L26

## BIOLOGICAL SMALL ANGLE X-RAY SCATTERING AT CEITEC-MU

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Biological Small Angle X-ray Scattering (Bio-SAXS) become mature and popular technique for structural studies of the macromolecules and macromolecular complexes in solution. Development of the software tools available and advances of synchrotron and „home“ X-ray sources brought Bio-SAXS to routine work-flow of number of structural biologists. Bio-SAXS is used for determination of the integral structural parameters, shape reconstruction, determination of the oligomeric and folding state, unraveling the quaternary architecture of the complexes, modeling of molecular flexibility and more. Bio-SAXS characterizes macromolecules in solution, *i.e.* close to their native and biologically relevant conditions. It is a low-resolution technique, but in combination with other techniques as X-ray crystallography, nuclear magnetic resonance, etc., the Bio-SAXS becomes powerful tool of the structural analysis of biological macromolecules.

The Core facility X-ray diffraction and Bio-SAXS of the CEITEC-MU located in Brno facilitates access to the state of art “in house” instrumentation for X-ray structural analysis. Besides the elementary collection of diffraction or scattering data, the facility offers assistance with data processing and interpretation. Year and half after the official opening of the laboratory the most typical Bio-SAXS case studies from users community are presented: *ab initio* shape reconstruction experiments, oligomeric state determination and oligomeric equilibrium studies, hybrid method approaches for quaternary structure model building of macromolecular complexes and studies of semi-flexible complexes and intrinsically disordered proteins.

# Friday, March 20, Session VII

L27

## THEORETICAL AND EXPERIMENTAL STUDY OF CHARGE TRANSFER THROUGH DNA: IMPACT OF MERCURY MEDIATED T-Hg-T BASE PAIR

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DNA-Hg complexes may play an important role in sensing of DNA defects or in detecting of Hg presence in the environment. A fundamental way of characterizing DNA-Hg complexes is to study the way how the electric charge is transferred through the molecular chain. The main goal of this contribution was to investigate the impact of a mercury metal cation that links two thymine bases in a DNA T-T mismatched base pair (T-Hg-T) on charge transfer through the DNA molecule. We compared the charge transfer efficiencies in standard DNA, DNA with mismatched T-T base pairs and DNA with T-Hg(II)-T base pair. For this purpose we measured the temperature dependence of steady-state fluorescence and UV-VIS of the DNA molecules. The experimental results were confronted with the results obtained employing theoretical DFT methods. Generally, the efficiency of charge transfer was driven by mercury changing the spatial overlap of bases.

L28

# THE INTERACTIONS OF SOIL NATURAL ORGANIC MATTER (NOM) AND POLYCYCLIC AROMATIC HYDROCARBON (PAH) WITH BIOLOGICAL INTERFACES STUDIED BY MD SIMULATIONS

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Soil is very complex compound which consists of different components such as mineral surfaces, organic and inorganic ions and natural organic matter thus many factors are important in adsorption and transportation processes in soil. Understanding the interactions of different compounds of soil not only needed to be addressed experimentally but also theoretically due to the fast growing the speed and capabilities of computers and computational methods. In order words, combined experimental and theoretical methods are more accurate to solve complex problems in soil science. One of most important components of soil, which has strong effect and play important role in the process of adsorption of different elements to the plants and complexation of many metal ions in the environment is natural organic matter (NOM). NOM and in particular humic acids, humates, and fulvic acids derived from dead plant and animal matter by partial decomposition, are present in environments and especially in aquatic environments. They play variety of important roles in soil [1-2] and their interactions with metal ions, minerals and organic species are important for environment.

Humic acids are able to interact with both organic and inorganic substances such as nutrients, metal ions, and hydrophobic organic compounds [3, 4]. Moreover, the interactions of humic acids with living surfaces have been detected by some experimental methods such as adsorption isotherms, electrophoretic mobility measurements, and transmission electron microscopy [5]. Humic acids and humates have heterogeneous and complex structures with different functional groups such as $NH_2$, COOH, OH, aromatic and aliphatic parts in which the non-covalent intermolecular interactions such van der Waals, charge-transfer, stacking interactions and hydrogen-bonding are the most important interactions [6]. Understanding their structure and interactions with ions and hydrophobic organic compounds can give us important information about their biodegradability, toxicity, and transport properties. NOMs are present in soil and water thus they can interact with natural macromolecules such peptide, proteins or enzymes, so that molecular modeling methods can give useful information about their interactions in molecular level.

In order to understand the solvation structure and interactions of NOMs in aqueous solution we have studied the solvation structure and surface propensity to the air/aqueous solution of building blocks of humic acids and we observed that they have strong surface propensity and decrease of surface tension [7] of water which is supported by surface sensitive spectroscopy [8]. Such as vibrational sum frequency generation spectroscopy.

