XVII Discussions in Structural Molecular Biology and 4th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Annual Meeting of the Czech Society for Structural Biology

Academic and University Center, Nové Hrady, March 19 - 21, 2020

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Martin Malý, Michal Strnad, Kristýna Adámková, Radek Kužel,Vladimír Sklenář

The event is organized by the Czech Society for Structural Biology, the Czech Infrastructure for Integrative Structural Biology, and the Institute of Biotechnology of the Czech Academy of Sciences.

Thursday, March 19, Session I

L1

STRUCTURAL STUDIES OF SORC FAMILY OF TRANSCRIPTIONAL REGULATORS

M. Nováková[1], J. Škerlová[1], M. Fábry[2], J. Brynda[1,2], P. Pachl[1], M. Babiak[3], J. Nováček[3], P. Řezáčová[1,2]

[1]Institute of Organic Chemistry and Biochemistry, CAS, v.v.i., Flemingovo nám. 542/2, Prague 6, 166 10, Czech Republic
[2]Institute of Molecular Genetics, CAS, v.v.i., Vídeňská 1083, Praha 4, 142 20, Czech Republic
[3]Central European Institute ofTechnology, Masaryk University, 62500 Brno, Czech Republic
rezacova@uochb.cas.cz

Transcriptional regulation is process crucial to metabolic control and development in all organisms. In bacteria, gene expression is controlled at the transcriptional level, through interactions between DNA operators and regulatory proteins. Recent studies of bacterial genomes led to the discovery of a large number of putative transcriptional regulators, with the assignment of new families and subfamilies. One of these is the SorC family, which includes proteins that control the expression of genes and operons involved in the metabolism of sugar substrates. SorC family repressors contain conserved helix-turn-helix domain DNA-binding (DBD) at their N-terminus and an effector-binding domain (EBD) at their C-terminus. The DBD recognizes palindromic operator sequence usually located downstream of the promotor. The C-terminal effector domain has a phosposugar binding function and also plays role in oligomerization.

We structurally characterized two members of SorC family, CggR and DeoR from *Bacillus subtilis.*

We determined structures using X-ray crystallography and cryo-electron microscopy to follow the DNA binding and allosteric changes induced by effector binding. Structural studies of these two representatives will provide information necessary for understanding the mechanisms of gene regulation by SorC repressors.

L2

UNUSUAL COVALENT BOND BETWEEN TRP AND HIS IN BILIRUBIN OXIDASE

J. Dohnálek[1], T. Kovaľ[1], L. Švecová[1, 2], L. H. Østergaard[3], T. Skálová[1], J. Dušková[1], J. Hašek[1], P. Kolenko[1,2], J. Stránský[1], M. Trundová[1]

[1]Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Biocev center, Průmyslová 595, Vestec, 252 50, Czech Republic

[2]Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague, Břehová 7,115 19, Praha 1, Czech Republic

[3]Novozymes A/S, Brudelysvej 26, DK-2880 Bagsværd, Denmark

jan.dohnalek@ibt.cas.cz

Covalent modifications of amino acids convey functionality, stability or interaction potential of a given protein. Many modifications are relatively well understood, such as glycosylation, disulphide bond formation, phosphorylation, methylation, co-factor covalent binding and other. Here we present a modification, relatively recently discovered and so far unique in proteins. The active site of bilirubin oxidase from the ascomycete plant pathogen *Myrothecium verrucaria* contains a covalent link between the side chains of tryptophan and histidine [1, 2]. Its presence was confirmed independently in several crystal structures and by mass spectrometry analysis [2, 3]. The role of this post-translational modification in substrate binding and oxidation is not sufficiently understood.

Protein bilirubin oxidase is used in medicine for determination of bilirubin level. Apart from bilirubin, this enzyme reacts strongly also with other organic and inorganic substrates, preferring different pH optima. The enzyme is also exploited in the textile industry, wood-processing industry, and, recently, also in nanotechnologies, especially in the construction of bio-fuel cells and biosensors.

The Trp396–His398 adduct is located at the T1 copper site of this multicopper oxidase and participates in oxidation of various types of substrates. The enzyme further transfers electrons from the T1 copper site to the trinuclear copper cluster in the protein interior, where reduction of O_2 to two H_2O molecules is catalysed.

