INSTRUCT ULTRA

3rd Structural biology meeting

November 14 - 15, 2019

Hotel Tatra, Bratislava

Hall „GRAND“

Námestie 1. mája 5, 811 06 Bratislava

ISBN 978-80-971665-1-9

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Programme

Thursday, November 14, 2019

12,30 -13,45 **Lunch**

13,45 – 14,00 **INSTRUCT-ULTRA H2020**
Miloš Hricovíni
Institute of Chemistry, Slovak Academy of Sciences, Bratislava

Chair: *Vladimír Pevala*

14,00 – 14,20 **Mechanics of single proteins**
Gabriel Žoldák
[1] Center for Interdisciplinary Biosciences, Technology and Innovation Park, P.J. Šafárik University, Košice, Slovakia

14,20 – 14,40 **Investigating the role of some key players of the human cytosolic iron-sulfur cluster assembly machinery**
Francesca Cantini [1,2], Vincenzo Maione[1], Lucia Banci[1,2]
[1]Magnetic Resonance Center (CERM) – University of Florence, Italy,
[2]Department of Chemistry - University of Florence, Italy

14,40 – 15,00 **Importance of Base-pair Probing for Mismatch Recognition**
Tomáš Bouchal[1,2], Ivo Durník[1,2], Petr Kulhánek[1,2]
[1]CEITEC – Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech Republic
[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic

15,00 – 15,20 **Comparative structural analysis of lectin family from *Photorhabdus spp.***
Josef Houser[1,2], Eva Fujdiarová[2], Gita Jančaříková[1,2], Filip Melicher[1], Michaela Wimmerová[1,2]
[1]CEITEC-Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

15,20 – 15,40 **Coffee break**

Chair: *Miloš Hricovíni*

15,40 – 16,00 **Micro-Electron Diffraction: A Powerful Tool For Protein And Small Molecule Structure Determination**

Radovan Spurny[1]
[1]*Thermo Fisher Scientific, Vlastimila Pecha 1282/12, Brno, Czech Republic*

16,00 – 16,20 **Local DNA structures and their importance in regulation of biological processes**

Václav Brázda
Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

16,20 – 16,40 **Weak Protein-Ligand Interactions: Advances in Saturation Transfer Difference (STD) NMR techniques**

Jesus Angulo[1]
[1]*School of Pharmacy, University of East Anglia (UEA), Norwich Research Park, NR4 7LU, Norwich, U.K.*

16,40 – 17,00 **Human cardiac ryanodine receptor and its association with arrhytmias - milestones, achievements and perspectives**

Vladena Bauerová
[1] *Institute of Molecular Biology SAS, Dubravska cesta 21, Bratislava, Slovak Republic*

17,00 – 17,20 **The exopolysaccharide produced by the cyanobacterium *Nostoc* sp. - a structural study**

Iveta Uhliariková[1], Mária Matulová[1], Vladislav Cepák[2], Jaromír Lukavský[2], Peter Capek[1]
[1] *Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 84538 Bratislava, Slovakia*
[2] *Institute of Botany, Centre for Algology, Academy of Sciences of the Czech Republic, Dukelská 135, 37901 Třeboň, Czech Republic*

18,00 **Dinner**

Friday, November 15, 2019

Chair: *Vladimír Frecer*

8,45 – 9,05 **Normal mode analysis as a routine part of a structural investigation**

Jacob Bauer
Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava

9,05 – 9,25 **Catalytic mechanisms of both inverting and retaining glycosyltransferases pass through oxocarbenium ion intermediates via SNi-like processes: Insights from DFT calculations.**

Juraj Kóňa
Department of Structure and Function of Saccharides, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84538 Bratislava, Slovakia

9,25 – 9,45 **Analysis of Protein Dynamics and Structure by nt-PaCS-MD and ProGA**

Vladimir Sladek [1,2,*], Ryuhei Harada[3], Yasuteru Shigeta[3]
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[3] *Center for Computational Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan*

9,45 – 10,05 **Study of the N-acetylglucosaminyltransferase-V (GnT-V) interactions with substrates using computational chemistry tools**

Barbora Stratilová[12], Stanislav Kozmon[1]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia*
[2]*Department of Physical and Theoretical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia*

10,05 – 10,25 **Photochemical properties of potential anticancer agents: *Anti-syn* isomerization of 2,3-disubstituted quinazolinones**

Michal Hricovíni,[1] James Asher,[2] Miloš Hricovíni[1]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 36 Bratislava, Slovak Republic*
[2] *Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovak Republic*

10,25 – 10,45 **Coffee break**

Chair: *Jesus Angulo*

10,45 – 11,05 **Building a center for research and teaching activities in structural biology for biomedicine and biotechnology – Interreg V-A Slovakia – Austria project StruBioMol**

Vladimír Pevala[1], Gabriela Ondrovičová[1], Veronika Kotrasová[1], Barbora Keresztesová[1], Nina Kunová[1], Lucia Martináková[1], Jelena Pavlović[1], Vladena Bauerová[1], Jacob A. Bauer[1], Július Košťan[2], Kristina Djinović-Carugo[2], Eva Kutejová[1]
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[2]*Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Campus Vienna Biocenter 5, A-1030 Vienna, Austria*

