INSTRUCT ULTRA

2nd Structural biology meeting

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INSTRUCT ULTRA

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Programme

Thursday, November 15, 2018

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

13,30 – 13,40 **INSTRUCT-ULTRA H2020**
Miloš Hricovíni
Institute of Chemistry, Slovak Academy of Sciences, Bratislava

Chair: *Radovan Fiala*

13,40 – 14,10 **Long-range NMR restraints in integrated structural biology**
Enrico Ravera[1], Giacomo Parigi[1] and Claudio Luchinat[1]
Magnetic Resonance Center (CERM), and Department of Chemistry, University of Florence, and Interuniversity Consortium for Magnetic resonance of Metalloproteins, Via L. Sacconi 6, 50019 Sesto Fiorentino (FI), Italy

14,10 – 14,30 **The function and structure of yeast phosphatidylinositol transfer protein Pdr17.**
Zuzana Pevalová[1], Vladimír Pevala[2], Nicolas J. Blunsom[3], Dana Tahotná[1], Roman Holič[1], Veronika Kotrasová[2], Barbora Keresztesová[2], Nina Kunová[2], Jacob A. Bauer[2], Július Košťan[4], Eva Kutejová[2], Shamshad Cockcroft[3], Peter Griač[1]
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[3]Department of Neuroscience, Physiology and Pharmacology, Division of Biosciences, University College London, London, United Kingdom
[4]Max F. Perutz Laboratories, Vienna, Austria

14,30 – 14,50 **The structure of the cell wall binding domain of phage endolysin solved by program ARCIMBOLDO**
Ľubica Urbániková[1], Martina Gerová[1], Jiří Brynda[2], Július Košťan[3], Nora Halgašová[1] and Gabriela Bukovská[1]
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14,50 – 15,10 **Mitochondrial nucleoid proteins**

Nina Kunová[1], Gabriela Ondrovičová[1], Vladimír Pevala[1], Jacob Bauer[1], Veronika Kotrasová[1], Barbora Keresztesová[1], Peter Baráth[2], Jana Bellová[2] and Eva Kutejová[1]
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[2]Department of Glycobiology, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

15,10 – 15,30 **Coffee break**

Chair: *Miloš Hricovíni*

15,30 – 16,00 **Hyperfine effects in paramagnetic NMR Spectroscopy**

Radek Marek
CEITEC – Central European Institute of Technology, Masaryk University Kamenice 5, CZ - 62500 Brno, Czechia

16,00 – 16,20 **A mystery of a through-space indirect NMR spin-spin coupling between two hydrogen atoms**

Olga L. Malkina
Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84536 Bratislava

16,20 – 16,40 **Molecular modeling of an enzymatic mechanism of glycosyltransferases which used a manganese ion co-factor for catalytic activity**

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

16,40 – 17,00 **Weak Te,Te interactions through the looking glass of NMR spin–spin coupling revisited**

Richard Szabó and Irina Malkin Ondík
Institute of Informatics, Information Systems and Software Engineering, Faculty of Informatics and Information Technologies STU in Bratislava, Ilkovičova 2, 842 16 Bratislava 4

19,00 **Dinner**

Friday, November 16, 2018

Chair: *Ľudovít Škultéty*

8,45 – 9,05 **Molecular determinants of juvenile hormone action as revealed by 3D QSAR analysis in *D r o s o p h i l a*.**
Denisa Beňová-Liszeková, Milan Beňo and Robert Farkaš
Laboratory of Developmental Genetics, Institute of Experimental Endocrinology, Biomedical Research Center,
Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia

9,05 – 9,25 **The structure, occurrence and biological role of G-quadruplexes**
Viktor Víglaský
[1]Department of Biochemistry, Institute of Chemistry, Faculty of Sciences, P. J. Šafarik University in Košice

9,25 – 9,45 **New acridine cytostatics – structure and properties**
Ján Imrich[1], Mária Vilková[1], Danica Sabolová[2], Michal Bečka[1]
[1]Department of Organic Chemistry and NMR laboratory, [2]Department of Biochemistry, Institute of Chemistry, Faculty of Science, P. J. Šafárik University, Šrobárova 2, 040 01 Košice, Slovakia

9,45 – 10,05 **The use of time-domain data for metabolite quantification**
Michal Kaliňák[1], and Martin Kopčík[1]
[1]Central laboratories, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia

