their functioning remain elusive. Crystallization and structural characterization of these transporters in different states with high resolution is needed to clarify the existing structural models and results of biochemical experiments.

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Posters - Macromolecular Complexes

P2-1

CLOSED CLEFT OF O-ACETYL SERINE SULFHYDRALASE (OASS) ACTIVE SITE FROM *BRUCELLA ABORTUS* IS RESPONSIBLE FOR LOSS CSC FORMATION

Sudhaker Dharavath, Gourinath Samudrala

Jawaharlal Nehru University, School of Life Sciences, New Delhi, 110067, India

Brucellosis also known as 'undulant fever', 'Mediterranean fever' or 'malta fever' is zoonosis, an infectious disease caused by bacteria called *brucella.* It can spread from animals to humans, when person comes into contact with an animal or animal products of infected with brucella. Brucellosis is considered a significant health treat in many parts of the world especially in Mediterranean countries of Europe, north and east Africa, the Middle East, South and Central Asia and Central and South America. Activation of NADPH oxidase and low ROS production needed for the bacterium to induce premature cell death of neutrophils without inducing pro inflammatory phenotypic changes. This event majorly achieved by important antioxidative property of Cystein, involved in survival and protection of pathogenic microbes from oxidative stress and is the precursors for biosynthesis of many metabolites like glutathione, tripanthione and taurine etc., involved in maintaining cellular homeostasis, as cysteine biosynthesis pathway is crucial for inhibitor designing. In bacteria, plants and most of the pathogenic protozoans, cysteine biosynthesis is a two-step pathway. Serine acetyl transferase (SAT) catalyzes the first step of the pathway in which it transfers the acetyl group from acetyl Coenzyme A to serine to form O-acetyl serine (OAS) and in second step O-acetyl serine sulfydralase (OASS) catalyzes the condensation of O-acetyl serine with sulfide to produce cysteine.

Cysteine biosynthetic pathway is regulated by formation of a decameric complex called cysteine synthase complex (CSC), plays an important role in maintaining intracellular cysteine level. The SAT C-terminal tail binds at the active site of OASS to form CSC, which was reported earlier in E.coli, Salmonella, Hemophilus, leishmania, and Arabidopsis. And there are few structural models for CSC, although no structural evidence has been given so far.

Here in *Brucella abortus* OASS, does not interacts with its cognate SAT C-terminal tail. We recently determined the crystal structure of native BaOASS at 2.2Å resolution. The detailed comparison of BaOASS crystal structure with OASS complexes with SAT C-terminal peptides from other organisms showed the two residues 96Q and 125Y of BaOASS present instead of M and G from all other OASS respectively, occupying the active site pocket and interfering the entry of SAT C-terminal tail into the active site pocket of OASS. Thus blacking the active site pocket for entry of SAT C-terminal peptide. Point mutation of these residues (Q96 and Y125) was done to generate three mutations (Q96A, Y125A and Q96A Y125A). The binding affinity of SAT C-terminal peptides were measured by fluorescence spectrometer for all three mutants, it was found that the binding affinity (Kd) of double mutant BaOASS (Q96A Y125A) is much stronger (1000 fold) and single mutants of BaOASS (Q96A), BaOASS (Y125A) about (10 fold stronger) up on titration with SAT C-terminal mimicking peptides compared to Native BaOASS.

The Surface Plasmon Resonance (SPR) has been done for both native BAOASS and mutant BaOASS (Q96A Y125A) with BaSAT protein, where double mutant can bind to BaSAT with about 7uM binding affinity while native protein can't bind to BaSAT. These observations clearly conforms the reason for not forming CSC in BaOASS and we have validated these observations by mutational studies.

P2-2

CHARACTERIZATION OF IMMUNE MODULATORY PROTEIN COMPLEX CD160-HVEM PARTICIPATING IN BIDIRECTIONAL SIGNALLING PATHWAYS

I. Nemčovičová[1,3], M. Nemčovič[2], M. Kúdelová[1], D. Zajonc[3]

[1]Biomedical Research Center and [2] Institute of Chemistry, Slovak Academy of Sciences, Bratislava
[2]Division of Cell Biology and Immune Regulation, La Jolla Institute, California, USA
viruivka@savba.sk

The majority of biological events involve the action of one macromolecule on another, thus triggering a series of recognition, signalling and modification events. The details of such macromolecular interactions are critical to our understanding of biological function and bestow greater knowledge than the three-dimensional structures of single macromolecules. Although substantial progress has been made in macromolecular docking, it still remains difficult to predict the mode of interaction between macromolecules even when the structures of the interacting partners are known. Natural killer (NK) cells express multiple activating receptors that, upon engagement, result in the rapid release of cytolytic and antiviral effectors required for host defence, notably against herpes viruses (-herpesvirus cytomegalovirus, CMV). The host is protected from the powerful inflammatory mediators produced by NK cells through the action of inhibitory receptors. It was shown that the UL144 mimic of herpesvirus entry mediator (HVEM) from the CMV binds exclusively to B and T lymphocyte attenuator (BTLA) but not to CD160, resulting in inhibition of NK cells. HVEM and the two Ig-superfamily member receptors that bind HVEM, CD160 and BTLA, are all expressed on NK cells. Here, we show the molecular characterization and preliminary crystallographic analysis of CD160 and HVEM and therefore CD160-HVEM complex formation. CD160 is a 27 kDa glycoprotein which was initially identified with the monoclonal antibody BY55. Its expression is tightly associated with peripheral blood NK cells. The cDNA sequence of CD160 predicts a cysteine-rich, glycosylphosphatidylinositol-(GPI)-anchored protein of 181 amino acids with a single Ig-like domain weakly homologous to KIR2DL4 molecule. It was found that HVEM preferentially engages CD160 trimer to costimulate activation, while a viral ortholog of HVEM specifically binds to BTLA to suppress this signalling. CD160 antigen is a protein that in humans is encoded by the CD160 gene. We have found that CD160 is expressed at the cell surface as a tightly disulphide-linked multimer. The homology model of atomic structure of CD160 antigen domain [Fig.1] shows cysteine-rich region that was found to be responsible for CD160 tight-timer formation even under reduced conditions. The crystallization of multimeric CD160-HVEM complex was accessed by advanced macromolecular crystallization methods while non-reducing conditions. CD160 trimer forms stable complex with HVEM, while monomeric form refused to binds its cognate ligand. Taken together, regulation of CD160 bidirectional binding may represent a common mechanism of immune regulation targeted by multiple pathogens, which by extension is a potential target for therapeutic manipulation.

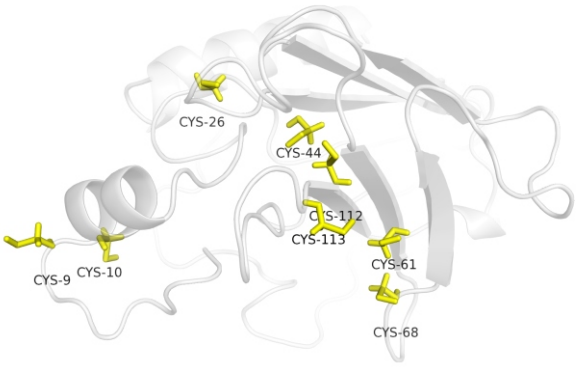

Figure 1. Homology model of CD160 structure with cysteine-rich intermolecular network.

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P2-3

EXPRESSION, PURIFICATION AND BIOPHYSICAL CHARACTERIZATION OF TRANSFER PROTEINS FROM THE GRAM-POSITIVE CONJUGATIVE PLASMID pIP501

Arumbakam Sridhar A.[1], Fercher C.[1], Grumet L.[1], Grohmann E.[2], Keller W.[1]

[1]Institute for Molecular Biosciences, Karl-Franzens-University Graz, Humboldtstr. 50/III, Graz, 8010, Austria; [2]Department of Life Sciences and Technology, Beuth University of Applied Sciences Berlin, Seestr. 64, 13347 Berlin, Germany

Conjugative plasmid transmission leads to an accelerated propagation of bacteria with multiple antibiotic resistances. In order to understand gram-positive conjugation on a molecular level, we are working on the functional characterization of individual transfer proteins derived from the conjugative plasmid pIP501. The respective transfer region is arranged in a single operon encoding fifteen putative transfer proteins which resemble a simplified type IV secretion system (T4SS) [1, 2]. So far, we could determine the high resolution structures of TraK, TraN as well as the C-terminal domain of TraM which exhibits a VirB8-like fold [3, 4]. Low resolution SAXS data were collected for soluble truncations of TraH and TraG, for the latter peptidoglycan degrading activity has been demonstrated thereby facilitating insertion of the T4SS machinery in the bacterial cell envelope [5].

Within this work, different biophysical and biochemical methods have been employed to functionally characterize the N–terminal domain of the bitopic protein TraM (TraM N) and the soluble truncations of TraF. The proteins have been successfully expressed, purified and biophysically characterized. The solution structure of the N-terminal domain was determined through SAXS; further NMR experiments are being conducted. Preliminary data has shown that TraM N binds to DNA, suggesting a direct involvement in the conjugative transfer of the T-DNA strand. Currently, NMR experiments are carried out and DNA band-shift experiments are performed to elucidate the functional aspects of TraM N. Crystallization trials are carried out in parallel for TraM N and TraF to determine their structure.