11,05– 11,25 **Amylolytic family GH126 - in silico analysis and preliminary experimental identification of potentially novel members**

Lenka Kerényiová*, Zuzana Šramková, Domenico Pangallo & Štefan Janeček
Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, Bratislava, Slovakia

11,25 – 11,45 **Bioinformatics analysis of acetylesterases from family CE16**

Ľubica Urbániková[1], Štefan Janeček[1,2]
[1] *Laboratory of Protein Evolution, Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovakia; lubica.urbanikova@savba.sk*
[2] *Department of Biology, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Nám. J. Herdu 2, 917 01 Trnava, Slovakia*

11,45 – 12,05 **Fragmentation analysis of O-specific bonded saccharides from bacterial samples using LC-MS and MALDI-MS**

Filip Pancik[1], Jana Valarikova[1], Alzbeta Cizova[1], Slavomir Bystricky[1], Peter Barath[1]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia*

12,05 – 12,25 **Pathogenicity of new *BEST1* variants identified in Italian patients with best vitelliform macular dystrophy assessed by computational structural biology**

Vladimir Frecer[1], Giancarlo Iarossi[2], Anna Paola Salvetti[3], Paolo Enrico Maltese[4], Giulia Delledonne[3], Marta Oldani[3], Giovanni Staurenghi[3], Benedetto Falsini[5], Angelo Maria Minnella[5], Lucia Ziccardi[6], Adriano Magli[7], Leonardo Colombo[8], Fabiana D'Esposito[9,10,11], Jan Miertus[12], Francesco Viola[13], Marcella Attanasio[14], Emilia Maggio[14] and Matteo Bertelli[4,11]

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[7] *University of Salerno, Salerno, Italy.*
[8] *San Paolo Hospital, University of Milan, Milan, Italy.*
[9] *Western Eye Hospital, Imperial College Healthcare NHS Trust, London, UK.*
[10] *University of Naples Federico II, Naples, Italy.*
[11] *MAGI Euregio, Bolzano, Italy.*
[12] *Genius n.o., Trnava, Slovakia.*
[13] *Fondazione IRCCS Cà Granda, Clinica Regina Elena, Milan, Italy.*
[14] *IRCCS-Ospedale Sacro Cuore Don Calabria, Negrar, VR, Italy.*

12,30 – 12,35 **Conclusions**

12,35 **Lunch**

Mechanics of single proteins

Gabriel Žoldák[1]

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One of the ultimate goals in protein biophysics is obtaining three-dimensional structural dynamics with nanosecond time-resolution and large enough dynamic range to sample rare events that only occasionally appear over long time intervals. On the simulation side, all-atom molecular dynamics simulations are already providing full structural dynamics information on ns timescales, and recently even ms timescales have been reported [1]. However, no experimental method can currently provide a similarly detailed picture. Single-molecule force spectroscopy, while still far from this goal, provides the possibility to measure structural dynamics along a single reaction coordinate with microsecond resolution over a timescale of hours, and thus can uncover fine details of the underlying principles of protein nanomachines.

Protein nanomachines can be thought of as being built like man-made machines: static parts form a basic scaffold for movable parts whose motion is fuelled by an energy source, e.g., thermal motion, ligand binding, concentration gradients or chemical reactions. Using single-molecule force spectroscopy, we are now able to identify the basic parts of these "machines" i.e., functional mechanical elements of proteins. This knowledge will enhance our understanding of the design principles of these highly complex nano-scale objects, eventually enabling us to design our own man-made nanomachines.

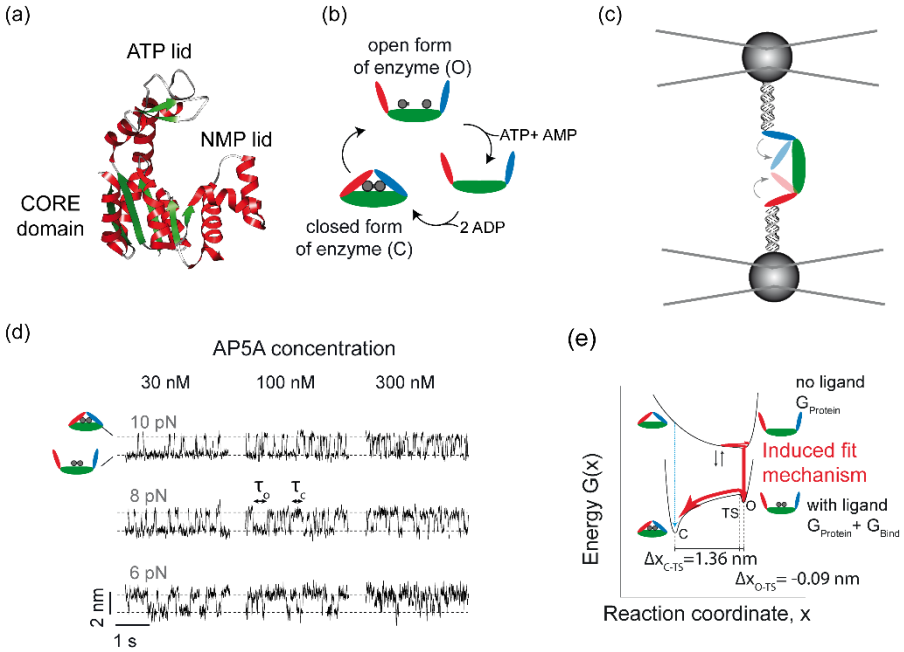

Fig.1. (a) Crystal structure of the open state of adenylate kinase (PDB accession code: 4X8H), showing the CORE domain, the ATP lid, and the NMP lid. (b) Simplified schematic of the conformational cycle of Adk. (c) Optical tweezers assay for monitoring conformational transitions between open and closed forms. (d) Single-molecule interconversion between open and closed forms of Adk during its interaction with Ap5A. (e) Energy profile of the particular forms of Adk, adapted from [2].

