Friday, September 23, Session V

L23

3D DOMAIN SWAPPING DIMERIZATION OF THE RECEIVER DOMAIN OF CYTOKININ RECEPTOR CRE1 FROM *ARABIDOPSIS THALIANA* AND *MEDICAGO TRUNCATULA*

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Cytokinins are phytohormones regulating many biological processes that are vital to plants. CYTOKININ RESPONSE1 (CRE1), the main cytokinin receptor, has a modular architecture composed of a cytokinin-binding CHASE (Cyclases/Histidine kinases Associated Sensory Extracellular) domain, followed by a transmembrane fragment, an intracellular histidine kinase (HK) domain, and a receiver domain (REC). Perception of cytokinin signaling involves (i) a hormone molecule binding to the CHASE domain, (ii) CRE1 autophosphorylation at a conserved His residue in the HK domain, followed by a phosphorelay to (iii) a conserved Asp residue in the REC domain, (iv) a histidine-containing phosphotransfer protein (HPt), and (v) a response regulator (RR). This work focuses on the crystal structures of the REC domain of CRE1 from the model plant *Arabidopsis thaliana* and from the model legume *Medicago truncatula*. Both REC domains form tight 3D-domain-swapped dimers. Dimerization of the REC domain agrees with the quaternary assembly of the entire CRE1 but is incompatible with a model of its complex with HPt, suggesting that a considerable conformational change should occur to enable the signal transduction. Indeed, phosphorylation of the REC domain can change the HPt-binding properties of CRE1, as shown by functional studies.

