Student abstracts

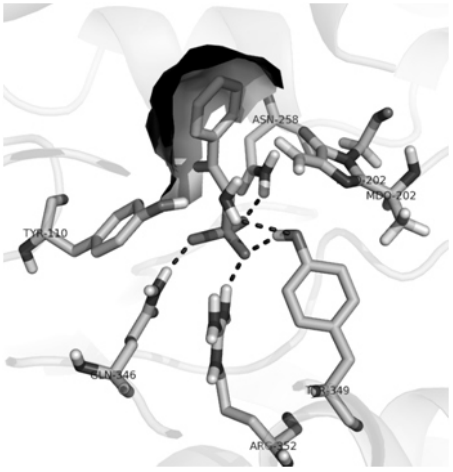
P1

EXPERIMENTAL AND COMPUTATIONAL CHARACTERIZATION OF AMINOPHOSPHONIC ACID MIO ENZYME INHIBITORS

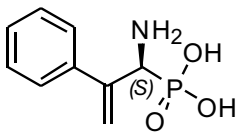
Zsofia Bata[1,2], Friedrich Hammerschmidt[3], Beata Vertessy [2,4], Laszlo Poppe[1]

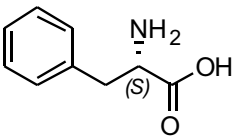
[1]BME VBK Department of organic chemistry and technology, Hungary
[2]MTA TTK Institute of Enzymology, Hungary
[3]University of Vienna, Organic Chemistry Institute, Austria
[4]BME VBK Department of Applied Biotechnology and Food Science

Aromatic amino acid ammonia-lyases and 2,3-aminomutases contain – as a common feature – the post-translationally formed prosthetic 3,5-dihydro-5-methylidene-4*H*-imidazol-4-one (MIO) group. The so called MIO-enzymes may be used – even at industrial scale – for the stereoselective synthesis of enantiopure - or -amino acids, hence making the chemical processes more environmentally friendly and more affordable. Even though there are a number of structures already available in the PDB for MIO enzymes, many of them are incomplete or are in a non-catalytically active conformation. Non-covalent competitive inhibitors may enhance crystallization in the catalytically active conformation, thus serving enhanced function-structure relationship studies on these proteins. This study describes the characterization of three aminophosphonic acid inhibitors designed for MIO enzymes with phenylalanine ammonia-lyase from *Petroselinum crispum* (*Pc*PAL) using enzyme kinetic measurements and isothermal titration calorimetry. From the tree molecules the novel 1-amino-2-phenylprop-2-en-1-ylphosphonic acid, shown at panel B of the graphical abstract, had the smallest K_i. Following a study with the racemic inhibitors, the *R* and *S* enantiomers were investigated individually as well. Unexpectedly, the *S* enantiomer – which is virtually the opposite enantiomer of natural substrate – caused the largest inhibitory effect. Accordingly, this aminophosphonic acid will be used in the following crystallization experiments of *Pc*PAL. Docking studies confirmed that the inhibitors bind in a similar way as the native substrate, and suggest the molecular mechanism of the inhibition. The lowest energy binding mode is shown on panel A of the graphical abstract. The inhibitory effect of these aminophosphonic acid compounds further support mechanism of these enzymes involving the *N*-MIO intermediate.



A





B

P2

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

The use of omega-3 polyunsaturated fatty acids (O3 PUFAs) 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 1.9 A 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.

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P3

STRUCTURE OF RNA DEPENDENT RNA POLYMERASE, 3Dpol FROM HUMAN AICHI VIRUS

A. Dubankova and E. Boura

Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., 160 00 Praha, Czech Republic

Viral RNA dependent RNA polymerases (RdRp) are enzymes which enable RNA viruses to replicate its genome and to prepare mRNA for translation of viral proteins. The RdRp catalyze creation of phosphodiester bond between ribonucleotides in RNA. All known RNA viruses use RdRp for its replication [1]. Nowadays are intensively studied structures of viral RdRp. So far, were solved several structures RdRp (3Dpol) of these viruses: Foot-and-mouth disease virus [2], Poliovius [3], Coxsackievirus [4], Enterovirus 71 [5], Encephalomyocarditis virus [6].