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P2-4

DEVELOPMENT AND APPLICATION OF METHODOLOGY FOR SUCCESSFUL CRYSTALLISATION OF MEDICALLY RELEVANT PROTEINS

Sean Kassen, Lata Govada, Naomi E Chayen

Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, London SW7 2AZ, United Kingdom
s.kassen@imperial.ac.uk

The production of high quality protein crystals for X-ray crystallography is a major obstacle to 3-D structure determination [1, 2], thus new methods to overcome this are continuously being designed. One such method is the "oil barrier" technique which enables the slowing down of the crystallisation process when poor crystals are formed due to crystallisation progressing too rapidly [3]. This method has been successful in improving crystal quality but has a few problems:

This technique involves manually placing a layer of oil (100-500 microlitre of oil) over the reservoir of vapour diffusion trials thereby consuming microlitre quantities of protein and reagents

i. The method does not work when using above 15% of polyethylene glycols (PEGs) and precipitants such as 2-methyl 2 4-pentanediol (MPD)
ii. The method is not amenable to high throughput trials

An improved oil barrier method that overcomes the limitations of the above method is presented. The principle of the oil barrier remains the same, but the key difference is dispensing the oil in quantities of between 100-250 nanolitres directly on top of the drop in vapour diffusion

trials. This creates a localised system of controlled evaporation between each drop and the precipitant reservoir. This improved method is easily customisable allowing for the use of nanolitre volumes of samples and making high throughput trials possible.

When tested on two medically related proteins – an antibody-peptide complex and alpha-crustacyanin; a noticeable difference in crystal number, size and quality was observed. Results show that this *"oil-on-drop"* method produces fewer crystals and these crystals grow to a larger size. Crystals of the antibody-peptide complex achieved diffraction of 3.6 Å compared to diffraction of 7 Å in previous studies. In addition, successful crystallisation of alpha-crustacyanin, which crystallises with 30% PEG-5000 as the main precipitant has produced fewer and larger crystals with the improved method that was not replicated with the traditional oil barrier method. Work with other proteins and precipitants is on-going.

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P2-5

STRUCTURAL STUDIES OF RNA-BINDING PROPERTIES OF ARCHAEAL Lsm PROTEINS FROM *METHANOCOCCUS VANNIELII* AND *SULFOLOBUS ACIDOCALDARIUS*

Natalia Lekontceva, Alisa Mikhailina, Vitaly Balobanov, Maria Nemchinova, Svetlana Tishchenko, Alexey Nikulin

Institute of Protein Research RAS, Pushchino, Russia

Archaeal Sm-like proteins belong to the large LSm family, which is characterized by the ability to adopt Sm fold. It is comprised of a 5-stranded ß-sheet and an N-terminal -helix. Despite the fact that they are structurally conserved, functions of the protein from archaea, bacteria and eukarya are dissimilar.

Eukaryotes contain at least 18 different Lsm proteins involved in mRNA splicing, telomere maintenance and mRNA degradation. In bacteria there is a single bacterial Lsm protein called Hfq. It acts as an RNA chaperone facilitating interaction between regulatory RNA and mRNA. Archaeal genomes encode one or two Sm-like proteins belonging to two subfamilies SmAP1 and SmAP2. Homology between SmAP1 protein sequences of various species is no less than 60%, while it does not exceed 30% between the SmAP1 and SmAP2 proteins of the same organism. It was shown that SmAP specifically recognizes poly(U) sequences and form complexes with tRNA but up to now the role of SmAP in the RNA metabolism or regulation translation in archaea studied rather poor.

In order to study functions of archaeal Lsm proteins SmAP2 from Methanococcus vannielii and SmAP from Sulfolobus acidocaldarius were chosen. The proteins were isolated and purified. Crystals of proteins and their complexes with ribonucleotides were obtained. Using the approach, which was developed in our group, we determined single-stranded RNA-binding sites on the surface of the proteins. In addition, AMP affinities to the proteins have been determined by measuring fluorescence changes during titration of the AMP-MANT solution by appropriate protein.

This work was supported by Russian Scientific Foundation (project 14-14-00496).

P2-6

CRYSTALLIZATION OF DC8E8 ANTIBODY TETRATOPE ON THE MOLECULE OF INTRINSICALLY DISORDERED PROTEIN tau

Ondrej Cehlár[1,2], Rostislav Škrabana[1,2], Mária Janubová[1], Michal Novák[1,2]

[1]Axon Neuroscience SE, Dvorakovo Nabrezie 10, 811 02 Bratislava, Slovakia
[2]Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia

Accumulation of intrinsically disordered protein tau in the form of insoluble aggregates is a common feature of neurodegenerative tauopathies. Tau protein is engaged in the regulation of microtubular dynamics in the neurons, however, under pathological conditions tau interacts with itself, escapes disordered state and forms toxic oligomers and aggregated filaments. Monoclonal antibody DC8E8 is able to inhibit tau-tau interaction and therefore it holds promise for the immunotherapy of Alzheimer's disease. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeat regions (MTBRRs) of tau. Unravelling the unique mode of recognition of DC8E8 can aid to reveal hindered structural features of tau protein and their implication for tau protein biology.

In the presented study we have crystallized the Fab fragment of DC8E8 antibody with the peptides covering its epitopes from all four MTBRRs of tau, where the antibody Fab fragment serves as a crystallization chaperon for the disordered tau peptide. Using biophysical measurements, namely surface plasmon resonance and ELISA, we have found that the antibody has the highest affinity against the second MTBRR. The affinity for particular repeat regions descends as follows: MTBR2 > MTBR1 > MTBR3 > MTBR4.

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P2-7

TOWARDS CRYSTALLIZATION OF THE ONCOGENIC TRANSCRIPTION FACTOR FROM THE T-BOX FAMILY, TBX2

S. Kimani[1,2], D. Ballim[1], T. Sewell[2,3], E. Sturrock[4], S. Prince[1]

[1]Department of Human Biology, University of Cape Town, Observatory 7725, South Africa
[2]Structural Biology Research Unit, University of Cape Town, Rondebosch 7700, South Africa
[3]Electron Microscope Unit, University of Cape Town, Rondebosch 7700, South Africa
[4]Integrative Biomedical Sciences department, University of Cape Town, Observatory 7925, South Africa
swkimani@gmail.com

The T-box transcription factor 2, TBX2, is upregulated in a number of epithelial cancers where it plays a key role in tumorigenesis by functioning as a potent growth promoting factor in the initial stages of cancer development [1]. Previous work in our laboratory has identified and validated TBX2 as a novel therapeutic target for treatment of malignant melanoma and advanced breast cancers, which are highly aggressive cancers with limited therapeutic options [2]. To take these findings further, we wish to carry out a structural analysis of the TBX2 DNA binding domain (T-box) in complex with various target genes DNA fragments, and use this information to computationally design novel inhibitors of the TBX2 oncogenic activity.

The TBX2 T-box domain from both the human and mouse have been cloned in a pET28b expression vector and the proteins over-expressed as N-terminal hexahistidine fusion proteins. Both proteins have been purified and crystallization screening for both the DNA-free and the DNA-complexed T-box proteins attempted using various screening kits and strategies. A number of crystalline forms have been obtained and optimization is underway to generate diffraction quality crystals. Progress in this work will be presented.

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P2-8

CRYSTALLIZATION BEHAVIOUR OF GLYCERALDEHYDE DEHYDROGENASE FROM *THERMOPLASMA ACIDOPHILUM*

Iu. Iermak[1,2], O. Degtjarik[1,3], F. Steffler[4], V. Sieber[5] and I. Kuta Smatanova[1,2]

[1]*Faculty of Science, University of South Bohemia in Ceske Budejovice, Branisovska 31, 37005 Ceske Budejovice, Czech Republic,*
[2]*Center of Nanobiology and Structural Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Zamek 136, 37333 Nove Hrady, Czech Republic,*
[3]*Weizmann Institute of Science, Department of Structural Biology, 76100 Rehovot, Israel*
[4]*Bio, Electro- and Chemocatalysis BioCat, Straubing Branch of the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Schulgasse 11a, 94315 Straubing, Germany,*
[5]*Chemistry of Biogenic Resources, Straubing Centre of Science, Technische Universitat Munchen, Schulgasse 16, 94315 Straubing, Germany*
julia.ermak90@gmail.com

The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (*Ta*AlDH) is a microbial enzyme that catalyses the oxidation of D-glyceraldehyde to D-glycerate in the artificial enzyme cascade designed for the conversion of glucose to the organic solvents isobutanol and ethanol [1, 2]. Various mutants of *Ta*AlDH were constructed by random approach followed by site-directed and saturation mutagenesis in order to improve enzyme properties essential for its functioning within the cascade [3].

For further improvement of *Ta*AlDH, it will be more effective to modify the enzyme at target positions *via* rational design. Since available *Ta*AlDH protein models are poor (homology < 40%), a *Ta*AlDH crystal structure would allow for distinct enzyme modifications with predictable impact on activity and stability.

Variously shaped crystals grew within one-two weeks after initial screening in 30 diverse conditions for *Ta*AlDH wild type and 24 different conditions for *Ta*AlDH F34M+S405N mutant. In order to obtain the best quality crystals optimization was carried out considering following parameters: (*a*) already known diffraction quality of crystals; (*b*) size and shape of crystals (big single crystals with sharp edges preferred); (*c*) visually different crystal forms (to check as many as possible different variants of protein molecules packing inside the crystal). Optimization, including variation of pH, protein and precipitant concentrations and ratios, resulted in diffracting crystals only from one condition for *Ta*AlDHwt and two conditions for *Ta*AlDH F34M+S405N. Crystals from other conditions were poorly reproducible and diffracted only to 8-10 Å resolution even after microseeding procedure.