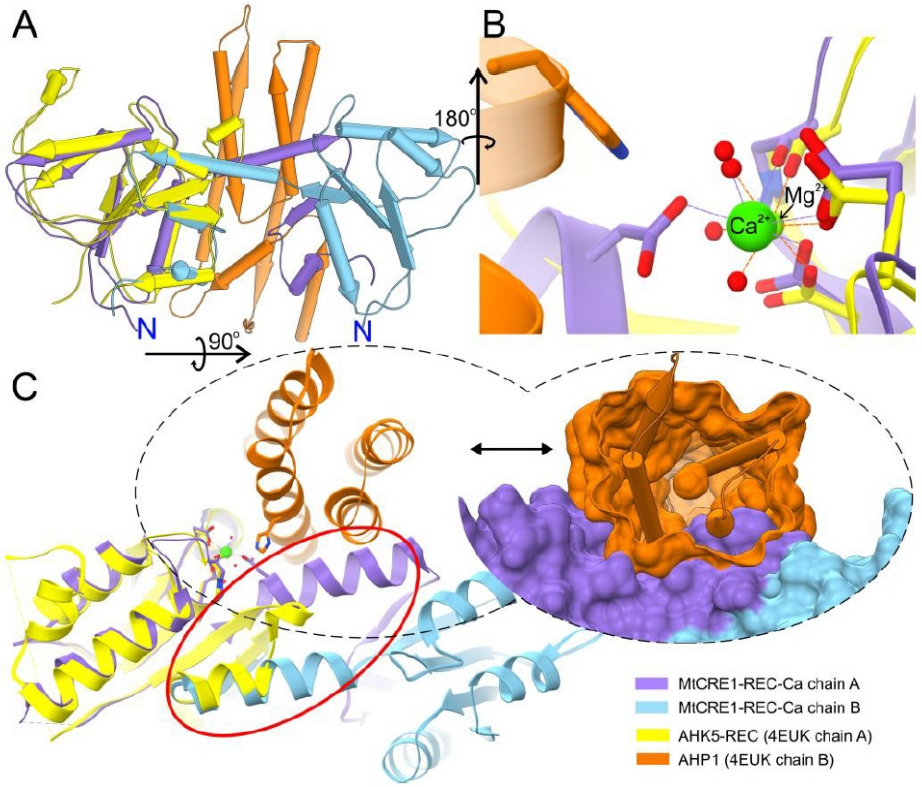

Figure 1. Superposition of the MtCRE1-REC dimer (purple/blue) onto the AHK5-REC (yellow) complex with AHP1 (orange) (A). MtCRE1-REC and AHK5-REC share a similar flavodoxin-like core structure. They also have a similar metal-binding site, shown in stick representation (B). While MtCRE1-REC binds Ca2+, AHK5-REC binds Mg2+. An attempt to superpose the two structures leads to severe clashes between AHP1 (orange surface) and MtCRE1-REC (purple surface), as shown in (C). The clashes occur because of the presence of the swapping domain (circled in red). In MtCRE1-REC, the purple a helix juts toward the second subunit (blue), while in AHK5-REC it flips back in the opposite direction (yellow)

L24

ADGRB2 AND ITS RESISTANCE TO AUTOPROTEOLYSIS

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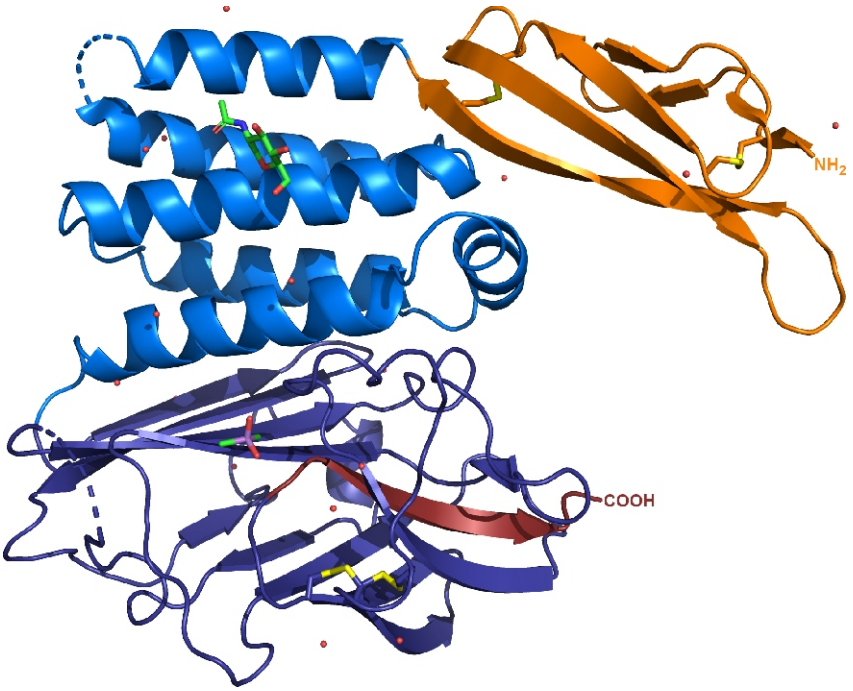
Adhesion G-protein coupled receptors (ADGRs) have garnered some notoriety as the second largest family of G-protein coupled receptors (GPCRs) with 33 homologues present in the human genome. While they were grouped together based on the phylogeny of their seven transmembrane (7TM) region, they are primarily distinguished from other GPCRs by their large and modular extracellular regions (ECRs) containing a variety of domains implicated in cell adhesion, which gave them their name. Except for GPR123 (ADGRA1, A1), which is missing an ECR entirely, all of them contain a GPCR autoproteolysis inducing (GAIN) domain, immediately adjacent to the 7TM region. The GAIN domain was identified by Araç *et al.* who showed that it contained the previously identified GPCR proteolysis site (GPS) motif, which is capable of cleaving itself at a conserved HLT/HLS triad. The role of the GAIN domain and its autoproteolysis as well as different modes of signal transduction by ADGRs have been a hot topic of discussion in recent years.

