Posters

P1

INFLUENCE OF SPECIFIC BORON DEFECTS ON BORON-DOPED DIAMOND CONDUCTIVITY

P. Ashcheulov[1,2], J. Šebera[1,6], V. Petrák[1,3], F. Fendrych[1], M. Nesládek[4], Z. Vlčková Živcová[5], O. Frank[5], L. Kavan[5], M. Dračínský[6] and I. Kratochvílová[1]

[1]Institute of Physics, Academy of Sciences Czech Republic v.v.i, Na Slovance 2, CZ-182 21, Prague 8, Czech Republic
[2]Faculty of Nuclear Physics and Physical Engineering, Czech Technical University in Prague, Zikova 1, CZ-160 00 Prague 6, Czech Republic
[3]Faculty of Biomedical Engineering, Czech Technical University in Prague, Sítná sq. 3105, 272 01 Kladno, Czech Republic
[4]Institute for Materials Research (IMO), Hasselt University, Wetenschapspark 1, B-3590 Diepenbeek, Belgium
[5]J. Heyrovský Institute of Physical Chemistry, AS CR, v.v.i., Dolejškova 3, 18223, Prague 8, Czech Republic
[6]Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo náměstí 2, CZ, 16610 Prague, Czech Republic

In this work we focused on a detailed description of specific boron defects and their impact on conductivity of the boron-doped diamond. We present experimental (Raman Spectroscopy, 2-point resistivity, Neutron Depth Profiling, Atomic Force Microscopy) and ab-initio (Density Functional Theory) study of the vibrational and electronic properties of boron (B) related defects (substitutional single B and B dimer) in the diamond lattice as a function of B concentration [1].

The structural and electronic calculations confirmed that single substitution defects are predominant when the concentration of boron in diamond lattice is low. For sufficiently concentrated single boron defects in the diamond lattice the charge carriers behave like in metallic materials. With an increase in boron concentration, i.e. heavily boron-doped diamond ([B] ~ 1021 at/cm^{-3}), the probability of creation more complex defects, e.g. boron dimers accompanied by the lattice deformation, is much higher [2]. From the point of view of the band structure, these complex boron defects make non-conductive states separated from the rest of the system (valence band). For such inactive states less effective conduction mechanisms are typical, i.e., the conductivity of heavily boron doped diamond attenuates with increase in B concentration. The calculated Raman spectra are in good agreement with experimental results and show specific features of single boron substitution and boron dimer: the experimentally observed "500 cm^{-1}" band in the heavily boron-doped diamond with high probability originates from boron dimer defects (B-B vibrations).

1. H. Okazaki, T. Arakane, K. Sugawara, T. Sato, T. Takahashi, T. Wakita, M. Hirai, Y. Muraoka, Y. Takano, S. Ishii, S. Iriyama, H. Kawarada, T. Yokoya, Journal of Physics and Chemistry of Solids 72, 582 (2011).
2. Rinat F. Mamin and Takashi Inushima, Phys. Rev. B 63 (2001) 033201.

P2

COMPUTATIONAL STUDY OF INTERACTIONS OF ORGANIC MATTER AND BIOMOLECULES WITH MINERAL SURFACES

H. Barvíková[1], M. Předota[1], O. Kroutil[1,2], Z. Chval[2] and B. Minofar[1,3]

[1]Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic
[2]Faculty of Health and Social Studies, University of South Bohemia, J. Boreckého 27, 37 011 České Budějovice, Czech Republic
[3]Institute of Nanobiology and Structural Biology of GCRC ASCR,v.v.i., Zámek 136, 373 33 Nové Hrady
h.barvikova@centrum.cz

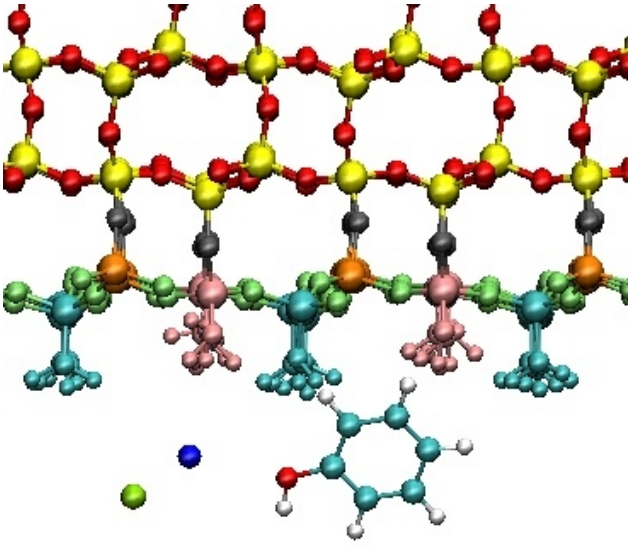
Humic acids and humates are characterized by heterogeneous and complex structures with different functional groups including acidic, hydrophilic and hydrophobic groups. Humic acids interact with both organic and inorganic substances such as nutrients, metals, hydrophobic organic compounds and mineral surfaces. Understanding their structure and interactions can provide important information about their degradability, toxicity and transport properties. These substances are also known as one of the major causes of so-called 'bio-fouling' of nanofiltration and reverse osmosis membranes used for water purification, desalination and wastewater treatment.

We carried out molecular dynamics simulations of interactions of quartz surfaces with aqueous solutions of ions and small organic molecules representing basic building blocks of larger biomolecules and functional groups of organic matter. As model molecules, benzoic acid, phenol, and salicylic acid were chosen.

Focusing our attention on leading interactions and roles of participating atoms and groups identified in the complexes, we studied interactions of molecules with surfaces for a set of surface charge densities corresponding to the experimentally or environmentally relevant ranges of pH values employing molecular mechanics, molecular dynamics and ab initio techniques. Simulated quartz surfaces covered the range of surface charge densities 0.00, -0.03, -0.06 and -0.12 C/m^2, approximately corresponding to pH values 4.5, 7.5, 9.5 and 11.

We found increasing water structuring within two water layers closest to the surface as well as sodium ions adsorption, both depending on increasing surface charge density. The adsorption of organic molecules is rather week, but specific binding patterns were identified.

Our future objective is modeling interactions of nucleic acid building blocks, polycyclic aromatic hydrocarbons, organic matter and mixtures of solvents with quartz and rutile surfaces applied on environmentally and technologically important systems.



We gratefully acknowledge support from the Grant Agency of the Czech Republic 13-08651S and P208/12/0622

P3

PROPOSED TRANSLOCATION CYCLE OF THE RESTRICTION-MODIFICATION SYSTEM ECOR124I

V. Bialevich[1,2], K. Shamayeva[1,2], D. Sinha[1,2], A. Guzanova[3], M. Weiserova[3], E. Csefalvay[1], J. Carey[1,4] and R. Ettrich[1,2]

[1]*Institute of Nanobiology and Structural Biology of GCRC, AS CR, 37333 Nove Hrady, Czech Republic*
[2]*University of South Bohemia in České Budějovice, 37333 Nove Hrady, Czech Republic*
[3]*Institute of Microbiology, AS CR, Vídeňská 1083, 142 20, Praha 4*
[4]*Department of Chemistry, Princeton University, New Jersey, USA*

Type I R-M systems are composed of three different subunits: one HsdS subunit is required for identification of target sequence and anchoring of enzyme complex on DNA; two HsdM subunits in MTase complex serve for host genome modification accomplishing protective function against self-degradation; two HsdR (or motor) subunits take part in ATP-dependent translocation and consequent cleavage of dsDNA [1, 2].

The crystal structure of the 120 kDa HsdR subunit of the Type I R-M system EcoR124I in complex with ATP was recently reported by our group [3]. HsdR is organized into four approximately globular structural domains in nearly square-planar arrangement: the N-terminal endonuclease domain, the recA-like helicase domains 1 and 2 and the C-terminal helical domain. The near-planar arrangement of globular domains creates prominent grooves between each domain pair. The two helicase-like domains form a canonical helicase cleft in which double-stranded B-form DNA can be accommodated without steric clash. The helical domain resembles HsdM and has strong interactions with helicase 2 domain [3].

Molecular mechanism of dsDNA translocation, cleavage and ATP hydrolysis has not been yet structurally investigated. Here we propose a translocation cycle of the restriction-modification system EcoR124I based on analysis of available crystal structures of superfamily 2 helicases, strutural modeling and complementary biochemical characterization of mutations introduced in sites potentially inportant for translocation in the HsdR motor subunit.

We gratefully acknowledge support from the Czech Science Foundation (project number GACR P207/12/2323).

1. Murray, N. E. *Microbiology and Molecular Biology Reviews*, 2000, Vol. 64, 412–434.
2. Dryden et al., *Nucl. Acids Res*., 2001, Vol. 29, 3728-3741.
3. Lapkouski M. et al, *Nat. Struct. & Mol.Biol*, 2009, 16, 94.

P4

EXTRACELLULAR CHITINOLYTIC ENZYMES OF *CLOSTRIDIUM PARAPUTRIFICUM* J4; SEPARATION AND CHARACTERIZATION

J. Dušková[1], G. Tiščenko[1], P. Kolenko[1], T. Skálová[1], J. Šimůnek[2], J. Dohnálek[1]

[1]Inst.of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského nám. 2, Praha 6, duskova@imc.cas.cz
[2]Inst. of Animal Physiology and Genetics, Acad. of Sciences of the Czech Rep., Vídeňská 1083, Praha 10,

Chitinases, glycosyl hydrolases, which catalyze degradation of chitin, are present in a wide range of organisms from primitive to higher types. They play an important role in physiology and metabolism of some bacteria by chitin digestion and utilization, in defense against infection by pathogenic fungi, in fungi hyphal growth and many others [1].

We focus on extracellular chitinases of a human intestinal bacterium *Clostridium paraputrificum* J4, especially on their isolation either from crude culture medium or production by recombinant protein expression in *Escherichia coli*, subsequent enzymatic characterization and structure-function analysis. Two enzymes with molecular weights 62.3 kDa (Chit62J4) and 87.0 kDa (ChitBJ4) were purified [2]. They possess chitinolytic activity toward 4-nitrophenyl--N,N'-diacetyl- -D-chitobioside and 4-nitrophenyl- -D-N,N',N''-triacetylchitotriose and a negligible activity toward 4-nitrophenyl-N-acetyl- -D-glucosaminide. Both enzymes belong to glycoside hydrolase family 18. Chit62J4 comprises a catalytic domain similar to chitinase D from *Bacilus circulans* [1], two domains with unknown function and a chitin binding domain. ChitBJ4, a sequence homolog to Chitinase B from *Clostridium paraputrificum* M21 [3] is composed of a catalytic domain, two Ig-like domains and a chitin binding domain. Further characterizations (MALDI, DLS, proteolytic analysis, kinetic studies) are discussed, crystallization of both enzymes is under way.