Crystals of *Ta*AlDHwt belong to monoclinic $P12_11$ space group with 8 molecules per asymmetric unit and diffracted to the resolution of 1.95 Å *Ta*AlDH F34M+S405N crystallizes in two different space groups: triclinic *P*1 with 16 molecules per asymmetric unit and monoclinic *C*121 with 4 molecules per asymmetric unit. These crystals diffracted to the resolutions of 2.14 and 2.10 Å for *P*1 and *C*121, respectively [4].

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P2-9

HIV-1 Vpr STEERS THE REPAIR ENZYME URACIL DNA GLYCOSYLASE TOWARDS DESTRUCTION: THE X-RAY STRUCTURE OF THE DDB1-DCAF1-Vpr-UNG2 COMPLEX

Guillermo Calero, Ying Wu, Xiaohong Zhu, Christopher Barnes, Aina Cohen, Angela Groneborn, Jingo Ahn

University of Pittsburgh, 3501 Fifth Ave, Pittsburgh, 15260 PA, USA

The HIV-1 accessory protein Vpr is required for efficient viral infection of macrophages and promotion of viral replication in T-cells. The biological activities of Vpr are closely tied to the interaction with DCAF1, a cellular substrate receptor of the Cullin4-RING E3 ubiquitin ligase (CRL4) of the host ubiquitin proteasome-mediated protein degradation pathway. At present, the molecular details of how Vpr usurps the protein degradation pathway have not been delineated. Here, we present the crystal structure of the DDB1-DCAF1-HIV-1-Vpr-Uracil-DNA glycosylase (UNG2) complex. The structure reveals how Vpr engages with DCAF1 and creates the special binding interface for UNG2 recruitment, distinct from how the related Vpx proteins recruit SAMHD1 for degradation. Vpr and Vpx utilize similar N-terminal and helical regions to bind the substrate receptor, while distinctly different regions target the specific cellular substrates. Furthermore, Vpr employs molecular mimicry of DNA by a variable loop for specific recruitment of the UNG2 substrate.

P2-10

INTEGRATED STRUCTURAL BIOLOGY STUDY OF THE FrpD PROTEIN FROM *NEISSERIA MENINGITIDIS*

E. Sviridova[1,2], L. Bumba[3], P. Rezacova[4,5], V. Veverka[4], G. Schenk[6], P. Sebo[3,7] and I. Kuta Smatanova[1,2]

[1]Faculty of Science USB CB, Branišovska 1760, 37005 Ceske Budejovice, Czech Republic
[2]Institute of Microbiology AS CR, Zamek 136, 37333 Nove Hrady, Czech Republic
[3]Institute of Microbiology AS CR, Videnska 1083, 14220 Prague, Czech Republic
[4]Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nam. 2, 16610 Prague, Czech Republic
[5]Institute of Molecular Genetics AS CR Flemingovo nam. 2, 16610 Prague, Czech Republic
[6]EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany
[7]Institute of Biotechnology AS CR, Videnska 1083, 14220 Prague, Czech Republic
eesviridova@gmail.com

FrpD is a highly conserved lipoprotein of *Neisseria meningitidis* anchored to the bacterial outer membrane. The *frpD* gene sequence contains two translation initiation sites, which give rise to production of the full-length FrpD protein ($FrpD_{271}$) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein ($FrpD_{250}$) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported $FrpD_{271}$ precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys_{25} residue, and sorted to the outer bacterial membrane [1]. The biological function of FrpD is unknown. It appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity (K_d = 0.2 nM) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of structural information on these proteins.

We present here the first crystal and solution structures of the FrpD protein and the NMR spectroscopy identification of the FrpD-FrpC interaction interface. According to the detailed structure analysis, the atomic structures of FrpD reveal a novel protein fold. Our work constitutes the first step in clarifying the molecular basis of FrpD-FrpC interaction and sets the base for further investigation of the role of FrpD in the virulence mechanism of *N. meningitidis* and for the functional and biochemical characterization of the high affinity interaction between the FrpD protein and the FrpC protein.

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P2-11

ANTIBODY-ASSISTED CRYSTALLIZATION OF A CELL SURFACE RECEPTOR

Galina Obmolova, Alexey Teplyakov, Jeffrey Luo, Gary L. Gilliland

Janssen Research & Development, LLC. 1400 McKean Rd, Spring House, PA 19477, USA

The presented crystallization case combines several practical approaches that should always be considered in difficult situations. Many cell surface receptors are multidomain molecules that are conformationally flexible but can be stabilized through interactions with the natural ligands or antibodies. We employed the latter approach to crystallizing the extracellular portion of a cell surface receptor alpha chain. Multiple expression constructs of the 3-domain molecule were tried. Based on the expression levels, the variant comprising domains 2 and 3 was chosen. Crystallization of this molecule in complex with the Fab fragment of the receptor-specific antibody was successful but yielded crystals that contained only the Fab. To avoid formation of Fab crystals, the smaller Fv fragment (scFv) was used. The receptor-scFv complex was formed but failed to yield any crystal hits. Therefore the complex was subjected to subtilisin treatment. Crystallization using microseed matrix screening resulted in the crystals of the complex. The structure was determined and analyzed to explain how the cleavage of the VL-VH linker in scFv promoted crystallization of the scFv complex.

P2-12

STRUCTURE-FUNCTIONAL CHARACTERIZATION OF THE MEMBER OF - HYDROLASES SUPERFAMILY - DpcA FROM *PSYCHROBACTER CRYOHALOLENTIS* K5

Katsiaryna Tratsiak[1]*, Ivana Drienovska[2], Pavlina Rezacova[3,4], Radka Chaloupkova[2], Michal Kuty[1,5], Jose A. Gavira[6] , Oksana Degtjarik[1,7], Jiri Damborsky[2] and Ivana Kuta Smatanova[1,5]

[1]Faculty of Science, University of South Bohemia in Ceske Budejovice Budweis, Czech Republic
[2]Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University Brno, Czech Republic
[3]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic v.v.i., Prague , Czech Republic
[4]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic
[5]Academy of Sciences of the Czech Republic, Institute of Nanobiology and Structural Biology GCRC, Nove Hrady, Czech Republic
[6]Laboratory for Crystallographic Studies (CSIC-UGR) University of Granada, Granada, Spain
[7]Weizmann Institute of Science, Department of Structural Biology, Rehovot, Israel
ktratsiak@gmail.com

The magnification of the anthropogenic influences of different pollutants on the biosphere, for instance halogenated alkanes, necessitate the searching of new methods and resources for their decontamination and degradation. Haloalkane dehalogenase (EC 3.8.1.5; HLDs) DpcA isolated from psychrophilic *Psychrobacter cryohalolentis* K5 is aimed on the solving of the issue mentioned above by catalysing the hydrolytic conversion of halogenated aliphatic compounds accomplished by cleavage of carbon-halogen bond with the subsequent releasing of the corresponding alcohol, halide ion and a proton.

DpcA has high enantioselectivity and the narrowest substrate specificity of all biochemically characterized HLDs with the highest activity of the enzyme toward 1-bromobutane and 1,3-dibromopropane at 25 °C what highlights it among the other HLDs.

The crystals of DpcA diffracted to the resolution 1.05Å beamline 14.2, detector Rayonics MX-225 CCD (BESSY II electron-storage ring, HZB, Germany), belonged to $P2_1$ space group and with one molecule in the asymmetric unit. The structure was solved by molecular replacement with the help of the coordinates of Xanthobacter autotrophicus (PDB code: 1B6G; 40% sequence identities).

The protein has a globular shape and is composed of two domains. The core domain contains eight parallel ß-strands that form central beta-sheet, within one is antiparallel (ß2). The central ß-sheet is flanked by four -helices on the one side and two are on the other side of the sheet. The highly conserved main domain is the scaffold- like for the catalytic residues, and a smaller helical cap domain, covering the active site, which has revealed the catalytic pentad essential for for the SN2 reaction mechanism: nucleophile D123, catalytic base H280, catalytic acid D250, halide-stabilizing W124 and W164.

For the structure analysis the crystals were soaked with the ligands: 1-bromohexane, 1-bromobutane, 1,2-dibromoethane and 2-bromopropane. The comparison of structure of DpcA and DpcA with 1,2-dibromoethane is carried

out for the understanding the psychrophilic properties of the enzyme.

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P2-13

PARALOGOUS CHEMORECEPTORS WITH DIFFERENT LIGAND SPECIFICITY MEDIATE CHEMOTAXIS TO AMINO ACIDS IN *PSEUDOMONAS AERUGINOSA*

J. A. Gavira[1], M. Rico-Jiménez[2], M. Conejero-Muriel[1], E. Pineda[1], T. Krell[2]

[1]Laboratorio de Estudios Cristalograficos, IACT (CSIC-UGR), Avda. Las Palmeras 4, 18100, Armilla, Granada, Spain.
[2] Department of Environmental Protection, EEZ, CSIC, C/ Prof. Albareda, 1, 18008 Granada, Spain
jgavira@iact.ugr-csic.es

Bacteria adapt to the variable environmental conditions through various signal transduction mechanisms achieved through the action of one-component systems, two-component systems and chemosensory pathways (1). The stimulation of the latter pathway is achieved by signal binding to the ligand binding domain (LBD) of the chemoreceptors, which in turn generates a molecular stimulus that modulates CheA autophosphorylation and consequently transphosphorylation of the CheY response regulator. Chemosensory pathways mediate either flagellum-based chemotaxis, type IV pili based motility or are involved in the regulation of alternative cellular processes.