RdRp has evolutionary conserved tertiary structure, named right hand structure. The structure includes individual fingers, palm and thumb [3]. These structural elements are common to all DNA dependent DNA polymerase, DNA dependent RNA polymerase and RdRp [7].

In this poster we present structure of the RdRp, 3Dpol of Aichi virus, which has not yet been solved. Aichi virus belongs to Kobuviruses from family of Picornaviruses. Aichi virus is a human pathogen that causes gastroenteritis [8]. Aichi virus is also used as a model organism for studying cognate viruses which virulence is more dangerous, for example: Rhinovirus, hepatitis A virus, hepatitis C virus, SARS virus, yellow fever and West-Nile virus. RdRp are relative evolutionary conserved and it makes them good target for a drug design.

The structure of RdRp 3Dpol from Aichi virus seems to be different from already solved structures RdRp. We had to solve problem with resolution, that we improved from initial 8Å to final (current) 3,8 Å using dehydration method. Because of the low level of structural similarity we

had also problem with use of molecular replacement. It is extremely difficult to solve the structure, thus we show just preliminary structure.

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P4

ELUCIDATING THE LONGE-RANGE SIGNAL TRANSDUCTION IN RED-LIGHT ACTIVATED DIGUANYLYL CYCLASES

G. Gourinchas, S. Etzl, and A. Winkler

Institute of Biochemistry, Graz University of Technology, 8010 Graz, Austria

Organisms that benefit from light as energy source have developed complex protein architectures enabling adaptation to their light environments. Photo-perception is allowed by a collection of modular photoreceptors that couple light sensing to signaling cascades critical for cell survival. Recently, photosensory domains coupled to enzymatic effectors have attracted special attention due to their potential for optogenetic applications [1]. Among them, red-light activated phytochromes are particularly promising due to deep-tissue penetration and low phototoxicity of red-light. However, the limited understanding of molecular mechanisms of light-signal transduction limits the rational design of innovative sensor-effector couples.

Naturally occurring red-light activated diguanylyl cyclases (PadC) are constituted of a canonical bacteriophytochrome [2], able to photointerconvert between an inactive state (Pr) and an active conformation (Pfr) by red-light illumination leading to isomerization of its biliverdin chromophore. The photosensory module is fused to a GGDEF domain [3] featuring diguanylyl cyclase activity, which is involved in synthesis of the bacterial second messenger bis-(3'-5') cyclic-dimeric-GMP.

Using biochemical and biophysical methods we obtained structural details of a full-length PadC in the Pr-state that supports the involvement of previously proposed important structural elements of phytochrome signaling [4]. Moreover, we highlight the importance of the sensor-effector linker element and show that its composition is fine-tuned for enabling the conformational dynamics required for signal transduction. Currently we are addressing structural details of the Pfr state to improve our understanding of molecular mechanisms involved in communication between the phytochrome, the sensor-effector linker and the functional output domain.

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P5

STRUCTURAL CHARACTERIZATION OF MUTANT FORMS OF CARBONIC ANHYDRASE II

M. Kugler[1], I. Sieglová[1], M. Fábry[2], P. Řezáčová[1]

[1]Department of Biochemistry and Molecular Biology, IOCB, Prague, Czech Republic
[2]Laboratory of Structural Biology, IMG, Prague, Czech Republic

Carbonic anhydrases (CA) are ubiquitous zinc metalloenzymes that catalyse interconversion between carbon dioxide and the bicarbonate ion. Humans express 15 CA isoforms, which differ in tissue distribution and subcellular localization. CA II is one of the most widespread of the CA isozymes and also with the highest catalytic activity. It is a cytoplasmic isoenzyme involve in many physiological functions like pH regulation, CO2 and HCO3- transport, and maintaining H2O and electrolyte balance. Until today the only know inherited deficiency of a carbonic anhydrase of clinical significance is the CA II deficiency syndrome (CADS). CADS is inherited as an autosomal recessive trait causing osteopetrosis in combination with renal tubular acidosis, cerebral calcification and mental retardation.

