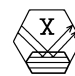

Heart-of-Europe Bio-Crystallography Meeting, HEC-18
Abstracts

Session I - Thursday, September 24

L1

SUBSTRATE INDUCED ACTIVATION OF THE PRO-PROTEIN CONVERTASE FURIN

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Furin is a serine endoproteinase belonging to the pro-protein convertase (PC) family. PCs are required for the activation and maturation of many secreted proteins, including peptide hormones, growth factors, matrix metalloproteases, blood coagulation factors, regulators of the cholesterol metabolism, bacterial toxins and viral capsid proteins. Therefore furin and other PCs are highly interesting targets for the treatment of many diseases, e.g. cancer as well as viral- and bacterial infections [1]. Development of furin inhibitors for pharmacological use requires a detailed structural understanding of the mechanisms involved in substrate-binding, catalysis and inhibition [2,3].

Here we present crystallographic structures of furin, a prototypical member of the PC protease family, showing its apo- and holo-form at 1.8Å and 2.0Å resolution, respectively. In combination with biochemical data we demonstrate a twofold activation mechanism of the protease: the switch from the inactive “off-state” to the active “on-state” is triggered by interactions of substrates/inhibitors at the substrate binding cleft. The catalytic residues adopt the catalytically active conformation only upon ligand binding, explaining also the widely observed “inactivity” of the apo-enzyme. Only tightly binding substrates or inhibitors can induce activity by structurally altering the active site, describing the high specificity of furin. This substrate-induced activation explains also energetically, how tight binding of substrates is compatible with high catalytic turnover as observed for the PCs. Our crystals with one molecule in the asymmetric unit are in addition highly suited for structure based inhibitor development approaches, e.g. specifically targeting the apo- and holo-conformation of furin in crystallography based fragment screening.

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L2

PSYCHROTROPIC -D-GALACTOSIDASE WITH UNUSUAL DOMAINS ARRANGEMENT

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The -D-galactosidase from *Paracoccus* sp.32d (*Par* DG) (EC 3.2.1.23) belongs to Glycosyl Hydrolase family 2 (GH2). It catalyzes the hydrolysis of terminal non-reducing -D-galactose residue in -D-galactosides. [1] DGs have been a focus of numerous researchers due to their utility in a variety of industrial technologies, especially dairy and pharmaceuticals production. [2]

The crystals of *Par* DG were obtained by hanging drop vapor-diffusion method in a form of very thin long needles. The best crystallization conditions contained dichloromethane, mPEG 2K and ammonium acetate as precipitants. The X-ray diffraction data were collected from one monocrystal at the PX13 Petra synchrotron beamline in Hamburg, Germany. Diffraction data were processed by XDS program at resolution of 2.4 Å.

Since neither the size nor the domain arrangement of the *Par*DG were similbar to the known galactosidases, the multiple models were used for the Molecular Replacement: 3HN3, 3FN9, 3CMG, 3BGA, 3GM8 and Multiple Domain Model from different PDBs (3HN3, 1T9K, 3FN9, 1Q33). Structure was solved in orthorhombic space group $P2_12_12_1$ with dimer in the asymmetric unit. The initial cycles of refinement were performed using PHENIX AutoBuilt which were followed by manual steps in Coot.

As a result the final model of a five domains was obtained. The *Par*DG active bsite is located close to the dimer interface. The catalytic site of the enzyme is constituted mainly by the amino acids from the central Domain 3 and completed by a few residues of Domain 4 from the symmetry related molecule. The unique in its structure, size and location Domain 5 additionally stabilizes the functional homodimer.

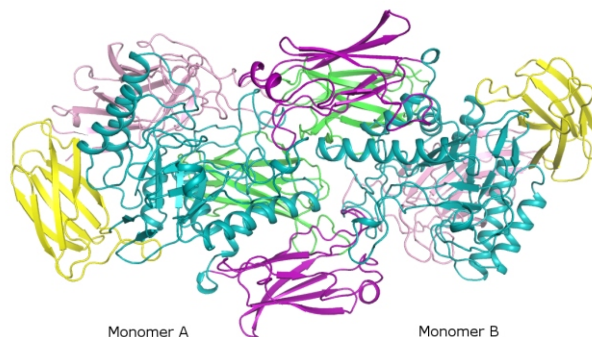

Figure 1. The homodimer of *Par* DG: domain 1 – pink, domain 2 – yellow, domain 3 – teal, domain 4 – violet, domain 5 - green.

