Friday, March 25, Session IV

L18

ZINC-DEPENDENT NUCLEASE FROM *STENOTROPHOMONAS MALTOPHILIA*: STRUCTURAL ANALYSIS OF THE ACTIVE SITE

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Zinc-dependent nucleases from S1-P1 family are relatively small globular proteins composed mostly of -helices, stabilized by disulfide bridges, and in the case of eukaryotic members they are also glycosylated. Gene coding an S1-P1 type nuclease (~25 % of sequence identity) can be found in many species, e.g. in plants, fungi, protozoan parasites, and also in some bacteria. Despite very similar active site composition and binding possibilities, these nucleases are able to cleave RNA, and single-stranded DNA as well as double-stranded DNA with different catalytic efficiency and substrate preferences [1].

The subject of our study is recombinant class I nuclease from *Stenotrophomonas maltophilia* (SmNuc1). *Stenotrophomonas maltophilia* is a Gram-negative aerobic bacterium from *Gammaproteobacteria.* It is an opportunistic human pathogen with a multiple antibiotic and stress resistance, infecting primarily severely immunocompromised patients and causing several nosocomial diseases [2]. Here we present novel structure of native SmNuc1 nuclease obtained at 1.4 Å resolution, followed by structures of complexes with 5'-mononucleotides and structures of mutants of SmNuc1.

Analysis of these high-resolution crystal structures revealed several interesting novel features. Near the active site, there is a flexible loop able to open and close, which brings up new questions about the catalytic mechanism. Our structure-activity study of SmNuc1 and its variants could shed light on some aspects of the cleavage mechanism of this whole family of nucleases.

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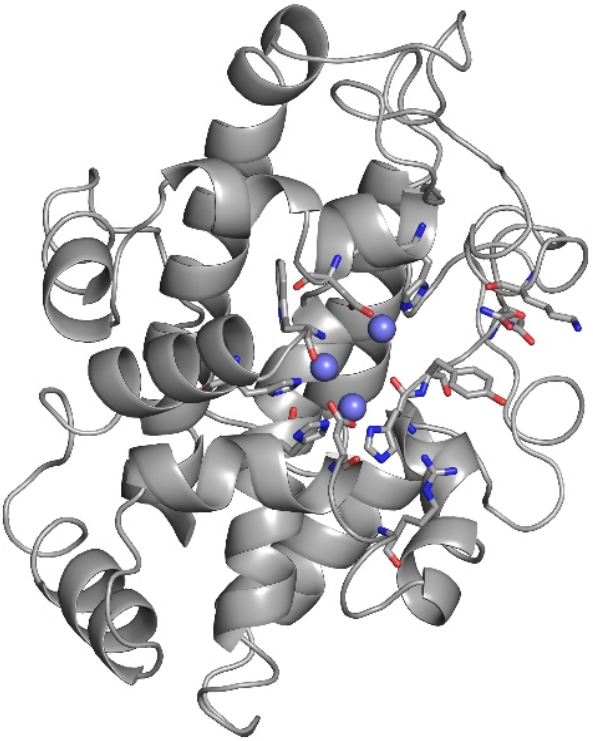

Figure 1 : Crystal structure of SmNuc1 nuclease. Residues of the active site are shown as sticks and zinc ions are shown as blue spheres. Graphics was created using PyMOL (Schrödinger).

L19

BIOPHYSICAL CHARACTERIZATION OF NOVEL MONOCLONAL ANTIBODIES TARGETING EPITOPES ON THE SARS-CoV-2 SPIKE PROTEIN

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Clinical trials suggest that antibody treatments can prevent deaths and hospitalizations among people with mild or moderate COVID-19. Considering high rate of SARS-CoV-2 escape mutations, a development of universal neutralization antibodies able to neutralize current and new variants of the virus is of a great importance. Employing hybridoma technology, a panel of anti SARS-CoV-2 Spike protein antibodies with a high affinity to the Spike receptor binding domain (RBD) has been generated (Fig. 1). Antibodies were selected based on the results of authentic virus neutralization assay. The antibodies AX290 and AX677, with non-overlapping epitopes on the Spike RBD (Fig. 2), showed excellent neutralization of an authentic SARS-CoV-2 virus representing strains circulating in Europe in spring 2020 and also the variants of concern B.1.1.7 (Alpha), B.1.351 (Beta) and B.1.617.2 (Delta). Unlike the majority of currently available therapeutic antibodies, AX677 is able to bind Omicron Spike protein just like the wild type Spike [1].