Several different mutations in the CA II gene causing CADS has been reported but only one mutation (H107Y) has been previously studied and characterized in detail on protein level [1]. We have selected three additional mutant forms of CA II (namely S29P, Q92P and G145R) [2, 3] for biophysical and structural characterization.

Recombinant expression in E. coli to obtain protein in a soluble form was optimized for mutants S29P and G145R, while optimization of expression conditions for Q92P are still in progress. Variants S29P and G145R were successfully purified by combination of ion exchange and size-exclusion chromatography with yields 6-8 mg/l of bacterial culture. No significant enzymatic activity was observed for S29P and G145R variants. Preliminary crystallization experiments are in progress. Further analysis by a variety of biochemical and biophysical techniques will provide insight into the molecular basis of the CADS.

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P6

STRUCTURAL STUDIES OF THE XcpYZ COMPLEX, A CORE PART OF AN INNER MEMBRANE PLATFORM OF THE TYPE II SECRTETION SYSTEM

A. Fulara[1, 2], J. Britch[3], I. de Moraes[3], S. Savvides[1, 2]

[1]Department of Biochemistry and Microbiology, Ghent University, Ledeganckstraat 53, 9000 Gent, Belgium
[2]Inflamation Research Centre, VIB, Technologiepark 927, 9052 Zwijnaarde, Belgium
[3]the Membrane Protein Laboratory, Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, Great Britain

Bacterial virulence strongly relies on the secretion of virulence factors across the cell envelope, such as exotoxins and proteolytic enzymes. *Pseudomonas aeruginosa*, an ubiquitous environmental bacterium that has evolved to be one of the most prominent causative agents of opportunistic human infections, often employs the type II secretion system (T2SS) for the secretion of its effector proteins. The T2SS is a multiproteinaceous machinery widespread in Gram-nagative bacteria, which spans the inner and the outer membrane to facilitate the transport of folded proteins to the extracellular environment.

Our study focuses on delineating the structure of XcpYZ, a core complex of the inner membrane platform of the T2SS. In parallel, we are trying to crystallize cytoplasmic domain of XcpY and the whole XcpYZ complex. Here we show an initial low resolution diffraction data of the membrane protein crystals. The crystals were exposed to an X-Ray beam in in situ set-up. Currently we are working on optimization of the crystals of the complex and scanning conditions for the crystallization of the cytoplasmic domain. *A.F. thank Instruct for funding in situ screening experiments at the Diamond synchrotron.*

P7

CHARACTERIZATION OF FAMILY 4 CARBOHYDRATE ESTERASE FROM *SCHIZOPHYLLUM COMMUNE*

Z. Šramková[1,2], B. Vidová[1,2], Ľ. Urbániková[2]

[1]*Department of Biology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, 917 01 Trnava, Slovak Republic*

[2]*Laboratory of Prokaryotic Biology, Department of Genomics and Biotechnology, Institute of Molecular Biology, Slovak Academy of Sciences, 845 51 Bratislava, Slovak Republic*

The genome of the wood-decaying fungus *Schizophyllum commune* encodes a diverse repertoire of degradative enzymes for plant cell wall breakdown. Secretomic analysis revealed that compared with three other fungi, this species produced a higher diversity of carbohydrate-degrading enzymes [1]. In our study, we focused on protein belonging to the carbohydrate esterase family 4 according to the classification of CAZy database [2], based on our analysis, using the program blastx. As this enzyme is meant to be a part of the secretome, we can assume that it occurs naturally in a glycosylated form. On one hand, natural glycosylation usually results in protein stabilization, but on the other hand, it frequently hampers crystallization efforts. In view of the envisaged preparation of crystals, which is necessary for the enzyme tertiary structure determination in order to reveal the mechanism of action as well as the enzyme specificity, the protein of our interest was prepared in non-glycosylated form using heterologous gene expression in E. coli. We managed to produce the CE4-homologous protein in a soluble form by optimization of isolation conditions.

