XIX Discussions in Structural Molecular Biology and 6th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Annual Meeting of the Czech Society for Structural Biology

Conference Centre of the Czech Academy of Sciences, Nové Hrady, March 23 - 25, 2023

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Kristýna Adámková, Radek Kužel

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 23, Session I

L1

EXCHANGING METALS IN Zn-DEPENDENT S1 NUCLEASE: EFFECTS ON STRUCTURE AND ACTIVITY

J. Hrubý[1,2], P. Kolenko[1,2], K. Adámková[2,3], B. Hus áková[2,3], M. Malý[1,2], L. H. Østergaard[5], T. Koval'[2], J. Dohnálek[2]

[1]Czech Technical University in Prague, Břehová 7, 115 19 Prague, Czech Republic
[2]Institute of Biotechnology of the Czech Academy of Sciences, Biocev, Průmyslová 595, Vestec,
[3]University of Chemistry and Technology Prague, Technická 5, Prague, Czech Republic
[4]Dept. of Agile Protein Screening, Novozymes A/S, Krogshoejvej 36, Bagsvaerd, Denmark
Corresponding authors: hrubyj18@fjfi.cvut.cz (JH); jan.dohnalek@ibt.cas.cz (JD)

Metalloenzymes constitute a large portion of known enzymes. The presence of metals in the active site of those enzymes can be crucial for both enzymatic activity and structural integrity [1].

S1 nuclease from *Aspergillus oryzae* is a Zn-dependent, single-strand specific enzyme with wide utilization in the biotechnology industry and research [2,3]. The active site of S1 consists of a zinc cluster containing three Zn^{2+} ions and 9 surrounding amino acid residues coordinating the cluster (Fig. 1). The shape of the active site resembles a pocket: Two Zn^{2+} ions (M1, M2) are buried at the bottom while the third Zn^{2+} ion is located at the pocket opening (M3), closer to the surface of the nuclease.

We studied the consequences of replacing Zn with various metals on the structure of the active site and the enzymatic activity towards the ssDNA substrate. For our work, S1 was first treated with a chelating agent ethylenediaminetetraacetic acid (EDTA). After the addition of various metals ($CuCl_2$, $CdCl_2$, $FeCl_3$, and $NiCl_2$), the resulting mixture was crystallised using the vapour diffusion method. Subsequently, X-ray diffraction experiments using multiple X-ray energies were conducted at Bessy II, Helmholtz Zentrum Berlin [4]. The activity towards ssDNA as a substrate was measured using precipitation of undigested nucleic acids and measurement of absorbance at 260 nm.

Using anomalous scattering at different X-ray energies, we proved that it is possible to artificially replace the Zn atoms in the active site (Fig. 2). Moreover, we discovered that all three positions are prone to exchange, with position M3 being the most frequent (Cu, Cd, and Ni were observed at this position). In all four cases, the exchange was incomplete with a maximum of two atoms replaced at a time, the remaining positions were occupied by Zn. The structure of the active site remained well conserved in comparison with native S1 (PDB ID 5FB9, [2]) with no significant structural changes.

The activity studies showed that, despite little to no structural changes, S1 remained inactive after the EDTA treatment and subsequent metal ion replacement using Cu, Cd, Fe and Ni. This suggests that the presence of three Zn ions is crucial for the activity and the metal replacement itself is sufficient for the complete inhibition of enzymatic activity.

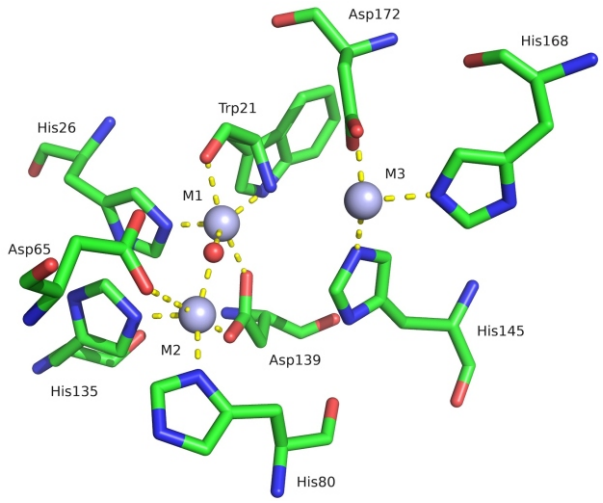

Figure 1. Active site of native S1 nuclease (PDB ID 5FB9, [2]). Zinc atoms and water molecule are represented using spheres in light blue and red, respectively. The surrounding structure is represented using sticks (C green, O red, N blue). Molecular graphics were created using PyMOL [5].

