Session IV, May 30, Tuesday

L15

MOLECULAR STRUCTURE AS A PREREQUISITE FOR FUNCTION – LABORATORY OF STRUCTURE AND FUNCTION OF BIOMOLECULES OF IBT

J. Dohnálek, T. Skálová, J. Dušková, P. Kolenko, K. Adámková, M. Trundová, L. Švecová, T. Kovaľová, M. Malý, J. Hrubý, B. Hus áková, J. Stránský, J. Hašek, T. Kovaľ

Institute of Biotechnology of the Czech Academy of Sciences, Průmyslová 595, 25250 Vestec, Czech Republic

dohnalek@ibt.cas.cz

Our understanding of function of a biological macromolecule requires information about its primary, secondary, tertiary, and quaternary structure. Targeted manipu-l ation of structural features then enables direct observation of functional consequences. The complete understanding of the structure-function relationship in biomolecules would provide us with a full view of the rules governing biological molecular systems and enable relatively easy control of their properties. Typically, this complete knowledge is never achieved as we are dealing with multivariable systems with incomplete description. However, we take the advantage of availability of a number of structural and biophysical experimental techniques, together with functional assays of the studied systems to form a picture which is as complete as possible and also precise and accurate.

Our structural analysis methods include single crystal X-ray crystallography, small-angle X-ray scattering (SAXS), and cryogenic electron microscopy (cryoEM). Crystallographic analysis of proteins, protein:protein, protein:ligand, or protein:nucleic acids complexes provides structural information at high or even atomic resolution, uncovering key interaction details, new types of three-dimensional protein structure, new types of bonds, intrinsic structural properties, identification of ligands or quantification of metal clusters occupancy. Application of SAXS results in information on low-resolution arrangement, protein oligomerization or induced structural changes. CryoEM single particle imaging is used to elucidate structure of difficult-to-crystallize multi-protein complexes.

Apart from structural analysis we utilize a range of methods of molecular biophysics. They provide priceless data on protein or complex stability (Differential Scanning Fluorimetry), structural state (Circular Dichroism), intermolecular interactions (Microscale Thermophoresis, Isothermal Calorimetry), size and aggregation/oligomerization of molecules (Dynamic Light Scattering, SAXS) or complex formation/size distribution (Mass Photometry).

In our studies of bilirubin oxidase we have uncovered the nature and role of a new covalent bond formed between two amino acids of the enzyme (Fig. 1) [1]. The structure-function study of the first representative of GH151 family of -l-fucosidases revealed not only an unexpected oligomerization pattern but also active site complementation [2]. Formation of an unprecedented complex between protein HelD and mycobacterial RNA polymerase helped explain HelD function and its emerging role in antibiotic resistance [3-4]. Our atomic-resolution study of S1 nuclease complexes with products of RNA cleavage provided insights into the intimate details of intermolecular interactions including dynamics of substrate/product binding [5]. The principle characteristics of protein:protein interactions between immune cell surface receptor NKR-P1 and its cognate ligand LLT1 pointing towards formation of larger zipper-like structures were uncovered in a recent collaborative study [6].

Mainly the structural analysis aspects of our studies will be presented to illustrate the power of this approach in explanation of functionality relevant for biomedical and biotechnological applications. Some of our contributions to the