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P8

IS THE WRENCH-LIKE ARCHITECTURE OF THE INTERLEUKIN-5 RECEPTOR PREFORMED

J-P. Scheide, T.D. Mueller

Department of Molecular Plant Physiology and Biophysics, Julius-von-Sachs Institute, University of Wuerzburg, 97082 Wuerzburg, Germany

Interleukin-5 (IL-5) together with IL-4 and IL-13 play an important role in many immune regulatory functions, but most importantly they are involved in the onset and progression of inflammatory diseases. IL-5 is produced mainly by T_H2 helper cells and mast cells. As a key regulator of eosinophilic granulocytes (eosinophils) it controls almost any aspect of the eosinophil's life, i.e. differentiation, migration, proliferation, survival and activation. As part of the immune response for the defence of antigens eosinophils are usually activated during helminth infection and allergic diseases. Activation of eosniophils leads to their degranulation and release of cytotoxic granule compounds, which then cause severe tissue and organ damage. IL-5 is also linked to hypereosinophilic diseases, which are associated with increased levels of eosinophils, and possibly also allergic asthma.

IL-5 signalling is characterized through a sequential interaction mechanism. In the first step IL-5 binds to its specific chain the IL-5 receptor (IL-5R). In the second step the common beta chain (c, CD131) is recruited to form a ternary complex. This ternary complex then activates mainly the JAK/STAT signalling cascade. Insights into the first step of receptor activation could be provided by previ-

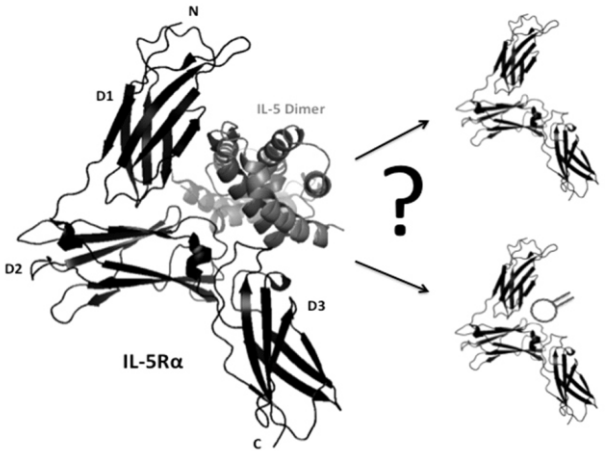

Figure 1. The wrench-like architecture of the IL-5R ectodomain in complex with IL-5 [1]. Possible fixed architecture of the unbound IL-5R ectodomain or bound to a small inhibitory IL-5 molecule. (PDB: 3QT2).

ously solving the structure of IL-5 bound to the ectodomain of the IL-5R via crystallography by our group [1]. Further investigations indicate that the wrench-like architecture of the IL-5R is likely preformed (Fig 1). For the design of small molecule-based IL-5 inhibitors the knowledge of possible conformational freedom in the IL-5R ectodomain is essential, as these inhibitors will target the free IL-5R .

We therefore aim to determine the structure of the IL-5R ectodomain in its unbound conformation or bound to such a small IL-5 inhibitor molecule. We optimized the expression/purification for the ectodomain protein to yield highly pure protein suitable for structural characterization. We have started screening 1000+ crystallization conditions to obtain crystals of the unbound IL-5R and in complex with an inhibitory peptide for structure determination by X-ray diffraction. First crystals have been obtained and optimization is in progress.

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P9

RECOMBINANT EXPRESSION OF RAT NATURAL KILLER CELL INHIBITORY RECEPTORS NKRP1B-Clrb

Ondřej Skořepa, Jan Bláha, Ondřej Vaněk

Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 2030, 12840 Prague 2, Czech Republic

Natural killer (NK) cells are an intensively studied part of immune system, possessing unique ability to recognize and induce death of tumor and virus-infected cells without prior antigen sensitization [1]. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell.This can be illustrated on the homodimeric rat inhibitory receptor NKRP1B and its ligand Clrb, which play a crucial role in the immunological response of NK cells to the infection with rat cytomegalovirus (RCMV), one of the most studied NK cell function model in rat model organism [2].