10,05 – 10,25 **Study of the Xyloglucan endotransglycosylase (XET) interactions with substrates using computational chemistry tools**
Barbora Stratilová[1,2], Stanislav Kozmon[1], and Eva Stratilová[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

10,25 – 10,40 **Coffee break**

Chair: *Radek Marek*

10,40 – 11,10 **Structural studies of non-B DNA by NMR spectroscopy**
Radovan Fiala[1], Martin Gajarský[1], Martina Lenarčič Živković[2], Janez Plavec[2], Šimon Džatko[1], Michaela Krafčíková[1], Lukáš Trantírek[1], Aleš Novotný[1], and Radek Marek[1]
[1]Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

11,10 – 11,30 **How Mutations to the N-Terminal Domain of the Human Cardiac Ryanodine Receptor Affect its Dynamics: A Molecular Dynamics Study**

Jacob Bauer[1*], Eva Kutejová[1], Vladena Bauerová[1]
[1]*Institue of Molecular Biology, Slovak Academy of Sciences, Bratislava Slovakia*

11,30 – 11,50 **Study of the substrate modification influence on the O-GlcNAc transferase reaction mechanism by QM/MM molecular dynamics.**

Stanislav Kozmon[1,2], Igor Tvaroška[1,2]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia*
[2] *Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5, 615 00 Brno, Czech Republic*

11,50 – 12,10 **Mass Spectrometry, a Tool for Discovery Proteomics**
Fishing for Specific Biomarkers, Virulence Factors, and Stress Related Proteins

Gabriela Flores-Ramirez, Maksym Danchenko, Katarina Palkovicova, Nikola Babitsova, Ludovit Skultety
Department of rickettsiology, Institute of Virology, Biomedical Research Center, Dubdravska c. 9, 845 05 Bratislava, Slovakia

12,10 – 12,15 **Conclusions**

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

Long-range NMR restraints in integrated structural biology

Enrico Ravera[1], Giacomo Parigi[1] and Claudio Luchinat[1]

[1] *Magnetic Resonance Center (CERM), and Department of Chemistry, University of Florence, and Interuniversity Consortium for Magnetic resonance of Metalloproteins, Via L. Sacconi 6, 50019 Sesto Fiorentino (FI), Italy*

Long range restraints such as RDC and PCS have proven valuable for monitoring and possibly improving the accuracy of a protein structure in solution by taking as a starting point the crystal structure and validating or "correcting" it. Crystal structures may indeed suffer from crystal packing forces, so that they may not be an accurate model for the protein structure in solution. Furthermore, atomic coordinates in crystal structures are affected by small but significant uncertainties, which are especially relevant for protons that are usually not directly observed. On the other hand, NMR structures are based on lower numbers of experimental restraints and their overall accuracy is often even lower than for X-ray structures. Therefore, interpreting as real the (modest) differences between X-ray and NMR structures is a risky attitude. We have proposed that the presence of real differences should be checked by a simultaneous refinement of the protein structure performed with both crystal and solution NMR data. If the two datasets are consistent with a single structural model, then the process provides a more accurate structure. To this end, the program REFMAC5 from CCP4 was modified (REFMAC-NMR) to allow the simultaneous use of X-ray crystallographic data and paramagnetic NMR data and/or diamagnetic residual dipolar couplings [1]. Obvious advantages of this approach are that an independently solved solution structure is not needed, and that the complementarity between X-ray and NMR data is fully exploited (Figure 1).

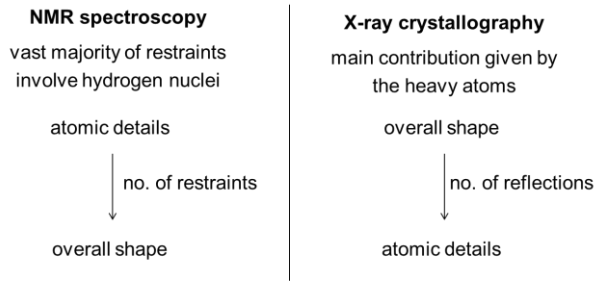

Figure 1. Increasing number of restraints improves structural quality in opposite directions for NMR and X-ray.