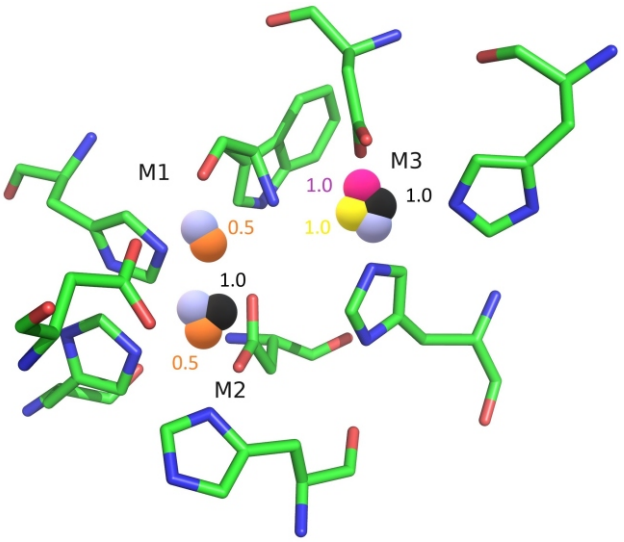

Figure 2. Schematic representation of all metal ion replacements in the active site. The position M3 (top right) is the most frequently replaced. Metal atoms are represented using spheres: Cu in pink, Cd in black, Fe in orange, Ni in yellow, and Zn (natively present in the active site) in light blue. Numbers represent the occupancy of substituent metals. Labels of the residues are omitted for clarity. Molecular graphics were created using PyMOL [5].

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This work was supported by the MEYS CR (projects CAAS – CZ.02.1.01/0.0/0.0/16_019/0000778 and ELIBIO – CZ.02.1.01/0.0/0.0/15_003/0000447) from the ERDF fund, by the Czech Academy of Sciences (grant No. 86652036), and by the Grant Agency of the Czech Technical University in Prague, grant No. SGS22/183/OHK4/3T/14. We acknowledge CMS-BIOCEV Crystallization and Diffraction, part of Instruct-ERIC, supported by the MEYS CR (LM2018127).

L2

CO-CRYSTALLIZATION STRATEGIES TO SUPPLEMENT THE STRUCTURE OF TAU PROTEIN FILAMENTS BY X-RAY CRYSTALLOGRAPHY

Klaudia Meskova[1], Katarina Martonova[1], Olga Parmar[2], Ondrej Cehlar[1], Rostislav Skrabana[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia
[2]Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Košice, Slovakia
klaudia.meskova@savba.sk

Alzheimer's disease (AD) is a neurodegenerative disorder, which represents the most common type of dementia. The main hallmark of AD is the accumulation of aggregated tau protein filaments in the cerebral cortex. The first atomic structures of AD filaments were solved by cryo-electron microscopy in 2017 and 2018, showing residues G304-E380 (Fig. 1) . However, it is known that the PHF core is longer and consists of residues I297-E391 , so, there are still unresolved parts in the structure of the core. In our work, we aim to solve these missing parts by X-ray crystallography, using specific recombinant antibodies. The monoclonal antibodies MN423 and DC11 recognize a conformational epitope on the PHF core (Fig. 1A) . The idea of crystallizing antibody-tau complexes is based on the hypothesis that the specific antibody could induce folding of the tau protein to mimic the folding in pathology . In our work, conformational antibodies MN423 and DC11 and other two helper antibodies DC8E8 and DC25 were used to co-crystallize with recombinant tau and form binary and ternary antibody-tau complexes. So far, we have crystallized eight different complexes, obtained crystals from six of them and diffraction data collected at the synchrotron sources of the X-ray radiation (DESY, Hamburg and PSI, Villigen). The crystals diffracted to 1.5-3Å resolution.

This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic (grant no. VEGA 02/0163/19) and APVV grant no. 21-0479.

