brno, Czech Republic
stre@chemi.muni.cz

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 together with MutL and MutS enzymes. A mismatch introduced during DNA replication is recognized by MutS enzyme, information about the mismatch is transferred through MutL to MutH enzyme. MutH specifically recognizes the GATC sequence on daughter DNA strand and cleaves this strand close to the G base. Wrongly paired base is removed and after that, the missing parts of DNA can 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 will 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 calcium ions present in crystal structures inhibit the cleavage reaction therefore these structures are assumed as pre-reaction complexes.

The cleavage mechanism is studied on semiempirical level (Amber 10 [3], PDDG/PM3 [4]) considering two possible nucleophiles (H_2O and OH^-). We are comparing two different models of the protein/DNA complexes with both inhibitors (Ca^{2+} ions) and activators (Mg^{2+} ions) of the cleavage reaction. Since the used level of theory gives only rough results, the major outcome of this study will be structure of the post-reaction complex, which has not been solved yet.

The presented work is supported by following grants: The Ministry of Education of the Czech Republic (contracts LC06030, MSM0021622413), Czech Science Foundation (301/09/H004), and the European Community's Seventh Framework Programme under grant agreement n° 205872. The access to the MetaCentrum supercomputing facilities provided under the research intent MSM 6383917201 is highly appreciated.

1. K.G. Au, K. Welsh, P. Modrich, *J. Biol. Chem*., **267**, (1992), 12142-12148.
2. J. Lee, J. Chang, N. Joseph, R. Ghirlando, D. Rao, W. Yang, *Mol. Cell*., **20**, (2005), 155-166.
3. D.A. Case, T. Darden, T. Cheatham, C. Simmerling, J. Wang, R. Duke, R. Luo, M. Crowley, Ross C. Walker, W. Zhang, K.M. Merz, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossváry, K. Wong, F. Paesani, J. Vanicek, X. Wu, S. Brozell, T. Steinbrecher, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, D. Mathews, M. Seetin, C. Sagui, V. Babin, P.A. Kollman, Amber 10, University of California, San Francisco (2008).
4. M.P. Repasky, J. Chandrasekhar, W.L. Jorgensen, *J. Comput. Chem.*, **23**, (2002), 1601-1622.

P36

CRYSTALLIZATION AND PRELIMINARY X-RAY ANALYSIS OF DhaA WILD TYPE AND DhaA13 PROTEINS FROM *Rhodococcus rhodochrous*

A. Stsiapanava[1], R. Chaloupkova[2], A. Jesenska[2], J. Brynda[3], J. Damborsky[2] and I. Kuta Smatanova[1,4]

[1]Institute of Physical Biology University of South Bohemia Ceske Budejovice, Zamek 136, 373 33 Nove Hrady, Czech Republic
[2]Loschmidt Laboratories, Institute of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5/A4, 62500 Brno, Czech Republic
[3]Institute of Molecular Genetics, Academy of Science of the Czech Republic, Flemingovo nam. 2, CZ-16637 Prague 6, Czech Republic
[4]Institute of Systems Biology and Ecology Academy of Science of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic

Haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064 is a bacterial enzyme showing catalytic activity for the hydrolytic conversion of highly toxic industrial pollutant 1,2,3-trichloropropane (TCP) [1,2]. DhaA13 protein variant, carrying mutation H272F in the catalytic histidine, was prepared to catch the protein in a complex with alkyl-enzyme intermediate. This mutant variant binds substrate to the active site, catalyses the first reaction step leading to the formation of the alkyl-enzyme intermediate, but it is not able to convert it

further to the product. The main goal of this project is to solve and compare structures of DhaA13 and DhaAwt proteins with two different ligands; environmental pollutant TCP and fluorescence probe coumarine.

DhaAwt and DhaA13 were crystallized using the sitting-drop vapor-diffusion technique [3]. Crystals of DhaAwt grew in the crystallization solution containing 6 % isopropanol and were measured at home diffractometer (IMG, Prague). These crystals diffracted to 1.7 Å. Other crystals of DhaAwt were grown in the solution containing 11 % isopropanol. These crystals and crystals of DhaA13 soaked in the solution with coumarine were used for synchrotron diffraction measurements at the beamline X12 (EMBL/DESY, Hamburg). Diffracted data for both DhaAwt and DhaA13 crystals were collected to the resolutions limit of 1.26 Å and 1.33 Å, respectively. Protein crystals of DhaAwt obtained in the solution containing TCP and crystals of DhaA13 soaked in the solution of TCP diffracted to ultrahigh resolutions of 1.04 Å and 0.97 Å, respectively. This diffraction data were collected at the beamline 14.1 (BESSY, Berlin). All structures were solved by molecular replacement in monoclinic space group *P1*. The refinement for DhaAwt and DhaA13 mutant models is currently in progress.