For proteins, the motion is fuelled by energy minimization. For example, the closure of adenylate kinase upon ligand binding is due to favorable pairwise interactions and binding to a pre-optimized solvated binding pocket[2]. Upon ligand binding to the open crevice of the active site, flexible lid parts cover the binding pocket. This is energetically favorable partly due to the direct interaction with ligand and partly as a result of the change of the local structure around the binding site changes to favor interaction with the lids.

While minimization of binding free energy drives many processes in cells, it is not sufficient to direct unidirectional mechanical work or reaction cycles. Therefore, highly complex mechanical machines use the energy released during exergonic chemical reactions for mechanical functions (e.g., ATP hydrolysis) that need directionality (e.g., physical motion) or controlled timings. Single-molecule force spectroscopy experiments can characterize these essential function-related motions in great detail. Several examples how mechanical studies can contribute to our understanding of proteins will be discussed.

ACKNOWLEDGEMENT

This work was supported by the research grants from the Slovak Grant Agency VEGA No 1/0175/19 and by the Slovak Research and Development Agency under contract no. APVV-18-0285

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Investigating the role of some key players of the human cytosolic iron-sulfur cluster assembly machinery

Francesca Cantini [1,2], Vincenzo Maione[1], Lucia Banci[1,2]

[1]Magnetic Resonance Center (CERM) – University of Florence, Italy,
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The CIA2A protein, in complex with CIAO1, has been proposed to be exclusively implicated in the maturation of cytosolic aconitase [1]. However, how the CIA2A-CIAO1 complex generates active aconitase is still unknown and the available structural information has not provided any crucial insights into the molecular function of CIA2A. We have characterized the Fe/S cluster binding properties of CIA2A and of the CIA2A-CIAO1 complex via NMR, UV−vis absorption and EPR spectroscopies and we have investigated how the Fe/S cluster is transferred to inactive aconitase/IRP1 protein. We found that an heterotrimeric species formed by two molecules of CIA2A and one of CIAO1 can bind one [4Fe-4S] cluster and that residue Cys90 of CIA2A is one of the cluster ligand. The holo trimeric complex is able to transfer the [4Fe-4S] cluster to apo-IRP1 thus generating the active form of aconitase. These findings, which highlight a functional role for CIA2A-CIAO1 complex in aconitase maturation, raises a broad interest and can have a high impact on the community studying metal trafficking and iron-sulfur protein biogenesis. The present study can provide solid bases for further investigation of the molecular mechanisms involving also other CIA machinery proteins.

ACKNOWLEDGEMENT
This work was supported by Instruct-ERIC, a Landmark ESFRI project, and specifically we thank the CERM/CIRMMP Italy Centre

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Importance of Base-pair Probing for Mismatch Recognition

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Thermodynamic stabilities of base pairs composed of 36 unique combinations of A, G, T, and C nucleobases in both *anti* and *syn* conformations in the central part of 13-nt long palindromic dsDNA were characterized by biased molecular dynamics simulations. The bias was introduced through two simple base-pair parameters, Opening, and Shear (1). In total, we identified and characterized over one hundred different free-energy minima. We found excellent agreement between calculated free energy minima and experimental structures of mismatches in both free DNA and DNA complexed with the MutS enzyme. The latter suggests that MutS evolved in such a way that the mismatch recognition is achieved by probing a mismatch towards the minor groove, where mismatches exhibit stable albeit energetically less favorable structures already in the free form while the canonical base pairs do not. We also found that opening of mismatch towards minor groove provides better discrimination from the canonical base pairs than previously suggested bending of DNA. This finding can be helpful in better understanding of sequence-dependent mutability (2) or designing chemical substances targeting damaged DNA.

ACKNOWLEDGEMENT

This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and the Grant Agency of the Czech Republic (GA16-11619S/2016). Computational resources were provided by the CESNET LM2015042, the CERIT Scientific Cloud LM2015085, and the IT4Innovations National Supercomputing Center LM2015070 provided under the program "Large Infrastructures for Research, Experimental Development and Innovations" by the Ministry of Education, Youth and Sports.

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Comparative structural analysis of lectin family from *Photorhabdus spp.*

Josef Houser[1,2], Eva Fujdiarová[2], Gita Jančaříková[1,2], Filip Melicher[1], Michaela Wimmerová[1,2]

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Photorhabdus is a genus of gram-negative bioluminescent bacteria living in a symbiosis with *Heterorhabditis* nematodes forming a highly entomopathogenic complex. Such a complex is used in agriculture as a nature-based insecticide. However, some members of this genus are human pathogens as well. Understanding the mechanisms that determine interaction between *Photorhabdus* and its symbionts/hosts could be highly beneficial not only in biotechnologies but also in clinical research and drug development.