Garrido et al. studied the structure, conformational changes and aggregation of Temple- Northeastern - Birmingham (TNB) [9] model of humic acid which was proposed by Sein et al. by molecular modeling approaches and they revealed water molecules stabilize the system specially when the model has bigger negative charge and in higher concentration of humic acid aqueous solution aggregation takes place [10].

High flexibility and presence of different functional groups such as carboxylic acid, alcoholic, phenolic and hydrophobic groups in humic acid make them to be favorable for interaction with metal ions and hydrophobic organic compounds. Such interactions make humic acids to be involved in metal ion complex formation and aggregation which are studied both theoretically and experimentally. Surface propensity and aggregation to the air/water occur for humates aqueous solutions in which hydrophobic parts of humates were moved towards air/water interface and hydrophilic parts towards the bulk of aqueous solution thus decrease of surface tension of such solution solutions was observed [11].

In order to understand the adsorption process of NOM on the surface of living organisms in aquatic environments we studied the interactions of NOM with biomembranes which is the crucial as weak interactions such as hydrogen bonding, van der Waals and hydrophobic interactions are the dominant interactions. As studying the interactions of biomembrane of living organisms is very complex due to the fact that they contain different lipids with different compositions thus study the interaction of model biomembrane can bring new insights to understand the process of adsorption and influence of the surface of biomembrane by NOM.

Weak interactions which are involved in the adsorption of NOM on the surface of living organisms can bring information to reveal the role of humic substances on the surface of biomembranes and their key interactions on metal toxicity when binding to the surface of living organisms in aquatic systems take place [5]. Moreover, studying the interaction of NOM with different biological membrane brings valuable information about the biodegradation process of hydrophobic organic pollutants such as polycyclic aromatic hydrocarbons (PAH) in aqueous environments [12].

This study brings new insights for the adsorption and interaction of different models of NOM with different biomembrane such as, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-palmitoyl -2- oleoyl - sn – glycero -3 - phosphocholine (POPC) as typical components of cell membrane. As both POPC and DPPC membrane have $N(CH_3)^{3+}$ groups in their structure thus electrostatic interaction between the negatively charged functional groups of the humic substances and the positively charged groups $N(CH_3)^{3+}$ of the biomembranes head groups and hydrophobic interactions between the NOM and membranes bilayer, and hydrogen bonding between the negatively charged functional groups of the NOM and the negatively charged groups of phosphate domain of the head groups are the most important interaction in the process of adsorption of humic substances on the surface of living surfaces.

To understand such complex phenomena as , we studied the surface propensity and interaction of TNB model of NOM with lipid bilayers such as both POPC and DPPC as by classical molecular dynamics (MD) simulations and revealed that the hydrophbic interaction between organic molecules and hydrophobic parts of humic acid is one of the most important factor for interactions and surface propensity to the air/aqueous solution interface. This study also has characterized the interactions of NOM with biological surfaces in order to understand the role of different functional groups such COOH, OH, aromatic and aliphatic parts of NOM with biologically relevant compounds.

1. Sposito, G. The Chemistry of Soils. Oxford University Press,New York, 1989.
2. Stevenson, F. J., Humus Chemistry Genesis, Composition,Reactions, 2nd ed. John Wiley & Sons: New York, 1994.
3. Tipping, E. Cation Binding by Humic Substances. Cambridge University Press, Cambridge. 2002
4. Buffle, J., Complexation Reactions in Aquatic Systems: An Analytical Approach, Ellis Horwood: Chichester.1981,8,2,4.
5. Campbell, P. G. C. Twiss, M. S. Wilkinson, K. J., Accumulation of natural organic matter on the surface of living cells: implications of the interaction of toxic solutes with aquatic biota. Can. J. Fish. Aquat. Sci., 1997,54, 2543–2554.
6. Simpson, A. J.; Kingery, W. L.; Hayes, M. H. B.; Spraul, M.; Humpfer, E.; Dvortsak, P.; Kerssebaum, R.; Godejohann, M.; Hofmann, M. Molecular structures and associations of humic substances in the terrestrial environment Naturwissenschaften 2002, 89 (2) 84–88.
7. Minofar, B.; Jungwirth, P.; Das, M. R.; Kunz, W.; Mahiuddin, S.: Jungwirth, P.:. Journal of Physical Chemistry C, 111 (2007) 8242.
8. Yi Rao, Mahamud Subir, Eric A. McArthur, Nicholas J. Turro, Kenneth B. EisenthalChemical Physics Letters 477 (2009) 241–244.
9. Sein LN, Varnum JM, Jansen SA. Environ Sci Technol 1999;33:546–52.
10. R.A. Alvarez-Puebla, C. Valenzuela-Calahorro, J.J. Garrido, Science of the Total Environment; 358 2006,243–254.
11. Čtvrtinková, A.; Kučerík, J.; David, J.; Drastík, M. Fresenius Environmental Bulletin, 2011, vol. 20, No. 7a, p. 1764-1771.12. Luke N. Ukiwe , Ubaezue U. Egereonu, Pascal C. Njoku, Christopher I. A. Nwoko, Jude I. Allinor, International Journal of Chemistry V(5) No. ( 4); 43-55; 2013.
12. Luke N. Ukiwe , Ubaezue U. Egereonu, Pascal C. Njoku, Christopher I. A. Nwoko, Jude I. Allinor, International Journal of Chemistry V(5) No. ( 4); 43-55; 2013.