1. T. Bosma, E. Kruizinga, E. J. D. Bruin, G. J. Poelarends & D. B. Janssen, *Appl. Environ. Microbiol*. **65**, (1999), 4575–4581.

2. J. F. Schindler, P. A. Naranjo, D. A. Honaberger, C.-H., Chang, J. R. Brainard, L. A. Vanderberg & C. J. Unkefer, *Biochemistry* **38**, (1999), 5772–5778.

3. A. Ducruix & R. Giegé, *Crystallization of Nucleic Acids and Proteins: A Practical Approach,* 2nd ed. Oxford: Oxford University Press, (1999).

This work is supported by the Ministry of Education of the Czech Republic (MSM6007665808, LC06010) and the Academy of Sciences of the Czech Republic (AV0Z 60870520).

P37

CRYSTALLIZATION STUDY OF THE IRON-REGULATED PROTEIN FrpD FROM *Neisseria meningitidis*

Ekaterina Sviridova[1], Ladislav Bumba[2], Pavlina Rezacova[3,4], Peter Sebo[2,5], Ivana Kuta Smatanova[1,6]

[1]*Institute of Physical Biology USB CB, Zamek 136, 373 33 Nove Hrady, Czech Republic*
[2]*Institute of Microbiology AS CR, Videnska 1083, 142 20 Prague, Czech Republic*
[3]*Institute of Organic Chemistry and Biochemistry, Flemingovo nam. 2, 166 10 Prague, Czech Republic*
[4]*Insitute of Molecular Genetics AS CR Flemingovo nam. 2, 166 10 Prague, Czech Republic*
[5]*Institute of Biotechnology AS CR, Videnska 1083, 142 20 Prague, Czech Republic*
[6]*Institute of Systems Biology and Ecology AS CR, Zamek 136, 373 33 Nove Hrady, Czech Republic*

Introduction:
Neisseria meningitidis is a highly diverse facultative bacterial pathogen. In most instances it colonizes its human host without causing disease. Its occasional invasion, however, can entail devastating diseases like septicemia or meningitis. Molecular basis of meningococcal virulence remains difficult to analyze. However, several traits potentially required for virulence of meningococci have been identified, including presence of several iron acquisition systems.

Under conditions of limited iron availability, *N. meningitidis* produces Fe-regulated proteins, 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 (Repeat in toxins) 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.

The iron-regulated protein FrpD was identified as a *N. meningitidis* outer membrane lipoprotein. FrpD is highly conserved in a set of meningococcal strains and its primary amino acid sequence does not exhibit any similarity to known protein sequences of other organisms. The *frpdD* gene has the potential to encode two protein variants the 271-residue long FrpD sequence ($FrpD_{271}$) and the 250-residue short sequence ($FrpD_{250}$). $FrpD_{271}$ is synthesized with a type II signal peptide for export across the cytoplasmic membrane. Then it is posttranslationally modified by a lipid molecule and targeted to the outer bacterial membrane. $FrpD_{250}$ lacks the signal peptide and possibly remains in the cytoplasm. The exported lipidated FrpD, as well as its recombinant non-exported $FrpD_{250}$ protein are both able to bind the N-terminal portion of FrpC (within first 300 residues) with very high affinity (apparent K_d = 0.2 nM). FrpD may serve as an accessory lipoprotein involved in anchoring of the secreted FrpC protein to the outer bacterial membrane.

Results and discussion:
This project is aimed to determine the structure of FrpD protein and to perform the structural characterization of FrpD. Our preliminary results showed the full version of $FrpD_{250}$ protein can not be crystallized. Therefore, we performed a specific truncation of 21 amino acid residues from N-terminus of $FrpD_{250}$ protein. The recombinant, truncated version (lacking the first 21 amino acid residues from N-terminus) $FrpD_{250}$ protein was expressed in non-methionine auxotrophic *Escherichia coli* BL21 DE3 cultivated on the media containing selenomethionine (Se-Met). Se-Met derived FrpD protein was purified using a combination of metal affinity and gel-filtration chroma-

tography. 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 ?. The diffraction data will be used to determine the structure of FrpD by single/multiple anomalous diffraction (SAD/MAD) method.