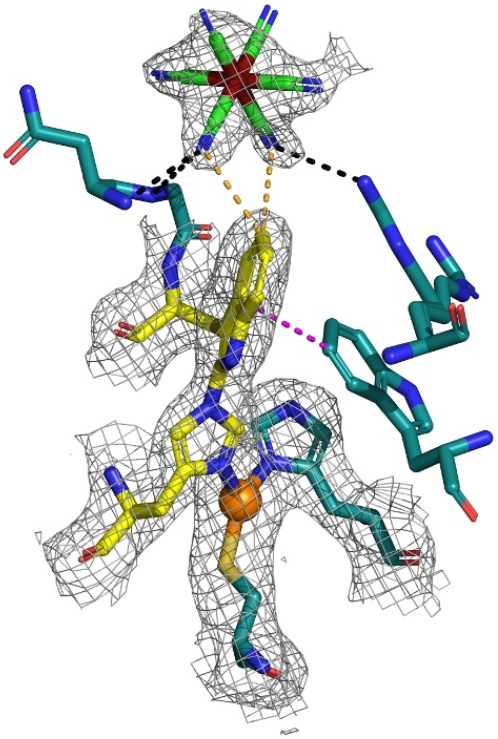

Figure 1. Detail of a crystal structure showing an unusual covalent bond between Trp and His in the surface active site of bilirubin oxidase from *Myrothecium verrucaria*, PDB ID 3I3J [1]. Coordination of T1 copper is represented as covalent bond. Colour coding: T1 site and oxidation site residues – carbon teal; covalent adduct Trp-His – carbon yellow; copper orange, iron brown; ferricyanide – carbon green. Interactions of $[Fe(CN)_6]^{3-}$ - black dashed line, in yellow VDW interaction with Trp396. Magenta dashed line – CH- interaction.

development of methods of structural analysis will be also mentioned [7-8].

1. T. Kovaľ, L. Švecová, L. H. Østergaard, T. Skalova, J. Dušková, J. Hašek, P. Kolenko, K. Fejfarová, J. Stránský, M. Trundová, J. Dohnálek J. *Sci. Rep.*, **9**, (2019), 13700.
2. T. Koval'ová, T. Kovaľ, J. Stránský, P. Kolenko, J. Dušková, L. Švecová, P. Vodičková, V. Spiwok, E. Benešová, P. Lipovová, J. Dohnálek. *FEBS J.*, **289**, (2022), 4998.
3. T. Kouba, T. Koval', P. Sudzinová, J. Pospíšil, B. Brezovská, J. Hnilicová, H. Šanderová, M. Janoušková, M. Šiková, P. Halada, M. Sýkora, I. Barvík, J. Nováček, M. Trundová, J. Dušková, T. Skálová, U. Chon, K. S. Murakami, J. Dohnálek, L. Krásný. *Nat. Commun.*, **11**, (2020), 6419.
4. P. Sudzinová, H. Šanderová, T. Kovaľ, T. Skálová, N. Borah, J. Hnilicová, T. Kouba, J. Dohnálek, L. Krásný. *FEMS Microbiol. Rev.*, (2022), doi: 10.1093/femsre/fuac051. Epub ahead of print.
5. K. Adámková, T. Koval', L. H. Østergaard, J. Dušková, M. Malý, L. Švecová, T. Skálová, P. Kolenko, J. Dohnálek. *Acta Crystallogr. D Struct. Biol.*, **78**, (2022), 1194.
6. J. Bláha, T. Skálová, B. Kalousková, O. Skořepa, D. Cmunt, V. Grobárová, S. Pazicky, E. Poláchová, C. Abreu, J. Stránský, T. Kovaľ, J. Dušková, Y. Zhao, K. Harlos, J. Hašek, J. Dohnálek, O. Vaněk. *Nat. Commun.*, **13**, (2022), 5022.
7. M. Malý, K. Diederichs, J. Dohnálek, P. Kolenko. *IUCrJ.*, **7**, (2020), 681.
8. P. Kolenko, J. Stransky, T. Koval', M. Maly, J. Dohnalek. *J. Appl. Cryst.*, **54**, (2021), 996.

This work was supported by MEYS (LM2018127, LM2023042), CSF (23-06295S, 18-10687S, 20-12109S), ERDF (CZ.02.1.01/0.0/0.0/16_013/0001776, CZ.02.1.01/0.0/0.0/15_003/0000447) and AS CR (86652036).

L16

CF DIFFRACTION TECHNIQUES IN CENTRE OF MOLECULAR STRUCTURE: IN-HOUSE X-RAYS FOR STRUCTURAL BIOLOGY

J. Stránský, J. Pavlíček, J. Dohnálek

Centre of Molecular Structure, Institute of Biotechnology, CAS v. v. i., Průmyslová 595, Vestec near Prague
Jan.stransky@ibt.cas.cz

The Centre of Molecular Structure (CMS) provides services and access to state-of-art instruments, which cover a wide range of techniques required by not only structural biologists. CMS operates as part of the Czech Infrastructure for Integrative Structural Biology (CIISB), and European infrastructures Instruct-ERIC and MOSBRI. CMS is organized in 5 core facilities: CF Protein Production, CF Biophysics, CF Crystallization of Proteins and Nucleic Acids, CF Diffraction Techniques, and CF Structural Mass Spectrometry.