L29

# ANALYSIS OF LIVING CELL 3D INNER STRUCTURES FROM HIGH-RESOLUTION BRIGHT-FIELD MICROSCOPY

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Knowledge of composition of biochemical structures (from organelles, bio-molecular assembles down to unique biomolecules) is important for understanding of structural-functional relationships inside a living cell and recognition its physiological state. The best imaging technique is provided by a classical microscopic technique without usage any contrast methods – bright-field light transmission microscopy. Provided we use an unlabelled, undyed cell, we obtain its most real image.

With regards to physical and physico-chemical process, which occur during passing light through a living cell, we developed a mathematical approach (Point Divergence Gain) for segmentation and analysis of three-dimensional structures down to size of a camera chip ($x$,$y$-coordinates) and scanning z-step from their light diffraction and emission patterns – object spread functions – as well as tracking organelles captured in a z-stack of micrographs [1].

Our results show necessity to consider the technical limitations of microscopy (i.e., precision of optics, image registration, size of scanning z-step and a camera pixel, speed of image acquisition and storage) in future building-up of a new microscope and relevant software.

1. R. Rychtáriková, T. Náhlík, R. Smaha, J. Urban, D. Štys Jr., P. Císař, D. Štys, in *ISCS 2014:Interdisciplinary Symposium on Complex Systems (Emergence, Complexity and Computation 14)*, edited by A. Sanayei, O.E. Rössler, I. Zelinka (Switzerland: Springer), 2014, pp. 261-267.

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L30

# THE ACTIVATION OF N-GLYCOSIDIC BOND CLEAVAGE OPERATED BY hOGG1 ENZYME CAN BE SPECIFICALLY CONTROLLED BY ELECTROPHILICITY/NUCLEOPHILICITY OF THE GLYCOSIDIC NITROGEN OF NORMAL/DAMAGED NUCLEOSIDES

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The role of lysine 249 (Lys 249) residue of base-excision repair enzyme hOGG1 in activation of N-glycosidic bond cleavage was studied by means of theoretical computations for 2'-deoxyguanosine (G), 8-oxo-2'-deoxyguanosine (OxoG) and N6-(2'- -D-deoxyribofuranosyl)-2,6- diamino-4-hydroxy-5-formamidopyrimidine (FapyG). The interaction sites of Lys 249 involved C1', N3, and N9 atoms of nucleosides that can be foreseen from available crystal structures.

The mechanistic pathway of nucleobase excision involving attack of lone-pair electrons at glycosidic nitrogen N9 to N -ammonium of Lys 249 resulted in specific activation of normal (G) and damaged (OxoG, FapyG) nucleosides owing to distinct electrophilic or nucleophilic character of the glycosidic nitrogen of normal and damaged nucleosides. Other pathways involving interaction of Lys 249 with N3 and C1' atoms in that regard appeared unspecific.

The chemical modification of normal G owing to damage resulted in alternation of electronic character of glycosidic nitrogen, strengthening of the C1'-N9 glycosidic bond and decrease of the aromatic character of five-membered ring of nucleobase. Particularly the nucleophilicity/electrophilicity of N9 seems to control specifi-

cally proton addition to nucleobase during its excision via deprotonation of N -ammonium of Lys 249. The checkpoint mechanism proposed theoretically is coherent with base-specific enzymatic repair of G, OxoG, and FapyG that was observed experimentally.

The activation operated by attack of lone-pair electrons at glycosidic nitrogen to N -ammonium of Lys 249 was efficient and specific with respect to normal and damaged nucleosides owing to electrophilicity (G)/nucleophilicity (OxoG, FapyG) of glycosidic nitrogen and owing to corrupted catalytic core that was obtained specifically only for the damaged nucleosides. The glycosidic nitrogen of OxoG and FapyG can donate lone-pair electrons capable of interaction with proton of N -ammonium of Lys 249 while highly delocalized lone-pair electrons at N9 of G can't interact efficiently with N -ammonium. The Lys 249 therefore seems to be not only key catalytic residue, but also the residue that is involved in recognition of damaged nucleobases.