We have crystallized several antibodies alone and in the complex with RBD and characterize them on a synchrotron X-ray source.

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The study was funded by AXON Neuroscience SE and AXON COVIDAX a.s. The synchrotron data was collected at beamline P13 operated by EMBL Hamburg at the PETRA III storage ring (DESY, Hamburg, Germany). We would like to thank I. Bento for the assistance in using the beamline and to J. Stransky for crystal characterization at Centre of Molecular Structure, BIOCEV, Czech Republic.

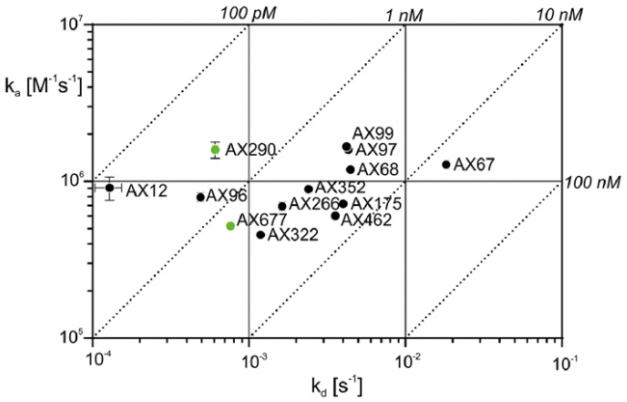

Figure 1. Kinetic characteristics of the interactions of RBD with selected neutralizing antibodies represented with isoaffinity lines (dotted) of the individual k_a, k_d and K_D affinity constants. The figure was adapted from ref [1].

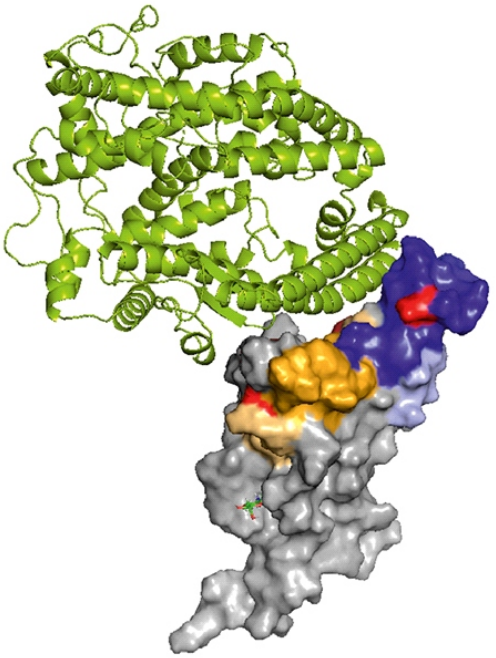

Figure 2. The positions of the peptides bound by the antibodies, highlighted in the structure of SARS-CoV-2 spike RBD bound to ACE2 (PDB ID 6M0J [2]). The AX290 binding site is represented by shades of blue, AX677 by orange and yellow. ACE2 is shown in a green cartoon model, RBD as a grey surface model. The figure was adapted from ref [1].

L20

INTEGRATIVE STRUCTURAL ANALYSIS OF ANTIBIOTIC-INACTIVATING ENZYME FROM *STENOTROPHOMONAS MALTOPHILIA*

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Stenotrophomonas maltophilia is an emerging pathogenic bacterium that causes an increasing number of serious infections worldwide. Mutations and the acquisition of antibiotic-resistance genes were shown to extend the broad antibiotic resistance of this species [1]. We performed a bioinformatic analysis of its available genomes to discover uncharacterised antibiotic-inactivating enzymes to carry out functional and structural analyses.