This work was supported by the Grant Agency of the Czech Republic (project no. 310/09/1407 and by the Ministry of Education, Youth and Sports of the Czech Republic (project BIOPOL, no. CZ.1.07/2.3.00/30.0029).

1. Y.C. Hsieh, Y.J. Wu, T.Y. Chiang, C.Y. Kuo, K. L. Shrestha, C.F. Chao, Y.C. Huang, P. Chuankhayan, W.G. Wu, Y.K. Li, C.J. Chen, *J. Biol. Chem.,* **285**, (2010), pp. 31603-31615.
2. J. Dušková, G. Tishchenko, E. Ponomareva, J. Šimůnek, I. Koppová, T. Skálová, A. Štěpánková, J. Hašek, J. Dohnálek, *Acta Biochim. Pol.,* **58**, (2011), pp. 261-263.
3. K. Morimoto, S. Karita, T. Kimura, K. Sakka, and K. Ohmiya, *J. Bacteriol.*, **179**, (1997), pp. 7306-731.

P5

BIPHASIC KINETIC BEHAVIOR OF FMN-DEPENDENT NAD(P)H:QUINONE OXIDOREDUCTASE WRBA FROM *E. COLI*: A MOLECULAR UNDERSTANDING

David Reha[1], Vasilina Zayats[1] , Balasubramain Harish[2] , Iryna Kishko[1] , Jannette Carey[2] and Rüdiger Ettrich[1]

[1]Institute of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic, Nové Hrady, ettrich@nh.cas.cz
[2]Department of Chemistry, Princeton University, Princeton, New Jersey, USA

The *E. coli* protein WrbA is an FMN-dependent NAD(P)H:quinone oxidoreductase that has been implicated in oxidative defense. Three subunits of the tetrameric enzyme contribute to each of four identical, cavernous active sites that appear to accommodate NAD(P)H or various quinones, but not simultaneously, suggesting an obligate tetramer with a ping-pong mechanism in which NAD departs before oxidized quinone binds. Steady-state kinetics results reveal that WrbA conforms to a ping-pong mechanism with respect to the constancy of the apparent V_{max} to K_m ratio with substrate concentration. Docking and energy calculations find that electron-transfer-competent binding sites for NADH and benzoquinone present severe steric overlap, consistent with the ping-pong mechanism. Unexpectedly, plots of initial velocity as a function of either NADH or benzoquinone concentration present one or two Michaelis-Menten phases depending on the temperature at which the enzyme is held prior to assay. The effect of temperature is reversible, suggesting an intramolecular conformational process. Preliminary sedimentation velocity analysis of WrbA indicates a large shift in size of the multimer with temperature, suggesting that subunit assembly coupled to substrate binding may underlie the two-plateau behavior. A novel approach of polarized QM/MM molecular docking of substrates to the dimar and tetramer demonstrates different binding affinities for the dimer and tetramer and supports the hypothesis that coupling of substrate binding to tetramer assembly may underlie the two-plateau kinetics.

Support from the Czech Science Foundation, no P207-10-1934, is acknowledged.

P6

PREDICTION AND STRUCTURAL STUDIES OF ACTIVE SITE OF SELECTED GLYCOSYL HYDROLASES FROM *PAENIBACILLUS THIAMINOLYTICUS*

Katarína Hlat-Glembová, Eva Benešová, Zita Purkrtová, Vojtěch Spiwok

Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Technická 3, Prague 6, 166 28, Czech Republic
hlatglek@vscht.cz

Glycosyl hydrolases -D-galactosidase and -L-fucosidase isolated from the bacterium *Paenibacillus thiaminolyticus* show a great potential because of their transglycosylation ability, which found a potential in synthesis of glycoconjugates. The aim of our research is to study active sites of these enzymes, as their crystal structures have not been solved yet. Our study is based on combination of theoretical and experimental methods. The structural model of each enzyme was predicted by using a homology modeling. According to the predicted structure of each enzyme, the catalytic residues were predicted. These residues were subjected to side direct mutagenesis to prove their catalytic role. Here we report the progress of mutagenesis experiments and kinetic characterization of mutants -D-galactosidase_mut157, which was already been purified, -D-galactosidase_mut233, which is now in the plasmid DNA state, L-fucosidase_mut186 and -L-fucosidase_mut239, which are in the state of PCR products.

P7

ISOLATION AND CRYSTALLIZATION OF THE RECOMBINANT PSBR PROTEIN OF HIGHER-PLANT PHOTOSYSTEM II

T. Holubeva[1,2], M. Plevaka[1,2], J.Heller[1,2], J. Ludwig[3], O. Sedo[4], Z. Zdrahal[4], I. Kuta Smatanova[1,5] and J. Kohoutova[1]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses and Institute of complex systems, Zamek 136, 373 33 Nove Hrady
[2]University of South Bohemia in Ceske Budejovice, Faculty of Science, Branisovska 31, 37005 Ceske Budejovice
[3]University of Bonn, IZMB / Molekulare Bioenergetik, Kirschallee 1, D-53115 Bonn, Germany
[4]Core Facility – Proteomics, CEITEC, Masaryk University, Kamenice 5,625 00 Brno
[5]Academy of Sciences of the Czech Republic, Institute of Nanobiology and Structural Biology GCRC, Zamek 136, 373 33 Nove Hrady

Photosystem II consists of more than 20 proteins, which catalyze the light-driven oxidation of water to molecular oxygen in cyanobacteria, algae, and plants. PSII can be divided into two functional domains: the electron transfer domain, comprising the integral membrane helices and cofactors and the oxygen evolving complex (OEC), located on the lumenal face of the complex including the extrinsic proteins PsbO (33 kDa), PsbP (23 kDa), PsbQ (17 kDa) and PsbR (10 kDa), the loop regions of several membrane proteins and the catalytic inorganic manganese, calcium, and chloride cluster [1].

PsbR is a low-molecular weight protein, which is found in higher-plant photosystem II and anticipated to play a role in water oxidation, yet the physiological significance, exact location and structure of the purified protein has remained obscure [1,2].

Therefore, further biochemical studies should elucidate the position of PsbR relative to other components of plant OEC. As primary structure of spinach PsbR protein is known, we were able to produce *psbR* gene from mRNA isolated from *Spinacia oleracea*, create an expression vector with its anchor as HisPsbR and without anchor as PsbR using molecular biology techniques. The further goal is to optimize purification conditions in order to get stable proteins that will be used for structural analysis.

This research was supported by the ME CR (COST LD 11011, CZ.1.05/2.1.00/01.0024), by the AS CR (AV0Z 60870520) and GAJU 170/2010/P.

1. Roose, J. L. et al., (2007). Photosynthesis Research 92, 369-387.
2. Suorsa, M. et al., (2006). Journal of Biological Chemistry 281, 145-150.

P8

COMBINATION OF ALCHEMICAL FREE ENERGY SIMULATION AND METADYNAMICS IN THE SIMULATION PACKAGE GROMACS

Petr Hošek, Vojtěch Spiwok

Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Technická 3, Prague 6, 166 28, Czech Republic
hosek@vscht.cz

In recent years, there have been a massive development of various computational method for prediction of binding constants of ligand and proteins, mainly for drug design application. One of these methods is free energy perturbation, method for calculating free energy difference between two chemical distinct systems. Free energies comparable with experimental values can be obtained employing a suitable thermodynamic cycle. The main drawback of free energy perturbation is necessity to know a suitable path connecting both terminal states and select several points on this path for intermediate simulations. This problem do not apply to metadynamics, however classical metadynamic method is not designed to change chemical properties of atoms. The aim of this study is to expand classical metadynamics by adding ability to change one atom into another. Unlike other metadynamics parameters, which can be calculated from coordinates of the studied system, lambda value needs to be handled differently. There are several approaches to handle lambda that ensure driving lambda to potential minima, the first evaluated approach used a simple gradient. Next task was to evaluate and handle associated problems such as the fact that the interval of allowed lambdas is closed or combination of lambda with standard parameters in a multidimensional metadynamics.

The results of this method were compared to experimental values and FEP results of several residue-side-chain solvation energies.

P9

MAGNESIUM BINDING TO THE RECEIVER DOMAIN OF CYTOKININ RECEPTOR CKI1RD FROM *ARABIDOPSIS THALIANA*

Dominik Hrebík, Olga Otrusinová, Blanka Pekárová, Lubomír Janda, Jan Hejátko, and Lukáš Žídek

National Centre for Biomolecular Research, Faculty of Science and Central European Institute of Technology, Masaryk University, Kamenice 5 , CZ-62500 Brno, Czech Republic

Two component signaling system serve as stimulus system to allow an organism to respond and sense various environmental changes and conditions. In the plant *Arabidopsis thaliana*, histidine protein kinase is phosphorylated upon signal recognition and forwards the phosphate group through histidine phosphotransfer proteins to a response regulator protein in nucleus, where responses take place. The input signal can be light, osmotic changes, or hormones.

We studied the receiver domain of histidine protein kinase CKI1 by nuclear magnetic resonance. The domain was expressed in *E. coli* and labeled with stable isotopes (^{13}C, ^{15}N). Resonance frequencies were assigned using the standart strategy. Effect of Mg^{2+}, a cofactor for signal transduction, has been studied in a series of titration experiments (2D ^{1}H-^{15}N HSQC spectra) and the most significantly affected residues were identified using secondary chemical shift mapping. The dissociation constant was determined.

This work was supported by grant from the Czech Science Foundation (grant No. P305/11/0756).

P10

Ca^{2+} BINDING PROTEINS INTERACT WITH THE N-TERMINAL REGION OF TRPM1

Michaela Jirku[1], Kristyna Bousova[1], Ladislav Bumba[2], Jan Teisinger[1]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic
[2]Institute of Microbiology, Academy of Sciences of the Czech Republic

TRPM1 or melastatin channel (MLSN) belongs to melastatin subfamily of transient receptor potential channels (TRPs). These non-selective ion channels are responsible for entry of mono- and divalent cationts into the cell. They participate on many processes e.g. sensitivity to high and low temperatures, taste, pressure, vision and pain. Intracellularly located N- and C-tails are responsible for regulation of TRP channels, which carry binding sites for signal molecules like calmodulin (CaM) or S100A1.