Šebera, Trantirek, Tanaka, Sychrovský, J. Phys. Chem. B 2012, 116, 12535-12544.

Šebera, Trantírek, Tanaka, Nencka, Fukal, Sychrovsky, RSC Advances 2014, 4, 83, 44043 – 44051.

**L31**

# USING NON-CRYSTALLOGRAPHIC-SYMMETRY TO SOLVE THE STRUCTURE OF A PERFECT MEROHEDRAL TWIN CRYSTAL OF AICHI VIRUS (AIV)

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Aichi virus (AiV) is associated with acute gastroenteritis and belongs to the family *Picornaviridae* of small (27-30nm in diameter), non-enveloped, positive-stranded RNA viruses [1-2]. The 2.3 Å resolution dataset collected from a cubic crystal of AiV particles displayed an apparent symmetry space group of I432 that truly revealed to be an I23 space group with a perfect merohedral twinning. A rotation-search using icosahedral 5-fold symmetry axes as probes in GLRF software [3] followed by a translation-search in the real space group have selected one of the possible twin orientations to position a capsid protein from *Poliovirus* as first template.

A twinning refinement method was used to determine the 'real' reflexion hkl contribution to the 'twinned' reflection '*hkl*'. Thus for each reflexion, calculated twinned intensity $I_{calc}$('*hkl*'), twin operator (TWOP) and twin fraction (*FRAC*(*hkl*)) were defined such as:

$$I_{calc}(\text{'}hkl\text{'}) = 0.5\ I_{calc}(hkl) + 0.5\ I_{calc}(TWOP[hkl])$$

$$FRAC(hkl) = I_{calc}(hkl)/I_{calc}(\text{'}hkl\text{'})$$

*FRAC*(*hkl*) was then applied to the observed intensity Iobs('hkl') as first approximation. Cycles of twinning refinement associated with real-space averaging of electron density map using Non-Crystallographic Symmetry were also required to manually build the model, improve phases and achieve the structure determination. The AiV capsid consists of a densely packed icosahedral arrangement of 60 protomers comprised of 3 polypeptides each.

1. Yamashita, T et al (1991) Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. J Infect Dis 164:954–957.
2. Yamashita, T et al (1998) Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. J Virol 72:8408-8412
3. Tong,T. & Rossmann,M.G (1972) The Locked Rotation Function. Acta Cryst. A46:783-792.

L32

# MOLECULAR ARCHITECTURE OF ENCAPSULIN NANOCOMPARTMENTS: AN IRON-SEQUESTERING SHELL THAT PROTECT CELLS FROM OXIDATIVE STRESS

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To store iron and regulate its metabolism, cells have developed ferritin-based organelles: classical ferritins and bacterioferritins that function in iron storage and Dps proteins that normally function in iron detoxification [1]. Bacteria additionally possess other kinds of protein-based organelles, which allow them to encapsulate enzymes and/or to sequester toxic or volatile products [2]. *Myxococcus xanthus* produces such protein-based particles that accumulate iron in its encapsulin nanocompartment, which is composed of the HK97-like shell protein EncA and three minor proteins, EncB, EncC and EncD [3]. These particles have dense iron-rich cores. We used cryo-electron microscopy and single particle reconstruction techniques to determine the structure of native encapsulin particles from *M. xanthus* and recombinant EncA shells produced in *E. coli*. The 3-D reconstruction of native particles shows them to have the same T=3 icosahedral shell as recombinant particles but filled with dense trilaminar material, which electron tomography shows to be composed of 11-19 dense granules, ~5.5 nm in diameter and not icosahedrally ordered. Based on STEM mass measurements, we estimated that the granules accommodate ~35,000 Fe atoms, as compared to a maximum of ~4,500 iron atoms in ferritin. In addition to T=3 capsids, recombinant EncA produces smaller particles, mainly T=1 icosahedra. These observations lead to a model for iron-sequestering encapsulin nanocompartments in which EncA encapsulates the minor proteins and EncB and EncC act as mineralizing centers for iron granule assembly.

1. Andrews, S.C. et al. (2010) *Biochim Biophys Acta* 1800: 691-705.
2. Kerfeld, C.A. et al. (2010) *Annu. Rev. Microbiol.* 64: 391-408.
3. McHugh C.A. et al. (2014) *EMBO J.* 33: 1896-1911.