1. N.E. Rosenshtein, B.A. Perkins, D.S. Stephens, T. Popovic, and J.M. Hughes, *The New England Journal of Medicine*, **344**, (2001), 1378-1388.

2. Y. L.Tzeng, D. S. Stephens, *Microbes and Infection*, **2**, (2000), 687–700.K. Prochazkova, R. Osicka, I. Linhartova,

3. P. Halada, M. Sulc, and P. Sebo, *The Journal of Biological Chemistry*, **280**, (2005), 3251-3258.

This project was supported by grants MSM6007665808 and LC06010 (Ministry of Education of the Czech Republic), AVOZ60870520 (Academy of Sciences of the Czech Republic) and GACR 310/06/0720.

P38

EXPLORING THE ACTIVE SITES OF PLANT AMINOALDEHYDE DEHYDROGENASES USING NATURAL AND SYNTHETIC SUBSTRATES

Jan Frömmel[1], Hana Moskalíková[1], Miroslav Soural[2], Martina Tylichová[1], David Kopečný[1] and Marek Šebela[1]

[1]Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic
[2]Department of Organic Chemistry, Faculty of Science, Palacký University, tř. 17. listopadu 1192/12, CZ-771 46 Olomouc, Czech Republic.
E-mail: marek.sebela@upol.cz

Aminoaldehyde dehydrogenases (AMADH, EC 1.2.1.19) catalyze the terminal step in polyamine catabolism by oxidizing compounds like 4-aminobutyraldehyde (ABAL) and 3-aminopropionaldehyde (APAL). This NAD^+-dependent oxidative reaction produces omega-amino acids related to the processes of cellular defense against stress events caused for example by increased drought and salinity (either as direct mediators or precursors). Based on indirect evidences, the enzyme also seems to participate in the production of carnitine in plants. AMADHs belong to the large enzyme superfamily of aldehyde dehydrogenases. In this work, isoenzymes 1 and 2 of plant AMADHs from pea, tomato and maize were obtained as pure recombinant proteins by expression of the respective genes in *Escherichia coli* followed by affinity purification. There were also eight mutants of the pea AMADH2 available, which had been prepared by site-directed mutagenesis of active-site residues. To characterize the difference in their substrate specificity, the enzymes were subjected to a large kinetic study with natural and synthetic aminoaldehyde and aldehyde substrates. The studied compounds comprised C2-C4 omega-aminoaldehydes and their *N*-methylated, hydroxy and guanidino analogs, plus *n*-alkyl aldehydes (C2-C7). For the activity measurements, there were also position isomers available of pyridine-carbaldehyde, 3-pyridinylpropanal and (pyridinylmethyl)lamino aldehydes together with a series of (9*H*-purin-6-ylamino) aldehydes, (7*H*-pyrrolo[2,3-d]pyrimidin-4-ylamino) aldehydes and (pyrimidin-2-ylamino) aldehydes. A majority of the studied AMADHs accepted APAL or ABAL as the best substrates and well oxidized their substitution derivatives. One of the mutants of pea AMADH2 behaved as a non-specific aldehyde dehydrogenase oxidizing preferentially capronaldehyde and other *n*-alkyl or aromatic aldehydes known as less effective substrates for the wild-type enzyme. Another enzyme showed a significant preference for aromatic aldehydes even though ABAL was the best substrate. The crystal structures of pea AMADH1 and AMADH2 together with structural models of the other enzymes were used to discuss the observed differences in substrate properties.

This work was supported by grant 522/08/0555 from the Czech Science Foundation.

P39

COMPLEXES OF -GALACTOSIDASE WITH LIGANDS

Andrea Štěpánková[1, 2], Tereza Skálová[1], Jan Dohnálek[1], Jarmila Dušková[1], Tomáš Koval[1], Jindřich Hašek[1], Petra Lipovová[3]

[1]Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovského nám. 2, 162 00, Prague 6, Czech Rep.
[2]Dept. of Solid State Physics, FNSPE, CTU, Trojanova 13, 120 00, Prague 2, Czech Republic
[3] Dept. of Biochemistry, ICT, Technická 5, 166 28, Prague 6, Czech Republic

The three-dimensional structures of enzyme -galactosidase [1] from an Antarctic bacterium Arthrobacter sp. C2-2 with bound ligands have been determined at resolutions 2.2 Å, 2.5 Å and 3.3 Å.