Inconsistencies between crystal structures and solution NMR data, if any, may be due either to structural rearrangements occurring on passing from the solid state to solution, or to a larger conformational heterogeneity [2,3]. In the case of multidomain or multisubunit systems, the NMR data can provide the correct reciprocal position of the domains in solution [4], but the restraints might be not enough to ensure the correct reconstruction of the tensors, and such a case might lead to a misinterpretation of the information from the NMR data. For this reason we have included in REFMAC-NMR the possibility of restraining either the orientation or the magnitude of any two tensors [5].

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The function and structure of yeast phosphatidylinositol transfer protein Pdr17.

Zuzana Pevalová[1], **Vladimír Pevala[2]**, Nicolas J. Blunsom[3], Dana Tahotná[1], Roman Holič[1], Veronika Kotrasová[2], Barbora Keresztesová[2], Nina Kunová[2], Jacob A. Bauer[2], Július Košťan[4], Eva Kutejová[2], Shamshad Cockcroft[3], Peter Griač[1]

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Many lipid transfer proteins operate at membrane contact sites where two membranes come close together to facilitate exchange of material and information. Yeast phosphatidylinositol transfer protein (PITP), Pdr17, is an essential component of a complex required for decarboxylation of phosphatidylserine (PS) to phosphatidylethanolamine at the non-mitochondrial location. This process involves transfer of PS from the endoplasmic reticulum to the Golgi/endosomes. We generated a Pdr17 mutant protein to better understand the mechanism by which Pdr17p facilitates inter-membrane transfer of PS at membrane contact sites. Pdr17 mutant protein is not capable of binding phosphatidylinositol (PI) by using permeabilized human cells and complementation assays *in vivo*. These mutations changed only the lipid binding cavity of Pdr17p and not the surface properties of the protein. In contrast to all other previously tested yeast PITPs, Sec14p, Pdr16p, and Sfh5p, the ability of Pdr17p to bind PI is not required for Pdr17p major cellular function in the inter-membrane transfer of PS.

ACKNOWLEDGEMENTS

Supported by grant VEGA 2/0111/15, APVV-15-0654, and the British Heart Foundation grant FS/15/73/31672.

The structure of the cell wall binding domain of phage endolysin solved by program ARCIMBOLDO

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Bacteriophage-encoded endolysins, enzymes showing bacteriolytic activity, are of growing interest for their applications as enzybiotics in veterinary and/or human medicines and various field of biotechnology, e.g. food safety. Bacteriophage BFK20 is a lytic phage of *Brevibacterium flavum* CCM 251 (gram positive corynebacteria), industrial producer of L-Lysine. The genome of the BFK20 has been sequenced and analyzed (EMBL accession no. AJ278322) [1]. The gene product of ORF24´ was identified as endolysin gp24´ (UniProt ID Q9MBI0). The protein is composed of two domains, the catalytic domain showing N-acetylmuramoyl-L-alanine amidase activity, and cell wall binding domain (gp24BD). The individual domains were cloned separately and the binding activity of the C-terminal region (81 aa) was proved [2]. Needle shaped crystals belonging to hexagonal space group P622 were grown overnight. Later, the crystals recrystallized directly in the drop. The newly obtained crystals belonged to tetragonal space group $P42_12$. Two sets of data have been collected, to 3.2 and 1.4 Å resolution using hexagonal and tetragonal crystals, respectively. The same protein sample stored for several weeks at 4 °C crystallized directly in the form of tetragonal crystals. PN-terminal sequencing revealed N-terminal truncation of the protein.

The 1.4 Å resolution data set was used for structure solution by the direct method using the program ARCIMBOLDO-LITE [3]. The overall structure revealed very loose bundle of three α-helices. One protein molecule was found in the asymmetric unit. The crystal symmetry gives four molecules in the unit cell which form very compact tetramer. As the oligomers have been previously determined also in the solution one can speculate about the oligomeric state as biologically active unit. The gp24BD is unrelated to any of the known cell wall binding domains of phage endolysins. The specificity of gp24BD binding on peptidoglycan substrate has not been determined so far. To answer the questions concerning the functioning of the binding domain and the whole endolysin, further work is needed, especially the structure of the whole molecule and the complexes with possible ligands should be solved.