Cell-cell interactions are frequently mediated by sugar-binding proteins – lectins. Based on the genome analysis, there has been identified potential lectins in number of *Photorhabdus* species. Recently, we examined two of them in further details, namely PLL from *P. laumondii* (formerly *P. luminescens*) [1] and PHL from *P. asymbiotica* [2,3]. Both lectins share the basic structural features, e.g. β-barrel fold with seven blades or presence of multiple binding sites per monomer. However, despite rather high sequence similarity, some non-marginal differences were detected: oligomeric state, binding site preferences and organization. This lead us to investigate this lectin family in further details.

We analyzed several homologues of proteins PLL and PHL from *Photorhabdus spp.* We managed to prepare some of them in recombinant form and perform basic analysis, as well as solve structure of these proteins in free form and in complexes with naturally occurring saccharide ligands. This research may not only reveal the differences in similar proteins from one family, but also answer the question, why single species of bacteria possess the ability to produce several similar lectins.

This work was supported by the Czech Science Foundation (project 13-25401S) and by the MEYS of the Czech Republic under the project CEITEC 2020 (LQ1601). CIISB research infrastructure project LM2015043 funded by MEYS CR is also gratefully acknowledged for the financial support of the measurements at the CF Biomolecular Interactions and Crystallization, CF X-ray Diffraction and Bio-SAXS and CF Proteomics at CEITEC (Brno, Czech Republic). We wish to thank the BESSY II (Berlin-Adlershof, Germany) and PETRA III (Hamburg, Germany) for access to their synchrotron data collection facilities and allocation of synchrotron radiation beam time.

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Micro-Electron Diffraction: A Powerful Tool For Protein And Small Molecule Structure Determination

Radovan Spurny

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X-ray crystallography experiments require large, well-ordered protein crystals for data collection. Growing such crystals is difficult, requires a lot of time and effort and it is sometimes even impossible. However, crystallographers are yielded quite often with plenty of microcrystals which are useless for conventional X-ray diffraction experiments. Moreover, large protein crystals are frequently imperfect and often suffer from different defects like high mosaicity. Small crystals are usually not affected by such defects and may yield in better data quality. These kind of small crystals could be used for high-resolution structure determination by the cryo-EM method called micro electron diffraction (MicroED).

In MicroED crystals are illuminated with electrons instead of X-rays, acquiring tomographic electron diffraction patterns. In some cases these small crystals are however too large for MicroED. Nevertheless, these protein crystals might be thinned by cryo-FIB milling, yielding in crystal lamella with desired thickness that are ideal for MicroED experiment.

MicroED is finding its significant role also in small molecule structure determination from small nanocrystals obtained directly from their powder form. This method offers fast track to determine the crystal structure of pharmaceutically important compounds in just few minutes.

Local DNA structures and their importance in regulation of biological processes

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Genome sequencing brings a huge amount of information regarding the genetic basis of life. While this information provides a foundation for our understanding of biology, it has become clear that the DNA code alone does not hold all the answers. Epigenetic modifications and higher order DNA structures beyond the double helix contribute to basic biological processes and maintaining cellular stability. Local alternative DNA structures are known to exist in all organisms. Negative supercoiling induces in vitro local nucleotide sequence-dependent DNA structures such as cruciforms, left-handed DNA, triplex and quadruplex structures etc. The formation of cruciforms requires perfect or imperfect inverted repeats of 6 or more nucleotides in the DNA sequence. Inverted repeats are distributed nonrandomly in the vicinity of breakpoint junctions, promoter regions, and at sites of replication initiation. Cruciform structures could for example affect the degree of DNA supercoiling, the positioning of nucleosomes in vivo, and the formation of other secondary structures of DNA. The three-dimensional molecular structure of DNA, specifically the shape of the backbone and grooves of genomic DNA, can be dramatically affected by nucleotide changes, which can cause differences in protein-binding affinity and phenotype. The recognition of cruciform DNA seems to be critical not only for the stability of the genome, but also for numerous, basic biological processes. As such, it is not surprising that many proteins have been shown to exhibit cruciform structure-specific binding properties [1] or G-quadruplex binding properties [2]. Contemporary we have developed easy accessible web tools for analyses of inverted repeats [3] and G-quadruplexes[4] and we have analyzed the presence of inverted repeats and G-quadruplexes in various genomic datasets, such as all sequences mitochondrial genomes [5], all bacterial genomes [6], in S.cerevisiae (in review), in human genome etc. A deeper understanding of the processes related to the formation and function of alternative DNA structures will be an important component to consider in the post-genomic era.

ACKNOWLEDGEMENT

This work was financed by The Czech Science Foundation (18-15548S).

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Weak Protein-Ligand Interactions: Advances in Saturation Transfer Difference (STD) NMR techniques

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Saturation Transfer Difference (STD) NMR is a powerful ligand-based NMR technique for weak ligand screening of protein targets and to gain quantitative structural information from biologically relevant protein-ligand complexes [1]. The approach is appropriate for small/medium-sized molecule binders of medium/weak affinity (dissociation constants from high nM to low mM), there is no upper limit for protein size, and labelling is not required. The technique is highly versatile and popular in the context of hit identification in drug discovery.