The GAIN domain of human BAI2 (*h*ADGRB2, *h*B2 did not live up to its name and was expressed and crystallized in an uncleaved state. The crystal structure of *h*B2-HG provided an as of yet unique perspective on the autoproteolysis behavior of ADGRs, highlighting the importance of properly positioning the nucleophile within the constraints of the catalytic triad. A set of interactions unique among human ADGRs was identified to be responsible for the misalignment of the GPS, hindering both the deprotonation of Ser912's hydroxyl group and most significantly its subsequent nucleophilic attack at Leu911's carbonyl carbon position as part of the autoproteolysis mechanism. Besides the unique configuration of its GAIN domain, the ECR of *h*B2 displayed an interesting dimerization behavior, which was charac-terized by small angle X-ray scattering, indicating a weak transient interaction consistant with a cis-interaction with a K_D in the micromolar range.



L25

STRUCTURAL ANALYSIS OF S1-LIKE NUCLEASE FROM OPPORTUNISTIC BIOFILM DWELLING PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

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S1-P1-like nucleases are a family of zinc-dependent enzymes cleaving phosphodiester bonds of nucleic acids. Members of this family from fungi and plants have already been studied and are widely used in biochemistry and biotechnology [1]. However, members from pathogenic organisms, such as some bacteria and protozoan parasites, have not yet been characterized and their function is not fully understood. Knowledge of their structure, active site composition, substrate preferences, and cleavage mechanism could be an important step towards exploiting their biotechnology potential.

The subject of our study is a zinc-dependent nuclease from *Stenotrophomonas maltophilia* (SmNuc1), small globular protein with high activity against single-stranded DNA, double-stranded DNA, as well as RNA. Bacterium *Stenotrophomonas maltophilia* is an emerging multi-drug-resistant Gram-negative aerobe causing severe nosocomial respiratory infections in humans, primary infecting immunocompromised patients. These infections are often complicated by the ability of this opportunistic pathogen to form highly resistant biofilm on various surfaces [2].

Here we present a novel structure of recombinant SmNuc1 nuclease obtained at 1.4 Å resolution, followed by structures of complexes with DNA and RNA cleavage products, and structures of SmNuc1 mutants and their complexes with 5'-mononucleotides. Analysis of these high-resolution crystal structures (1.2 – 2.0 Å) combined with activity studies has expanded our knowledge of the active site composition and the impact of individual residues on the activity and substrate preferences. This study also revealed several interesting features, such as a flexible loop near the active site (we termed this loop "R-loop" because of the presence of the active-site-forming Arg74) capable