TRPM1 is present in human melanocytes and retina. It seems that loss of TRPM1 correlates with increased aggressiveness in melanoma. TRPM1 is localized in bipolar cells in retina and participates in processes connected to vision. Mutations of TRPM1 gene are associated with congenital stationary night blindness in humans. There is currently a scarcity of structural / functional data on TRPM1 channel.

We studied possible interactions between CaM and S100A1 and the N- and C-termini of rat TRPM1. Using bioinformatic approach we identified CaM and S100A1 binding site in region 242-344 within the rat TRPM1 N-terminus (NT). The domain L_{242}-E_{344} on NT was cloned into the pET32b vector and verified by sequencing. This construct was expressed in bacteria *E. coli* Rosetta cells and purified in two-step purification protocol using affinity chromatography and HPLC gel chromatography. Amino acid sequence was checked by MS MALDI-TOF. Steady-state fluorescence anisotropy and surface plasmon resonance measurements were used to test the binding of CaM and S100A1 to L_{242}-E_{344}. We determined several positive and hydrophobic residues to be responsible for binding of TRPM1-NT L_{242}-E_{344} to CaM and S100A1. The results of the experiments also suggest that CaM and S100A1 bind to the same or overlapping binding site.

This project was supported by Grants GACR 301/10/1159, GACR 207/11/0717 and GACR - Project of Excellence in the Field of Neuroscience P304/12/G069.

P11

STUDY OF BSOBI ENDONUCLEASE BY MOLECULAR DYNAMICS

I. Kabelka[2], J. Štěpán[1,2], J. Koča[1,2] and P. Kulhánek[1,2]

[1]CEITEC - Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

BsoBI is a type II restriction endonuclease belonging to the EcoRI family. The enzyme was isolated from the bacteria *Geobacillus stearothermophilus* and crystallized in complex with short DNA duplex [1]. The interesting discovery about this homodimeric enzyme was the formation of long tunnel for DNA binding and recognition. The DNA molecule is completely encircled inside the tunnel. Considering the fact that BsoBI does not break up into monomers while is still capable to cleave circular DNA molecules, some conformational change is needed to enable the DNA binding. The most probable scenario is the separation of two domains, which would cause a formation of a gap large enough to enable the DNA get inside the active site. This scenario is supported by experimental evidence indicating significant conformational changes during the DNA binding [2].

In our project we have been focusing on investigating the process of opening of the BsoBI enzyme using molecular dynamics simulations. Our starting point was the crystallographic structure of the enzyme in complex with short DNA molecule [1]. We have divided the project into two parts: a) the study of the BsoBI-DNA complex and b) the study of the unbound enzyme. Because there is no experimental structure for free enzyme it was prepared artificially by the removal of the DNA molecule from the crystallographic structure. The sampling of opening process was improved by accelarated molecular dynamics employing Metadynamics [3] approach. This method enabled us to model proposed conformational changes and qualitatively evaluate associated free energy differences among various stable protein conformations.

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

puting facilities provided under the programme Center CERIT Scientific Cloud, part of the Operational Program Research and Development for Innovations, reg. no. CZ. 1.05/3.2.00/08.0144 is appreciated..

1. M.J. van der Woerd, J.J. Pelletier, S. Xu, A.M. Friedman, Structure, 9, (**2001**), 133–144.
2. J. Dikić,http://geb.uni-giessen.de/geb/volltexte/2009/7301, (**2009**).
3. A. Laio, M. Parrinello, P. Natl. Acad. Sci. USA , 99, (**2002**), 12562–12566.

P12

MAPPING OF THE INTERACTION SURFACE OF PHOSDUCIN USING HDX-MS

Miroslava Kacirova[1,2], Petr Man[1,3], Alan Kadek[3] and Tomas Obsil[1,2]

[1]Faculty of Science, Charles University in Prague, 12843 Prague, Czech Republic
[2]Institute of Physiology, Academy of Sciences of Czech Republic, 14220 Prague, Czech Rep.
[3]Institute of Microbiology, Academy of Sciences of Czech Republic, 14220 Prague, Czech Rep.
mirkakacirova@seznam.cz

Phosducin (Pdc), a regulatory and highly conserved phosphoprotein, plays an important role in the regulation of the G protein signaling by modulating an amount of the G_t heterotrimer through a competition with the G_t subunit for binding to the G_t complex [1]. Besides its well-established role in the regulation of the G protein signaling, Pdc is also involved in the transcriptional control and the modulation of blood pressure. The function of Pdc is regulated through its phosphorylation and a binding to the regulatory 14-3-3 protein [2]. The 14-3-3 proteins are scaffolding molecules that regulate the function of other proteins through a number of different mechanisms. The exact role of the 14-3-3 protein in the regulation of Pdc function is, however, still elusive.

We have recently shown that the 14-3-3 protein interacts with and reduces the flexibility of both the N-terminal and the C-terminal domains of doubly phosphorylated Pdc (dpPdc). We also observed that the phosphorylation alone has a significant effect on the structure of the whole Pdc molecule [3]. In order to gain additional insight into the interaction of Pdc with the 14-3-3 protein, we decided to use hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) to map their interaction interface. Therefore, the HDX kinetics of the 14-3-3:dpPdc and 14-3-3:Pdc complexes as well as free 14-3-3, dpPdc and Pdc proteins were measured. The exchange kinetics of Pdc regions were followed on 72 peptides from the pepsin digest, together covering 100 % of its sequence, while the exchange kinetics of 14-3-3 regions were followed on 94 peptides covering 100 % of its sequence. Our data revealed that several dpPdc and 14-3-3 peptides exhibit significant change in the deuteration kinetics upon the complex formation suggesting that these regions either form the binding interface or undergo a structural change upon the interaction. The slower deuterium incorporation was observed for 14-3-3 peptides 48-59, 60-66, 155-174, 191-199. These peptides map to the surface of helix H3 as well as outside the central channel of the 14-3-3 protein dimer. In the case of dpPdc the slower deuterium incorporation was observed for peptides 4-28, 117-135, 152-157, 208-212, 221-242. These peptides map to the surface of both domains. Interestingly, HDX-MS data also show significant differences between dpPdc and Pdc confirming that phosphorylation alone has significant effect on Pdc structure.

This work was funded by Grant P305/11/0708 of the Czech Science Foundation, by Research Project MSM 00216 20857 and by Research Project AV0Z50110509 of the Academy of Sciences of the Czech Republic.

1. R. Gaudet, A. Bohm, P. B. Sigler, *Cell* 87, (1996), 577-588.
2. B. Y. Lee, C. D. Thulin, B. M. Willardson, *J. Biol. Chem.* 279, (2004), 54008-54017.
3. L. Rezabkova, M. Kacirova, M. Sulc, P. Herman, J. Vecer, M. Stepanek, V. Obsilova, T. Obsil, *Biophysical J.* 103, (2012), 1960-1969.

P13

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

Mikalai Lapkouski[1], Tatsiana Baikova[1,2], Ladislav Csefalvay[1], Dhiraj Sinha[1,2], Alina Kevorkova[1,2], Vyas Ramasubramani[3], Alena Guzanova[4], Abdul Samad[1], Morteza Khabiri[1], Pavel Janscak[5], Ivana Kutá Smatanova[1], Santosh Panjikar[5], Marie Weiserova[4], Jannette Carey[3], Eva Csefalvay[1], Rüdiger Ettrich[1,2]

[1]Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences of the Czech Republic, Nove Hrady, Czech Republic
[2]Faculty Of Sciences, University of South Bohemia, Czech Republic
[3]Chemistry Department, Princeton University, Princeton, New Jersey, USA
[4]Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[5]EMBL Hamburg Outstaion, c/o DESY, Hamburg, Germany
[6]Institute of Molecular Cancer Research, University of Zurich

The recent structure of the first intact motor subunit of a Type I restriction enzyme suggested a mechanism by which stalled translocation triggers DNA cleavage. Cleavage assays in vivo, ATPase assays in vitro, computational modeling and the crystal structures of active and inactive mutants now reveal how interdomain engagement brings DNA cleavage under the control of translocation.

We gratefully acknowledge support from the Grant Agency of the Czech Republic (P207-12-2323), and joint Czech - US National Science Foundation International Research Cooperation (ME09016 and INT03-09049), Additionally, A.K. was supported by the University of South Bohemia, grant GAJU 170/2010/P.

P14

YFID FROM *E.COLI* AS A PFL REPAIR PROTEIN

P. Kolenko[1,3], C. Doberenz[2], L. Beyer[2], G. Sawers[2], M. T. Stubbs[3]

[1]Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovského nám. 2/1888, 162 06 – Prague 6, Czech Republic
[2]Institut für Mikrobiologie, Martin-Luther Universität, Kurt-Mothes-Straße 3, 06 120 Halle (Saale), Germany
[3]Institut für Biochemie und Biotechnologie, Martin-Luther Universität, Kurt-Mothes-Straße 3, 06 120 Halle (Saale), Germany
petr.kolenko@gmail.com

Facultative anaerobic bacteria like *E.coli* are able to undergo transitions between aerobic and anaerobic environments. The bacteria have mechanisms to adapt their metabolism according to the level of oxygen in the environment [1]. Many of the adaptive mechanisms are still not fully understood.

YfiD protein in *E.coli* is known as a "quick re-activator" of protein PflB, a glycyl radical enzyme that undergoes oxygenolytic cleavage of the polypeptide chain at the site of the radical. A molecular mechanism of the reactivation of PflB by YfiD was suggested after sequence analysis of both proteins. The proteins share high sequence identity in a roughly sixty amino acid-long C-terminal amino acid stretch [2].

We have cloned, expressed, and purified YfiD and three genetically truncated variants of protein PflB. We have performed analysis of the proteins and their interactions using size exclusion chromatography, ITC, pull-down assays, and NMR. We have also performed crystallization trials and NMR measurements for structural studies.

1. E.W. Trotter, M.D. Rolfe, A.M. Hounslow, C.J. Craven, M.P. Williamson, G. Sanguinetti, R.K. Poole, J. Green, *PLoS ONE*, **6**, (2011), e25501.