During the infection of RCMV the target cell downregulates cell surface expression of Clrb, thus decreasing the inhibitory signal transmitted through NKRP1B to the NK cell, which would ideally lead to NK cell activation and lysis of the infected cell. However, RCMV carries a gene for "decoy" surface receptor – RCTL that mimics Clrb and thus escapes the immunological response of NK cells. Moreover, while this escape strategy was demonstrated in the rat strain WAG, it has been shown that the NKRP1B homologue from rat strain SD ligates only Clrb and does not recognize RCTL. Thus the SD rat strain is less susceptible to the RCMV infection [2].

This research aims to elucidate the molecular basis of the NKRP1:Clr receptor ligand recognition, based on previous successful results with homologous human NKRP1: LLT1 receptor ligand pair[3]. For protein crystallization, it was found out that the best recombinant expression system for production of soluble extracellular domains from this family of receptors is transiently or stably transfected HEK293S GnTI- human cell line possessing homogeneous N-glycosylation profile. To increase the yields of recombinant proteins, we have optimized transposon-based doxycycline inducible mammalian cell expression system piggyBac [4] within HEK293S GnTI- cell line using Clrb soluble expression construct as target protein.

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P10

STRUCTURAL CHARACTERIZATION OF HALOALKANE DEHALOGENASES ISOLATED FROM EXPREMOPHILES

K. Šarmírová, J. Damborský, R. Chaloupková

Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment RECETOX, Faculty of Science, Masaryk University, Kamenice 5/A13, 625 00 Brno, Czech Republic

Haloalkane dehalogenases (HLDs) are microbial enzymes catalyzing hydrolytic cleavage of a carbon-halogen bond in broad range of halogenated alkanes. Structurally, HLDs belong to the superfamily of -hydrolases and consist of conserved main domain and variable cap domain. The active site is buried at the interface between these two domains . Phylogenetic analysis uncovered that HLD family can be divided into three subfamilies of which each has typical composition of a catalytic pentade . Most of characterized HLDs have originated from HLD-II and HLD-I subfamilies. HLD-III has remained the most unexplored subfamily whose members originated predominantly from extremophilic organisms. Up to now, no structure of HLD-III enzymes has been solved by X-ray crystallography or NMR spectroscopy with atomic resolution, making structural analysis of these proteins very attractive and challenging.

Herein presented project is focused on structure-functional relationships of DhmeA (HLD-III) from halophilic archea *Haloferax mediterranei* ATCC 3350, DhcA (HLD-III) from halophilic marine bacterium *Hahella chejuensis* KCTC 2396 and DsvA (HLD-I) from thermophilic bacterium *Saccharomonospora viridis* DSM 43017. All three HLD enzymes were biochemically characterized, together with determination of their secondary and quaternary structure. Size-exclusion chromatography equipped with static light scattering, refractive index, ultraviolet and differential viscometer detectors revealed that DsvA exists in the solution as a monomer, while both DhmeA and DhcA exists predominantly as high molecular weight oligomers with approximate size 1.3 MDa. Oligomeric state and size of the proteins will be further investigated by using of SAXS and analytical ultrafiltration. Initial crystallization trials of all there enzymes were performed using various commercial crystallization kits, however, no monocrystals suitable for X-ray diffraction experiments were observed until today.