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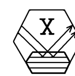

L3

STRUCTURAL CHARACTERIZATION OF GLYCERALDEHYDE DEHYDROGENASE FROM *THERMOPLASMA ACIDOPHILUM*

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The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (*Ta*AlDH) is a part of an artificial cell-free enzyme cascade for production of isobutanol and ethanol from glucose. *Ta*AlDH catalyzes the oxidation of D-glyceraldehyde to D-glycerate in this synthetic pathway [1]. Various mutants of *Ta*AlDH were constructed by random approach followed by site-directed and saturation mutagenesis in order to improve the enzymes' properties essential for its functioning within the cascade. Further optimization of *Ta*AlDH requires structural information about the enzyme for which crystallization followed by X-ray diffraction analysis was employed [2].

Diffracting quality crystals of *Ta*AlDH wild type and mutant *Ta*AlDH F34M+S405N were obtained after optimization of condition H6 of the Morpheus screen (Molecular Dimensions Ltd., UK) and No. 1 of PEGs Suite (Qiagen, the Netherlands), respectively. Full data sets were collected on the BL 14.1 operated by the Joint Berlin MX-Laboratory at the BESSY II electron-storage ring (Berlin-Adlershof, Germany) at 1.95Å and 2.10Å resolution for *Ta*AlDHwt and *Ta*AlDH F34M+S405N. *Ta*AlDHwt crystals belong to monoclinic $P2_1$ space group with unit cell parameters of a = 95.29 Å, b = 152.35 Å, c = 149.90 Å, = = 90.0°, = 92.19° and 8 molecules per asymmetric unit. Crystals of *Ta*AlDH F34M+S405N belong to monoclinic space group $C2$ with unit cell parameters of a = 108.1 Å, b = 158.4 Å, c = 130.1 Å, = = 90°, = 91.6°. Matthews coefficient (V_M = 2.5 $Å^3$ Da^{-1}) suggests 4 molecules in the asymmetric unit with solvent content of 50.47%.

The structure of *Ta*AlDHwt was solved by molecular replacement using the coordinates of betaine-aldehyde dehydrogenase from *Pseudoalteromonas atlantica* T6c (sequence identity 38%, PDB ID 3K2W). The final model contains two tetramers in the asymmetric unit that are related by non-crystallographic symmetry with differences observed in regions participating in crystal contacts. The *Ta*AlDHwt homotetramer consists of two homodimers that display a very tight connection through the formation of an extended beta-sheet between monomers of the dimer. Structure of *Ta*AlDH F34M+S405N was solved using *Ta*AlDHwt structure as a model for molecular replacement and refinement is now in progress.

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L4

CRYSTAL STRUCTURE OF INORGANIC PYROPHOSPHATASE FROM A HIGHER PLANT

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Inorganic pyrophosphatase (PPase) is a ubiquitous cytosolic enzyme that catalyzes the hydrolysis of inorganic pyrophosphate (PPi) to orthophosphate (Pi). The first crystal structure of inorganic pyrophosphatase from *Arabidopsis thaliana* (AtPPA1) has been solved by Molecular Replacement using the coordinates of archaeal PPase from *Pyrococcus furiosus* (PDB 1twl) as the template. The structure has been refined to an R-factor of 15.6% at 1.93Å resolution using synchrotron radiation X-ray diffraction data.

PPases are oligomeric enzymes that are active as homohexamers, composed of ~20 kDa subunits in prokaryotes. In contrast, eukaryotic PPases act as homodimers of 30-35 kDa subunits. The plant AtPPA1 protein is an exception as it forms a homotrimer with a total mass of 75 kDa. The structure of AtPPA1 represents an OB Fold (Oligonucleotide/Oligosaccharide-Binding) which overlaps with other known structural models of bacterial and yeast inorganic pyrophosphatases. In the AtPPA1 structure, one sodium-binding site per protomer is found. Analysis of the AtPPA1 sequence using PsiPred (signal peptide predictor) revealed that it possesses a putative N-terminal transit peptide of mitochondrial targeting, and a possible cleavage site at Val31. *In vitro* cleavage of a short (4 kDa) N-terminal fragment is observed during protein storage. Two aspartic acid mutations, D98N or D103N, have been shown to delay the autoproteolysis compared to the wild type protein. The crystal structure and protein N-terminal sequencing confirm that the cleaved N-terminal fragment corresponds to the predicted mitochondrial targeting peptide.