ACKNOWLEDGEMENT

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Mitochondrial nucleoid proteins

Nina Kunová[1], Gabriela Ondrovičová[1], Vladimír Pevala[1], Jacob Bauer[1], Veronika Kotrasová[1], Barbora Keresztesová[1], Peter Baráth[2], Jana Bellová[2] and Eva Kutejová[1]

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Mitochondrial nucleoids are the intricate nucleo-protein complexes formed of mitochondrial DNA and two sets of proteins, DNA-binding core-proteins involved in mtDNA organization, maintenance and transcription, and a range of peripheral factors acting as components of various signalling pathways. The molecular interactions of nucleoid components with the organellar network and cellular metabolism are still unclear and continue to be studied widely. Our study focuses on ATP-dependent protease Lon which degrades oxidatively damaged, partially unfolded, and short-lived regulatory proteins in mitochondria. Lon was found within the core nucleoid region, though little is presently known about its role there. Recently, Lon's involvement in regulating the levels of TFAM[1], the most abundant mtDNA structural factor in humans, was determined. On such basis, we began to study other potential Lon substrates from among the proteins that play their respective roles in key processes of mtDNA metabolism. Our work is comparing the *in vitro* digestion profiles of the *Saccharomyces cerevisiae* TFAM functional homologue Abf2, the yeast mtDNA maintenance protein Mgm101, and two human mitochondrial proteins, Twinkle helicase and the large ribosomal subunit protein MrpL32[2]. Firstly, we examined how presence of protein or nucleic acid agent influences Lon's enzymatic activities, and then how Lon's recognition ability alters when substrates are bound to that nucleic acid. The study shows an interesting correlation between proteins' *in vivo* function and their recognition by Lon indicating that such regulatory mechanism might facilitate the dynamic changes to mitochondrial nucleoids and ribosomes.

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HYPERFINE EFFECTS IN PARAMAGNETIC NMR SPECTROSCOPY

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NMR spectroscopy is one of the most powerful tools for exploring the chemical structure of any new compound. However, the NMR signals of the individual atomic nuclei in open-shell systems can be significantly shifted and broadened by the *nucleus-electron(s) hyperfine* interaction which complicates the measurement and interpretation of the NMR spectra.

The hyperfine contribution to the total NMR shift is temperature dependent and can be calculated using knowledge of the electronic **g** and hyperfine **A** tensors [1]. Theoretical calculations of the EPR and NMR parameters can significantly facilitate an interpretation of the experimental observations. According to the physical mechanism, the hyperfine interaction can be broken down to the contributions of Fermi-contact, spin-dipole, and paramagnetic spin-orbit terms [2,3].

The "through-bond" *Fermi-contact* contribution is generally very isotropic as it originates in the Fermi-contact interaction between the nuclear spin and the difference in α/β electron spin density at the spectator nucleus. An interplay between the through-bond hyperfine and substituents effects in coordination compounds will be discussed [4]. The *spin-dipole* hyperfine contribution stems from the direct "through-space" interaction between the nuclear and electron magnetic moments and has traditionally been used as a source of information about the distance between a paramagnetic center and NMR spectator atom. Analysis of the spin-dipole contribution to the hyperfine NMR shift as an approach to investigating the structure of supramolecular host-guest complexes between ruthenium compounds and macrocyclic cavitands will be demonstrated [5].

Acknowledgment: This work has received support from the Czech Science Foundation (Grant No. 18-05421S).

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A mystery of a through-space indirect NMR spin-spin coupling between two hydrogen atoms

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Indirect nuclear spin-spin coupling constants are amongst the most important magnetic resonance parameters, invaluable in establishing molecular structure from NMR spectroscopy. Their detailed understanding in terms of molecular and electronic structure is thus of central importance in many fields of research and has been pursued since the beginnings of NMR spectroscopy. Nowadays quantum-chemical calculations can offer a variety of tools for the interpretation of couplings including visualization of spin-spin coupling pathways by real-space functions [1].

In this presentation we will show how visualization of NMR spin-spin coupling pathways has been used for interpretation of the experimentally detected “through-space” indirect spin-spin couplings between protons formally separated by 18 covalent bonds.[2]

Acknowledgment.