The first structure of bilirubin oxidase in complex with one of its products, ferricyanide ion, shows interaction with the modified tryptophan side chain, Arg356, and with the active site-forming loop 393-398 [3]. Our structural and mutational studies also confirm that the adduct modifies T1 copper coordination and is important for the substrate binding and oxidation site [3]. The effects of the presence of the adduct vary with the type of the substrate being oxidized. The results imply that structurally and chemically distinct types of substrates, including bilirubin, utilize the Trp–His adduct mainly for binding and to a smaller extent for electron transfer [3].

The results have implications for technological applications of bilirubin oxidase and related enzymes.

This work was supported by the European Regional Development Fund (CZ.02.1.01/0.0/0.0/15_003/0000447, CZ. 02.1.01/0.0/0.0/16_013/0001776, and CZ.1.05/1.1.00/ 02. 0109), institutional support of the Institute of Biotechnology of the Czech Academy of Sciences, v. v. i. (RVO: 86652036), by the Ministry of Education, Youth, and Sports of the Czech Republic (LM2015043, support of Biocev-CMS).

1. Murao, S. & Tanaka, N. *Agric. Biol. Chem.* **45**, (1981), 2383–2384.
2. Akter, M., Tokiwa, T., Shoji, M., Nishikawa, K., Shigeta, Y., et al. *Chemistry* **24**, (2018), 18052–18058
3. Kovaľ, T., Švecová, L., Østergaard, L.H., Skalova, T., Dušková, J., et al.. *Sci Rep.* **9**, (2019), 13700.

L3

ADVANTAGES OF COMPLETE CROSS-VALIDATION IN PAIRED REFINEMENT

M. Malý[1,2], K. Diederichs[3], J. Dohnálek[1], P. Kolenko[2,1]

[1]Institute of Biotechnology of the Czech Academy of Sciences, Biocev, Průmyslová 595, 252 50 Vestec, CR
[2]Czech Technical University in Prague, Faculty of Nuclear Sciences and Physical Engineering, Břehová 7, 115 19 Prague, Czech Republic
[3]University of Konstanz, Box M647, 78457 Konstanz, Germany
malymar9@fjfi.cvut.cz

In macromolecular crystallography, a high-resolution cutoff is usually applied to the diffraction data to avoid inclusion of noisy information in structure refinement. Suitability of its estimation can be later checked performing the *paired refinement* protocol [1]. Refinement is usually carried out against the majority of reflections in a *working set,* whereas a little fraction (often 5 %) is randomly selected and set aside in a *free set*. The latter data are not involved in the calculations but provide *cross-validation* to monitor the process [2].

In our calculations, we analysed a possible dependence of paired refinement results on the use of a particular *free* reflection selection. For this purpose, we chose crystal structure of cysteine-bound complex of cysteine dioxygenase from *Rattus norvegicus* (CDO) [1]. Diffraction data were processed up to 1.42 Å resolution and atomic coordinates and ADPs of the input structure model were perturbed. Then, paired refinement using *REFMAC*5 [3] was performed for each of all 20 *free sets* individually, *i.e.* the complete *cross-validation* protocol. The following high-resolution cutoffs were analysed: 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, and 1.42 Å.

Obtained results confirmed the supposed dependence of paired refinement results on *free* reflection set. The differences between the used *free* sets were remarkable, especially in high resolution. The high-resolution cutoff estimation varies from 1.7 Å (one *free set*) through 1.6 Å (a few *free sets*) and 1.5 Å (half of the *free sets*) to 1.42 Å (a few *free sets*). The averaged results, that are statistically more significant, suggested cutting the data at 1.5 Å resolution. This case demonstrates that the complete *cross-validation* protocol provides more relevant information across the whole data set than a commonly-used single *cross-validation* protocol.

1. P. A. Karplus & K. Diederichs, *Science*, **336**, (2012), pp. 1030–1033.
2. A. Brünger, *Nature*, **355**, (1992), pp. 472–475.
3. G. N. Murshudov, A. A. Vagin, E. J. Dodson, *Acta Cryst. D*, **53**, (1997), pp. 240–255.

This publication was supported by the MEYS CR (projects CAAS – CZ.02.1.01/0.0/0.0/16_019/0000778 and BIOCEV – CZ.1.05/1.1.00/02.0109) from the ERDF fund, by the Czech science foundation (18-10687S) and by the GA CTU in Prague (SGS19/189/OHK4/3T/14).