In this talk the investigation by STD NMR of molecular recognition processes of ligands by biologically relevant protein receptors will be presented [2-5]. Although the technique has demonstrated its broad applicability to study many different protein-ligand systems, particularly in the field of Fragment Based Drug Design, the talk will keep a focus on the application to specific cases of recognition of glycans by proteins. Protein-glycan interactions are very relevant protein-ligand interactions in Nature and are processes typically falling within the range of fast chemical exchange (weak affinity) but still showing high specificity [6]. These protein-glycan systems will allow to introduce along the talk novel methodological developments in STD NMR produced in our lab, as the identification about how the fast ligand rebinding process can affect the determination of accurate dissociation constants by STD NMR [7], as well as the development of the recent method "*DiffErential EPitope mapping STD NMR* (DEEP-STD NMR)" [8] that allows for the first time to identify the nature of the protein-ligand contacts in the bound state from STD NMR approaches.

ACKNOWLEDGEMENT

JA acknowledges funding from the Biotechnology and Biological Sciences Research Council (BBSRC) through a New Investigator grant (BB/P010660/1).

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Human cardiac ryanodine receptor and its association with arrhythmias – milestones, achievements and perspectives

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The cardiac Ryanodine receptor (RyR2) is one of the largest known ion channels. Its primary role is to release Ca^{2+} ions from the sarcoplasmic reticulum of myocytes into the cytoplasm, thereby triggering heart cell contraction [1]. Electron microscopy studies of the whole channel have shown that these channels are roughly mushroom-shaped, with a cytosol-facing cap and a stalk embedded in the SR membrane [2]. The *RYR2* gene has been found to harbor three mutation "hot spots", one at the N-terminus, one lying in the central region, and one in the C-terminal channel domain. Mutations in these regions are associated with heritable cardiac arrhythmias (ARVD2, CPVT1); these are often fatal after severe emotional or physical stress.

In our laboratory, we have solved the crystal structure of the N-terminal domain (NTD) of human RyR2 (a fragment containing residues 1–606) under near-physiological conditions [3]. This structure showed that the NTD can be divided into three domains, A, B, and C, surrounding a central helix. The interfaces between these domains revealed a number of interactions involving residues whose mutations are implicated in arrhythmias. In particular, the central helix hosts 5 residues giving rise to 7 mutations which are associated with CPVT1 or ARVD2. We therefore began a systematic examination of the structural and biophysical consequences of these seven mutations. We have cloned, expressed and purified the I419F, R420W, and I419F-R420W mutations in the laboratory. We found that the I419F mutant was unstable, while the R420W and I419F-R420W double mutant had stability comparable to the wild-type. We carried out molecular dynamics studies on the R414L, I419F, and R420W mutants in order to understand how these mutations might disrupt the ability of the channel to open and close correctly. The mutations do not appear to greatly change the overall structure of the NTD, but do alter its dynamics [4]. In particular, the mutations alter the motion of domains A and C with respect to on e another, thereby possibly disrupting a proposed gating mechanism [5] suggested to strengthen the closed conformation of the receptor.

ACKNOWLEDGEMENTS
The authors would like to thank Ing. Eva Kutejová, DrSc. for support and helpful discussions. This work was supported by VEGA research grant 2/0140/16 from the Slovak Grant Agency.

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The exopolysaccharide produced by the cyanobacterium *Nostoc* sp. - a structural study

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Cyanobacteria (blue-green algae) are the oldest photosynthetic prokaryotic microorganisms, which colonize all biotopes and territories of our planet. Factors such as light, pH, temperature, salinity and nutrient availability affect their photosynthesis, biomass production and ultimately the biochemical composition of their cells. During the life cycle, cyanobacteria produce low or high molecular weight metabolites of a great biotechnological and industrial importance. They can be located in cells, on their surface or released into their environment [1]. Exopolysaccharides (EPSs) are one of the most important excreted compounds, due to their special physico-chemical properties and biological activities. In dependence on the type of cyanobacteria, they are produced in the form of a pure polysaccharide, proteoglycan or glycoprotein conjugates. Detailed structural studies of cyanobacterial EPSs were rarely investigated so far. Whereas EPSs are often viscous very complex branched heteropolysaccharides, consisting of differently linked six and more monosaccharide units, it is difficult to determine their structure [2, 3].

In this study, we have analysed water-soluble EPS isolated from a culture medium of the freshwater cyanobacterium *Nostoc* sp. Conventional methods of isolation (ethanol precipitation) and fractionation (ion-exchange chromatography) were used. Obtain fractions were further characterised by chemical composition (protein, carbohydrates, uronic acids content), monosaccharide compositional analysis and by NMR spectroscopy.

ACKNOWLEDGEMENT

This work was financed by the Slovak Research and Development Agency (Grant no. APVV-15-0410) and by the Slovak Grant Agency VEGA (Grant no. 2/0051/18).