2. A.F. Volker Wagner, S. Schultz, J. Bomke, T. Pils, W.D. Lehmann, J. Knappe, *Bioch. Biophys. Res. Commun.*, **285**, (2001), 456-462.

This work was supported by the GRK1026 of the Deutsche Forschungsgemeinschaft, and by the Ministry of Education, Youth and Sports of the Czech Republic (grant No. CZ.1.07/2.3.00/30.0029).

P15

STRUCTURAL CHARACTERIZATION OF TWO MAIZE ALDEHYDE DEHYDROGENASES FROM FAMILY 2

Radka Končitíková[1,2], Martina Kopečná[1], David Kopečný[1], Solange Moréra[3], Armelle Vigouroux[3], Jan Frömmel[1,2], Marek Šebela[1]

[1]Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research and
[2]Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic;
[3]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, F-91198 Gif-sur-Yvette Cedex, France

Aldehyde dehydrogenases (ALDHs) constitute a protein superfamily of $NAD(P)^+$-dependent enzymes (EC 1.2.1). They have been considered as general detoxifying enzymes, which eliminate biogenic and xenobiotic aldehydes to the corresponding carboxylic acids. Under conditions of oxidative stress, ALDH enzymes act as aldehyde scavengers by metabolizing reactive aldehydes produced in consequence of the oxidative degradation of lipid membranes, also known as lipid peroxidation. Up to date, 13 ALDH families have been described in plants, but only a small number of the enzymes have been functionally characterized despite the existence of a large number of coding genes. In this work, we focused on ALDH gene family 2 in maize (*Zea mays*). The ALDH2 family expanded significantly during evolution of terrestrial plants and the number of family members varies substantially between species. The maize ALDH2 includes six genes coding for mitochondrial and cytosolic enzymes. Some ALDH2 family members were originally identified as genes restored fertility in plants (called RF2) i.e., gene that have the ability to suppress the male-sterile phenotype and restore the production of pollen to plants carrying the deleterious mitochondrial genome. Two genes (*RF2C* and *RF2F*) coding for ALDHs were cloned and expressed in T7 *E. coli* cells. The recombinant enzymes were thoroughly characterized and their identity was verified by MALDI-TOF peptide mass fingerprinting. Both ZmALDH2 enzymes utilize NAD^+ but not $NADP^+$ as a coenzyme. They are able to oxidize wide range of aldehydes but prefer aliphatic aldehydes. The crystal structure of ZmALDH2 (ALDH2C1, also RF2C) has been solved up to 2.25 Å resolution with R_{cryst} and R_{free} values of 18.2% and 22.5%, respectively, and represents the first structure of plant ALDH2 family member.

This work was supported by grant P501/11/1591 from the Czech Science Foundation and IGA grant PrF_2012_012 from Faculty of Sciences, Palacky University.

P16

HOW CALCIUM AND BMH1 ACTIVATE YEAST NEUTRAL TREHALASE NTH1?

Miroslava Kopecka[1], Lenka Rezabkova[1], Eva Macakova[1], Petr Man[2], Veronika Obsilova[1]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic v.v.i., Videnska 1083, 14220 Prague, Czech Republic
[2]Institute of Microbiology, Academy of Sciences of the Czech Republic v.v.i., Videnska 1083, 14220 Prague 4, Czech Republic

Yeast neutral trehalase (Nth1, EC 3.2.1.28) is a highly conserved enzyme which was found in many organisms. Nth1 from the *Saccharomyces cerevisiae* hydrolyses the cytosolic disaccharide trehalose (1- -D-glucopyranosyl -D-glucopyranoside) into two molecules of glucose. Trehalose serves as a carbon and energy source as well as a universal stress protectant against adverse conditions like dehydration, heat or oxidation. The activity of Nth1 is regulated by PKA protein phosphorylation, yeast 14-3-3 (Bmh) protein binding and by calcium [1,2]. Specific EF-like motif D^{114}TDKNYQITIED^{125} is located in the N-terminus of Nth1. This motif is conserved in many Ca^{2+}-binding proteins. Residues D^{114} and D^{125} are probably responsible for Ca^{2+}-binding and I121 is important for a correct conformation of the motif [3]. Therefore we prepared four Nth1 mutants with one-point mutation in this motif. For our study we used analytical ultracentrifugation, enzyme-kinetic measurements and hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) to reveal how the Ca^{2+} and Bmh1-binding affect the activity of Nth1. Our kinetic measurements revealed that Nth1 mutants D^{114} and D^{125} are inactive and that the Bmh1-dependent activation of Nth1 is significantly more potent than the Ca^{2+}-dependent one. From the HDX-MS measurements we suggest that regions surrounding the buried active site of pNth1 directly interact with Bmh1. These regions undergo a structural change and thus enable easier substrate and products entry and departure. The Ca^{2+}-dependent structural changes of Nth1 revealed that region containing putative Ca^{2+}-binding site and segments from the vicinity undergo a significant structural change in the presence of calcium. This might reflect an interaction between the catalytic domain and upstream

located Ca^{2+}-binding domain and suggest an explanation for the Ca^{2+}-dependent activation of Nth1. According to HDX-MS data another three point mutations were designed to test the effect on the Nth1 activity. Sedimentation velocity measurement was used to check the oligomeric status of the Nth1 mutants.

Supported by the Grant P207/11/0455 of the Grant Agency of the Czech Republic.

1. Veisova D, Rezabkova L, Stepanek M, Novotna P, Herman P, Vecer J, Obsil T, Obsilova V. *Biochemistry* 2010; **49**: 3853 – 3861.
2. Veisova D, Macakova E, Rezabkova L, Sulc M, Vacha P, Sychrova H, Obsil T, Obsilova V. *Biochem J* 2012; **443**: 663 – 670.
3. Franco A, Soto T, Vicente-Soler J, Paredes V, Madrid M, Gacto M, Cansado J. *Biochem J* 2003; **376**: 209 – 217.

P17

BIOPHYSICAL STUDY OF THE COMPLEX BETWEEN THE 14-3-3 PROTEIN AND THE KINASE DOMAIN OF ASK1

Dalibor Kosek[1,2], Lenka Rezabkova[1,2], Veronika Obsilova[2] and Tomas Obsil[1,2]

[1]Faculty of Science, Charles University in Prague, 12843 Prague, Czech Republic
[2]Institute of Physiology, Academy of Science of Czech Republic, 14220 Prague, Czech Republic
kosek@natur.cuni.cz

ASK1 (Apoptosis signal-regulating kinase, MAP3K5) plays a critical role in the regulation of the apoptosis triggered by the cellular oxidative stress, immune response or anticancer agents. So far it has been connected with the development of several neurodegenerative or cardiovascular diseases, diabetes and cancer. It is a homodimeric serine/threonine kinase from MAP3K family and its activity is tightly regulated by phosphorylation, oligomerization and protein-protein interaction. Once activated, ASK1 phosphorylates MAP2K4/7 or MAP2K3/6 that in turn activates JNK or p38 signaling pathways triggering apoptosis. The 14-3-3 protein has been identified as one of its most important physiological regulators. It binds to the Ser967 at the C-terminus of the kinase domain and holds ASK1 in an inactive state preventing the iniciation of the signaling. It has been shown that ASK1 is activated after dephosphorylation and dissociation of 14-3-3 in the presence of reactive oxygen species but the molecular mechanism of this interaction is still unknown.

Here we report the inicial biophysical characterization of the complex between 14-3-3 protein and kinase domain of ASK1. We optimized expression, purification and phosphorylation protocols for the preparation of recombinant enzymatically active human ASK1 catalytic domain. Purification protocol for the preparation of the 14-3-3 protein had been developed previously. Interaction with the 14-3-3 protein was verified using native electrophoresis and analytical ultracentrifugation. Determination of stochiometry and apparent K_d of the complex were performed using sedimentation velocity analysis.

This work was supported by the Grant Agency of Charles University in Prague (Grant 568912).

P18

STRUCTURE OF MULTIFUNCTIONAL PLANT NUCLEASE TBN1

T. Koval[1], J. Stránský[1, 2], T. Podzimek[3], P. Lipovová[3], J. Matoušek[4], K. Fejfarová[1], P. Kolenko[1], J. Dušková[1], T. Skálová[1], J. Hašek[1] and J. Dohnálek[1]

[1]Institute of Macromolecular Chemistry, AS CR, v.v.i., Heyrovského nám. 2, 162 06 Praha 6, Czech Republic
[2]Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University, Břehová 7, 115 19 Praha 1, Czech Republic
[3]Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic
[4]Institute of Plant Molecular Biology, Biology Centre, AS CR, v.v.i., Branišovská 31, 370 05 České Budějovice, Czech Republic
koval.tomas@gmail.com

Plant nuclease TBN1 (UniProt sequence accession no. Q0KFV0) from *Solanum lycopersicum* (tomato) is a Zn^{2+}-dependent glycoprotein with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated). TBN1 belongs to plant nuclease I family and plays an important role in specific apoptotic functions, vascular system development, stress response and tissue differentiation in plants [1]. In addition, TBN1 exhibits anticancerogenic properties [2].

Two recombinantly expressed variants of TBN1 (wild type and hypoglycosylated mutant N211D) were used in our study. Datasets for structural analysis were collected at the synchrotron radiation source BESSY II (Helmholtz-Zentrum Berlin). Presence of zinc in the protein was confirmed by X-ray fluorescence and an absorption edge scan. The phase problem was solved using combination of MAD phasing and molecular replacement [3, 4].

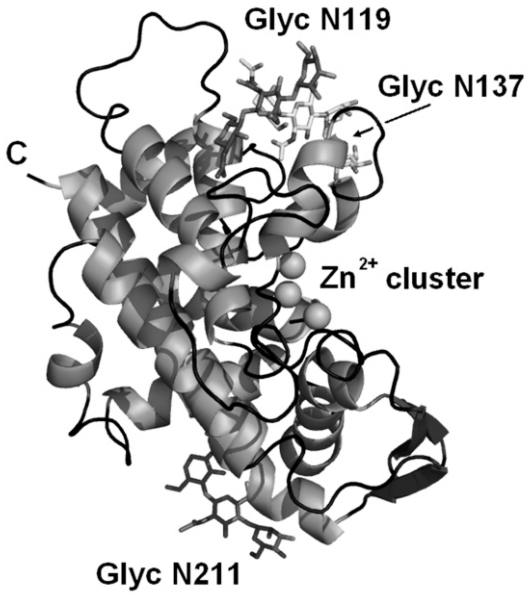


Figure 1 Fold, secondary structure and main features of TBN1 [5].