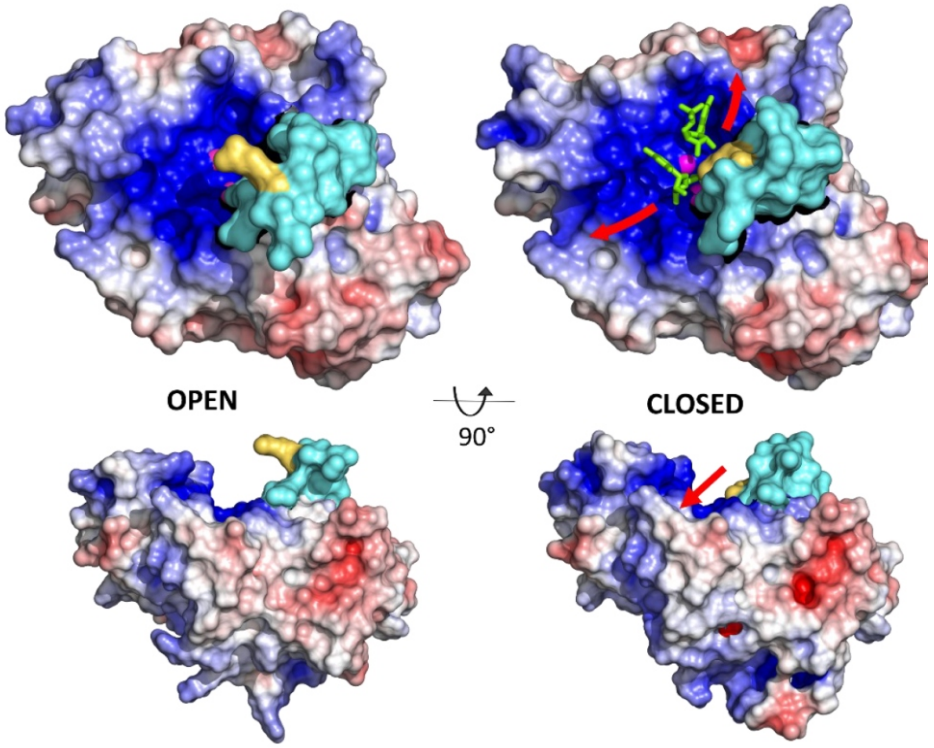

Figure 1: SmNuc1 solvent accessible surface. The cyan part of the surface indicates R-loop near the substrate binding site, with Arg74 shown in yellow, and the rest of the surface is coloured by electrostatic potential (-5 kT/e – 5 kT/e). Open R-loop is shown on the left and closed R-loop on the right. Red arrows indicate the direction of nucleic acid binding, ligands are shown in green sticks and zinc ions as magenta spheres. All graphics was created using *PyMOL* (Schrödinger).

of significant remodeling of substrate binding site, which brings up new questions about the catalytic mechanism. This information could shed light not only on some aspects of the SmNuc1 behavior, but also help us better understand the entire S1-P1 nuclease family.

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L26

LIQUID-LIQUID PHASE SEPARATION AND ITS ROLE IN Z-DISC FORMATION

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Mobility is vital for all animals. Striated muscles, allow animals to voluntarily move as well as involunteered movement of the cardiac muscle. This is achieved by converting chemical energy into mechanical one. The smallest contractile subunit of striated muscles is called the sarcomere. The boundaries between each sarcomere are called Z-disc. [1,2] They allow force transmission and are also a hub for cell signaling. These functions are possible thanks to a plethora of proteins that interact with each other in a highly ordered manner, appearing almost paracrystalline. [1] In this project we want to contribute to a better understanding of myofibrilogenesis the process in which the sarcomeres form. According to the premyofibril theory, the myofibrillogenesis starts from small punctate structures, called Z-bodies, which fuse laterally together. [3-5] Recently we discovered that one of the Z-Body associated Proteins, FATZ-1, can undergo Liquid-liquid phase separation (LLPS) [6], raising for the first time the question of whether FATZ-1 can create an interaction hub for Z-disk proteins through membrane-less compartmentalization during the initial stages of sarcomere assembly. Experiments to answer this question proved that FATZ-1 droplets have liquid-like properties *in vitro* and that -actinin 2 is colocalizes and is enriched in those droplets. Furthermore, -actinin 2 alter the phase diagram of the droplets by the size and even dissolving them, indicating a regulatory mechanism. [6] Recently, the group of Travis Hinson was able to generate a cell model with human induced pluripotent stem cell cardiomyocytes (hiPSC-CM´s) in which they observed Z-Bodies upon troponin T knock out. [7] This project aims not only to expand our study on the localization and influence of the other Z-bodies protein on FATZ-1 phase separation but also to prove this hypothesis *in vivo*. Unrevealing how such a highly ordered structure like the Z-Disc emerges would lead to a better understanding of how muscles actually form on a molecular level and may reveal how pathogenic protein variants lead to myopathies.