Chemotaxis of the human pathogen *Pseudomonas aeruginosa* to amino acids has been previously studied and is mediated primarily by three paralogous chemoreceptors, termed PctA, PctB (2) and PctC (3). While PctA responds to 18 different proteinogenic L-amino acids, PctB binds preferentially to L-glutamine, which is one of the two amino acids that is not recognized by PctA. PctC binds with high preference to gamma-aminobutyrate (GABA), which is a compound omnipresent in nature and that exerts multiple biological functions such as human neurotransmitter, plant hormone or growth substrate for bacteria.

We report here high resolution structures of the LBDs of these three paralogous receptor in complex with several of their ligands. These 3 domains adopt a double CACHE fold and ligand recognition occurs in the membrane distal CACHE moiety. These structures are different to the 4-helix bundle type of LBD of the *E. coli* chemoreceptors, which are the traditional models in the study of chemoreceptors. The ligand specificity of LBD homologues in other species cannot be inferred by overall LBD sequence comparison. However, the molecular detail of ligand recognition, as revealed in our study, will provide the basis for the development of specific bioinformatic algorithms to identify homologues with similar ligand profiles. All crystals were obtained using the capillary counter-diffusion technique. The use of capillaries allowed us to obtain the PctA-Met complex from the PctA-Ile crystal by diffusing

an excess of methionine within a capillary containing the crystals of the complex.

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P2-14

SMALL ANGLE SOLUTION SCATTERING STUDY OF THE COMPLEX FORMATION BETWEEN XANTHINE OXIDOREDUCTASE AND NOVEL PURINE ANALOG

Markéta Šmídková[1], Borislav Angelov[2], Vasyl M. Garamus[3], Petr Jansa [4] and Helena Mertlíková-Kaiserová[1]

[1] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague,
[2] Fyzikalni ustav, ELI Beamlines, AVCR, 25241 Dolní Břežany, Czech Republic,
[3]Helmholtz-Zentrum Geesthacht, Centre for Materials and Coastal Research, D-21502 Geesthacht, Germany,
[4]Gilead Sciences, Inc, 333 Lakeside Drive, Foster City, CA 94404, USA
Smidkova@uochb.cas.cz; Borislav.Angelov@eli-beams.eu

The development of novel therapeutic drugs targeting the active site of a protein requires detailed knowledge of the molecular structure. Using small angle X-ray (SAXS) or neutron scattering it is possible to reconstruct the structure of the targeted protein at low resolution before and after complexation with the drug. Here SAXS was employed to study the structural changes of the xanthine oxidoreductase (XOR) under complexation with novel purine analog as effective inhibitor of the XOR enzyme activity (Ki =0.55 microM). XOR is a key enzyme of purine catabolism, which is involved in the pathogenesis of hyperuricemia, gout and oxidative stress-related cardiovascular diseases [1, 2]. Currently, there are two XOR inhibitors available at the market – purine based allopurinol and nonpurine febuxostat. Since these both drugs possess significant side effects [3], the search for new XOR inhibitors continues.

The initial results revealed that the studied purine analog influenced the structure of XOR thus binding affinity was rather high. This effect can be explained by considering the geometry of the active site of XOR and the overall shape of the small purine analog. Further experiments are planned to clarify mechanism of XOR inhibition by this purine analog. Based on these structure-activity relationships, improved analogs will be designed.

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P2-15

STRUCTURE OF THE C345c DOMAIN OF MURINE COMPLEMENT COMPONENT C4

Trine A. F. Gadeberg, Sofia Mortensen, Gregers R. Andersen

Department of Molecular Biology, Aarhus University, Aarhus, Denmark
trineg@mbg.au.dk

Complement is a central part of the innate immune system. It acts as a danger-sensing system and its activation initiates a proteolytic cascade, resulting in a strong inflammatory response [1]. There are three different complement activating pathways; the classical pathway, the lectin pathway and the alternative pathway. The pathways converge in the formation of the proteolytic C3 and C5 convertases. In the classical and lectin pathway, the convertases contain C4b as the substrate recognising subunit, and C2a as the proteolytic subunit. In the classical pathway, C3b and Bb are found as these respective subunits.

In the lectin and classical pathway, activation is triggered when recognition molecules detects danger signals in the form of carbohydrates or antibodies. MASP-2 and C1s are serine proteases found in complex with the recognition molecules. Upon activation they bind and cleave C4 into C4b and C4a [2, 3]. The binding occurs through the C-terminal C345c domain of C4 interacting with an exosite between two CCP domains of the protease (Fig. 1) [4].

Over-activation of complement is the cause of several diseases [5, 6], and inhibitors of complement activation are interesting to find. It has been found that the C4 C345c domain can act as an inhibitor of the classical and lectin pathways. Future experiments based on this finding would be conducted in a mouse based animal model. Therefore, the structural similarity of the murine C4 C345c domain to its human counterpart is of interest. Here, I present the crystal structure of the murine C4 C345c domain at 2.1Å resolution (Fig. 2). The structure reveals a positively charged patch on the C4 C345c domain where it interacts with the CCP exosite of MASP-2.

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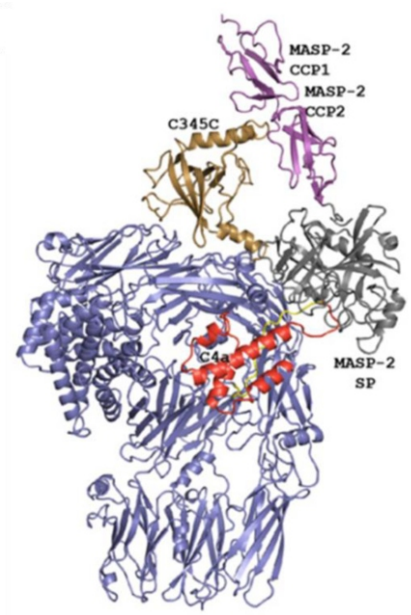

Figure 1. The crystal structure of the C4-MASP2 complex. C4 is blue expect for C4a (red) and the C345c domain (brown). The MASP2 CCP domains (magenta) interact with the C4 C345c domain [4].

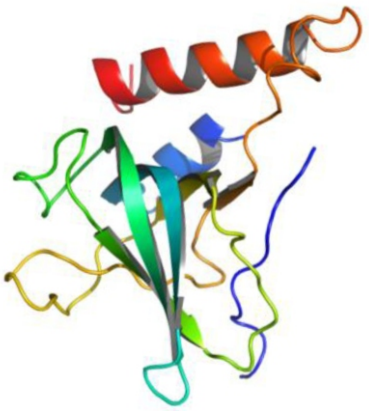

Figure 2. The crystal structure of the murine C4 C345c domain in the same orientation as in figure 1. The schematics are colored from blue (N terminus) to red (C terminus).

P2-16

STRUCTURES OF THE FeSI and FeSII (SHETHNA) PROTEINS OF *AZOTOBACTER VINELANDII*

B. V. Kabasakal, C. A. R. Cotton, L. Lieber, J. W. Murray

Department of Life Sciences, Imperial College London, Exhibition Road, London, SW7 2AZ, England
burak.kabasakal11@imperial.ac.uk

Nitrogen fixing *Azotobacter vinelandii* has two [2Fe-2S] proteins, FeSI and FeSII, Shethna protein I and II. In this study, we present the 2.11 Å, and 2.34 Å resolution X-ray crystal structures of FeSI and FeSII, respectively. FeSII has been shown as a protective enzyme for nitrogenase against oxygen-mediated inactivation. However, there is no known relation of *A. vinelandii* FeSI with nitrogenase, even though it is homologous to the [2Fe-2S] ferredoxin from *Clostridium pasteurianum*, which has been shown to interact with the nitrogenase MoFe protein. FeSI reveals a homodimer with [2Fe-2S] cluster coordinated by the side chains of surrounding conserved cysteine residues. It is highly similar to the structure of [2Fe-2S] protein from *Aquifex aeolicus*, including the positions of [2Fe-2S] clusters and conserved cysteine residues. On the other hand, dimeric FeSII reveals five monomers per asymmetric unit.

All four copies are in an "open" conformation which may allow the [2Fe-2S] cluster to interact with nitrogenase, whereas one copy is in a "close" conformation which has a less accessible [2Fe-2S] cluster. The conformational changes may be related to oxidation and reduction states of the [2Fe-2S] cluster. These structures will provide crucial information for understanding the nitrogenase oxygen protection mechanism, and elucidating of the relations of iron-sulphur proteins in both structural and evolutionary aspects.

P2-17

ISOLATION AND CRYSTALLIZATION OF PLANT PSII

Jaroslava Kohoutová[1], Magda Vojtová[1], Estela Pineda Molina[2], Zbyněk Zdrahal[3], Ondřej Šedo[3], David Potěšil[3], Jose A. Gavira[4],Tatyana Prudnikova[1] and Ivana Kutá Smatanová[1]

[1]University of South Bohemia in České Budějovice, Faculty of Science, Branišovská 31, 37005 České Budějovice
[2]Academy of Sciences of the Czech Republic Center for Nanobiology and Structural Biology, Institute of Microbiology , Zámek 136, 373 33 Nové Hrady
[3]Core Facility – Proteomics, CEITEC, Masaryk University, Kamenice 5,625 00 Brno
[4]Laboratorio de Estudios Cristalográficos, Edf. López Neyra, P.T. Ciencias de la Salud, Avenida del Conocimiento s/n, 18100 Armilla (Granada) Spain

Photosystem II (PSII) is the heart of photosynthetic process. This multisubunit complex is embedded in the thylakoid membrane of plants, algae and cyanobacteria. The function of the photosystem II in different organisms is identical while the composition of subunits is different [1]. Recently the 3D X-ray structures of cyanobacterial PSII were determined to the maximum resolution of 1.9 Å [2]. Compared to plant PSII cyanobacterial PSII consists of different extrinsic proteins and also the light-harvesting complex is not bound in thylakoid membrane to PSII core as it is in plant PSII.