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Molecular modeling of an enzymatic mechanism of glycosyltransferases which used a manganese ion co-factor for catalytic activity

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Most glycosyltransferases with the GT-A structural fold use the transition metal Mn^{2+} ion cofactor for a proper catalytic activity. In the presence of other ions as Mg^{2+}, Ca^{2+} or Zn^{2+} the enzymatic reaction is not effective. Using quantum mechanics methods (DFT-QM/MM, FMO-PIEDA) we modeled a part of catalytic cycle of several glycosyltransferases (Kre2p[1], GTB[2] and β4Gal-T1[3]) to analyze influence of different ion cofactors on a progress of the enzymatic reactions as well structures of transition states, intermediates and interaction energies. The DFT-QM/MM calculations indirectly indicate that Mn^{2+} is not only essential for the biochemical enzymatic process. It may be important also for other biophysical processes of the catalytic cycle of GT-A glycosyltransferases as binding or releasing of substrates and conformation changes of the active sites.

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Weak Te,Te interactions through the looking glass of NMR spin–spin coupling revisited

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J(^{125}Te,^{125}Te) spin–spin coupling is a highly sensitive probe into the electronic and geometric structure of 1,8-*peri*-substituted naphthalene tellurium derivatives. The coupling is related to the onset of multicenter bonding in these systems.

In this presentation we will show how a combination of NMR and DFT techniques has been used to study the interactions between formally nonbonded, but spatially close Te atoms. Weak donor–acceptor interactions in the *peri*-naphthalene system, which mark the onset of 3c4e bonding, reinforce the Te,Te couplings and lead to unusually large *J*(^{125}Te,^{125}Te) values. This property turns out to be a sensitive probe ("looking glass"), not only into the electronic structure underlying the bonding situation, but also into the particular conformations that ensue. In the broader context of well-known through-space spin–spin coupling, this conformational aspect is a new facet worth exploring. [1] In addition, we will present a new tool for analyzing computational data from the data science perspective.

Acknowledgment.

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Molecular determinants of juvenile hormone action as revealed by 3D QSAR analysis in *D r o s o p h i l a*.

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Postembryonic development, including metamorphosis, of many animals is under control of hormones. In *Drosophila* and other insects these developmental transitions are regulated by the coordinate action of two principal hormones, the steroid ecdysone and the sesquiterpenoid juvenile hormone (JH). While the mode of ecdysone action is relatively well understood, the molecular mode of JH action remains elusive. To gain more insights into the molecular mechanism of JH action, we have tested the biological activity of 86 structurally diverse JH agonists in *Drosophila melanogaster*. The results were evaluated using 3D QSAR analyses involving CoMFA and CoMSIA procedures. Using this approach we have generated both computer-aided and species-specific pharmacophore fingerprints of JH and its agonists, which revealed that the most active compounds must possess an electronegative atom (oxygen or nitrogen) at both ends of the molecule. When either of these electronegative atoms are replaced by carbon or the distance between them is shorter than 11.5 Å or longer than 13.5 Å, their biological activity is dramatically decreased. The presence of an electrondeficient moiety in the middle of the JH agonist is also essential for high activity. The information from 3D QSAR provides guidelines and mechanistic scope for identification of steric and electrostatic properties as well as donor and acceptor hydrogen-bonding that are important features of the ligand-binding cavity of a JH target protein. In order to refine the pharmacophore analysis and evaluate the outcomes of the CoMFA and CoMSIA study we used pseudoreceptor modeling software PrGen to generate a putative binding site surrogate that is composed of eight amino acid residues corresponding to the defined molecular interactions.

The structure, occurrence and biological role of G-quadruplexes

Viktor Víglaský

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G-rich regions in nucleic acids able to form G-quadruplexes have been identified in various living organisms, even in viruses. The existence of G-quadruplexes *in vivo* has also been confirmed. It is generally believed that this structural motif regulates multiple biological processes. G-quadruplex formation can serve both beneficial and regulatory roles in cells such as forming the capping structure of telomeres, the specification of DNA replication origins in vertebrates, and deleterious effects as they can impede the progression of replicative DNA polymerases [1]. Biological impacts are for example cell senescence, cell differentiation leading to either carcinogenesis or apoptosis. The bioinformatics analysis of many animal genomes has revealed that the location of G-rich loci is non-random, they correlate with functionally important genomic regions. It is not surprising that their location is also non-random in viral systems.