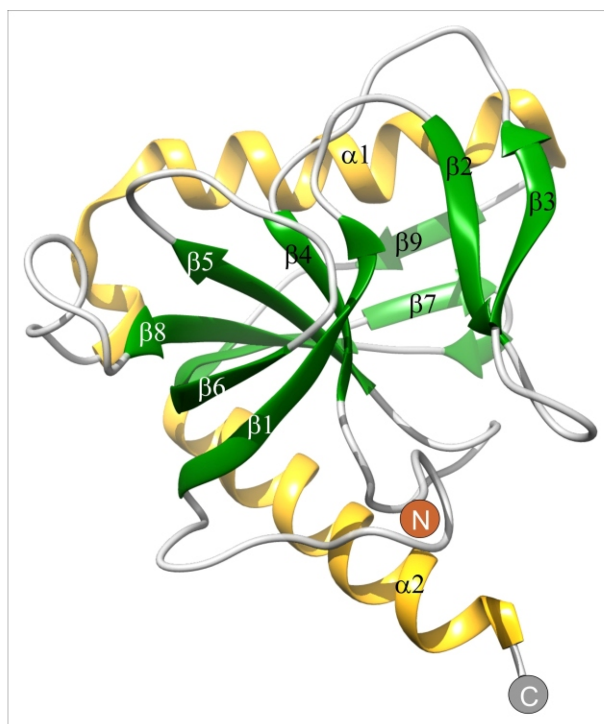
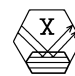


Figure 1. Overall structure of AtPPA1 protomer.



L5

X-RAY STRUCTURE OF BILIRUBIN OXIDASE FORM *MYROTHECIUM VERRUCARIA* WITH LIGAND IN THE ACTIVE SITE

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Bilirubin oxidase from plant pathogen *Myrothecium verrucaria* (BO, EC 1.3.3.5) is a blue multicopper oxidoreductase (MCO) catalyzing oxidation of a wide spectrum of aromatic substrates (consisting of tetrapyrrole, diphenols and aryl diamines) and some inorganic compounds with accompanying reduction of molecular oxygen to water. BO contains four copper centres classified into three types, type I Cu (T1), type II Cu and a binuclear type III Cu forming a trinuclear copper cluster (TNC). The T1 Cu cation in the substrate binding site is coordinated by one cysteine, two histidine residues and one methionine and accepts an electron from substrate. Four electrons are transferred from T1 via cysteine-histidine pathway to TNC coordinated by eight histidine residues, where O_2 is reduced to water. BO's capability to oxidize a great variety of organic substrates suggests many applications in industry (e.g. pulp bleaching, delignification, drug detection, degradation of herbicides or development of biosensors and biofuel cells [1,2,3]).

Two non-liganded structures of BO have been already published in PDB (2XLL [3], 3ABG [4]). The mechanism of substrate binding and the way of the electron transport from substrate to the T1 site in MCOs have been studied for many years and some key parameters are still to be determined. We determined two structures of BO - a native structure at 2.3Å resolution and a structure with a small ligand in the substrate binding site at 2.6Å resolution. On the basis of our results we suggest possible electron transfer routes from substrate to the T1 centre.

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This work is supported by the project „BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund and institutional support of IBT CAS, v.v.i. RVO: 86652036.", by the Ministry of Education, Youth and Sports of the Czech Republic (grant No. EE2.3.30.0029 and No. LG14009), by the Grant Agency of the Czech Technical University in Prague, grant No. SGS13/219/OHK4/3T/14, by Grant Agency of the Czech Republic, grant No. 15-05228S.

L6

LATEST DEVELOPMENTS IN EQUIPMENT FOR DIFFRACTION AND SCATTERING FROM RIGAKU OXFORD DIFFRACTION

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With the recent formation of Rigaku Oxford Diffraction there have been a number of exciting and valuable advances in equipment for diffraction and scattering of macromolecules. The new developments will be presented.

L7

LATEST DEVELOPMENTS IN HOME-LAB MACROMOLECULAR CRYSTALLOGRAPHY AND BIO-SAXS

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Recent Developments in X-ray instrumentation in the *D8 VENTURE* Systems have provided an opportunity to redefine their role in the Structural Biology workflow.

The introduction of the novel *METALJET* X-Ray source technology enables smaller X-ray beams of higher intensity to be produced than was previously possible with existing technologies. New *Active Pixel Sensor* detector technology finally makes available home-lab detectors that combine a large area, high sensitivity and low noise and fast readout for continuous data collection.

In situ crystallography has become popular at synchrotrons as a method to identify target quickly and efficiently. The *ISX Stage* is designed to be mounted on the *KAPPA* stage and is compatible with any source. Being fully motorised and software controlled, it finally makes the full benefits of *in situ* crystallography available in the home-lab.

The *NANOSTAR* is a compact SAXS beamline that can be mounted independently or on the second-port of an existing source. The beamline design uses a large two-dimensional photon counting VANTEC 2000 detector to capture all the scattered X-rays and maximise signal-to-noise.

An overview of the new technologies will be presented along with examples of users results which lead one to describe his instrument a "*pocket synchrotron*".

Figure 1. D8 VENTURE with NANOSTAR: Complete Home-Lab System for macromolecular crystallography and bio-SAXS.