L4

UPDATES TO INSTRUMENTATION IN THE CENTRE OF MOLECULAR STRUCTURE IN BIOCEV

J. Stránský, J. Pavlíček, T. Charnavets, P. Pompach, P. Vaňková, Ľ. Škultétyová, M. Schneiderová, J. Dohnálek

Centre of Molecular Structure, Institute of Biotechnology, Academy of Sciences of the Czech Republic, Průmyslová 595, 252 50 Vestec u Prahy
Jan.stransky@ibt.cas.cz

The Centre of Molecular Structure offers wide range of methods of structural biology, which are provided via Czech Infrastructure for Integrative Structural Biology (CIISB) and Instruct-ERIC. CMS offers unique opportunity for deep macromolecule characterization using setate-of-art instruments at one place. The instrument portfolio is constantly improved to keep the latest techniques and techonology available to our users. The latest notable additions, are crystal dehumidification system (HCLab, Arinax) for X-ray diffractomemter, and new crystallization robot (NT8, Formulatrix). HCLab is designed to precisely control humudity around the protein crystal before its vitrification, which can lead to improved diffraction quality of the crystal. The technique is traditionally available at synchrotons, however the usage there is suboptimal due to the time constrains. The NT8 crystallization robot operates in humidity controlled environemnt, which enables usage of crystallization drops down to 50 nl. The robot is also equipped for setting lipidic cubic phase (LCP) experiments.

Centre of Molecular Structure is supported by projects LM2015043 and LM2018127 from Ministry of Education, Youth and Sports, CIISB for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) and project Structural dynamics of biomolecular systems (CZ.02.1.01/0.0/ 0.0/15_003/0000447) from the ERDF.

AQS3 SEE CHANGE IN PROTEIN CHARACTERIZATION

Patrick King

Specion/Redshift

Infra-Red (IR) analysis has been long accepted as a powerful tool in protein characterization, particularly in the Amide I band (~1600 - 1700 cm-1), which gives detailed secondary-structural information that can be critical in determining protein structure-activity relationships, stability, batch-to-batch comparisons and in formulation studies as a few examples. Technologies traditionally used for secondary structure analysis, such as benchtop Fourier Transform IR (FTIR) or Circular Dichroism (CD), suffer from a number of issues that have prevented their routine use in this area, preventing this application from reaching its full potential. These include concentration and buffer restrictions, incompatibility with a range of excipients, a lack of automation, low spectral reproducibility and for FTIR, water subtraction problems.

Microfluidic Modulation Spectroscopy (MMS) is a new key technology that was brought to market in 2019 by RedShift Bioanalytics. It focuses on the IR Amide I region to produce exceptionally high data quality and reproducibility that aim to solve the aforementioned issues encountered with traditional technologies. It is fully automated, running samples from 24- and 96-well plates, compatible with a very broad concentration range (0.1 to >200 mg/ml), and is also compatible with a wide range of complex buffer systems and excipients, including those that absorb in the amide I region, surfactants and organic solvents. The platform includes a powerful software package that facilitates data analysis, and can be included in the automation procedure. This presentation highlights the technical benefits of MMS and its application in the protein structural workflow, giving relevant application examples.

Thursday, March 19, Session II

L5

STRUCTURAL ALPHABET FOR STRUCTURAL ANALYSIS OF NUCLEIC ACIDS

Bohdan Schneider and Jiří Černý

Institute of Biotechnology of the Czech Academy of Sciences, Prumyslova 595, Vestec, 252 50, Czech Republic

A newly developed structural alphabet [1] is the first alphabet universally applicable to both types of nucleic acids, RNA and DNA. In the talk, I will demonstrate some of the applications of the alphabet. It can be used to statistically weight sequence preferences of different conformational forms and to discover RNA and DNA structural motifs. The alphabet is based on 96 dinucleotide conformational classes, NtC, that were identified by analysis of structures of ~60 thousand RNA and ~60 thousand DNA steps. The resulting automated assignment of the conformational classes to any nucleic acid structure is available at the website dnatco.org [2] and is an extension of our previously published algorithm assigning only DNA structures [3].

1. Božíková et al., to be published 2020.
2. Černý et al., Nucleic Acids Research 2016, 44, W284.
3. Schneider et al., Acta Cryst. D 2018, 74, 52-64.