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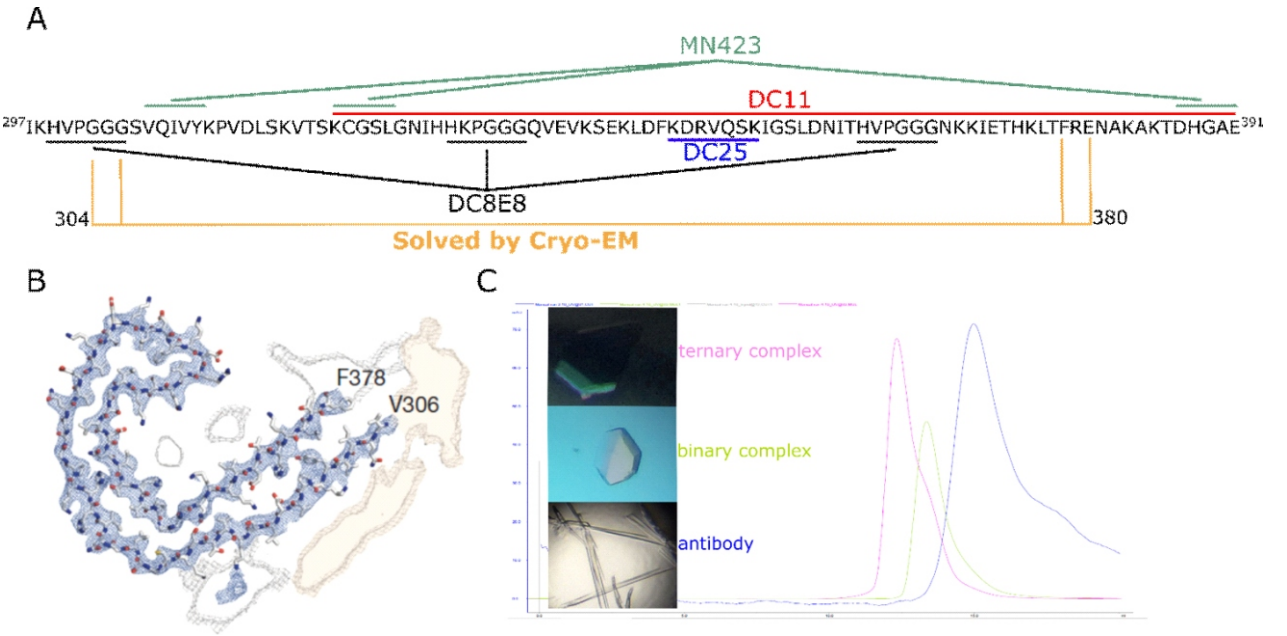

Figure 1. (A) Epitopes of the specific antibodies used for the co-crystallization with recombinant tau fragments. (B) AD protofilament solved by cryo-EM . (C) Purification of antibody-tau complexes and example of obtained crystals.

L3

STRUCTURAL STUDY OF RNA-PREFERRING NUCLEASE SmNuc1 FROM *STENOTROPHOMONAS MALTOPHILIA*

K. Adámková[1, 2], T. Kovaľ[1], M. Trundová[1], B. Hus áková[1], J. Dohnálek[1]

[1]*Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Průmyslová 595, 252 50 Vestec, Czech Republic*
[2]*University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Technická 5, 166 28 Prague 6, Czech Republic*
adamkovak@ibt.cas.cz

Zinc-dependent nuclease from pathogenic bacterium *Stenotrophomonas maltophilia* (SmNuc1) is a highly active RNA-preferring nuclease from the S1/P1 family (EC 3.1.30.1). Members of this family, especially from fungi and plants, have already been characterized and their structures were solved [1], but many questions remained unanswered. As they are widely used in biotechnology and biochemistry, new, more detailed information on their cleavage mechanism, substrate preferences, and active site composition may lead to an expansion of their potential applications.

Here we present a structure of recombinant SmNuc1 at 1.4Å resolution followed by high-resolution (1.20 - 1.85 Å) structures of complexes with RNA cleavage products (mononucleotides). These complexes show possible binding of products to the active site of SmNuc1 after cleavage and also their binding in novel, inhibitor-like modes. Also, we were able to capture some intermediate states of RNA cleavage process (product leaving), and this helped us to suggest a binding mode of longer RNA oligonucleotide substrates. Interestingly, close to the active site we detected a mobile segment, previously unseen in this family, containing the active-site-forming Arg74 and capable of remodeling the substrate binding site, which raises some new questions, such as is this remodeling the main mechanism for substrate specificity?

Structural and kinetic studies of SmNuc1 brought new insights into the cleavage mechanism and in combination with the already known facts about S1/P1 nucleases we are closer to clarifying some aspects of substrate preferences.