Interestingly, occurrence of G-rich regions is either very high or sporadic depending on the type of viruses. For example DNA herpetic viruses consist of huge amount of G-rich regions in comparison to papilloma viruses [2]. The frequency of such loci in RNA integrative retroviruses (HIV and SIV) and non-integrative filoviruses (e.g. Ebola and Marburg) is comparable to human genome [3]. Viral G-quadruplexes also offer other possibilities influencing both gene expression of virus itself and its host cell. Therefore, the viral G-quadruplexes can pose another molecular target for specific antivirus chemical compounds inhibiting a viral infection.

However, G-rich sequence may not necessarily form G-quadruplex structure; it can form another non-canonic fold [4]. Therefore, we have developed a new, simple and cost effective methodology based on combination of various electronic spectroscopies and specific fluorescent ligand increasing G-quadruplex stability to unambiguously confirm G-quadruplex formation of unknown DNA sequences derived from viral genome [5].

This work was supported by the Slovak Research and Development Agency under Contract no. APVV-0280-11, European Cooperation in Science and Technology (COST CM1406), Slovak Grant Agency 1/0131/16, and internal university grant (VVGS-PF-2017-251)

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New acridine cytostatics – structure and properties

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New 3-(acridin-9-yl)methyl-2-imino-1,3-thiazolidin-4-ones were regiospecifically synthesized from unstable (acridin-9-yl)methyl thioureas and methyl bromoacetate (MBA) or bromoacetyl bromide. Unexpected formation of only one thiazolidine regioisomer with the both reagents indicated the mechanism involving a transient spiro 9,10-dihydroacridine intermediate. UV-Vis titrations, CD spectra, fluorescence quenching, thermal denaturation, and gel electrophoresis showed the new products to intercalate into calf thymus (CT) DNA and displace ethidium bromide (EB) from a CT DNA–EB complex. The strongest binder was 2-(4-methoxyphenylimino)thiazolidinone. [1]

Eight novel spiro acridine-isoxazolines were prepared and characterized by NMR, IR, UV-Vis, and CD spectra which implied that the drugs inhibited topoisomerase I and interacted with CT DNA via intercalation. The Stern-Volmer quenching constants were of the size 10^4 M^{-1}. [2]

Acridine thiosemicarbazones prepared from aromatic isothiocyanates, hydrazine and acridin-9-carbaldehyde afforded with MBA and DEAD new acridine-thiazolidinone regioisomers which upon standing in DMSO-d_6 spontaneously isomerized. All compounds were characterized by multinuclear NMR, MS, and X-ray crystallography. The ^{13}C and ^{15}N chemical shifts, coupling constants J_{HC} and J_{HN}, and NOESY experiments disclosed the configuration of the original as well as transformed stereoisomers. [3]

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The use of time-domain data for metabolite quantification

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Metabolite quantitation is usually done by the integration of the area under the peak in the frequency-domain spectrum, including the use of various deconvolution methods. Recently, a new method called CRAFT was proposed [1] that extracts quantitative data in the form of amplitudes from time-domain FID. It was proposed to circumvent subjective errors in phasing and baseline correction.

We have compared both approaches and determined the advantages and disadvantages of each. Using the artificial sample, the CRAFT method showed better accuracy with well resolved peaks but failed in areas of substantial overlap. However, the conversion of amplitudes into concentrations is not very straight-forward for many metabolites and their peak clusters and therefore we advise the use of CRAFT for data reduction and multivariate statistical analysis.

We have shown that using real-life samples (wine and rat heart extracts), the CRAFT analysis gives better or at least very comparable results to spectral fitting methods by Chenomx NMR Suite software [2]. However, it is much faster than the manual fitting and the only bottleneck is the need for spectral alignment.

The possible use of CRAFT in 2D spectra will be discussed.