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P11

STRUCTURAL STUDIES ON ENANTIOCOMPLEMENTARY-TRANSAMINASES FOR THE DEVELOPMENT OF A NEW BIOCATALYTIC TOOLBOX FOR THE SYNTHESIS OF ENANTIOPURE CHIRAL AMINES

F. Ruggieri[1,2], D. Logan[1], P. Berglund[2], L. van Langen[3], B. Walse[1]

[1]SARomics Biostructures AB, SE-223 63 Lund, Sweden
[2]Kungliga Tekniska Högskolan, Department of Industrial Biotechnology, 114 28 Stockholm, Sweden
[3]Viazym , 2629 Delft, The Netherlands

-transaminases (EC 2.6.1) are PLP-dependent enzymes that catalyze the reversible enantiospecific transfer of an amino group from a chiral amine substrate onto a prochiral ketone cosubstrate to yield the corresponding prochiral ketone and chiral amine [1]. The increasing number of characterized R- and S- -transaminases has strongly promoted active research towards the development of both mono- and multi-enzymatic systems for the synthesis or the deracemization of commercially interesting chiral amino compounds via both kinetic resolution and asymmetric synthesis [2]. The potentially interesting coupling of enantiocomplementary -transaminases in multi-enzymatic systems is limited by the narrow spectrum of common substrates.

In accordance with common knowledge about the catalytic mechanism of PLP-dependent enzymes, R- and S- -transaminases are supposed to follow the same reaction mechanism. Differences in substrate scope and stereospecificity have been explained with differences in steric and electronic properties in the enzymes' active sites. Indeed, R- and S- -transaminases belong to two distinct fold classes among the seven known for PLP-dependent enzymes, namely IV and I, respectively [3]. So far structural information is available for a few -transaminases

belonging to both fold classes in the apo-forms, holo-forms and in some instances in complex with the inhibitor gabaculine [4, 5].

Structural studies on R- and S- -transaminases in complex with different substrates are being attempted in order to identify the structural determinants responsible for their different substrate spectra and stereoselectivity. This information might help in enzyme redesign to obtain a toolbox of enantiocomplementary -transaminases for biocatalytic de-racemization of high-value chiral amines. Structures of two S-transaminases in complex with two substrates have already been determined. Studies on an R-transaminase are currently ongoing.

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P12

PREPARATION OF HUMAN NK CELL ACTIVATION RECEPTOR NKp80 AND ITS LIGAND AICL

B. Kalousková, J. Nový, J. Bláha, O. Vaněk

Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 2030, 12840 Prague 2, Czech Republic, kalouskb@natur.cuni.cz

Natural killer cells (NK cells) play a key role in innate immunity. Their function is to recognize and kill infected, stressed or malignantly transformed cells. A range of surface receptors promotes this recognition. Cytotoxic mechanisms lead to induction of apoptosis in the target cell [1]. Activating and inhibitory NK cell receptors (NCRs) can be subdivided into immunoglobulin-like family and C type lectin-like family. NCR NKp80 and its myeloid-specific activating ligand AICL are both C type lectin-like receptors (CTLRs) with C-type lectin-like domain (CTLD) [2]. Immunocomplex NKp80-AICL promotes lysis of malignant myeloid cells, mediates crosstalk between NK cells and monocytes, is engaged in cytokine release and contributes to initiation of immune response during inflammation [3].

AICL ectodomain contains odd cysteine which is believed to be responsible for formation of dimers on the cell surface. However, this cysteine in position 87 is not conserved, compared to other CTLD receptors. AICL ectodomain was expressed in E. coli BL21 Gold (DE3) strain as inclusion bodies and we have found out that upon mutation of this cysteine to serine (C87S mutation) the yield of subsequent in vitro refolding and purification as well as the stability of prepared protein are greatly enhanced compared to wild-type construct.

We used mammalian expression system of modified human embryonic kidney cell line 293 (HEK293) to produce glycosylated NKp80 ectodomain. Unfortunately, transient transfection was not successful. Using pOPINTTGneo plasmid as well as PiggyBac cloning system we were able to create stable cell lines expressing soluble extracellular parts of NKp80 in constitutive or inducible way. Thanks to these approaches, we are able to produce both proteins in sufficient amount to initiate structural studies using analytical ultracentrifuge, dynamic light scattering and finally crystallization of this immunocomplex.