No crystal structure of PSII from higher plants is available until now and to get structural data of PSII, crystallization of the PSII from higher plants employing several crystallization techniques and testing the role of different additives were reported. After testing of several crystallization conditions and techniques, the 3D crystals of PSII were obtained from PSII complexes isolated from *Pisum sativum* at the resolution of about 10.0 Å [3], from *Nicotiana tabaccum* at 7.0 Å [4] and from *Spinacia oleracea* at 6.5 Å [5].We report here new optimized protocol for isolation of plant PSII with reproducible crystallization conditions and using of new crystallization techniques and testing methods. Obtained green crystals were analyzed by LC/MS and content of selected proteins from PSII was confirmed.

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P2-18

CRYSTALLIZATION AND STRUCTURE DETERMINATION OF HUMAN ALDEHYDE OXIDASE AND COMPLEXES

C. Coelho[1], T. Santos-Silva[1], A. Foti[2], S. Leimkühler[2] and M.J. Romão[1]

[1]UCIBIO@REQUIMTE – Departamento de Química, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[2]Institute of Biochemistry and Biology, Department of Molecular Enzymology, University of Potsdam, D-14476 Potsdam, Germany
c.coelho@fct.unl.pt

Aldehyde oxidase (AOX) is a xanthine oxidase (XO) related enzyme with emerging importance in the metabolism of drugs and xenobiotics [1]. The hAOX holoenzyme is a homodimer (2x150 kDa) and each monomer is constituted by three domains: the N-terminal domain with two distinct [2Fe-2S] clusters (FeSI and FeSII), the central FAD binding domain and the C-terminal catalytic Moco domain. We report the first crystal structure of human AOX in the substrate-free form (2.6Å resolution) and in complex with the substrate phthalazine and the inhibitor thioridazine (2.7Å resolution) (Fig 1-A) [2].

Crystals of hAOX were obtained through the vapour-diffusion technique using polyethylene glycol 4000 as precipitant. The crystals are very sensitive to manipulation and grew to their maximum size within 24 h (Fig 1-B). Extensive trials and modified protocols were per-

formed in order to promote better crystallization conditions, improve crystal quality and allow co-crystallization and soaking experiments with bound substrate and/or inhibitors.

Crystals with bound molecules were only obtained when using large amount of the substrate phthalazine as co-crystallization agent, followed by soaking of the obtained crystals with a thioridazine hydrochloride harvesting solution. Analysis of the protein active site combined with steady-state kinetics highlight the unique features that characterize hAOX as an important drug-metabolizing enzyme, which include binding and substrate orientation at the active site [2].

The structure of the complex with the non-competitive inhibitor thioridazine revealed a new, unexpected and fully occupied inhibitor-binding site, structurally conserved among mammalian AOXs and XO (Fig 1-C). The new structural insights into the catalytic and inhibition mechanisms of human AOX now reported will be of great value for the rational analysis of clinical drug interactions involving inhibition of AOX1 as well as for predicting and designing AOX-stable putative drugs [2].

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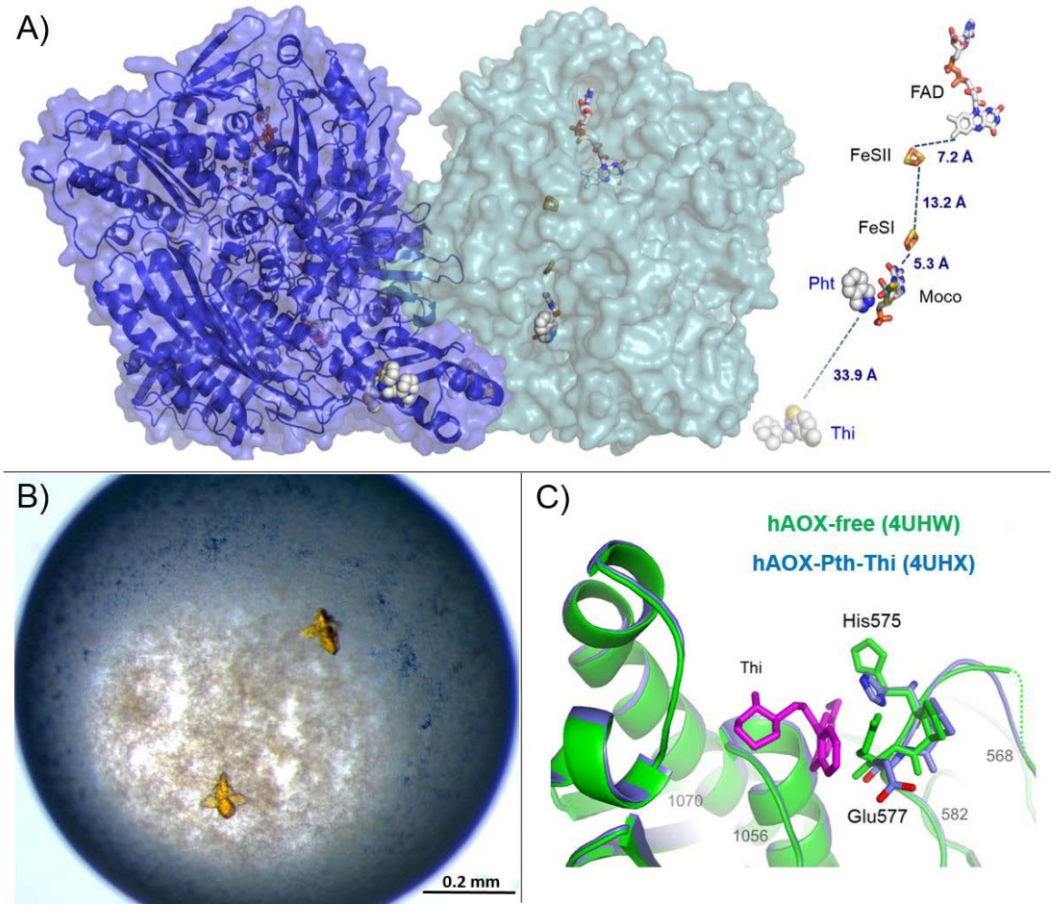

Figure 1. (A) Surface representation of the hAOX crystal structure and different protein cofactors and distances (FAD, FeSII, FeSI and Moco). The phthalazine (Pht) and thioridazine (Thi) molecules from different monomers (light green and blue) are represented in space-filling mode; (B) Crystals of hAOX with approximate dimensions of 0.1x0.15x0.15 obtained using polyethylene glycol 4000; (C) Close-up of the thioridazine molecule (pink) binding site pocket with the structures of hAOX-free (green) and hAOX-Pht-Thi (blue) superimposed.

P2-19

CRYSTALLOGRAPHIC STUDIES OF A TRANSCRIPTIONAL METALLOREGULATOR PROTEIN CUER IN COMPLEX WITH Hg^{2+}

Ria Katalin Balogh[2], Valéria Bugris[1], Veronika Harmat[1], Sándor Brockhauser[1], Attila Jancsó[2]

[1]X-Ray crystallography laboratory, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[2]Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary
bugris.valeria@brc.mta.hu

In bacteria the optimal concentration level of metal ions is controlled by transcriptional metalloregulators. The CueR (Cu efflux Regulator) protein regulates the intracellular amount of CuI-ion in several strains of bacteria. This member of the MerR protein family also operates by an activation mechanism based on a conformational change of the DNA-bound protein upon metal ion coordination. This also affects the structure of the DNA and therefore, RNA polymerase can initiate the transcription of the regulated genes leading to the formation of proteins the role of which is the removal of metal ion from the cell. The CueR protein gives a transcriptional response only for singly charged transition metal ions (Cu^{I}, Ag^{I} and Au^{I}).

The purpose of our work is to solve the structure of the CueR protein in complex with an unflavoured divalent metal ion, the strong binder Hg^{II}. In that case we could compare the structural differences with the already available protein structures containing monovalent metal ions. Thus would help the understanding of the structural details of bacterial metal ion regulatory mechanisms on molecular basis. For this purpose we have optimized the conditions of the CueR protein expression and purification in *E. coli*, and set up a crystallization screen with similar conditions as described in the literature. The results of these experiments are presented.

P2-20

STRUCTURE AND FUNCTION STUDIES OF RNA-BINDING PROTEINS WITH FAST MOTIFS AND A RAP DOMAIN

J. Kutner, M. Merski, A. Jurska, M. Gorna

Structural Biology Group, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, 101 Zwirki and Wigury St, 02-089 Warsaw, Poland
jkutner@chem.uw.edu.pl

The FASTK family (Fas-activated Serine/Threonine Kinase) contains six human proteins which localize to the mitochondria and have been functionally linked to cellular respiration and with a rare mitochondrial disease. While human FASTK was initially annotated as an atypical Ser/Thr kinase later studies dispute this annotation [1]. Structurally, FASTKD proteins contain an N-terminal mitochondrial targeting signal, a pair of FAST motifs and a C-terminal RAP domain (Figure 1).The N-terminal part is predicted to be highly globular but with small disordered regions. The FAST motifs are putative RNA binding domains with a novel -helical repeat fold that has no sequence similarity to any other known helical repeat motifs. Interestingly, the RAP domain is found in many members of the recently identified class of octotricopeptide repeat (OPR) proteins, which are abundant in plants and green algae and is believed to play a role in chloroplast RNA biology [2]. The OPR proteins have been shown to bind RNA with preference for some substrates [3], but their structure or RNA binding specificity is unknown. The RAP domain is also overrepresented in *Plasmodium*, and hence structural information of this domain is relevant to the field of malaria.