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FT7

STRUCTURAL ANALYSIS OF THE BisI FAMILY MODIFICATION DEPENDENT RESTRICTION ENDONUCLEASES

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The BisI family of restriction endonucleases is unique in requiring multiple methylations or hydroxymethylations within a short cytosine rich recognition sequence (GCNGC), and in cleaving directly within this sequence, rather than at a distance. Here, we report that the number of modified cytosines that are required for cleavage can be tuned by the salt concentration. We present crystal structures of the catalytic domain of Eco15I alone and at higher resolution of NhoI, with quadruple methylated DNA. The structures show that NhoI senses and recognizes modified cytosines in the context of double-stranded DNA without base flipping. Each protomer has two pockets for the 5' (internal, G5mCNGC) and 3' (external, GCNG5mC) methyl/hydroxymethyl groups. In our NhoI-DNA co-crystal structure, the internal methyl groups interact with the side chains of an (H/R)(V/I/T/M) diamino acid motif near the C-terminus of the domain. The external methyl groups make mostly main chain contacts, and in case of NhoI, interact also with a non-conserved arginine two residues downstream of the QXK motif (suggesting tight coupling of 5mC recognition and cleavage). Surface plasmon resonance analysis shows that for Eco15I, the internal and external methyl binding pockets contribute about equally to methylcytosine sensing.

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FT8

CRYSTALLOGRAPHIC STUDY REVEALS AN UNEXPECTED SUBSTRATE FOR S1-P1 NUCLEASE

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S1-P1 nucleases are metal-dependent nucleases cleaving both DNA and RNA. While the S1-P1 nucleases are produced by fungi, plants, trypanosomatids or bacteria, including human pathogens, they are not present in mammals. They found usage in biotechnological application, predominantly nuclease S1 from *Aspergillus oryzae* . Their activity can also lead to a decrease of tumor growth thus they represent potential tools for antitumor therapy . Recently, it was reported that S1-P1 nucleases from *Leishmania* can be a part of the pathogen defence against the host immune system as they are able to degrade neutrophil extracellular traps (NETs) .

Human opportunistic bacterium *Stenotrophomonas maltophilia* can induce serious complications to immunocompromised patients and to patients with cystic fibrosis . The growing numbers of multi-drug resistant isolates lead to the search for the new therapeutic approaches in treatment of *S. maltophilia* infections. One of the potential targets is the S1-P1 nuclease from *S. maltophilia* SmNuc1 which was produced in *E. coli*, characterized and crystallized.

The crystals of SmNuc1 diffracting to high resolution enable fragment screening and ligand binding studies in search for SmNuc1 specific inhibitors or new binding sites. Soaking of cyclic diguanylate (c-di-GMP) led to the discovery of its cleavage as only GMP was identified in the difference maps of electron density. To the best of our knowledge it is the first time the hydrolysis of cyclic dinucleotides was reported in the S1-P1 nuclease family. These results suggest a possible role of the nuclease SmNuc1 in the host-pathogen interaction.

This project was supported by the ERDF fund (CZ.02. 1.01/0.0/0.0/16_013/0001776 and CZ.02.1.01/0.0/ 0.0/ 15_003/0000447), Czech Science Foundation, project 20-12109S, and financial support from specific university research (MSMT-No-21-SVV/2020), by the Ministry of Education, Youth and Sports of the Czech Republic (LM2018 127, support of BiocevCMS – core facilities Biophysical Methods, Crystallization of Proteins and Nucleic Acids and Structural Mass Spectrometry of CIISB, part of Instruct-ERIC).

FT8

EFFECTS OF THE Zn^{2+} TO Cd^{2+} EXCHANGE ON THE STRUCTURE AND ACTIVITY OF S1 NUCLEASE

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S1 nuclease from *Aspergillus oryzae* is a single-strand specific nuclease widely utilized in biotechnology industry for biochemical analysis of nucleic acids [1,2]. It is a globular protein with its secondary structure predominantly composed of -helices (Fig. 1). Its activity depends on the presence of three Zn^{2+} ions in the pocket-shaped active site: Two Zn^{2+} ions of the cluster are buried at the bottom of the active site, the third Zn^{2+} ion is situated closer to the surface of the nuclease. The zinc cluster is coordinated by nine amino acid residues.