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Study of the Xyloglucan endotransglycosylase (XET) interactions with substrates using computational chemistry tools

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Xyloglucan endtransglycosylase (XET) is an enzyme primary catalysing the cleavage of xyloglucan or cellulose followed by transfer of polysacharide fragment with former nonreducing end to another molecule of xyloglucan or cellulose. This work is focused on the form of XET from germinating seeds of *Tropaeolum majus* (nastrium) with wide acceptor specificity, TmXET6.3. Based on primary sequence alignment (V5ZEF7, UniProtKB) with all known xyloglugan endotransglycosylase/hydrolases from UniProtKB and homology modeling His94, Ala104, Gln108, Lys234 and Lys237 were identified as amino acid residues most probably responsible for enzyme specificity related to neutral saccharides. This was proved also experimentally by site directed mutagenesis. Result of bioinformatics analysis shows that acceptor unspecificity of XETs in plants can be more widespread as was previously expected. Acceptor substrate docking was performed in the active site of TmXET6.3 model and its acceptor specific template PttXET16-36 followed by molecular dynamics simulations and binding free energy calculations using MM(GB/PB)SA method. These calculations proved increasing stability of complexes with increasing length of oligosaccharide main chain (celooligosaccharide acceptors) as well as with increasing length of side chains (xyloglucanoligosaccharides with various galactosylation of side chains). Simulations with xylooligosaccharides as acceptors proved their instability in active site of acceptor specific PttXET16-36. These results were in agreement with experimental activity assays of PttXET16-36 and TmXET6.3.

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Structural studies of non-B DNA by NMR spectroscopy

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NMR spectroscopy has been an indispensable tool in chemical laboratories for decades. In structural biology, NMR has an advantage in its ability to study the compounds in their natural environment, namely in water solution, and at temperatures at which they perform their biological roles. Attempts are being made to study the structures of proteins and nucleic acids inside the living cells. NMR is also suitable for studies of complexes and can provide information about intramolecular dynamics.

DNA is a biopolymer occurring in all living organisms, where it functions in encoding, transmitting, and expressing genetic information. Based on sequence composition and environmental conditions, DNA can adopt diverse conformations, which predefine its physiological function. Aside from double helical B-DNA, DNA is capable of adopting alternative conformations, including parallel/antiparallel duplexes, various hairpins, triplexes and tetraplexes, and branched architectures. These so-called non-B DNA structures have been demonstrated to participate in cellular regulation, and their formation has also been associated with a number of human diseases [1].

Undoubtedly, most of the attention in non-B DNA-forming sequences currently is paid to G-rich DNA, particularly that displaying the potential to form G-quadruplexes. However, we recently reported the formation of a stable G-hairpin from a natively occurring DNA sequence 5-d(GTGTGGGTGTG)-3, corresponding to the most abundant sequence motif in irregular telomeric DNA from *Saccharomyces cerevisiae* (yeast) [2].

DNA sequences with stretches of cytidines are abundant in eukaryotic genomes and are overrepresented in biologically important genomic regions such as centromeres, telomeres, and/or the promoter regions of (onco)genes. Under specific in vitro conditions, these sequences can form the so-called i-motif, a four-stranded structure consisting of two parallel DNA duplexes zipped together by the intercalation of protonated cytosine–cytosine (C.C+) base pairs. Using state-of-the-art in-cell NMR spectroscopy, we provided direct experimental evidence for the existence of these structures in vivo and evaluated the stabilities of i-motif structures in the complex cellular environment [3].

Alternating $d(GA)_n \cdot (TC)_n$ ($n>15$) sequences are unusually abundant in mammalian genome. It was shown that some proteins are able to selectively bind d(GA) repeats, e.g. PGB protein found in human fibroblasts [4]. In-depth description of the d(GA) duplex structure using NMR is complicated by its dynamics. To overcome this problem a C_3 tract was inserted in the sequence. In acidic conditions, the cytosine tract forms an i-motif that acts like an anchor restraining the flexibility of the system.

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How Mutations to the N-Terminal Domain of the Human Cardiac Ryanodine Receptor Affect its Dynamics: A Molecular Dynamics Study