The obtained structures and functions of these proteins may have relevance to drug design therapeutic strategies, particularly of cancer and inflammation and will likely reveal new folds of RNA binding domains thus contributing to the general knowledge of the rules that govern RNA recognition. Our project aims to provide for the first time structural and novel biochemical information about the relatively understudied FASTK family.

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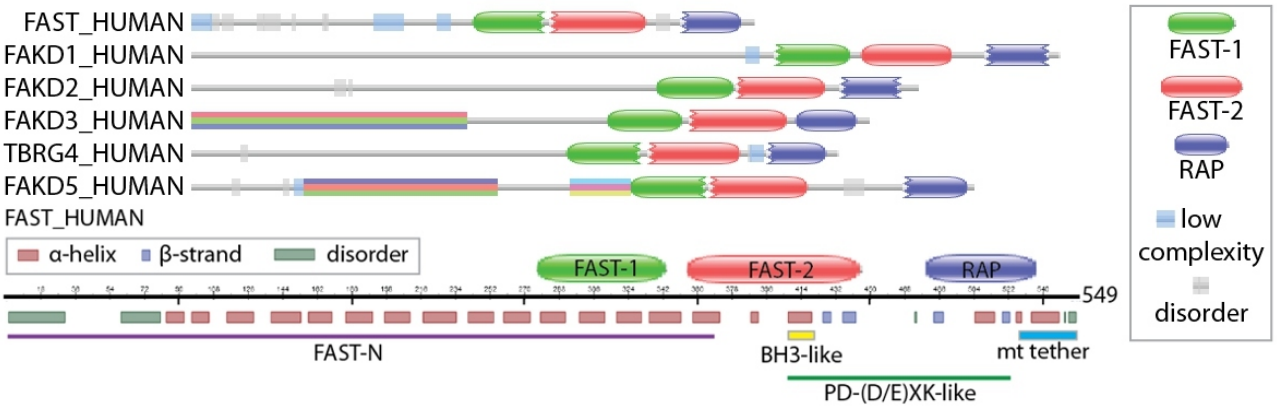

Figure 1. Domain composition and secondary structure prediction of the human FASTK family (Pfam: PF08373).

P2-21

CRYSTAL POLYMORFISM OF MULTIFUNCTIONAL PLANT NUCLEASE TBN1

J. Stránský[1, 2], T. Kovaľ[1], T. Podzimek[3], P. Lipovová[3], J. Matoušek[4], A. Týcová[4,5], J. Dohnálek[1]

[1]Institute of Biotechnology CAS, v.v.i., Průmyslová 595, 252 50 Vestec, Czech Republic
[2]Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University, Břehová 7, 115 19 Praha 1, Czech Republic
[3]Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic
[4]Institute of Plant Molecular Biology, Biology Centre, CAS, v.v.i., Branišovská 31, 370 05 České Budějovice
[5]Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 370 05 České Budějovice, Czech Republic
stransky@ibt.cas.cz

Tomato multifunctional nuclease (TBN1; UniProt accession no. Q0KFV0), which belongs to the nuclease type I family, plays an important role in specific apoptotic functions, vascular system development, stress response, and plant tissue differentiation [1]. Furthermore, TBN1 exhibits anticancerogenic properties [2]. The enzyme possesses endonuclease and exonuclease-like activity on single stranded and double stranded RNA and DNA and on structured RNA, with production of mono- and oligonucleotides from 3'-end of nucleic acids [3]. Based on the crystal structure of TBN1, the phospholipase activity of the enzyme was discovered [4]. TBN1 consists of 277 amino-acids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated).

Three crystal structures of TBN1 were solved in our group: one structure of wild type TBN1 and two structures of mutant N211D [4,5]. The common motif conserved among all known structures is formation of super-helices, where protein molecules are related by 3_1 screw axis. The contacts are provided by the active site of one molecule and a surface loop (SDR-loop) of a neighboring molecule. However, packing of super-helices to final crystal differs across different crystallization conditions. The conserved formation of intermolecular contacts in crystals suggests the way of assembly of molecules into oligomers in solution, which were observed by dynamic light scattering. The interaction of the active site and the surface loop is best resolved in the currently reported structure, where the active centre at the zinc cluster is occupied by phosphate ion. It correlates with behavior of TBN1 in phosphate buffer. The phosphate ion binds differently than corresponding ions in known structures of AtBFN2 from *Arabidopsis thaliana* [6].

Properties of mutants, designed to modify dimerization and activity of TBN1, suggest that deliberate disruption of the loop-active site contacts limits expression of the active enzyme. Therefore formation of TBN1 oligomers together with phosphate binding are hypothesized to have regulatory roles in apoptotic-like and senescense processes in plant cells.

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P2-22

CRYSTALLIZATION AND X-RAY STRUCTURAL ANALYSIS OF RESURRECTED ANCESTOR OF HALOALKANE DEHALOGENASE AND *RENILLA* LUCIFERASE

R. Chaloupkova[1,2], J. Waterman[3], E. Sebestova[1], V. Liskova[1,2], J. Damborsky[1,2]

[1]*Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment RECETOX, Masaryk University, Brno*
[2]*International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*
[3]*Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot, United Kingdom*
radka@chemi.muni.cz

Ancestral sequence reconstruction allows resurrection of ancient enzymes based on the sequences predicted by a phylogenetic analysis. Starting from an alignment of modern sequences, the phylogenetic tree is inferred, and statistical methods are used to predict the most likely ancestral sequences at the internal nodes of the tree. Genes that encode the inferred ancestral sequences can then be synthesized and expressed in cultured cells; allowing the structure, function and biophysical properties of each resurrected protein to be experimentally characterized [1,2]. The main objective of this project is to provide structural insights into catalysis of resurrected ancestor of two functionally distinct enzymes, hydrolytic haloalkane dehalogenase (HLD) LinB from *Sphingobium japonicum* UT26 [3,4] and luciferase Rluc from *Renilla reniformis* [5,6], belonging to oxidoreductases. Design, construction and activity testing of the resurrected ancestral enzyme ancHLD-RLuc revealed correct folding, enhanced thermodynamic stability up to 20 °C compared to the present-day enzymes and the most importantly promiscuous hydrolase and oxidoreductase activity. The ancestral enzyme was successfully crystallized by the sitting-drop vapour diffusion method. Two different crystal forms of ancHLD-RLuc were obtained at 292 K in Morpheus, JSCG (Molecular Dimensions, UK) and Wizard (Rigaku Reagents, USA) screens. The crystals size and shape was further optimized by varying the buffer pH and PEG concentration. Diffraction data for optimized crystals of ancHLD-RLuc grown from different crystallization conditions were collected to resolutions ranging from 1.5 to 1.9 Å. The crystals belonged to the orthorhombic space group $P2_12_12$ and monoclinic space group $P12_11$. Structure of the ancestral enzyme was solved by molecular replacement using the coordinates of RLuc [6]. X-ray structural analysis revealed that ancHLD-RLuc can adopt two different conformations: monomeric conformation, very similar to that of LinB and RLuc monomers, and domain-swapped dimer conformation which has never been detected before for any HLD member or RLuc. Both monomer and dimer conformations of ancHLD-RLuc were identified in solution together with small portion of tetramer, confirming that the domain-swapped dimer is not a crystallization artefact. The structural data, together with kinetic and molecular modelling data, will provide insight into structural basis of dual hydrolase and oxidoreductase activity of resurrected ancestor.

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P2-23

STRUCTURE OF A KETOSYNTHASE-CHAIN LENGTH FACTOR OF A POLYUNSATURATED FATTY ACIDS SYNTHASE AND ITS ROLE IN THE ARCHITECTURE AND FUNCTION OF THE SYNTHASE COMPLEX

Santín-Martínez O. and Moncalián G.

Departamento de Biología Molecular e Instituto de Biomedicina y Biotecnología de Cantabria, Universidad de Cantabria-Consejo Superior de Investigaciones Científicas-SODERCAN, C/ Albert Einstein 22, 39011, Santander, Spain
santino@unican.es

The use of omega-3 polyunsaturated fatty acids (O3PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), has increased in recent years because of its benefits for human health. For the production of these O3PUFAs, marine bacteria such as moritella marina or colwellia psychrerythraea (DHA producers) or Shewanella baltica (EPA producer) possess large enzyme complexes called PUFA synthases (Pfa), which resemble bacterial antibiotic production proteins known as polyketide synthases (PKS).

For growing PUFA carbon chain, some essential enzyme modules are needed to produce the first decarboxylation and successive condensations rounds. These modules consist of heterodimers of keto synthases (KS) and acyltransferase (AT) that, in coordination with acyl carrier proteins (ACPs), incorporate acyl groups to the growing chain, performing the so-called claisen condensations. In cases where long-chain PUFAs are synthesized, the chain elongation is guided by a special heterodimeric Ketosynthase-Chain length factor (KS-CLF) domain. In contrast to homodimeric KS-KS domains found in regular fatty acid synthases, the active site cysteine is absent from the C-terminal subunit of this KS-CLF heterodimer. A hydrophobic tunnel that holds the growing chain is a critical determinant of the final polyketide chain length. Besides determining the final length of PUFAs, KS-CLF heterodimer can decarboxylate malonyl units, thus "seeding" the acyl chain in the first cyclization of the polyketide and placing the new molecule within the KS-CLF tunnel. We have recently obtained crystals of a 100 kDa Ketosynthase-Chain length factor heterodimer from a PUFA synthase. These crystals were diffracted at ALBA synchrotron in Barcelona (Spain) and the protein structure was solved by molecular replacement at 1.9Å resolution. This first solved structure of a Pfa KS-CLF heterodimer resulted to be very informative to understand the role of this domain in O3PUFA synthesis. Moreover, biochemical studies in combination with modeling of the other Pfa modules allowed us to propose a model of the overall Pfa architecture. These structural studies could be used for the modification and optimization of O3PUFA synthesis in different microorganisms.