Three of the obtained crystals with dimensions 100 – 400 μm were soaked in different ligands and used for X-ray diffraction data collection. D-galactonolactone and isopropyl -D-thiogalactopyranoside (IPTG) are inhibitors and D-galactose is a product of the catalyzed reaction.

X-ray diffraction data were collected at the beam-line ID14.1 of the source of synchrotron radiation ESRF in Grenoble and on an in-house rotating anode diffractometer. The data were processed using HKL2000. All the three crystals belong to the same space group $P2_1$ but packing of hexamers in the crystals differs.

Binding of the reaction product confirms behavior similar to the mesophilic enzyme from E.coli whereas D-galactonolactone interaction with the enzyme brings up some interesting structural changes and related questions. The molecule of inhibitor D-galactonolactone was bound in the active site as D-galactono-1,5-lactone, which is its the least probable isomer [2], despite the fact that the crystal was soaked in an isomer D-(-)-galactono-1,4-lactone. This isomer creates specific bonds to Glu442 and ion Na^+.

The enzyme -galactosidase (EC 3.2.1.23) belongs to the enzyme class called glycosylases which catalyze hydrolysis of the terminal -D-galactosyl moiety of -D-galactosides. It is attractive for research and industry because of its wide range of biotechnological applications (treatment for lactose intolerance, prevention of crystallization in sweet products and increase of sweetening power of saccharides, simpler fermentation during production of soured milk products, etc.). Unlike the more studied -galactosidase from *Escherichia coli* [3], which forms tetramers, the enzyme from Arthrobacter sp. C2-2 forms hexamers with a molecular weight of 660 kDa. Each monomer consists of five domains and contains 1023 residues.

1. Skálová, T., Dohnálek, J., Spiwok, V., Lipovová, P., Vondráčková, E., Petroková, H., Dušková, J., Strnad, H., Králová, B., Hašek, J. (2005). Cold-active -galactosidase from *Arthrobacter* sp. C2-2 forms compact 660 kDa hexamers: Crystal structure at 1.9 Ĺ resolution. *J. Mol. Biol*., **353**, 282-294.
2. Bierenstiel M., Schalf M.: *Eur. J. Org. Chem*., 2004/7,1474 (2004).
3. Jacobson, R. H., Zhang, X.-J., DuBose, R. F., Matthews, B. W. (1994). Three-dimensional structure of -galactosidase from *E. coli*. *Nature*, **369**, 761-766.

This work was supported by GA ČR, project 305/07/1073.

P40

INFORMATION ENTROPY AND BIOLOGICAL MICROSCOPY

Jan Urban, Jan Vaněk, Petr Císař, Tomáš Náhlík and Dalibor Štys

Institute of Physical Biology, University of South Bohemia, Zámek 136, Nové Hrady 3333, CZ

In cell biology experiments there are increasingly popular time-lapse movies created from sequences of thousand of images, captured by digital device using different microscopy techniques in predefined time interval. The captured images become as input for evaluation of their content in preprocessing phase of analysis. Parameters, observed and described in image may produce relevant information in some mathematical model for cell life cycle. Unfortunately there is no general image segmentation method able to recognize cell or even the cell organells, properly in significant number of case. Each experiment require own parametrisation and/or manual selection of important points in image matrix. Those operations are time consuming and may differ to each other by selected order of parameters thresholding or by independent observer.

One of the promising ways for automation whole process of proper parameters selection is using equations for information entropy, defined by Shannon as measure of surprise, generalised by Rényi for conditional probability distributions or Tsalis, Havrda and Charvát (THC) in non-extensive cases. This method is rational also since it properly represents the stochastic nature of the observed signal which in each case is an incomplete representation of inherently stochastic Gibbs energy or, rather, a Rényi- or THC- type of distribution function. In the literature are described many algorithms based on Shannon entropy for one dimensional thresholding and filtering. The more theoretically justified Rényi or THC type distributions are seldom considered. Main practical reason for that is the computational intensiveness.

This work was supported by South Bohemia University grant GAJU 091/2008/P and HCTFOOD A/CZ0046/ 1/ 0008 of EEA funds.