CF Diffraction Techniques employs two laboratory X-ray instruments equipped with high flux MetalJet X-ray sources: a single crystal diffractometer D8 Venture (Bruker) and a small angle X-ray scattering instrument SAXSpoint 2.0 (Anton Paar). The configurations of both instruments represent the top tier of possibilities of laboratory instrumentation. Apart from standard applications, the instruments are also extended for advanced experiments: the diffractometer is equipped with the stage for in-situ crystal diffraction, X-ray flourscence detector and crystal dehydration, SAXS is equipped with in-situ UV-Vis spectroscopy and a liquid chromatography system for SEC-SAXS. The setups enable easy access and fast turn-around of samples under different conditions, but also collection of high quality end-state data without further need for synchrotron data collection in many cases. CF Diffraction Techniques provides services in synergy with the other CFs on-site, therefore scientific questions can be quickly answered as they emerge from the experiments.

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042); project Czech Infrastructure for Integrative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447), and MOSBRI from EU Horizon 2020 (No. 101004806).

L17

LABORATORY OF STRUCTURAL BIOLOGY OF NEURODEGENERATION – INSTITUTE OF NEUROIMMUNOLOGY

O. Cehlar, R. Skrabana

Institute of Neuroimmunology of Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia

ondrej.cehlar@savba.sk, rostislav.skrabana@savba.sk

The Laboratory of Structural Biology of Neurodegeneration focusses in the first place on describing the physiological and pathophysiological role of intrinsically disordered tau protein in Alzheimer's disease using the approaches of structural biology and biophysics. The second interest consists in examination of general principles of the conformation behaviour of disordered proteins.

Primary experimental approach represents the crystallization of tau polypeptides in complexes with Fab fragments of monoclonal antibodies created mostly at the Institute of Neuroimmunology by hybridoma technology. Back in 2007, the first structural information about the Alzheimer's disease tau filaments was revealed following crystallization of the filament core C-terminal hexapeptide in complex with the core-specific monoclonal antibody MN423 [1]. Since then, other complexes of Fab targeting different tau regions were crystallized [2].

The second approach represents the biophysical characterisation of tau monomeric and polymerized forms and their interaction with antibodies and other binding partners. The laboratory has a long history in using surface plasmon resonance (SPR) and serves as an unformal reference lab for this technique. Other available methods for proteins include isothermal titration calorimetry (ITC), differential scanning calorimetry, dynamic light scattering and Fourier transform infrared spectroscopy. Recently we have published an interaction study characterising the binding of C-terminal specific tau antibody to 12 various tau proteins using SPR, microscale thermophoresis and cross-linking mass spectrometry [3].

The third approach represents the characterisation of tau conformations using molecular dynamics simulations and the comparison of conformations obtained with simulations of tau peptides in free state in solution with conformation of respective tau peptide from the complex with antibody Fab fragment.

During last twenty years the lab members participated in more than 15 different projects with several Slovak and European scientific teams, procuring the interaction and structural parameters of various biological complexes. *E.g*., collaboration with the company Axon Neuroscience R&D Services SE contributed to the development of passive [4] and active Alzheimer's disease immunotherapy and to the evaluation of affinity maturation of antibodies generated during the phase II clinical trial. The antibody candidates neutralizing SARS CoV 2 were also characterized by SPR [5].

We are committed to continue sharing our expertise. Currently running collaboration projects examine innate immunity player lactoferrin, regulation of plasminogen activation or universal adaptor protein 14-3-3.

1. Sevcik J, Skrabana R, Dvorsky R, Csokova N, Iqbal K, Novak M., *FEBS Lett.*, **581**(30), (2007), 5872.
2. Cehlar O, Skrabana R, Kovac A, Kovacech B, Novak M., *Acta Crystallogr Sect F Struct Biol Cryst Commun.*, **68**, (2012), 1181.
3. Hornakova L, Sinsky J, Janubova M, Mederlyova A, Paulenka Ivanovova N, Piestansky J, Kovac A, Galba J, Skrabana R, Cehlar O., *FEBS Lett.,* **596**(9), 1178.
4. Weisova, P., Cehlar, O., Skrabana, R., Zilkova, M. et al., *Acta neuropathol commun* **7***,* 129 (2019).
5. Kovacech, B., Fialova, L., Filipcik, P., Skrabana, R. et al., *eBioMedicine* **76**, (2022).