The chosen target enzymes were expressed in *Escherichia coli* and successfully purified. To confirm the expected function – enzymatic inactivation of antibiotics, kinetic assays were performed. An enzyme catalysing the antibiotic-inactivation reaction was crystallized and diffraction images were collected. The dataset exhibits severe anisotropy: an estimated resolution limit in *Aimless* [2], according to the criterion to $I/\ (I) > 1.5$, varied from 2.69 Å to 1.96 Å for different directions in reciprocal lattice. The phase problem was solved with *MoRDa* [3] and the structure model was refined in *REFMAC*5 [4].

Surprisingly, the crystal structure consists of a homodimer covalently linked via two cysteine bridges. However, consequent integrative structural analysis using SAXS, MS, MX and DLS indicates a monomeric state in solution. Nevertheless, the determined atomic structure reveals a spatial arrangement of the active site in detail. This provides an important knowledge for the development of antibiotic treatment strategies, as well as for further structural analysis – *in vitro* or *in silico* – of complexes with antibiotics or potential enzyme inhibitors.

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L21

2.4Å STRUCTURE OF THE DOUBLE CONCENTRIC RINGED LIGHT HARVESTING COMPLEX FROM PHOTOTROPHIC BACTERIUM *GEMMATIMONAS PHOTOTROPHICA*

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Anoxygenic photosynthesis has previously been described in the bacterial phyla; Cyanobacteria, Proteobacteria, Chlorobi, Chloroflexi, Firmicutes and Acidobacteria. Recently we described a phototrophic bacterium belonging to phylum Gemmatimonadetes [1]. This organisms *Gemmatimonas phototrophica* evolved the ability to use solar energy following horizontal transfer of photosynthesis-related genes from an ancient phototrophic proteobacterium.

Thus, we used the cryo-EM technique to investigate its detailed structure. The complex contains the type-2 reaction center surrounded by two concentric rings of light harvesting antennae. The outer LH ring consist of 24 subunits and contains 72 bacteriochlorophyll (BChl) molecules. The inner ring consists of 16 subunits, with 32 BChls. The function of the light harvesting complexes was investigated using fs-spectroscopy. It was found that the LH rings serve as an energy funnel with the pigments at higher excitation energy placed at the periphery and the pigments with lower energy in the centre. The excitation energy migrates within picoseconds down the energy gradient and is finally trapped in the RC [2].

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L22

THERMO SCIENTIFIC™ TUNDRA CRYO-TEM: 100kV CRYO-TEM DEDICATED FOR SINGLE PARTICLE ANALYSIS

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Single Particle Analysis (SPA) application of cryo-electron microscopy (cryo-EM) has become a widely used method for determination of the 3D structure of broad types of proteins and protein complexes, to study the mechanism of their function [1]. As the popularity of this technique increases, so does the need for greater efficiency and accessibility from not only microscopy experts but also from broader audience with little to no cryo-EM experience.

The Thermo Scientific Tundra Cryo-TEM is a new transmission electron microscope operating at 100kV acceleration voltage dedicated to SPA [2] which has been developed especially for new Cryo-EM users.

To load the sample into the microscope, Tundra Cryo-TEM brings a novel semi-automated loading technology (SAL). Sample loading operation is supported by fully guided workflow on the on-screen display (OSD) and in a few minutes allows to load the vitrified sample to the TEM column for people with very limited Cryo-EM expertise.

For 100kV accelerate voltage a new scintillator-based camera CETA-F with speed enhancement was developed. CETA-F is dedicated for low dose application and brings the possibility of dose fractionation mode as is implemented on the Falcon camera. This allows to store image frames for correction of beam induced motion in a post processing pipeline.

A new objective lens was developed for Tundra Cryo-EM to decrease the spherical and chromatic aberrations at 100-kV acceleration voltage and boost signal at high resolution frequencies.