1. I. Glauche, R. Lorenz, D. Hasenclever, I. Roeder (2008): A novel view on stem cell development: Analyzing the shape of cellular genealogies, Cell Proliferation.
2. Yuguang Xiong, Padmini Rangamani1, Benjamin Dubin-Thaler, Michael P. Sheetz, RaviIyengar; A three-dimensional stochastic spatio-temporal model of isotropic phase of cell spreading; *Nature Preceedings* : doi:10.1038/npre.2007.62.2 : Posted 3 Dec 2007.
3. Carpenter et al.: „CellProfiler: image analysis software for identifying and quantifying cell phenotypes “, *Genome Biology*, **7**:R100, 2006.
4. A. Rényi (1961). "On measures of information and entropy". Proceedings of the 4th Berkeley Symposium on Mathematics, Statistics and Probability 1960: 547-561.
5. P.Jizba, Toshihico Arimitsu; The World according to Renyi: Thermodynamics of multifractal systems. Annals Phys.312:17-57,2004.
6. A.O. Hero; B. Ma; O.Michel, and J. Gorman: Alpha-Divergence for Classification, Indexing and Retrieval; Communications and Signal Processing Laboratory Technical Report CSPL- 328 2001.
7. Beucher S.: „Applications of mathematical morphology in material sciences: A review of recent developments“, International Metallography Conference, pp. 41-46, 1995.
8. Atkins P., de Paula J., Physical Chemistry, Oxford University Press 2006.
9. Gokcay, D.; Bowers, D.; Rochardson, C.; Desai, A.; Use of local entropy changes as a measure for identification of facial expressions; IEEE International Conference on Processing, 2000. ICASSP Volume 6, Page(s):2334 - 2337 vol.4.
10. Urban J., Vanek J., Štys D. Preprocessing of microscopy images via Shannon's entropy, In Proc. of Pattern Recognition and Information Processing, pp.183-187, Minsk, Belarus, (2009).

P41

C-TERMINAL SEGMENT OF YEAST BMH PROTEINS EXHIBITS DIFFERENT STRUCTURE COMPARED TO OTHER 14-3-3 PROTEIN ISOFORMS

D. Veisova[1], L. Rezabkova[1,2], P. Herman[3], J. Vecer[3], T. Obsil[1,2], V. Obsilova[1]

[1]Institute of Physiology Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic
[2]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, 12843 Prague, Czech Republic
[3]Faculty of Mathematics and Physics, Institute of Physics, Charles University, 12116 Prague, Czech Republic
obsilova@biomed.cas.cz

Yeast 14-3-3 protein isoforms BMH1 and BMH2 posses a distinctly variant C-terminal tail which differs them from the isoforms of higher eukaryotes. Their C-termini are longer and contains polyglutamine stretch of unknown function. It is now well established that the C-terminal segment of 14-3-3 proteins plays an important regulatory role by functioning as an autoinhibitor which can occupy the ligand binding groove and blocks the binding of inappropriate ligands [1-3]. Whether the same holds true or not for the yeast isoforms is unclear. Therefore, we investigated the conformational behavior of the C-terminal segment of BMH proteins using various biophysical techniques. Dynamic light scattering, time-resolved fluorescence anisotropy decay and size exclusion chromatography measurements showed that the molecules of BMH proteins are significantly bigger compared to the human 14-3-3 zeta isoform. On the other hand, the sedimentation equilibrium analysis confirmed that BMH proteins form dimers. Time-resolved tryptophan fluorescence experiments revealed no dramatic structural changes of the C-terminal segment upon the ligand binding. Taken together, the C-terminal segment of BMH proteins adopts very open and extended conformation that increases their apparent molecular size. It seems, therefore, that the C-terminal segment of BMH proteins does not function as an autoinhibitor and does not block their ligand binding grooves.

1. A.B. Truong, S.C. Masters, H. Yang, H. Fu, *Proteins*, **49**, (2002), 321.
2. V. Obsilova, P. Herman, J. Vecer, M. sulc, J. Teisinger, T. Obsil, *J. Biol. Chem.*, **279**, (2004) 4531.
3. J. Silhan, V. Obsilova, J. Vecer, P. Herman, M. Sulc, J. teisinger, T. Obsil, *J. Biol. Chem.*, **279**, (2004), 49113.

This work was funded by Grant IAA501110801 of the Grant Agency of the Academy of Sciences of the Czech Republic, by Research Project MSM0021620857 and Centre of Neurosciences LC554 of the Ministry of Education, Youth, and Sports of the Czech Republic, and by Research Project AV0Z50110509 of the Academy of Sciences of the Czech Republic.