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Normal mode analysis as a routine part of a structural investigation

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Normal mode analysis (NMA) is a technique that can be used to describe the flexible states accessible to a protein about an equilibrium position. These states have been shown repeatedly to have functional significance. NMA is probably the least computationally expensive method for studying the dynamics of macromolecules, and advances in computer technology and algorithms for calculating normal modes over the last 20 years have made it nearly trivial for all but the largest systems. Despite this, it is still uncommon for NMA to be used as a component of the analysis of a structural study. The nature of NMA, its advantages and limitations, the most commonly used techniques for reducing its computational cost, and some of its applications will be described, and recent examples where NMA served as a valuable component of analysis of a structural study will be reviewed [1].

ACKNOWLEDGEMENT

This work was financed by VEGA Grant number 2/0140/16.

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Catalyses of inverting and retaining glycosyltransferases pass through oxocarbenium ion intermediates via S_Ni-like mechanism: Insights from DFT-QM/MM calculations

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Glycosyltransferases (GTs) catalyze transfer of a monosaccharide from a donor substrate activated by nucleotide on different acceptor substrates. In such nucleophilic substitution reactions an alpha-glycosidic bond is broken and a new glycosidic bond, either beta, in case of the inverting GTs or alpha in case of the retaining GTs is formed. Using quantum mechanics methods (DFT-QM/MM, FMO-PIEDA) we modeled a part of catalytic cycle of an inverting glycosyltransferase, bovine inverting β-1,4-galactosyltransferase-1(β4Gal-T1) with a native and thio donor substrate (UDP-5' thio galactose) to analyze differences in enzymatic kinetics and explain influence of the oxygen versus sulfur ring atoms in the donor substrate on a progress of the enzymatic reactions as well structures of transition states, intermediates and interaction energies. Using several DFT methods, the calculations predicted S_Ni-like mechanism via oxocarbenium (thiocarbenium) ion intermediates for both reactions with native and thio donor substrates. This mechanism is identical with that found for the retaining GTs in previous studies [1-3] and is in contrast with previously proposed S_N2-like mechanisms for the inverting GTs [4-6]. I will explain why both inverting and retaining GTs use the same S_Ni-like mechanism and why it was not found in the previous studies [4-6].

ACKNOWLEDGEMENT

This work has been supported by the Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences (projects VEGA 2/0035/16 and VEGA 2/0031/19).

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Analysis of Protein Dynamics and Structure by nt-PaCS-MD and ProGA

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The prediction of folding pathways for proteins with atomistic models presents a severe theoretical and computational challenge. One of the challenges is the definition of coordinates (collective variables) in such way, that one would need as few as possible and at the same time one would sufficiently describe the complexity of protein conformations. Recently, we proposed a set of such collective variables that address these issues; the moving root mean square deviation of atomic positions (mRMSD) and the inner products of backbone dihedral angles Φ, Ψ [1]. We will show how the use of these collective variables enables the formulation of an efficient and effective algorithm enhancing the efficiency of the previous version of the non-targeted Parallel Cascade Selection Molecular Dynamics (nt-PaCS-MD) protocol [2].

An additional problem related to the analysis of protein structure and/or dynamics is the definition of a metric quantitatively describing the folding state of a protein. We extend the application of the Estrada folding degree [3], to show that this parameter is very useful in the analysis of peptide dynamics, and is not restricted to be used on native structures (as it was originally tested).

The presented methods are implemented in the ProGA package and are freely available [4].

ACKNOWLEDGEMENT

This work has been supported by the Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences (projects VEGA 2/0035/16 and VEGA 2/0031/19).

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Study of the N-acetylglucosaminyltransferase-V (GnT-V) interactions with substrates using computational chemistry tools

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N-acetylglucosaminyltransferase-V (GnT-V) is an enzyme altering structure of specific N-glycans by transfering N-acetylglucosamine (GlcNAc) residue from high-energy UDP-GlcNAc donor to the α1-6-linked mannose of N-glycans via β1-6 linkage [1]. Increased productions of these branching structures is reported to contribute to cancer growth and metastasis [2]. Model of GnT-V was constructed using crystal structures 5ZIB [3] and 5ZIC as templates. Molecular docking of donor and acceptor substrates was performed using Induced Fit protocol allowing optimization of enzyme side chains in close proximity of substrates. Obtained enzyme-donor-acceptor complex was used for molecular dynamics simulations. These simulations were performed with various numbers of water molecules inside cavity in close proximity of substrates. Complex without any water molecules inside cavity proved to be most stable. Optimized structure obtained from this simulation will be in future used for QM-MD study of reaction mechanism.

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Photochemical properties of potential anticancer agents: *Anti-syn* isomerization of 2,3-disubstituted quinazolinones

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EPR and NMR experiments of a quinazolinone-based Schiff's base in DMSO solution showed that irradiation with UV light (365 nm) led to photochemically-induced isomerization from the *anti*- to the higher-energy *syn*-form around the −N−N= linkage. The *anti*- to *syn*-isomerization was relatively fast and the maximum amount of conversion (25%) was detected within 10 min; the thermodynamic equilibrium re-established in about 15 min.