This work was supported by the grant number APVV-21-0479.

L18

STRUCTURAL CHEMISTRY LABORATORY AT THE UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE

LABORATOŘ STRUKTURNÍ CHEMIE NA JIHOČESKÉ UNIVERZITĚ V ČESKÝCH BUDĚJOVICÍCH

Barbora Kascakova, Petra Havlickova, Anna Koutska, Michal Kuty, Ivana Kuta Smatanova*

Department of Chemistry, Faculty of Science, University of South Bohemia in Ceske Budejovice, 37005, Czech Republic

ivanaks@seznam.cz

The University of South Bohemia (USB) Chemistry Department laboratories are well equipped for a broad spectrum of research. Structural analysis of different viral proteins, enzymes, protein-inhibitor complexes, and large multiple-domain proteins is performed using X-ray crystallography or cryo-EM microscopy. In addition, molecular docking complemented by molecular dynamics is implemented to simulate possible interactions of the tested proteins.

In our laboratory, selected proteins or protein complexes are cloned into vectors suitable for bacterial, baculovirus or mammalian transient protein expression systems. We are currently working with bacterial competent cells: ArticExpress (DE3) Competent Cells, SHuffle® T7 Competent E. coli, BL21-AI™ One Shot™ Chemically Competent E. coli, One Shot™ BL21(DE3)pLysS Chemically Competent E. coli, BL21(DE3) Competent Cells, BL21-CodonPlus (DE3)-RIPL Competent Cells, Rosetta-gami™ 2(DE3) Competent Cells and many more. We also work with the Bac-to-Bac Baculovirus and MultiBac™ expression systems (High Five™, Sf9 or Schneider 2 cell lines). Bhk-21 cells, Hela cell line, Vero cells and others are used for protein expression in mammalian cells. Well-equipped proteomics laboratories with high-capacity orbital shakers (Eppendorf Innova 44r), high-volume centrifuges (Heraeus Multifuge X3R) and ultracentrifuges (Beckman Coulter Optima XPN90) together with well-equipped cell culture and BSL2 facilities are suitable for expressing proteins at sufficient concentrations for further purification.

Two AKTA pure 25 chromatography systems (room temperature or 4 °C conditions) are used to purify the target proteins. Many commercial chromatography columns are available that are suitable for both systems. In addition, the room temperature size exclusion chromatography system is complemented by Dawn Heleos II and Otilab T-rEX detectors, which are used for accurate analysis of purified samples by measuring multi-angle static light scattering (MALS) and refractive index. The most commonly used

Figure 1. Working life in the structural biochemistry laboratory at the University of South Bohemia in České Budějovice.

techniques in the proteomics laboratory are affinity chromatography, anion exchange chromatography, heparin chromatography, and SEC-MALS of small globular proteins to large macromolecular complexes.

Structural analysis of proteins is performed by basic X-ray crystallography. Standard crystallization techniques (vapor diffusion, microdialysis and free-interface diffusion) and advanced techniques (counter diffusion, capillary and in gel crystallization,) are used to prepare diffracting quality crystals. In case of problematic crystallization, seeding, co-crystallization with ligands or crystallization in living cells are used. Before each crystallization experiment, a dynamic light scattering instrument (unless SEC-MALS is used) is implemented to confirm the homogeneity of the molecules. The Oryx Nano (and soon Oryx 8) crystallization robotic system and several incubators with different temperatures are at disposal to screen crystallization conditions to ensure that the best possible conditions for crystal growth are found. Diffraction measurements are performed on synchrotron-based macromolecular beamlines at BESSY (Berlin, Germany), DESY (Hamburg, Germany) or ESRF (Grenoble, France) according to standard application procedures. Data processing and further structure solution steps are performed on computational hardware and software located in the Structural Biochemistry Laboratory and operated by trained personnel. A new crystallographic method, crystallization in living cells, is beginning to be incorporated into the methods used in our laboratory.

These researches are supported by European Regional Development Fund-Project, MEYS (No. CZ.02.1.01/0.0/ 0.0/ 15_003/0000441); by the Grant Agency of the Czech Republic (Grant No. 19-14704Y) and by the Grant Agency of the University of South Bohemia (grant No. 04-046/ 2022/P).