By using all these new features Tundra Cryo-TEM achieved using apo-ferritin 2.6Å resolution of a reconstructed 3D map (Figure 1). At this resolution, *de novo* protein structures can be determined, and important biological

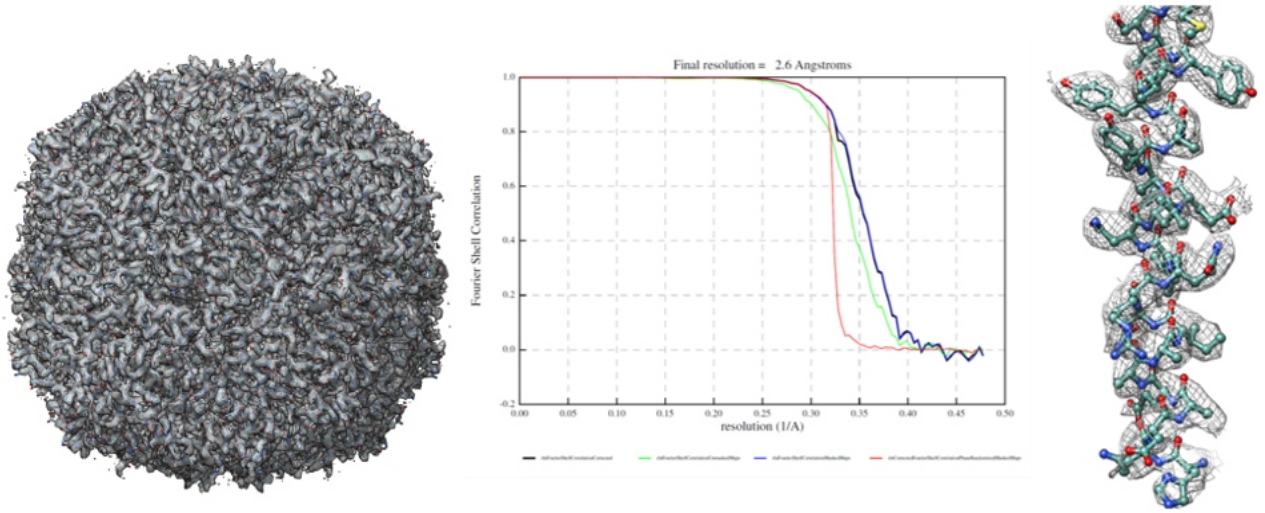

Figure 1. Structure of Apoferritin protein determined at 100 keV. a) 3D reconstruction of apoferritin at 2.6Å resolution, b) Gold-standard FSC plot corresponding to the calculated map, showing the correlation between the phase-randomized (red), unmasked (green) and masked (blue) half-maps, c) Electron density of the 2.6 Å resolution map showing the apoferritin -helix

questions answered. Data was collected with pixel size of 0.75Å, using aberration free image shift (AFIS) technology for less than 4 hours and processed using Relion 3.1 [3]. The data was collected using Thermo Scientific™ EPU™ software with pre-defined settings, were a new functionality in EPU was used – to automatically checks and refines optical alignments and provides system status for high-quality data acquisition.

All these new features that are introduced within the Tundra Cryo-TEM to achieve relevant resolution of their biological samples while keeping an accessible price point. This would make cryo-TEM accessible to many scientists across all life science branches.

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Friday, March 25, Session V

L23

ON THE IMPORTANCE OF PHYSICALLY CORRECT MODELS FOR PROTEIN-LIGAND BINDING

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Understanding protein-ligand binding in atomistic details is key to success in structure-based drug design. I will discuss two recent approaches: i) effective electronic polarization scheme for classical molecular dynamics (MD) [1] and ii) parametrized semiempirical quantum mechanics (SQM)-based scoring function [2, 3]. The former method includes polarisation in classical additive force fields via charge scaling. We have shown its power in correct structural description of the bridging Ca^{2+} ions in lectin/carbohydrate complex (Fig. 1A, B) [1].

A universal method, which includes also other quantum effects, such as charge transfer or -hole bonding, is quantum mechanics. We have developed an SQM-based scoring function which, due to its chemical generality, outperforms standard academic and commercial packages in sampling, ranking and virtual screening. In summary, developing and applying physically correct models of protein-ligand binding heads toward an unrivalled qualitative and quantitative enhancement of the predictive power of computer-aided drug design.

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