TBN1 is mainly -helical with a trinuclear Zn^{2+} cluster placed in the active site in the center of the wide groove. Three oligosaccharides bonded on the surface serve primarily as a shielding of the hydrophobic regions and therefore contribute to solubility and stability of the enzyme (Figure 1). TBN1 acts as phosphodiesterase cleaving the bond between phosphorus and 3' hydroxyl group in both single stranded and double stranded forms of DNA and RNA and shows 3'-nucleotidase activity. Moreover, phospholipase C-like activity of TBN1 was detected using artificial and native substrates for bacterial phospholipase C. Hydrolysis of the phosphodiester bond is caused by a nucleophilic attack of the activated water (hydroxide) molecule followed by creation of penta-coordinated transition state and its breakup into the products.

The work on this project was supported by the Czech Science Foundation, projects no. P302/11/0855, 202/06/0757 and 521/09/1214, by the EC under ELISA grant agreement number 226716 (synchrotron access, projects 09.2.90262 and 10.1.91347), by the Institution research plan AV0Z50510513 of the Institute of Plant Molecular Biology, Biology Centre. We acknowledge support of the Ministry of Education, Youth and Sports of the Czech Republic (grant No. CZ.1.07/2.3.00/30.0029). The authors wish to thank Dr. U. Müller of the Helmholtz-Zentrum Berlin, for support at the beam line.

1. J. Matousek, P. Kozlova, L. Orctova, A. Schmitz, K. Pesina, O. Bannach, N. Diermann, G. Steger, D. Riesner, *Biol. Chem*., **388**, (2007), 1–13.
2. J. Matousek, T. Podzimek, P. Pouckova, J. Stehlik, J. Skvor, P. Lipovova, J. Matousek, *Neoplasma,* **57** (2010), 339-348.
3. T. Koval, P. Lipovova, T. Podzimek, J. Matousek, J. Duskova, T. Skalova, A. Stepankova, J. Hasek, J. Dohnalek, *Acta. Cryst.* **F67**, (2011), 124-128.
4. J. Dohnalek, T. Koval, P. Lipovova, T. Podzimek, J. Matousek, *J. Synch. Rad.,* **18**, (2011), 29-30.
5. T. Koval, P. Lipovova, T. Podzimek, J. Matousek, J. Duskova, T. Skalova, A. Stepankova, J. Hasek, J. Dohnalek, *Acta. Cryst.* **D69**, (2013), 213-226.

P19

MOLECULAR MODELING OF A FUNGAL HEXOSAMINIDASE FROM *T. FLAVUS* WITH HIGH SUBSTRATE PROMISCUITY

Natalia Kulik[1], Kristyna Slamova[2], Vladimir Kren[2], Rudiger Ettrich[1]

[1]Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic, kulik@nh.cas.cz
[2]Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4, Czech Republic

Beta-N-acetylhexosamnidases (HEX) are glycoside hydrolases from the family 20 (EC 3.2.1.52). They cleave terminal glucose/galactose residue from di-/oligosaccharides by a retaining mechanism. Apart of the ability to cleave hexoses specifically, fungal HEX tolerate a variety of substrate modifications [1-3]. This feature together with the ability to hydrolase the transglycosilation reaction makes these enzymes useful in biotransformation to produce modified carbohydrates with defined structures [2-3]. A homology model of the newly sequenced fungal hexosaminidase (Hex) from *T.flavus* has been generated. The new *T.flavus* Hex has a similar topology to *A.oryzae* and conserved active site amino acids, participating in the electrostatic interaction with the substrate. Differences are mainly in sequence and length of loops close to the active site. The model of *T.flavus* Hex has been refined by energetic modeling and molecular dynamics, and we now have a stable and convincing model in our hands.

The standard substrate, P-NP-GlcNAc, which is used in in vitro enzymatic assaying, the natural substrate di-N-acetylchitobiose, as well as NAG-thiazoline were docked in the active site of *T.flavus* Hex and their interaction energies were compared with experimental data. Differences from earlier models of *A.oryzae*, exclusive features of the *T.flavus*, as well as implications of the docking results are discussed.

Support from the Czech Science Foundation, no P207/11/0629, is acknowledged.

1. Carmona AT, Fialova P, Kren V, Ettrich R, Martinkova L, Moreno-Vargas AJ, GonzalesC, RobinaI. 2006. Cyanodeoxy-glycosyl derivatives as substrates for enzymatic eeactions. Eur. J. Org. Chem. 8:1876–1885.
2. Fialova P, Carmona AT, Robina I, Ettrich R, Sedmera P, Prikrylova V, Petraskova-Husakova L, Kren V. 2005. Glycosyl azide—a novel substrate for enzymatic transglycosylations. Tetrahedron Letters. 46(5): 8715-8718.
3. Ogata M, Zeng X, Usui T, Uzawa H. 2007. Substrate specificity of N-acetylhexosaminidase from *Aspergillus oryzae* to artificial glycosyl acceptors having various substituents at the reducing ends. Carbohydr Res. 342(1):23-30.
4. Bojarova P, Slamova K, Krenek K, Gazak R, Kulik N, Ettrich R, Pelantova H, Kuzma M, Riva S, Adamek D, Bezouska K and Kren V. 2011. Charged hexosaminides as new substrates for -*N*-acetylhexosaminidase-catalyzed synthesis of immunomodulatory disaccharides. Adv. Synthesis & Catalysis, 353:2409–2420.

P20

PRACTICAL ASPECTS OF PROTEIN CRYSTALLOGRAPHY

Ivana Kuta Smatanova[1, 2], Jakub Jung[1], Pavel Malcher[1], Pavel Nemec[1], Martin Papez[1], Jan Tvaroh[1], Oksana Degtjarik[1], Jaroslava Kohoutova[1], Tatyana Prudnikova[1], Katsiaryna Tratsiak[1], and Michal Kuty[1, 2]

[1] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, CENAKVA and Institute of Complex Systems, Zamek 136, 373 33 Nove Hrady
[2] Academy of Sciences of the Czech Republic, Institute of Nanobiology and Structural Biology GCRC, Zamek 136, 373 33 Nove Hrady

The protein crystallography is one of the powerful techniques used for studying of protein structures and description their mechanism and function. This is possible only in the case of obtaining diffractable monocrystals. Different crystallization techniques such as standard, advanced and alternative methods are used to crystallize soluble and membrane proteins and protein complexes. Research projects are designed for graduate students and postgraduates who work on these projects on different levels. Finally developed methods and obtained crystallization and crystallographic data are compiled and results are published in prestigious journals.

Nowadays more than 20 proteins, protein complexes and their mutant variants are systematically studied. The main target is focused on research of (1) soluble haloalkane dehalogenases e.g. DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DbeA of *Bradyrhizobium elkani* USDA94, LinB of *Sphingobium japonicum* UT26 or new haloalkan dehalogenases DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marynobacter* sp ELB 17, as well as (2) membrane photosynthetic proteins isolated from different higher plants.

This research is supported by the ME CR (COST LD11011 and CZ.1.05/2.1.00/01.0024), GACR (P207/12/0775 and P207/11/0717), and by the AS CR.

P21

TOWARDS A STRUCTURE OF GREEN PLANT PHOTOSYSTEM II

Michal Kutý[1,4], Jaroslava Kohoutová[1], Jiří Heler[1,2], Marie Plevako[1,2], Tatsiana Holubeva[1], Daryna Kulik[1], Ondrej Šedo[3], Zbyněk Zdrahal[3], Ivana Kutá Smatanová [1,4]

[1] University of South Bohemia in České Budějovice, Institute of complex systems FFPW, Zámek 136, 373 33 Nové Hrady
[2] University of South Bohemia in České Budějovice, Faculty of Science, Branišovská 31, 37005 České Budějovice
[3]Core Facility – Proteomics, CEITEC, Masaryk University, Kamenice 5,625 00 Brno
[4] Academy of Sciences of the Czech Republic, Inst. of Nanobiology and Structural Biology GCRC, Zámek 136, 373 33 Nové Hrady

Photosystem II (PSII) is a pigment-protein complex of thylakoid membrane in cyanobacteria, algae and higher plants. PSII performs series of light driven reactions resulting in charge separation and subsequently in a reduction of an electron-transport chain and water oxidation. Primary site of the light to chemical energy conversion is located in so-called reaction center (RC).

PSII is a multisubunit complex embedded in the thylakoid membrane of plants, algae and cyanobacteria The function of the PSII in different organisms is identical while the composition of their subunits is different. Re-

cently the 3D X-ray structures of cyanobacterial PSII were determined to the maximum resolution of 2.9 Å. Cyanobacterial PSII consists of different extrinsic proteins compared with plant PSII and also the light-harvesting complex is not bound in thylakoid membrane to PSII core as it is in plant PSII.

Nowadays we are developing model of thylakoid photosynthetical membrane (glycolipid membrane instead of our previous octane layer) to build complex model of PSII RC.

In a paralell direction to theoretical approach we are focused on hydroponic plant growth hunder controlled conditions and optimization of reproducible purification protocol of homogeneous sample suitable for crystallization. Few types of higher plants like model organisms were chosen (tobacco, spinach, pea, haricot and soya). It was shown that the type of detergent and conditions of solubilization of thylakoid membranes are critical steps, which should be carefully chosen.

P22

NOVEL BINDERS DERIVED FROM AN ALBUMIN-BINDING DOMAIN SCAFFOLD TARGETING PROSTATE SECRETORY PROTEIN-94

Lucie Marečková[1], Hana Petroková[1], Milan Kuchař[1], Jiří Černý[2] and Petr Malý[1]

[1]Laboratory of Ligand Engineering and [2]Laboratory of Biomolecular Recognition, Institute of Biotechnology AS CR, v. v. i. Vídeňská 1083, 142 20 Prague, Czech Republic
Contact: lucie.mareckova@img.cas.cz, petr.maly@img.cas.cz

PSP-94, also known as -microseminoprotein (MSMB), is a small cysteine-rich protein, mainly found in human seminal plasma. Recently it has been well documented that the expression of PSP-94 is decreasing during the development of prostate cancer (PC). Currently used test for PC diagnosis relies on the detection of serum level of PSA (prostate-specific antigen known also as KLK3) in ELISA but this examination is insufficient due to high numbers of false positives leading to a useless therapeutic intervention, i.e. risky and high-cost prostate biopsies. The principle problem is that diagnosis of high levels of PSA does not well distinguish between a malignant form and benign prostate hyperplasia. Therefore, the development of tools for more precise assessment of PC biomarkers in blood serum can significantly improve the accuracy and validity of the novel diagnostics. Besides standard commercial one-type antibody–based ELISA test for PC exploration, a biosensor with multiple biomarkers might be a promising solution. We plan, therefore, to develop novel binders raised against human PSP-94 seminal fluid protein biomarker. While for KLK-family members the increase in serum levels might be an indicator of PC progression, a combination with serum level decrease for PSP-94 can be confirmative. The aim of this study was to generate novel binders (called PAB variants), derived from albumin-binding domain scaffold, targeting human PSP-94 protein and to select them using ribosome display approach. We demonstrate here that from the collection of 34 sequence-verified clones, seven candidates PAB019, PAB036, PAB037, PAB042, PAB044, PAB046 and PAB050 were identified as promising binders of human PSP-94 and currently are being characterized in more detail.