P2-24

STRUCTURAL CHARACTERISATION OF THE HUMAN PHOSPHATIDYLINOSITOL 4-KINASE II ALPHA

Baumlova A.[1], Chalupska D.[1], Różycki B.[2], Jovic M.[3], Wisniewski E.[3], Klima M.[1], Dubankova A.[1], Kloer D.[4], Nencka R.[1], Balla T.[3], Boura E.[1]

[1]Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic
[2]Institute of Physics Polish Academy of Sciences, Warsaw, Poland
[3]Section on Molecular Signal Transduction, Program for Developmental Neuroscience, NICHD NIH, Bethesda, MD, USA
[4]Syngenta Jealott's Hill Internation Research Centre, Bracknell, UK
boura@uochb.cas.cz

Phosphatidylinositol 4-kinase II (PI4K II) is one of the four mammalian lipid kinases that catalyse the conversion of phosphatidylinositol to phosphatidylinositol 4 phosphate, a major precursor of higher phosphoinositides and a key lipid molecule in a receptor-mediated signalling pathway [1]. Since PI4K IIa is a constitutively membrane bound protein, its enzymatic activity is modulated indirectly via changing the lipid composition and affecting the enzyme diffusion in a phospholipid bilayer [2]. The functional role of PI4K II in a cell physiology is still not fully understood. Here, we present the crystal structure of human PI4K II in complex with ATP [3]. The structure revealed a bi-lobal character (N- and C-lobes) of the catalytic domain with the ATP binding pocket positioned in between. Furthermore, a unique lateral hydrophobic pocket with a second ATP bound was found in the C-lobe of the catalytic domain. Molecular dynamic simulations and mutagenesis analysis revealed the membrane binding mode and a putative function of a lateral hydrophobic pocket.

Altogether, these results provided the insight into a functional state of PI4II when bound to the membrane.

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P2-25

UNRAVELING THE *B. PSEUDOMALLEI* HEPTOKINASE WcbL: FROM STRUCTURE TO DRUG DISCOVERY

M. Vivoli[1], M.N. Isupov[1], R. Nicholas[1], A. Hill[1], A.E. Scott[2], P. Kosma[3], J.L. Prior[2], N.J. Harmer[1]

[1]Department of Biosciences, University of Exeter, Henry Wellcome Building, Stocker Road, Exeter EX4 4QD, UK
[2]Defence Science and Technology Laboratory, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK
[3]University of Natural Resources and Life Sciences-Vienna, Muthgasse 18, 1190 Vienna, Austria
n.j.harmer@exeter.ac.uk.

Bacterial cells display a variety of polysaccharides and glycoconjugates in their cell walls and external structures. Gram-negative bacteria utilize heptoses as part of their repertoire of extracellular polysaccharide virulence determinants. The polysaccharides of particular relevance for infection are the lipopolysaccharides (LPS) and capsular polysaccharides (CPS). The Gram-negative bacterium *B. pseudomallei* requires CPS for full virulence [1]. This pathogen is the causative agent of melioidosis, the most common cause of community-acquired septicemia in many regions of South-East Asia [2, 3]. Mortality from septicemia ranges from 14 % to 50 %, depending on treatment, and up to 90 % if untreated [4]. The organism is also considered to be a significant threat for bioterrorism [5] and is currently classed by the US Centers for Disease Control and Prevention as a tier-1 agent [6]. New treatments are being urgently sought by the USA and UK defense agencies. Disruption of heptose biosynthesis offers an attractive target for novel antimicrobials. A critical step in the synthesis of heptoses is their 1-*O* phosphorylation, mediated by kinases such as HldE or WcbL.

Here, we present the structure of WcbL from *Burkholderia pseudomallei*. We report that WcbL operates through a sequential ordered Bi-Bi mechanism, loading the heptose first and then ATP. We show that dimeric WcbL binds ATP anti-cooperatively in the absence of heptose, and cooperatively in its presence. Modeling of WcbL suggests that heptose binding causes an elegant switch in the hydrogen-bonding network, facilitating the binding of a second ATP molecule.

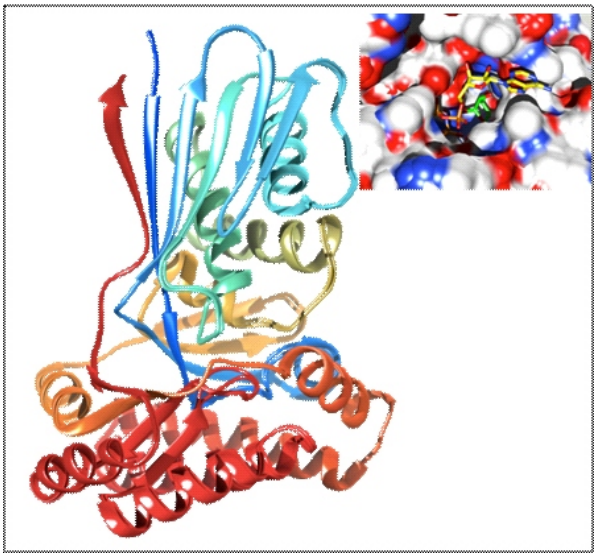

Figure 2. Monomeric crystal structure of WcbL at 1.76 Å. The backbone is shown in cartoon representation, rainbow colored from red (N terminus) to blue (C terminus). Molecular surface of WcbL coloured by charge density (red: negative; blue: positive) with bound **AMPPNP** and **D-mannose**.

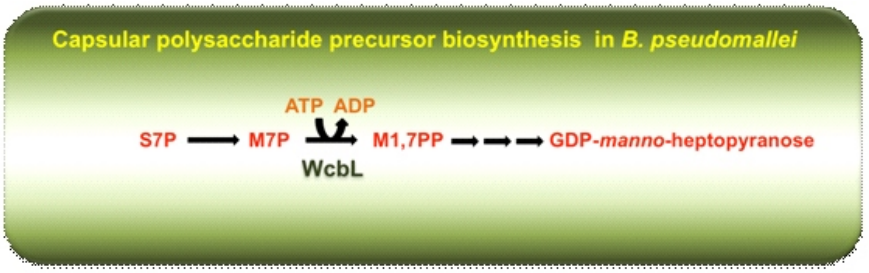

Figure 1. Schematic representation GDP-linked pathway for the capsular polysaccharide biosynthesis, highlighting the kinase activity of WcbL. S7P, sedoheptulose-7-phosphate, M7P, D-*manno*- ,-D-heptose-7-phosphate; phosphorylation of D- -D-heptose-7-phosphate to D- -D-heptose-1,7PP (M7PP) using ATP.

Finally, we screened a library of drug-like fragments, identifying hits that potently inhibit WcbL. Based on the structural and kinetic data, we have proposed a mechanism for the action of WcbL, and show that small drug-like fragments are competent in binding to the active site and inhibiting WcbL in competition with ATP. Given that WcbL knockouts present a very strong phenotype, these data strongly suggest that WcbL would be an excellent target for adjunct therapies to prevent the formation of protective surface polysaccharides in Gram-negative bacteria.

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P2-26

CRYSTALLIZATION OF THE MET EXTRACELLULAR DOMAIN WITH CRYSTALLIZATION CHAPERONES

T. Meyer, H. H. Niemann

Structural Biochemistry, Department of Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany
timo.meyer@uni-bielefeld.de

The human receptor tyrosine kinase MET is activated upon binding of the hepatocyte growth factor (HGF), its endogenous ligand. MET-activation plays an essential role in development and wound healing and induces diverse responses in several cell types, such as proliferation, motility, morphogenesis and angiogenesis. The structure of the complete dimerized MET ectodomain together with HGF is still unknown. The MET ectodomain consists of the six subdomains SEMA, PSI, and the Ig-like domains Ig1, Ig2, Ig3 and Ig4. While a rigid-body model could be calculated from SAS data [1], the crystallization of MET constructs containing Ig2, Ig3 and Ig4 still remains challenging, most likely due to high interdomain flexibility.

A promising approach to overcome this issue and solve the structure of MET is the use of interaction partners as crystallization chaperones. This technique was initially successfully applied for the MET SEMA-domain together with the HGF -chain [2]. Moreover, the pathogenic bacterium *Listeria monocytogenes* also binds to MET via its surface protein InternalinB (InlB), essentially hijacking the MET signalling pathway and inducing the uptake of *Listeria* into otherwise nonphagocytic cells. Recombinant InlB could be utilized to stabilize the SEMA-, PSI- and Ig1-domain for crystallographic analyses [3, 4].

In our most recent studies, we focused on this approach and were able to obtain a more defined structure of the Ig2 domain. In addition, we identified a wider set of MET binding proteins for further crystallization trials to finally solve the structure of the whole MET extracellular domain.