P42

EFFECT OF NONPLANARITY ON ^{3}J-COUPLINGS IN NUCLEIC ACID BASES

Z. Vokáčová[1], L. Trantírek[2], V. Sychrovský[1]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic, Praha, Czech Republic
[2]Department of Chemistry, Utrecht University, The Netherlands
zuzana.vokacova@uochb.cas.cz

The glycosidic torsion angle is used as a measure of the nucleobase orientation with respect to sugar ribose in nucleosides and it is one of the major determinants of nucleic acid structure. The deformations from planar geometry arrangement in the deoxy-adenosine (dA), deoxy-cytidine (dC), deoxy-guanosine, and deoxy-thymidine (dT) nucleosides due to variation of the glycosidic bond orientation were investigated with the computational methods and the calculated trends were correlated with the X-ray data [1].

One of the methods often used for determination of the torsion is NMR spectroscopy. Correlation between value of ^{3}J-coupling and corresponding torsion angle is described by Karplus equation [2]. Our study showed that angular argument of Karplus equations for the ^{3}J(C8/6-H1') and ^{3}J(C4/2-H1') couplings assigned to the torsion, must reflect deformation from ideal tetrahedric and planar spatial arrangement of atoms around carbon C1' and/or nitrogen N9/1 involving in the torsion, respectively (Conception of ideal tetrahedric and planar spatial arrangement consist in using of constant phase shift in Karplus equation.[3]). Whereas, the calculated magnitude of the deformations revealed to be large at the side of the glycosidic nitrogen N9/1 (pyramidalization), the deformation of the arrangement around the carbon C1' (sugar side) was relatively smaller. The pyramidalization depends significantly on the torsion, namely the orientation of the pyramidalization (up and down). The magnitude of pyramidalization is also slightly different for molecules with different sugar conformation.

The deformation of the spatial arrangement of atoms around the carbon C1' and degree and orientation of the pyramidalization at the glycosidic nitrogen N9/1 affect mostly the phase factor in the Karplus equation for structural interpretation of the ^{3}J(C8/6-H1') coupling. The ^{3}J(C4-H1') coupling is effected slightly and only by deformation at the C1' arrangement.

The calculated dependence of pyramidalization on torsion for all deoxy-nucleosides shows similar behaviour, including the trends due to the sugar pucker.

1. V. Sychrovský, S. Foldýnová-Trantírková, N. Špačková, K. Robeyns, L. Van Meervelt, W. Blankenfeldt, Z. Vokáčová, J. Šponer, L. Trantírek, *Nucleic Acid Res.*, **37**, (2009), 7321.
2. M. Karplus, *J.Am.Chem.Soc.*, **85**, (1963), 2870.
2. S. S. Wijmenga, B. N. M. van Buuren, *Prog. NMR Spectrosc.*, **32**, (1998), 287.

This work was supported by the Grant Agency of the Czech Republic, grants no. P208/10/P398 and P205/10/0228.

P43

STRUCTURE AND FUNCTIONS OF TRANSIENT RECEPTOR POTENTIAL A1: HOMOLOGY MODELLING

V. Zayats[1,2], A. Samad[2], R. Ettrich[1,2]

[1]Laboratory of Structural Biology, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zámek 136, CZ-37333 Nové Hrady, Czech Republic
[2]Institute of Physical Biology, University of South Bohemia, Zámek 136, 37333 Nové Hrady, Czech Republic

Transient receptor potential (TRP) channels are a large superfamily of nonselective cation channels. TRPA1 is a candidate for mechanically gated transduction channels potentially mediating the sensations of hearing, touch, and some forms of pain. Human TRPA1 is a 127.4 kDa protein comprised of 1119 amino acids. Like other TRPs also TRPA1 has six predicted membrane-spanning domains (S1 to S6) and the pore between S5 and S6 [2]. In this work we focus on homology modeling of its for TRPs unusually long N-terminal intracellular region containing 18 predicted ankyrin repeats. Ankyrin repeats have been implicated in protein-protein interactions, provide elasticity and make molecular springs [3]. Also a calcium-binding domain, EF-hand, was indicated at the N-terminus, consisting of 12 residues involved in Ca-dependent activation [1].