Funding by the grant FR-TI4/667 MPO ČR and the institutional research concept AV0Z5052070.

P23

COMPARISON OF THE ANTICANCER ACTIVITY WITH ELECTRONIC PROPERTIES AND THERMODYNAMIC AND KINETIC PARAMETERS OF THE INTERACTION OF SELECTED PLATINUM(II) DERIVATIVES WITH GUANINE

Petra Matunová and Jaroslav V. Burda*

Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 121 16 Prague 2, Czech Republic
**author whom correspondence should be addressed*

Three potential anticancer agents (trans-[$PtCl_2$(NH3)(thiazole)], cis-[$PtCl_2$(NH_3)(piperidin)], and $PtCl_2$(NH_3)(cyclohexylamine) (JM118)) were explored and compared with cisplatin and the inactive $[PtCl(dien)]^+$ complex. Basic electronic properties, bonding and stabilization energies were determined and thermodynamic and kinetic parameters for interaction with guanine were estimated at the B3LYP/6-311++G(2df,2pd) level of theory. Since the interaction with nucleotides can represent one of the basic processes, the obtained rate constants can be compared with the experimental IC_{50} values for several tumor cells. Despite the fact that the processes in which the drugs are involved and in which they affect cells are very complex, some correlation can be deduced.

P24

RATIONAL DESIGN OF HIGH-AFFINITY VARIANTS OF INTERFERON-GAMMA RECEPTOR 1

Pavel Mikulecký, Jiří Černý, Lada Biedermannová, Peter Šebo, Bohdan Schneider

Institute of Biotechnology AS CR, v.v.i., Vídeňská 1083, CZ-142 20 Prague, Czech Republic.
pavel.mikulecky@img.cas.cz, bohdan@img.cas.cz

Protein-protein interactions play an essential role in many biological processes and our goal is to elucidate specificity of the interactions between human interferon gamma receptor 1 (hIFNgR1) and its natural ligand interferon gamma (hIFNg), an important protein of innate immunity. We searched for mutations within the receptor molecule to increase its affinity to hIFNg by computational analysis of existing crystal structures of hIFNg/hIFNgR1 (PDB IDs 1fg9 and 1fyh) and modeling using empirical force field implemented in the software FoldX. All selected hIFNgR1 variants were expressed in *Escherichia coli* and successfully purified to homogeneity. Kinetics of interactions between hIFNg and hIFNgR1-wild type as well as mutants was determined by surface plasmon resonance (SPR). The first set of mutations was designed on the interface of the hIFNg/hIFNgR1 complex and according to SPR measurements the affinity of most receptor variants had virtually the same affinity as wild-type receptor, a few had affinity slightly decreased, but a few variants bound hIFNg with significantly higher affinity. The second set of mutations of hIFNgR1 included residues that were not directly involved in binding of hIFNg and were supposed to fill up cavities inside the receptor molecule. SPR measurements showed that the affinities of these new single mutants were practically unchanged but enhanced in combination with the previously tested mutation at the interface. Our results indicate that rational design by relatively simple and accessible computational methods is capable of predicting hIFNgR1 variants with significantly increased affinity. These new high-affinity binders help in better understanding of forces governing protein-protein interactions and could be developed into a new diagnostic tool.

Support from grant P305/10/2184 from the Czech Science Foundation is greatly acknowledged. All authors are supported by the institutional grant AV0Z50520701.

P25

DIGITAL METADYNAMICS: NEW BIASED SIMULATION CONCEPT AND ITS TESTING ON THE CONFORMATIONAL EQUILIBRIUM OF AC-ALA-NHME

Pavel Oborský, Vojtěch Spiwok

Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Technická 3, Prague 6, 166 28, Czech Republic
oborsky@vscht.cz

According to the Moor's law, computer performance doubles every 18 month. Despite that the computer performance is the main limitation for simulation methods in chemistry. Various events occur in timescales larger than accessible through the computing. Instead of waiting for better computers, sophisticated methods are being developed to enhance efficiency of calculations, such as metadynamics. Metadynamics uses collective variables, which are relevant for the studied event, and during the simulation disfavours them with the bias potential in the manner the system is forced to escape from visited values of the collective variables. This causes faster sampling of molecule's conformations. The negative picture of inserted bias potential is the free energy surface in the space of collective variables. Improvement of metadynamics, called digital metadynamics, uses reference structures of the molecule instead of the collective variables. The bias potential is inserted during the simulation in reference structures based on similarity with the simulated molecule. Retroactively inserted bias potential influence dynamics of the molecule through repulsion from reference structures where the bias potential was added. For testing digital metadynamics, a model of peptide bond Ac-Ala-NHMe was used. The result was a Ramachandran plot in an agreement with the same plot obtained from classic metadynamics.

P26

COMPUTATIONAL SIMULATION OF TRIMER ROTATION IN HEXAMERIC ARGINE REPRESSOR FROM *E.COLI*

Saurabh Pandey[1], Chris Shuck[2], Jannette Carey[2], David Reha[1] and Rudiger Ettrich[1]

[1]Faculty of Science, University of South Bohemia in České Budějovice and Institute of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic, Nové Hrady, pandey@nh.cas.cz
[2]Department of Chemistry, Princeton University, Princeton, New Jersey, USA

Argine Repressor ArgR from *E.coli* is the master feedback regulator of transcription in bacterial L-arginine metabolism. Molecular dynamics simulations reveal that conserved Arg and Asp sidechains in each L-arginine binding pocket promote rotational oscillation of apoArgR trimers by engagement and release of salt bridges [1]. However, this rotation of trimers in earlier simulations using the gmx force field occured on a very short timescale during the equilibration phase of the simulations, which made a proper study of the dynamics of this transition very difficult. In new simulations using the Amber99SB forcefield we succeeded to shift the rotation to the production phase. The whole transition takes now more than 20ns reaching full equilibrium around 50 ns. The degree of rotation corresponds to the 13 degrees described earlier, and all six salt-bridges are formed. This allows to study in detail the molecular changes during the transition, in which Arg110 competes with the solvent for an interaction with Asp128, leading to the consequent rotation. These simulations now help to adress the question how the rotation is propagated throughout the hexameric structure and if all six subunits act in a concerted manner. Additionally, unbinding of L-Arg from ArgR was explored by umbrella sampling and PMF calculations.

Support from the Czech Science Foundation, no 13-21053S, is acknowleged.

R. Strawn, M. Melichercik, M. Green, T. Stockner, J. Carey, R. Ettrich, PLOS Computational Biology, 2010, 6: 6. e1000801.

P27

GATEWAY-BASED SYSTEM FOR OPTIMIZATION OF RECOMBINANT PROTEIN EXPRESSION IN K. LACTIS

Pavlíček J., Škultétyová Ľ., Daniel P., Bařinka C.

Institute of Biotechnology AS CR, Vídeňská 1083,142 20 Prague 4, Czech Republic

Preparation of sufficient amounts of functional recombinant proteins is typically a major bottleneck in structural biology projects. Here we report the construction and evaluation of a set of Gateway-compatible destination vectors comprising the combination of various N- and C-terminal tags. Recombination of the destination vectors with entry clones of interest results in a series of complementary expression vectors. These can be then used to test and optimize expression levels and solubility of a given protein in the contexts of several expression tags in yeast Kluyveromyces lactis.

P28

SELECTION AND CHARACTERIZATION OF ANTICALIN-BASED BINDERS TARGETING HUMAN GLUTAMATE CARBOXYPEPTIDASE II

Jakub Ptacek, Antonia Richter, Arne Skerra, Cyril Barinka

Laboratory of Structural Biology, Institute of Biotechnology AS CR, v. v. i., Videnska 1083, 142 20 Prague 4, Czech Republic

Anticalins are members of growing family of designed non-immunoglobulin proteins with binding properties similar to antibodies. Glutamate carboxypeptidase II (GCPII) is a membrane bound metallopeptidase highly upregulated on the surface of prostatic carcinomas and the neovasculature of solid tumors and it thus represents an excellent diagnostic target for detection of most solid cancers. Here we report our initial experiments aimed at the selection and characterization of Anticalin-based binders targeting GCPII. A randomized library of approximately 10^{10} different combinatorial variants derived from human lipocalin Lcn2 was panned against FLAG-tagged human GCPII and variants tested for GCPII specificity and affinity following the four rounds of selection. Best binders exhibit nanomolar affinity for GCPII in the ELISA setup and can be easily expressed and purified in milligram quantities from the prokaryotic expression system. These candidates will be further subjected to directed evolution to optimize their biophysical properties.

P29

BIOLOGICAL APPLICATIONS OF QM/MM CALCULATIONS WITH POLARIZED EMBEDDING

David Řeha, Vasilina Zayats, Dhiraj Sinha, Rüdiger Ettrich

Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences of the Czech Republic Zamek 136, Nove Hrady, 373 33, Czech Republic

Hybrid quantum mechanical/molecular mechanics (QM/MM) calculation can be widely used for the study of the biological systems. The effect of polarization on the charge distribution is usually included in the QM part only and MM part is often neglected due to difficulties of the implementation. The MM polarization can however play an important role. Therefore we have introduced simple approach to treat the polarization of the MM part of the model. The approach is based on the treatment of polarization by induced atomic charges instead of induced dipoles [1]. The main advantage of this approach is the easy implementation to the existing MM programs. We have implemented the method in various QM/MM programs and we have applied it for the theoretical study of the numerous biological systems.