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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]. Higher-resolution crystal structures of the first 544 residues of the N-terminal domain (NTD) have also been determined, which show that this fragment can be divided into three domains, A, B, and C, surrounding a central helix [3, 4]. Mutations to RyR2 can disrupt its ability to close and open correctly. We used molecular dynamics simulation (MD) to study how the R414L, I419F, and R420W mutations affect the structure and dynamics of the human RyR2 NTD. We selected likely solutions structures from the MD simulation, and carried out principal components analysis (PCA) of the trajectories to infer how the mutations might change its dynamics. The mutations do not appear to greatly change the overall structure of the fragment, but do alter the position of a loop-and-helix motif in domain C (Cα3; residues 463–487). PCA showed that the largest characteristic motion of the wild-type protein (23% of the total) is a rotation of domains A and C towards and away from each other while domain B remains relatively stationary. In the R414L and I419F mutants, this motion remains, but is greatly reduced comprising only 9% and 7%, respectively, of the total motion. Both of these mutations appear to make the NTD more rigid overall. In contrast, the R420W mutation becomes more flexible. The wild-type characteristic motion becomes 38% of the total and has changed in character: domain B has now become flexible and moves together with domain A. All three of these mutants change the dynamics of the Cα3, which forms part of a three-way junction between NTD and two other domains. Moreover, by altering the motion of domains A and C with respect to one another, these changes would also disrupt a proposed gating mechanism [5] suggested to strengthen the closed conformation of the receptor.

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Study of the substrate modification influence on the O-GlcNAc transferase reaction mechanism by QM/MM molecular dynamics.

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Carbohydrates are ubiquitously present in all cells in the variety of forms e.g. glyco-conjugates, playing a pivotal role in a plethora of the biological processes. These glyco-conjugates are formed by glycosyltransferases, which add saccharides onto proteins, lipids, sugars, etc. The inverting glycosyltransferase O-GlcNAc transferase (OGT) post-translationally modifies a variety of proteins. The misregulation of O-GlcNAc-ylation is linked to a wide range of diseases, so knowing its reaction mechanism is very significant.

In the present study, experimentally proposed mechanisms with native UDP-GlcNAc, sulphur analogue UDP-5-S-GlcNAc donor substrate and amino analogue of the acceptor serine were investigated at the DFT QM/MM level. The sophisticated theoretical approaches such as hybrid QM/MM DFT Carr-Parinello ab initio molecular dynamics combined with metadynamics and a string method for reaction path optimization were used to investigate whether changes in the reaction substrates can influence the O-GlcNAc reaction mechanism. Obtained results suggest that the non-native substrates can influence the reaction path and energetics of the enzymatic reaction.

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Mass Spectrometry, a Tool for Discovery Proteomics
Fishing for Specific Biomarkers, Virulence Factors, and Stress Related Proteins

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Coxiella burnetii (C. burnetii), the obligate intracellular, acidophilic, highly pleomorphic Gram-negative bacterium is causative agent of a widespread zoonosis - Q fever. This organism has a huge infectious potential due to remarkably low infectious dose. Clinically, it is manifested mainly as a flu-like illness, which is accompanied by fever, headache, chills and fatigue. However, it may also present as pneumonia or granulomatous hepatitis. It is estimated that approximately 5% of acute infections of Q fever develops into a more serious chronic form, which is often accompanied by endocarditis.

Proteomic analysis based on mass spectrometry (MS) have confirmed the presence of enzymes related to the biosynthesis and metabolism of LPS I that were found only in virulent form of the bacterium and therefore they might be assigned as proteins associated with virulence. Furthermore, using MS technology we have discovered several specific spectral markers related to inter - and intrastrain variation of C. burnetii that could be useful for topologization activities. We also recognized many immunogenic proteins with great value in diagnosis and proteins that are present on the cell surface. Among them not only the membrane proteins and lipoproteins can be found, but also proteins that form complexes with molecules anchored to the bacterial membrane and also effector proteins translocated by the type IV secretion system [1]. The group of special interest is formed by proteins related to bacterial defenses and its adaptation or survival in extreme conditions of the parazitoforous vacuole at the low pH. It was found that C. burnetii persist in this environment due to basic protein and protein with positively charged amino acid residues , which limit the active proton transport through the membrane [2]. These recent proteomic discoveries lead us to propose the biosynthesis pathway of the unique biomarker - C3-methylated deoxy sugar of C. burnetii – virenose. Since, this pathway might have a significant impact on development of new therapeutic agents specifically effective in treatment of Q fever [3].

Using advanced proteomic technologies, we also identified a biomarker of spontaneous regression of hematologic malignancies and recognize its immunoreactive epitopes [4]. We have also contributed to clarifying the processes occurring under stress conditions in different organisms of plant origin [5]. Thus, all the achieved knowledge and progress have made, provide deep insight into metabolic and signaling pathways of the studied organisms and provide broader elucidation of the processes, which subsequently led to formation of numerous hypotheses, that indicates future direction of our research.

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