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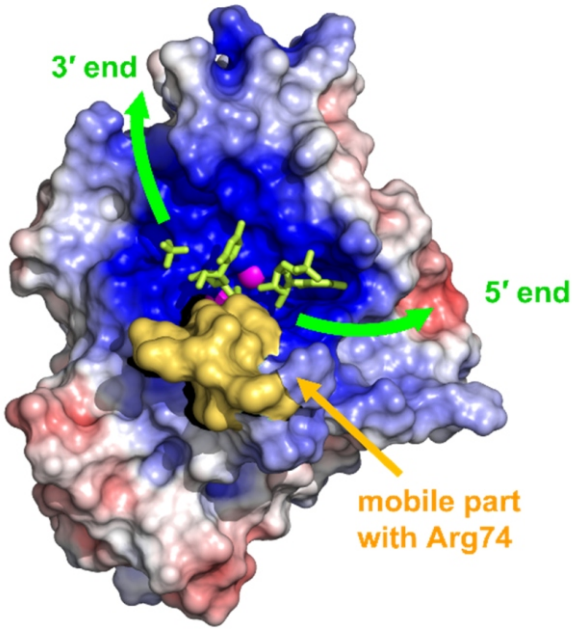

Figure 1. SmNuc1 surface coloured by electrostatic potential from red (-) to blue (+). The yellow surface indicates the mobile segment with Arg74 close to the substrate binding site. Green ar-

The work was supported by the institutional support of IBT CAS, v.v.i. (RVO: 86652036), CSF (23-06295S), ERDF (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), MEYS CR (CZ.02.1.01/0.0/0.0/16_019/0000778 and LM2018127, support of Biocev-CMS – core facilities Crystallization of Proteins and Nucleic Acids, and Structural Mass Spectrometry of CIISB, part of Instruct-ERIC).

L4

STRUCTURAL CHARACTERIZATION OF LECTIN FROM *PHOTORHABDUS LAUMONDII*

Filip Melicher[1,2], Josef Houser[1,2], Michaela Wimmerová[1,2,3]

[1]Central European Institute of Technology, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic
[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic
[3]Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic
melicherfilip@mail.muni.com

Lectins are proteins and glycoproteins able to reversibly bind saccharide moieties of glycoconjugates with high specificity. Lectins are involved in many physiological processes and play crucial roles in cell-cell communication or recognition of the host by a pathogen [1]. The research is focused on the lectins produced by the Gram-negative bacteria *Photorhabdus laumondii*. Bacteria of *Photorhabdus genus* live in symbiosis with *Heterorhabditis nematodes*. This symbiotic complex can be found in soil, where it searches for insect prey [2].

Unusual dual behaviour makes *Photorhabdus* bacterium a compelling organism for further study of its biomolecules. Besides functional characterization, structural information is essential for discovering the number of binding sites, the key residues involved in interaction and the orientation of the binding partner. For this purpose, protein crystallography was used to determine the 3D structure of lectin PLU1 and its complex with binding partners in atomic resolution. Examination of the PLU1 structure revealed a unique binding pocket, which significantly impacts the binding properties of the PLU1.

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This work was supported by the Czech Science Foundation (21-29622S) and CIISB research infrastructure project LM2018127 funded by MEYS CR.

L5

APPLICATION OF MONOLITH AND PANTA BIOPHYSICAL PLATFORMS IN THE STRUCTURAL CHARACTERIZATION OF THE RNA MODIFYING ENZYMES

Jakub Jezowski, Ting-Yu Lin, Jakub Nowak, Sebastian Glatt

Malopolska Centre of Biotechnology (MCB), Jagiellonian University, Krakow, Poland

RNA, one of essential components of life, is composed of four simple nucleotide building blocks that can assemble into complex forms. The basic nucleotides, namely U, A, G, C, can be decorated by various moieties that expand the structural and functional capacities. RNA modifications are frequently present in many different types of RNA. In our work we combine cryoEM, biophysical, and biochemical tools with the aim to structurally characterize the basis of selected RNA modifications as well as the protein complexes responsible for the modifications.

The intent of this presentation is to introduce our biophysical platform consisting of the Prometheus Panta and Monolith series of instruments from NanoTemper Technologies. The presentation will cover the biophysical background of the Monolith, used routinely in our laboratory to study interactions of the protein-RNA complexes. In addition, I will introduce the Prometheus Panta technology and its application to characterize protein properties. This technology allows us to characterize colloidal and conformational protein properties; assessing not only the quality of our protein purification pipeline but also effects of clinical point mutations on the protein conformational stability. The presentation will include an example of our structural and biophysical characterization of the protein-RNA complex involved in writing a commonly occurring RNA modification.