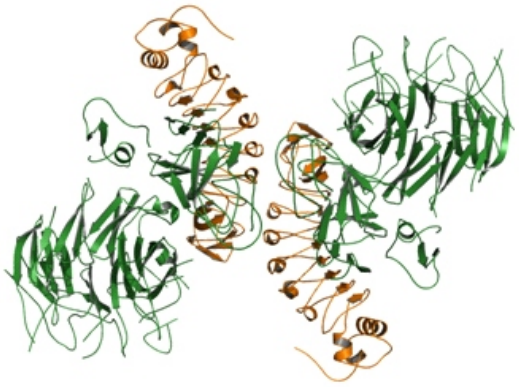

Figure 1. MET741 (green) together with InlB321 (orange).

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P2-27

CRYSTALLIZATION AND PRELIMINARY STRUCTURE DETERMINATION OF *E. COLI* ARGINYL-TRNA SYNTHETASE-tRNA (arg) COMPLEX

Ming Zhou[1,A], Sheng Ye[2,A], Preyesh Stephen[1,A], Rongguang Zhang[2,3,*], En-Duo Wang[3] & Sheng-Xiang Lin[1,3,*]

[1]*Laboratory of Molecular Endocrinology, CHU Research Center and Laval University, Québec, Canada*
[2]*National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, China*
[3]*Shanghai Institutes of Biochemistry and Cell Biology, SIBS, China*
[A]*Co-first authors*

Aminoacyl-tRNA synthetases (aaRSs) catalyze the first reaction in the biosynthesis of proteins. The *E. coli* arginyl-tRNA synthetase (ArgRS) has been crystallized in complex form with $tRNA^{Arg}$ (*B. stearothermophilus*), at pH 5.6 using ammonium sulfate as a precipitating agent.

For crystallization of the ArgRS:$tRNA^{Arg}$ complex, protein and tRNA were mixed at a 1:1.3 molar ratio and crystallized in hanging drops. Reservoirs contained 0.1 M Na citrate and 1.95 M $(NH4)_2SO_4$ at pH 5.6. Di-pyramid shaped crystals measuring $0.2 \times 0.2 \times 0.6$ mm^3 were grown over 2–3 days. All crystals were flash-frozen in liquid nitrogen for X-ray diffraction data collection at 100° K. For the ArgRS:tRNAArg complex, data were recorded at an Quantum 315r charge-coupled device detector (ADSC, Poway, CA) at beamline 19-ID of the Advanced Photon Source.

The space group of EcoArgRS:tRNA crystals is P65, which could not be determined until solving the phase problem. For phasing data of ArgRS:tRNA crystals, molecular replacement was performed with PHENIX.phaser. The structure of SceArgRS in complex with tRNA was employed as the search model. The space group P65 provided a reasonable phase solution. The final model was manually adjusted in COOT and refined with PHENIX.

The solved structure demonstrates several determinant interactions between tRNA and the synthetase in the D-stem of the tRNA. The *E coli* ArgRS is an alpha-helix rich (about 60%) structure having an active site built on a Rossmann beta-sheet scaffold. The tRNA spreads over the active site with the 3' end spanding over Ins-2 and D-loop over Add-1 and the anticodon arm pointing toward Add-2.

P2-28

PURIFICATION, CRYSTALLIZATION, AND X-RAY CRYSTALLOGRAPHIC STUDIES ON PseH, THE *CAMPYLOBACTER JEJUNI* N-ACETYLTRANSFERASE INVOLVED IN PSEUDAMINIC ACID BIOSYNTHESIS

W. S. Song, B. Namgung, M. S. Nam., S. I. Yoon

Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University, Chuncheon 24341, Republic of Korea
sungil@kangwon.ac.kr

Campylobacter jejuni colonizes in the intestinal tract of various animal species and causes acute gastroenteritis worldwide in humans. *C. jejuni* moves into intestinal mucosa using flagella for colonization on the mucosal epithelia. The flagellar filament is composed of flagellin that is O-linked glycosylated with pseudaminic acid (PseAc). *C. jejuni* PseH (cjPseH) is responsible for the third step of the pseudaminic acid biosynthesis pathway and is indispensable for flagellar formation and bacterial motility. cjPseH is an N-acetyltransferase that transfers an acetyl group from acetyl coenzyme A to the amino group of UDP-4-amino-4,6-dideoxy-N-acetyl- -L-altrosamine to generate UDP-2,4-diacetamido-2,4,6-trideoxy- -L-altropyranose. As a first step to provide the structural mechanism for cjPseH-mediated catalysis, recombinant cjPseH protein was overexpressed by the *Escherichia coli* expression system and purified to homogeneity by Ni-NTA affinity chromatography, cation exchange chromatography, and gel filtration chromatography. cjPseH was initially crystallized in PEG 300 solutions and its crystallization condition was improved to obtain diffraction-quality crystals via various screening and optimization strategies (Figure 1). As a result, X-ray diffraction data to 2.0 Å resolution were collected from the cjPseH crystal that belonged to space group $P2_12_12$ with one molecule in the asymmetric unit.

Crystals produced by streak-seeding and feeding methods at 25% PEG MME 550, 0.1 M phosphate-citrate pH 4.2 (D) Crystals produced by streak-seeding and feeding methods in the presence of 10 mM reduced glutathione and 10 mM oxidized glutathione at 24% PEG MME 550, 0.1 M phosphate-citrate pH 4.4.

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1 A2053497 to SIY).

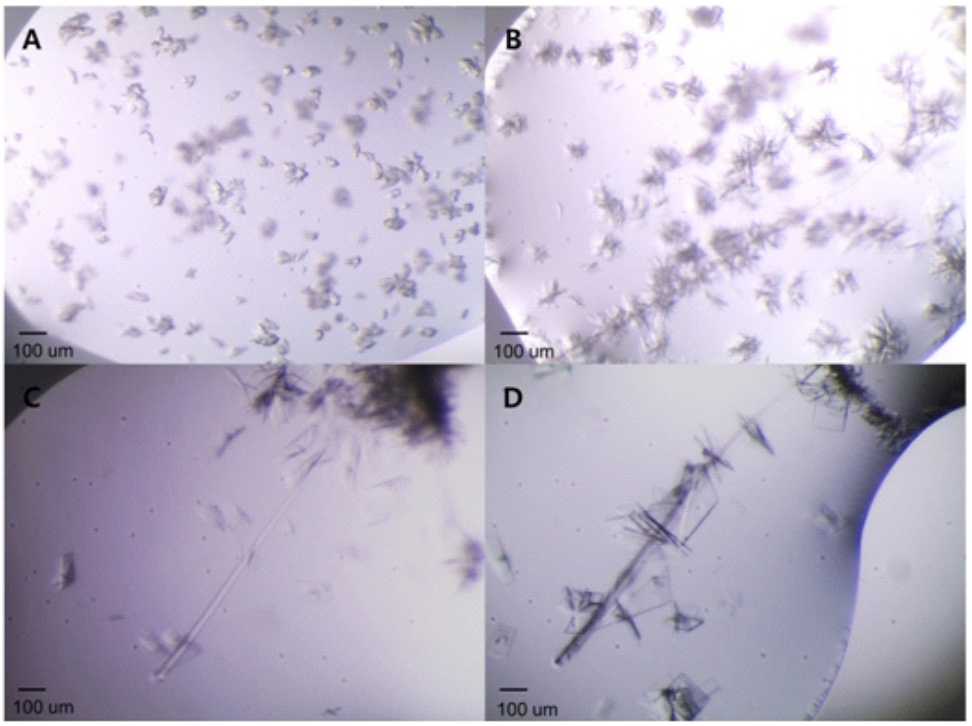

Figure 1. Optimization of cjPseH crystallization. (A) Initial crystals obtained at 34% PEG 300, 0.1 M phosphate-citrate pH 4.0. (B) Crystals produced by streak seeding at 31% PEG MME 550, 0.1 M phosphate-citrate pH 4.6. (C) Crystals produced by streak-seeding and feeding methods at 25% PEG MME 550, 0.1 M phosphate-citrate pH 4.2 (D) Crystals produced by streak-seeding and feeding methods in the presence of 10 mM reduced glutathione and 10 mM oxidized glutathione at 24% PEG MME 550, 0.1 M phosphate-citrate pH 4.4.

P2-29

PURIFICATION, CRYSTALLIZATION, AND X-RAY CRYSTALLOGRAPHIC STUDIES OF *BACILLUS* MazG-LIKE PROTEIN, BC1531

Meong il Kim, Chungdoek Lee, and Minsun Hong*

Division of Biological Science and Technology, Yonsei University, 1 Yonseidae-gil, Wonju-si, Ganwon-do, 220-710, Republic of Korea
minsunhong@yonsei.ac.kr

Bacillus cereus has been a useful model for studying *Bacillus anthracis*, an obligate pathogen, due to their evolutionary closeness. Among a large number of homologous proteins, the putative MazG-like protein, BC1531, is absolutely conserved with 100% sequence identity in *B. cereus*, *B. anthracis*, and other *Bacillus* species. Recombinant BC1531 protein was expressed in *Escherichia coli* cells and was purified by Ni-affinity and size-exclusion chromatography. Size-exclusion chromatography analysis indicated that the purified BC1531 protein exists as a tetramer in solution. BC1531 was crystallized in sodium citrate solution and was diffracted to 2.74Å resolution. The diffraction images were indexed in either the $P3_1$ or $P3_2$ space group with unit cell parameters, a = 90.84 Å, b = 90.84 Å, c = 97.41 Å. Future structure-based comparative functional assays on BC1531 would make a significant contribution to understanding the cellular function of MazG in life threatening *B. anthracis*. Here, we present the expression, purification, and crystallization of BC1531 as a first step toward revealing the structural and biochemical features of *Bacillus* MazG-like protein.