Simulations of the dynamic behavior of tree-dimensional all-atom models let us describe structural and functional properties of the system. Structural models are build using Modeller, for visual analyzing and energy minimization of the created models Yasara is used, and molecular dynamics simulations are carried out in GROMACS (molecular dynamics simulation package). The general aim is to embed the results of this work later into an all-atom model of the channel the membrane to get a stable tetrameric overall structure of fully functional TRPA1 in its natural environment.

1. J. F. Doerner, G. Gisselmann, H. Hatt, and C. H. Wetzel *J. Biol. Chem.* (2007) **282**, 13180-13189.
2. J. Garcia-Anoveros, K. Nagata Handb. *Exp. Pharmacol.* (2007) **179**:347-362.
3. P. Michaely, D.R. Tomchick, M. Machius, R.G. Anderson *EMBO J.* (2002) **21**:6387-6396.

This research was supported by the Ministry of Education of the Czech Republic (LC06010 and MSM6007665808) nd by the Academy of Sciences of the Czech Republic (AV0Z60870520).

P44

BARLEY LIPID TRANSFER PROTEIN 1 WITH A COVALENTLY ATTACHED LIPID-LIKE MOLECULE

Jitka Žídková[1], Jozef Hritz[2], Michaela Matejková[3], Lukáš Žídek[3], Vladimír Sklenář[3], Janette Bobáľová[1]

[1]Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Veveří 97, 60200 Brno, Czech Republic
[2]Department of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, NL-1081 HV Amsterdam, The Netherlands
[3]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic
zidkova@iach.cz

Lipid transfer protein 1 (LTP1) from barley is a small protein able to transfer lipids across a membrane *in vitro*. Its physiological role is not clear, it has been proposed to be involved in cutin synthesis and plant defense mechanism. LTP1 is also important commercially, as it has an effect on beer foam formation. Interestingly, LTP1 isolated from barley flour is almost completely covalently modified with 9-hydroxy-10-oxo-12-Z-octadecenoic acid, bound to Asp 7 via an ester bond. The lipid modification alters physicochemical properties of the protein and contributes to its unusual heat stability. An X-ray structure of the lipid-modified protein (LTP1b) has been published recently (3GSH.PDB). In our study, we clarify some ambiguities related to the resolution of the bound lipid in the X-ray structure and present complementary NMR data illustrating dynamic behavior of the protein. In addition, we describe a computational protocol used to predict the 3D structure of LTP1b without a knowledge of the X-ray structure, based on a docking approach.

This work was supported by the Grants 1M0570, MSM 0021622413, and LC06030 of the Ministry of Education, Youth, and Physical Culture of the Czech Republic, and by Grant AV0Z40310501 of the Academy of Sciences of the Czech Republic.

PROGRAMME

Thursday, March 18

12:00 - 13:15 Registration

13:30 - 14:30 **Open forum about the future of structural biology in Europe**
chair person: *V. Sklenář*

Introduction
L1 p. 3
Jan Dohnálek
INSTRUCT – An Integrated Structural Biology Infrastructure for Europe. Do we need formal professional organization?

14:30 - 15:00 Coffee break

15:00 - 15:05 **Opening of the conference**

15:05 - 17:05 **Lecture session I**, chair person: *B. Schneider*

L2 p. 4
Zdeněk Tošner
Solid-state NMR of biological samples
L3 p. 4
Pavel Srb
Dynamical analysis of WT M-PMV matrix protein and its single point mutant R55F with respect to their different oligomerization properties
L4 p. 5
Jan Prchal
Interaction of the M-PMV matrix protein with phosphatidylinositol bisphosphate
L5 p. 6
Vladimír Kopecký
Structure differences between protein crystals and solution structures monitored by Raman spectroscopy – case study of PsbP protein
L6 p. 6
Valery Andrushchenko
Structure of guanine octamers d(G)8 determined by combination of VCD spectroscopy and theoretical computations
L7 p. 7
Irena Kratochvílová
Charge transport in DNA oligonucleotieds with various base-pairing patterns

17:05 - 17:20 Coffee break

17:20 - 19:20 **Lecture session II**, chair person: *J. Damborský*
L8 p. 8
Pavel Jungwirth
The interface between oxidized phospholipid bilayers and aqueous solutions
L9 p. 8
Babak Minofar
Aqueous salt solution of organic solvents as a media for enzymatic reactions
L10 p. 9
Veronika Štěpánková
Effect of organic solvents on structure and function of haloalkane dehalogenases
L11 p. 10
Tomáš Mozga
Biochemical and structural characterization of novel haloalkane dehalogenase DbeA from *Bradyrhizobium elkani* USDA94 possesing two halide-binding sites
L12 p. 11
Rudiger Ettrich
Was binding of free amino acids an early innovation in the evolution of allostery?