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

The method was also applied to study the mechanisms of NADH:quinone oxidation reduction reaction in flavoprotein WrbA. During the enzymatic reaction, the NADH is oxidized to NAD^+ and quinone is reduced to hydroquionone. The reaction proceeds via FMN acting as an enzyme cofactor. We have used molecular docking improved by our approach based on QM/MM calculation with MM polarization to estimate the positions and relative binding energies of the substrates of the WrbA protein. The results of calculations supports the experimental evidence of the hopping mechanisms, where in the first step, the NADH is oxidized to NAD^+ by FMN (which is reduced to $FMNH_2$) and in the second step (after the NAD^+ leaves to active site), the quinone is reduced to hydro-quinone by $FMNH_2$ (which is oxidized back to FMN). Furthermore, our calculations helps to explain the unusual position of NAD in crystal structure of Andrade *at al* [2]. The crystal structure represents the product (NAD^+) leaving the active site after the reaction.

Finally, the method was applied for the calculation of the conformational changes connected to the coupling of translocation and endonuclease activity in the restriction-modification system EcoR1241. We have calculated the binding energies of ATP with the aminoacid residues in the active site. The results showed that LYS313, ARG688 and ARG691 have major contributions in ATP binding. The strongest interactions are with the phosphate part, the adenine ring itself has a minor contribution to the overall binding energy. The contact with LYS220 has a negligible contribution in terms of the whole ATP binding itself, however it has some significance if just the interaction between adenine ring and protein is taken in to account.

The all applications of QM/MM calculation with polarized embedding demonstrated the importance of the implementation of our method to study the biological problems.

Authors gratefully acknowledge financial support from grant Kontakt ME09016 and the grant GACR P207/12/2323.

1. Ferenczy, G.G.; Reynolds, C.A. *J. Phys. Chem. A* **2001**, *105*, 11470–11479.
2. Andrade, S.L.; Patridge, E.V.; Ferry, J.G.; Einsle, O. *J. Bacteriol.* **2007**, *189*, 9101-9107.

P30

THEORETICAL STUDY OF METAL ION BINDING IN MODIFIED AND NATURAL CYTOSINE-CYTOSINE BASE PAIRS

Jakub Šebera[1,2], Kumiko Sugiyama[3], Akira Ono[3], Jos Mulder[4], C. Fonseca Guerra[4], F. Matthias Bickelhaupt[4], Yoshiyuki Tanaka[5], Vladimír Sychrovský[2]

[1] Institute of Physics, Academy of Sciences Czech Republic v.v.i, Na Slovance 2, CZ-182 21, Prague 8, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Flemingovo nám. 2, 166 37 Prague, Czech Republic
[3]Department of Material and Life Chemistry, Faculty of Engineering, Kanagawa University,3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama, Kanagawa 221-8686, Japan
[4]Department of Theoretical Chemistry and Amsterdam Center for Multiscale Modeling VU University Amsterdam, De Boelelaan 10831081 HV Amsterdam, The Netherlands
[5]Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aza-Aoba, Aoba-ku, Sendai 980-8578, Japan

We present here the optimized geometries and stabilization energies of metal-mediated base pair C_A-M-C_A, where C_A is 4-N-carboxymethylcytosine and M is Ag^+,Ni^{2+} and Cu^{2+}. The second part of our work includes the optimized geometries, relative energies of individual isomers and conformers of metal-mediated base pair C-Ag^+-C and the calculation of spin-spin coupling ^{1}J(N,Ag).

The calculations were performed using the DFT CAM-BLYP or B3LYP functional employing the continuum model of water solvent. The NMR spin-spin coupling constant ^{1}J(N,Ag) in C-Ag^+-C base pair was calculated using the DFT-ZORA (Zero Order Regular Approximation) approach with the BLYP and B3LYP functionals.

We described several structurally possible metal binding modes of C_A-M-C_A base pairs. The C_A-Cu^{2+}-C_A was found to be the most stable metal-mediated base pair as regards the calculated stabilization energy, which was in agreement with the melting temperature experiment for doping the DNA duplex 5‘-GTGACCA**C$_A$**TGCAGTG-3‘: CACTGGT**C$_A$**ACGTCAC-5‘ with the three different metals. At the second part of our study, we found that the most stable isomer of C-Ag^+-C base pair possessed the N3-Ag^+-N3 linkage and the calculated ^{1}J(N,Ag) spin-spin coupling was in good agreement with experiment.

This work was supported by the Young Investigator’s Grant of the Human Frontier Science Program (HFSP), Grant Agency of the Czech Republic P205/10/0228, Technological Agency of the Czech Republic Grant no. TACR TA01011165, and by the Czech Science Foundation Grant No. P304/10/1951. The access to the MetaCentrum computing facilities is appreciated.

P31

STRUCTURAL PROPERTIES OF NK RECEPTORS AND LIGANDS WITH C-TYPE LECTINE-LIKE FOLD

T. Skálová[1], P. Kolenko[1], J. Dušková[1], T. Kovaľ[1], J. Hašek[1], J. Stránský[1,2], J. Dohnálek[1]

[1] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, v.v.i., 16206 Praha 6, Czech Republic
[2]Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University, Břehová 7, 115 19 Praha 1, Czech Republic
t.skalova@gmail.com

Natural killer cells (NK cells) are blood corpuscles, more precisely, a sort of lymphocytes. They comprise 5-10% of lymphocytes in blood and their role in the immune system is to discover and kill cancer cells and cells infected by viruses.

This work is aimed at a class of NK receptors, i.e. receptors on the surface of NK cells, which have a special fold: C-type lectin-like fold (CTL fold, (1)). More generally, other receptors, not only NK receptors, but with CTL fold, will be mentioned included in the presented analysis. The role of NK receptors with CTL fold is to mediate contact with other cells, in order to kill infected or cancer cells. NK receptors with CTL fold interact with protein ligands,

which are of the same, CTL, fold, and are located on surface of partner cells.

During recent years, we have solved structures of several receptors/ligands with CTL fold (high resolution structure of human CD69 (2), mouse NKR-P1A (3, 4), mouse Clr-g (5)), and other structures are in progress. These structures inspire us to study 1) CTL fold, its characteristics and its variability, 2) Types of oligomerization of CTL receptors and ligands (Figure 1), and 3) Rules of formation of CTL receptor-ligand complexes.

It was found that the dimerization mode of CTL proteins is very variable, while complexation of structurally known CTL protein-protein complexes happens in the same area of monomers, in the part distant to N and C terminal region.

This work was supported by the Czech Science Foundation (P302/11/0855), and the Ministry of Education, Youth and Sports of the Czech Republic (CZ.1.07/2.3.00/30.0029).

1. A. N. Zelensky and J. E. Gready, The C-type lectin-like domain superfamily, *FEBS Journal,* **272,** (2005), 6179–6217.
2. P. Kolenko *et al.,* The high-resolution structure of the extracellular domain of human CD69 using a novel polymer, *Acta Crystallogr.,* **F65**, (2009), 1258-1260.
3. P. Kolenko *et al.,* Molecular architecture of mouse activating NKR-P1 receptors, *J. Struct. Biol.,* **175(3)**, (2011), 434-441.
4. P. Kolenko *et al.,* Structure of the H107R variant of the extracellular domain of mouse NKR-P1A at 2.3 Å resolution, *Acta Crystallogr.,* **F67**, (2011), 1519-1523.
5. T. Skalova *et al.,* Mouse Clr-g, a Ligand for NK Cell Activation Receptor NKR-P1F: Crystal Structure and Biophysical Properties, *J. of Immunology*, 189(10), 2012, 4881-4889.

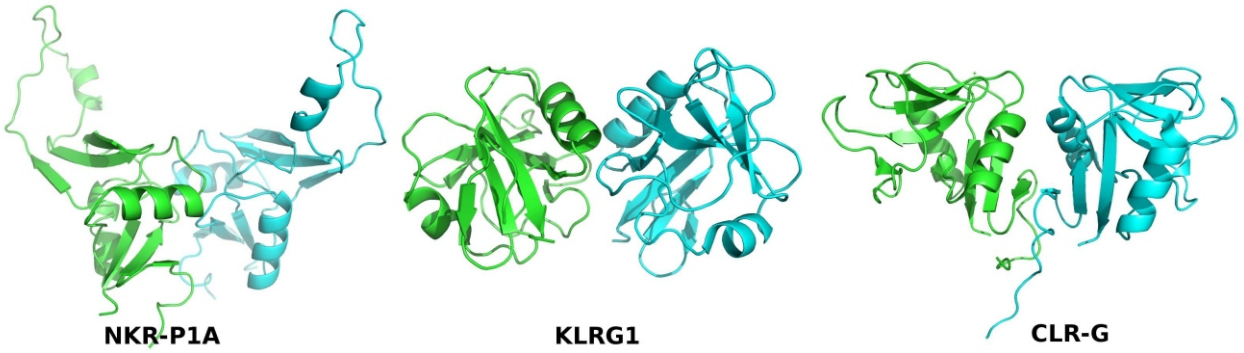


Figure 1. Comparison of dimerization modes of three mouse CTL proteins: NK receptor NKR-P1A (PDB code 3M9Z), NK receptor Klrg1 (3FF9), and a ligand for NK receptor NKR-P1F: Clr-g (3RS1).

P32

VALIDATION OF A HIGH-THROUGHPUT SETUP FOR MANUAL ASSEMBLY OF NANOLITRE VAPOUR-DIFFUSION PROTEIN CRYSTALLIZATION SCREENS

R. Skrabana[1,2] , O. Cehlar[1], M. Novak[1,2]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia
[2]Axon Neuroscience SE, Grosslingova 45, 811 09 Bratislava, Slovakia

Nanolitre-sized drops are characteristic of high-throughput protein crystallization screening. Traditionally, reliable nanolitre drop dispensing has required the use of robotics. We have developed a protocol for the reproducible manual assembly of nanolitre-sized protein vapour-diffusion crystallization trials in a 96/192-drop format. The protocol exploits the repetitive-pipetting mode of handheld motorized pipettes and saves precious protein material without sacrificing the effectiveness of the screening process. The manual dispensing of solutions with variable viscosity in the range of 0.2 – 0.5 µl by a motorized multichannel pipette was comparably reliable as dispensing by robotics [1] or by a handheld nanoject pipettor [2]. We have tested the repeatability and reproducibility of proposed method by repeating successful crystallization trials of an antibody Fab fragment in sitting drops on 96-well plates with variable precipitant volume and comparing motorized and manual mode of pipetting. We were able to produce crystals repeatedly in 0.5 and 0.3 µl drops and in a 0.2 µl drop assembled by a manual pipette. Summarizing, developed protocol allows for reproducible manual formation of nanolitre protein crystallization drops in high-throughput format. We believe that the method could be useful especially for small crystallographic groups since it needs no additional expensive instrumentation [3].