DFT calculations were performed on the investigated compound and small model systems, and reproduced the experimental fact of the *anti*-conformer being lower in energy than the *syn*. Theoretical analysis of excited states, including visualisation of natural transition orbitals, suggests that the photoisomerization process depends upon the size of the molecular system: in smaller systems the lowest n–π* transition (S_1) would provide a route to isomerisation; but the n–π* excitation changes its energy profile with size, and larger π-systems have π-π* transitions below the n-π* transition. The investigated compound probably photoisomerises through S_3, a π–π* excitation which is lower than the n–π* excitation. There are thus limits to the utility of small model calculations for investigating the excited states.

ACKNOWLEDGEMENT
This work was financed by the Slovak Research and Development Agency (Grant no. APVV-15-0726) and by the Slovak Grant Agency VEGA (Grant no. 2/0022/18).

Building a center for research and teaching activities in structural biology for biomedicine and biotechnology – Interreg V-A Slovakia – Austria project StruBioMol.

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Biomedicine and biotechnology are modern science disciplines whose development contributes to improving the health of the population and the environment. Structural biology identifies the nature of serious illnesses and forms the basis for the design of potential drugs. It also allows to search for potentially harmful substances and study their impact on the environment. What is lacking in our region is a common educational and scientific research base that would train and educate professionals capable of transferring modern knowledge in this area into practice. Within the framework of this project will be set up a joint cutting-edge structural and biology education and research center in the region to educate PhD students, researchers and practitioners in the Bratislava-Vienna region to build a broad scientific and technical base capable of making significant advances in research into biomedical and biotechnology applications to improve the quality of life. In building of the center we will use the experience of both partners as well as well established international cooperation. The lectures and training seminars will be organized alternately in both workplaces to attract the widest possible community and to encourage mutual contacts between PhD students and researchers in Vienna and Bratislava with the perspective of further cooperation. The individual objectives of the project include: a) strengthening the long-term and sustainable basis for the development of structural biology in the region; b) education of university students, PhD students, researchers and practitioners; (c) building a research center of excellence in structural biology for the study of human diseases. The long-term benefits will be to create a base for high quality education, providing guidance and excellent research in the field of biomedicine and biotechnology, which will contribute to increasing the competitiveness of the region and will attract foreign experts' interest in cooperation and the transfer of acquired knowledge into practice.

ACKNOWLEDGEMENTS

StruBioMol is co-financed by the European Regional Development Fund (ERDF) under the Interreg V-A Slovakia-Austria program (www.sk-at.eu).

Amylolytic family GH126 - in silico analysis and preliminary experimental identification of potentially novel members

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The glycoside hydrolase (GH) family 126 was established based on the X-ray structure determination of the amylolytic enzyme CPF_2247 from *Clostridium perfringens* ATCC 13124 genome [1]. Its original identification as a putative carbohydrate-active enzyme was based on its low, yet significant sequence identity to members of the family GH8, which are inverting endo-β-1,4-glucanases [2]. As the family GH8 forms the clan GH-M with GH48 [3], the CPF_2247 protein also exhibits similarities with members of the family GH48 [1]. The screening of the CPF_2247 on carbohydrate substrates demonstrated its activity on glycogen and amylose, thus classifying this protein as an "α-amylase" [1,3]. It should be pointed out, however, there are apparent inconsistencies concerning the exact enzyme specificity of the "amylase" CPF_2247, since it exhibits both the endo- and exo-fashion of action [1]; moreover, there is a difficulty of understanding how a retaining α-amylase could share the catalytic mechanism with above-mentioned inverting β-glucanases [4]. The family GH126 currently counts more than 800 amino acid sequences solely from Bacteria. The present study delivers a detailed *in silico* analysis of over 350 selected amino acid sequences from the family GH126, featuring sequence comparison, locations of highly conserved sequence regions and illustrating their evolutionary relationships. Part of the study is focused on a comparative structural analysis of the "amylase" CPF_2247 with representatives of other GH families in the CAZy database [3] that adopt the same catalytic $(\alpha/\alpha)_6$-barrel fold in an effort to reveal potential evolutionary homologues. The experimental part of the study describes our findings of four novel protein sequences isolated from *Bacillus subtilis*, *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus rhamnosus* samples. All exhibit a high level of nucleotide and amino acid sequence similarity with existing members of the family GH126. Subsequent biochemical characterization of the four sampled proteins is still in progress, with the protein isolated from *B. subtilis* being the most suitable candidate for further analysis of its enzymatic activity and substrate specificity.

ACKNOWLEDGEMENT

This work was supported by grants No. 2/0146/17 from the Slovak Grant Agency VEGA.

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Bioinformatics analysis of acetylesterases from family CE16

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Hemicellulosis are polysaccharides (xylan, glucomannan, arabinoxylan etc.) presenting along with cellulose in almost all plant cell walls. Very often they are decorated with various side substituents, including acetyl groups, which sterically hinder their cleavage by glycoside hydrolase. On the other hand, to ensure complete degradation of plant biomass, microorganisms and fungi that break down plant cell walls usually produce an enzyme cocktail containing also several types of acetylesterases, which differ in properties, and substrate- and regio- specificities [1,2]. The presented bioinformatics analysis was focused on acetylesterases classified in **C**arbohydrate **A**ctive En**zy**mes database (CAZy) in the carbohydrate esterase (CE) family CE16. Since the catalytic domain of CE16 enzymes contains a GDSL/Y motif containing a catalytic serine, they belong to the SGNH hydrolase superfamily [3,4]. By the usage of catalytic triad Ser (nucleophile) / His (base) / Asp (acid) they resemble serine hydrolases.