19:30 - 22:30 Banquet

Friday, March 19

7:00 - 8:45 Breakfast

9:00 - 10:50 **Lecture session III**, chair person: *T. Obšil*

L13 p. 12
Jaroslav Koča
Computational methods as a component of modern structural biology
L14 p. 12
Vojtěch Spiwok
Free Energy Modelling of Substrate Distortion in the Enzyme Active Site: An Example of Influenza Neuraminidase
L15 p. 13
Radka. Svobodová-Vařeková
SiteBinder – software tool for superimposing multiple structural motives
L16 p. 14
Jiří Černý
Improving protein binding, in silico approach

10:30 - 10:50 Coffee break

11:05 - 12:15 **Lecture session IV**, chair person: *J. Koča*

L17 p. 14
Ladislav Benda
Computational analysis of the stabilization effects in successive U-Hg-U base pairs
L18 p. 15
Crina Maria Ionescu
Fast methods of atomic charge calculation: parameterization of EEM for applicability to metal containing proteins
L19 p. 15
Jan Alán
Employment of free energy calculations to estimation of carbohydrates affinities towards PA-IIL superfamily lectins

L20 p. 16
Imre Barák
Molecular and structural mechanisms of cell division site recognition in *Bacillus subtilis*

12:10 - 13:45 Lunch

14:00 - 16:00 **Lecture session V**, chair person: *J. Dohnálek*

L21 p. 17
Tomáš Obšil
Structure of the 14-3-3/FOXO4 complex derived from the fluorescence spectroscopy data
L22 p. 18
Kateřina Procházková
Structural and molecular mechanism for autoprocessing of MARTX toxin of Vibrio cholerae at multiple sites
L23 p. 18
Pavel Mader
Human carbonic anhydrase complexes with isoquinoline inhibitors
L24 p. 19
David Kopečný
Structural analysis of plant aminoaldehyde dehydrogenases
L25 p. 19
Julie Wolfová
Defining structural features of the family of tetrameric flavoproteins WrbA
L26 p. 20
Tomáš Klumpler
Solving phase problem using a Se-Met derivative of the flavoenzyme NAD(P)H:acceptor oxidoreductase (FerB)

16:00 - 16:30 Coffee break

16:30 - 18:20 **Lecture session VI**, chair person: *R. Ettrich*

L27 p. 24
Bohdan Schneider
Bioinformatic analyses of the first solvation shell of nucleic acids
L28 p. 24
Jindřich Hašek
Using the Cambridge Structure Database of Organic and Organometalic Compounds in Structure Biology
L29 p. 26
Martin Kuba
MetaCentrum - On the Way from Distributed Computing to Cyber Infrastructure for Research
L30 p. 27
Dalibor Štys
Three dimensional information structure of living cell fate

18:30 - 19:30 Dinner

19:45 - 21:00 **First plenary meeting of NFSB**
chair person: *B. Schneider*
Organization of the NFSB
National forum for structural biology
Legal framework
Membership
Election of the NFSB committee

21:00 - 23:00 **Poster session**

Saturday, March 20

7:00 - 8:45 Breakfast

9:15 - 10:45 **Lecture session VII**, chair person: *R. Štefl*

L31 p. 28
Veronika Motáčková
Structure and dynamics of RNA polymerase delta subunit from *Bacillus subtilis* determined by NMR spectroscopy
L32 p. 28
Fruzsina Hobór
The role of Nrd1-Nab3 complex in transcription termination
L33 p. 29
Karel Kubíček
Structural insights into recruitment and dissociation of RNA polymerase II termination factors

10:20 - 10:45 Coffee break

10:45 - 12:05 **Lecture session VIII**, chair person: *J. Vondrášek*

L34 p. 29
Václav Veverka
Structural insight into regulation of the Wnt/b-catenin signalling pathway by sclerostin: implications for osteoporosis treatments
L35 p. 30
Michaela Wimmerová
High-affinity lectins from pathogens: the potential targets for antiadhesive drug design
L36 p. 30
Richard Dvorský
From Structural Biology to the Drugs against Inflammatory Bowl Diseases

12:05 - 12:07 Concluding remarks

12:15 - 14:00 Lunch

POSTERS