This work was supported by the Slovak Research and Development Agency under the contract No. LPP-0038-09 and by the Slovak Grant Agency VEGA grant No. 2/0163/13.

1. Walter, T. S., Diprose, J., Brown, J., Pickford, M., Owens, R. J., Stuart, D. I. & Harlos, K. (2003). Journal of Applied Crystallography 36, 308-314.
2. Cherezov, V. & Caffrey, M. (2005). Journal of Applied Crystallography 38, 398-400.
3. Skrabana, R., Cehlar, O. & Novak, M. (2012). J. Appl. Cryst. 45, 1061–1065.

P33

CHARACTERIZATION OF THE SELECTED HALOALKANE DEHALOGENASES CRYSTALS SPECIFIC ACTIVITY TOWARDS TO THEIR SUBSTRATES

Katsiaryna Tratsiak[1,2],Oksana Degtjarik [1,2], Ivana Drienovska [3], Lukas Chrast [3], Pavlina Rezacova [4,5], Michal Kuty [1,6], Radka Chaloupkova [3], Jiri Damborsky [3], Jose A. Gavira [7] and Ivana Kuta Smatanova [1,6]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, CENAKVA and Institute of Complex Systems, Zamek 136, 373 33 Nove Hrady
[2]University of South Bohemia in Ceske Budejovice, Faculty of Science, Branisovska 31, 370 05 České Budějovice, Czech Republic
[3]Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice 5/A4, 625 00 Brno, Czech Republic
[4]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic v.v.i., Videnska 1083, Prague 4, Czech Republic
[5]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic v.v.i., Flemingovo nam. 2, 166 37 Prague, Czech Republic
[6]Academy of Sciences of the Czech Republic, Institute of Nanobiology and Structural Biology GCRC, Zamek 136, 373 33 Nove Hrady, Czech Republic
[7]Laboratory for Crystallographic Studies (CSIC-UGR) University of Granada, Av. de las Palmeras, 4, 18100 Armilla - Granada, Spain
tratsiak@frov.jcu.cz

Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are microbial enzymes with catalytic activity for the hydrolytic conversion of xenobiotic and highly toxic halogenated aliphatic compounds to the corresponding alcohols (J. Damborsky et al, 2001, Janssen et al. 2005). These enzymes are able to convert a wide spectrum of substrates including halogenated alkanes, cycloalkanes, alkenes, ethers, alcohols, ketones, and cyclic dienes (E. Chovancova et al.2007).

Enzymes DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marinobacter sp.* ELB 17 were used to obtain crystals in optimized crystallization conditions for carrying out further HLD activity reaction according to the Iwasaki method, which is based on observing the intensity of color of a dark red iron-thiocyanate complex that is proportional to the concentration of halogen ions in the sample. Crystal of DpcA enzyme diffracted to the 1.05 Å resolution and belonged to the primitive monoclinic space group $P2_1$ and crystals of DmxA diffracting to the resolution of 2.49 Å belonged to centered orthorhombic $C222_1$ space group. Both crystals were cross-linked by 8% gluteraldehyde and specific activity towards to 1-bromoheaxane and 1,2-dichloroethane at different time were examined.

The research of the represented data was supported by GA CR (P207/12/0775) and ME CR (CZ.1.05/2.1.00/01.0024)

P34

HUMAN IL-23 RECEPTOR ANTAGONISTS DERIVED FROM AN ALBUMIN-BINDING DOMAIN SCAFFOLD INHIBIT IL-23-DEPENDENT *EX VIVO* EXPANSION OF IL-17-PRODUCING T-CELLS

Lucie Vaňková[1], Milan Kuchař[1], Hana Petroková[1], Jiří Černý[1], Radim Osička[2], Ondřej Pelák[3], Bohdan Schneider[1], Tomáš Kalina[3], Peter Šebo[1], Petr Malý[1]

[1]Institute of Biotechnology AS CR, v. v. i. and [2] Institute of Microbiology AS CR, v. v. i., Vídeňská 1083, 142 20 Prague, Czech Republic
[3]Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
lucie.vankova@img.cas.cz, petr.maly@img.cas.cz

Interleukin-23 (IL-23), a heterodimeric cytokine of covalently bound p19 and p40 subunits, has been recently described to play a pivotal role in the development of chronic autoimmune diseases.

As an alternative to antibody-based drugs, we engineer novel protein binders suppressing IL-23-mediated signaling. Library of three-helix bundle variants of albumin-binding domain (ABD) from streptococcal protein G (Ahmad et al, 2012) was used to identify binders of extracellular human.

IL-23R via ribosome display selection. To screen the library of ABD variants for high affinity binders of IL-23 receptor, we produced bacterial extracellular N-terminal poly-his-modified IL-23 receptor.

The collection of variants (REX series) was tested for binding affinity in ELISA and selected clones were modified by a C-terminal AviTag for *in vivo* biotinylation. Finally we identified a group of clones that inhibited binding of p19-subunit of IL-23 to refolded IL-23R or glycosylated IL-23R Fc chimera, secreted by murine NSO-derived cells.

We further demonstrate that REX variants bind to K-562, Jurkat and THP-1 human cell lines and this binding correlates with IL-23R cell-surface expression. As binding of the REX clones to THP-1 cells can be substantially diminished by a high dose of p19 protein and the three best-performing REX binders inhibited the IL-23-driven expansion of IL-17-producing primary human $CD4^+$ T-cells, we conclude that unique IL-23R antagonists derived from the ABD scaffold were generated that might be useful in designing novel anti-inflammatory biologicals.

Funding by the grant GACR P303/10/1849 and the institutional research concept AV0Z50520701.

Novel high-affinity binders of human interferon gamma derived from albumin-binding domain of protein G. Jawid N. Ahmad, Jingjing Li, Lada Biedermannová, Milan Kuchař, Hana Šípová, Alena Semerádtová, Jiří Černý, Hana Petroková, Pavel Mikulecký, Jiří Polínek, Ondřej Staněk, Jiří Vondrášek, Jiří Homola, Jan Malý, Radim Osička, Peter Šebo, Petr Malý. *Proteins: Structure, Function, and Bioinformatics.* vol. 80, no.3 pages 774–789, March 2012. DOI: 10.1002/prot.23234.

P35

NMR INVESTIGATION OF UNSTRUCTURED MALARIA SURFACE PROTEIN MSP2, ISOFORM 3D7

M. Zachrdla[1], J. Nováček[1], L. Žídek[1], V. Sklenář[1], R. S. Norton[2]

[1]NCBR, Faculty of Science and CEITEC, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic
[2]The Walter and Eliza Hall, Institute of Medical Research, 1G Royal Parade, Parkville 3050, Australia
324489@mail.muni.cz

Malaria is one of the most important infectious diseases resulting in two million deaths worldwide. Merozoite surface protein 2 (MSP2) belongs to potential malaria vaccine candidates. In this study, we focused on the MSP2 isoform 3D7. The aggregation and fibril formation propensities of 3D7 are lower than those of the other isoform FC27. The goal of the study is to describe structural differences between the isoforms. This task is complicated by the fact that both proteins are intrinsically disordered. Nuclear magnetic resonance (NMR) has been chosen as a method well suited to describe structural features of molecules lacking a well-defined structure. The first step of the study was assignment of NMR frequencies to individual atoms of the protein. In order to overcome a severe peak overlap, 5D non-uniformly sampled NMR experiments were employed. Frequencies of backbone atoms of all 217 non-proline residues were successfully assigned. The obtained data was used to calculate secondary structure propensity of the protein.

P36

MOTIONS OF BIOMOLECULES MONITORED BY SPECTRAL DENSITY MAPPING

V. Zapletal, P. Kadeřávek, R. Fiala, V. Sklenář, L. Žídek

NCBR, Faculty of Science and CEITEC, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic
vojtis@mail.muni.cz

Nucleic acids, proteins, saccharides and other biologically important molecules exhibit a broad variety of motions ranging from femtosecond vibrations to very slow processes. Description of the internal dynamics is necessary for correct understanding of the physiological functions of the studied molecules. A detailed picture of molecular motions is obtained by measuring and analyzing the NMR relaxation rates of suitable nuclei in the studied molecules. A suite of methods extending applicability of a straightforward analysis relaxation data, known as spectral density mapping, is presented. The methodology was tested on samples presenting a considerable challenge for the standard approaches, including a partially disordered protein, a uniformly ^{13}C,^{15}N-labeled RNA hairpin, and a disaccharide with a selectively ^{13}C-labeled bridging methylene group.

P37

GLYCOPROTEINS AND PROTEIN GLYCATIONS IDENTIFIED IN BARLEY GRAIN AND MALT BY 2D-HPLC AND MS/MS

J. Žídková[1], I. Petry-Podgórska[2], M. Laštovičková[1], J. Bobáĺová[1]

[1] *Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Veveří 97, 60200 Brno, Czech Republic*
[2] *Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Vídeňská 1083, 14660 Prague 4, Czech Republic*
zidkova@iach.cz

Carbohydrate moieties represent typical post-translational modifications of barley proteins. Binding of mono- and oligosaccharides to polypeptide chain can be either a physiological reaction, glycosylation, or an artificial process occurring during industrial processing, glycation. Both types of modification have important consequences for consumers of barley products. Glycation changes physical and chemical properties of malt and other products, and several glycoproteins were described as important allergens. Two-dimensional liquid chromatography (ion-exchange and reversed-phase) with off-line mass-spectrometric detection (MALDI-TOF-MS/MS) has been used to analyze glycoproteins and glycated proteins in barley grain and malt. Seven glycoproteins were identified by partial sequencing based on fragmentation MS/MS spectra. Glycations were observed for five out of 16 unambiguously assigned proteins in malt extract, while no glycation was detected in barley grains. The results document that the proposed analytical procedure is well suited for monitoring carbohydrate-modified proteins in cereal products and their end foodstuff products.

This work was supported by the Czech Science Foundation (grant no. P503/12/P395), and by institutional support RVO 68081715 of Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, v.v.i.