We studied the possibility of replacing Zn^{2+} with various metals and effects of such exchange on the structure and enzymatic activity. Here we present the results of the Zn^{2+} to Cd^{2+} exchange. S1 nuclease was mixed with chelating agent ethylenediaminetetraacetic acid (EDTA) in molar ratio 1:5, respectively, and dialysed, resulting in EDTA-treated S1. A mixture of EDTA-treated S1 with $CdCl_2$ in molar ratio 1:10 was successfully crystallized using the vapour diffusion method. The obtained crystals were of sufficient quality for the diffraction experiment on the synchrotron radiation source Bessy II, Helmholtz Zentrum Berlin [3]. Activity of fully EDTA-treated S1 with $CdCl_2$ towards ssDNA as a substrate was measured using precipitation of undigested nucleic acids and measurement of absorbance at 260 nm.

The diffraction data were collected at three different X-ray energies with the aim of differentiating the presence of Zn^{2+} or Cd^{2+} ions in the active site using anomalous scattering. The structural data showed that the conformation of the surrounding residues of the active site remained conserved. Only one of the inner Zn^{2+} ions remained intact. The other two Zn^{2+} ions were successfully replaced by Cd^{2+} ions. The key data collection statistics are summarized in Table 1. The anomalous difference map is shown in Fig. 2.

The activity studies showed that EDTA-treated S1 is entirely inactive (less than 1% activity of untreated S1). Only minor restoration of the activity was observed after adding $CdCl_2$, approximately 3% of the activity of untreated S1 activity.

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Table 1. Data collection statistics

Dataset name	Cd-peak	Zn-low	Zn-peak
X-ray energy [keV]	6.600	9.630	9.680
Resolution [Å]	44.71 - 2.30	44.66 - 2.60	44.66 - 2.70
Rpim	0.033	0.074	0.064
$CC_{1/2}$	0.999	0.996	0.996
Mean I/ (I)	18.3	10.4	10.8
Avg. anomalous multiplicity	11.9	13.7	13.9
Anomalous completeness [%]	99.2	100.0	100.0

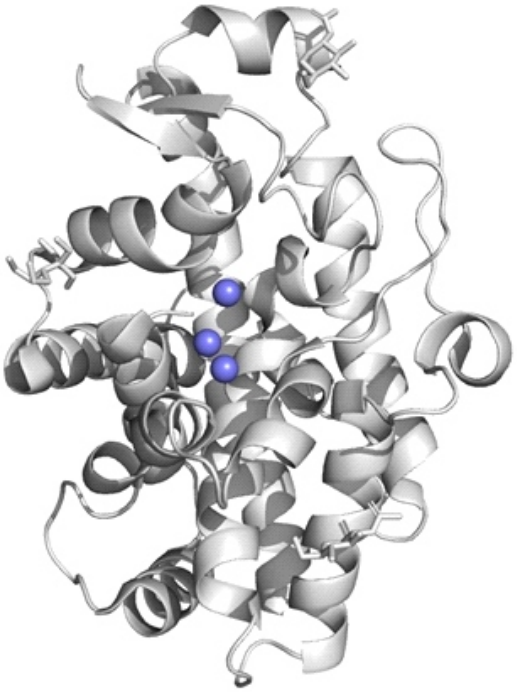

Figure 1. Secondary structure of S1 nuclease (PDB ID 5FB9). Zinc atoms are represented using blue spheres. Graphics created using PyMOL [4].

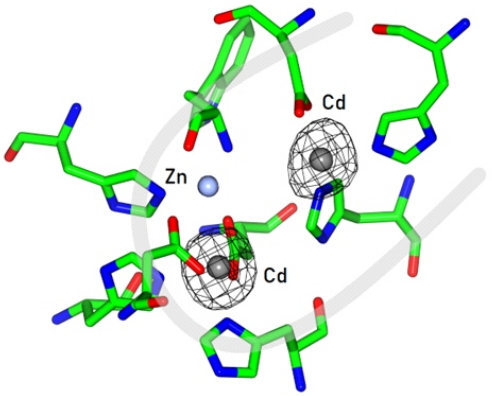

Figure 2. Anomalous difference map (Cd-peak) at a level of 5 . Two distinct peaks prove the presence of Cd^{2+}. The pocket shape of the active site is highlighted by the grey line. Graphics created using CCP4MG [5].