The studied set of CE16 acetylesterases was complemented by: (i) characterized members of the CAZy families, CE2, CE3, CE6 and CE12, which also belong to the SGNH hydrolase superfamily; and (ii) sequences of proteins with various activities but known tertiary structure belonging to the same Pfam families (PF00657 and PF13472) as CE16 acetylesterases. Four conserved sequence regions (CSRs) carrying catalytic and oxyanion-hole residues were identified in all 143 studied sequences. Besides this, the CSR-IV was identified as a region unique to CE16 family members. Sequence alignment showed specific features of each of the CE families. Also, an evolutionary analysis based on the alignment of four CSRs (CSR-I, CSR-II, CSR-III and CSR-V) has clearly distinguished between the individual CAZy families. The structures of active sites of structurally characterized members have been also compared and analyzed. Another unique feature of CE16 family is that aspartic acid from the catalytic triad is replaced by an asparagine in many members, indicating two or more different possible catalytic machineries. Relationships between CAZy acetylesterases, as well as between the structurally characterized members of related Pfam families (PF00657 and PF13472), similarities and differences with implications to catalytic mechanism will be also presented. The analysis of the different types of acetylesterases makes it possible to understand the structure-function relationship and to use this knowledge for targeted protein design for the needs of biotechnology.

ACKNOWLEDGEMENT
This work was financially supported by the projects Nos. 2/0190/14 and 2/0146/17 from the Slovak Grant Agency VEGA

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Fragmentation analysis of O-specific bonded saccharides from bacterial samples using LC-MS and MALDI-MS

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Despite scientific and medical progress cholera is still a constant threat today, especially in developing countries or countries affected by natural disasters or wars (for example: Bangladesh, Haiti, Kenya, Malawi or DRC) [1, 2]. The symptoms of this disease are mainly abdominal pain, dehydration, vomiting, fever and watery diarrhea (known also as "rice water") [3]. Thus, the persistent occurrence of this disease is the reason why the structural analysis of the pathogen *Vibrio cholerae,* which is its major carrier, is still emphasized. New knowledge of the structure of this bacterium can lead to the preparation of new vaccines and original approaches to treatment of this disease.

In previous work we focused on the isolation, purification, characterization and direct conjugation of OSP (O-specific polysaccharide core) from *Vibrio cholerae O139* [4].

As in previous work, we have separated the samples with HPLC separation method using porous graphitic carbon chromatography (PGC) coupled to mass spectrometry. In addition to the separation, we have employed fragmentation analysis using various dissociation modes to confirm the structure of individual OSP forms. The data were complemented by the analysis of individual fractions using the MALDI-TOF/TOF method. The overall structural data could help us obtain new knowledge about *Vibrio cholereae O139*. Obtained information might be useful in preparation of new glycoconjugate vaccines against this epidemic disease or to its better treatment in affected countries.

ACKNOWLEDGEMENT

This work was financed by National Institutes of Health in pursuance of grant No.: R01 AI106878 and also by the Scientific Project Agency – VEGA project 2/0093/17.

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Pathogenicity of new *BEST1* variants identified in Italian patients with best vitelliform macular dystrophy assessed by computational structural biology

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Best vitelliform macular dystrophy (BVMD) is an autosomal dominant macular degeneration. The typical central yellowish yolk-like lesion usually appears in childhood and gradually worsens. Most cases are caused by variants in the *BEST1* gene which encodes bestrophin-1, an integral membrane protein found primarily in the retinal pigment epithelium. We describe the spectrum of *BEST1* variants identified in a cohort of 57 Italian patients analyzed by Sanger sequencing. In 13 cases, the study also included segregation analysis in affected and unaffected relatives. We used molecular mechanics to calculate two quantitative parameters related to calcium-activated chloride channel (CaCC composed of 5 BEST1 subunits) stability and calcium-dependent activation and related them to the potential pathogenicity of individual missense variants detected in the probands. Thirty-six out of 57 probands (63% positivity) and 16 out of 18 relatives proved positive to genetic testing. Family study confirmed the variable penetrance and expressivity of the disease. Six of the 27 genetic variants discovered were novel: p.(Val9Gly), p.(Ser108Arg), p.(Asn179Asp), p.(Trp182Arg), p.(Glu292Gln) and p.(Asn296Lys). All *BEST1* variants were assessed *in silico* for potential pathogenicity. Our computational structural biology approach based on 3D model structure of the CaCC showed that individual amino acid replacements may affect channel shape, stability, activation, gating, selectivity and throughput, and possibly also other features, depending on where the individual mutated amino acid residues are located in the tertiary structure of the bestrophin-1. Statistically significant correlations between mean logMAR best-corrected visual acuity (BCVA), age and modulus of computed BEST1 dimerization energies, which reflect variations in the in CaCC stability due to amino acid changes, permitted us to assess the pathogenicity of individual *BEST1* variants. Using this computational approach, we designed a method for estimating BCVA progression in patients with BEST1 variants.

References

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