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P13

RELATION OF NKp30 GLYCOSYLATION AND C-TERMINAL CHAIN LENGTH TO ITS STRUCTURE

Samuel Pažický[1], Barbora Kalousková[1], Sami Kereiche[2], Michal Rosůlek[1,3], Jan Bláha[1], Ondřej Vaněk[1]

[1]Charles University in Prague, Faculty of Science, Department of Biochemistry,Hlavova 2030, 12840 Prague 2, Czech Republic

[2]Charles University in Prague, First Faculty of Medicine, Institute of Cellular Biology and Pathology, Albertov 4, 12801 Prague 2, Czech Republic

[3]Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 14220 Prague 4, Czech Republic

spazicky@gmail.com

Immune system is able to recognize tumor cells and subsequently to eliminate them. Special sort of lymphocytes -natural killer (NK) cells are able to provide this function by causing apoptosis of tumor cells using Fas ligand binding or granzymes. These processes are activated when signals from their inhibitory receptors are decreased and on the other hand, signals from activating receptors are increased. NKp30 is an activation receptor of NK cells with one Ig-like extracellular domain. Out of its ligands, two of them are stimulating NK cells through NKp30 when bound to membrane: B7-H6, a membrane protein with two Ig like domains, which is present on the surface of some tumor cells; and BAG-6, a large multifunctional protein normally found in cytoplasm or nucleus which is transported onto the cell surface under stress condition by an unknown way [1].

The structure of the B7-H6 ligand bound to NKp30 produced in E. coli has already been solved [2]; however, the structure of the BAG-6 ligand is yet to be elucidated. Moreover, the glycosylation and length of C terminal chain of NKp30 extracellular domain as well as its oligomerization status influence its ability to bind ligands [3]. The structural basis of these effects is not known.

For our studies the extracellular domains of NKp30 and B7-H6 have been cloned into the pTW5sec vector with C-terminal histidine tag. To study the effect of C-terminal region of extracellular domain of NKp30, shorter and longer constructs have been cloned. Both proteins have been produced in human HEK293S GnTI- cell line possessing homogeneous N glycosylation profile, purified by TALON affinity column and size exclusion chromatography. Glycosylation of NKp30 was confirmed by mass spectrometry and formation of its oligomers was observed by analytical ultracentrifugation and transmission electron microscopy. Impact of glycosylation and C-terminal length of NKp30 construct was measuredusing analytical ultracentrifugation. Crystallization screens of the complex with glycosylated NKp30 constructs have been set up, too.

Library of 47 plasmids of BAG-6 was generated by cloning constructs of various lengths into vectors with TriEx plasmid backbone. Production of these constructs is to be screened in E. coli, as well as in Sf9 insect and HEK293S GnTI- human cell lines.

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P14

AMINO ACID HYDROXYLASES: STRUCTURAL CHARACTERIZATION AND ANALYSIS OF SUBSTRATE SPECIFICITY

R.S. Cordeiro[1], J. Enoki[1], F. Busch[1], C. Mügge[1], E. Hofmann[2] and R. Kourist[1]

[1]Junior Research Group for Microbial Biotechnology, Ruhr-University Bochum, Universitätstr. 150/44780 Bochum, Germany
[2]Department of Biophysics, Protein Crystallography Group, Ruhr-University Bochum, Universitätstr. 150/44780 Bochum, Germany

Biocatalysis as an alternative to organic syntheses often has the great advantage of excellent enantio- and regioselectivity [1]. This project focuses on the development of a novel enzyme cascade for the synthesis of enantiopure hydroxy amino acids and amino alcohols that are valuable building blocks for the pharmaceutical industry. Isoleucine dioxygenase from Bacillus thuringiensis (IDO) and a new enzyme from Anabaena variabilis (AvH), identified by similarity with Leucine 5-hydroxylase (LdoA) from Nostoc punctiforme, are involved in the synthesis of enantiopure hydroxy amino acids [2]. On the other hand, Lysine Decarboxylase (LDC) from Streptomyces Coelicolor a3(2) will be involved in the second step of the cascade; the decarboxylation of the formed hydroxy amino acids [3]. IDO and AvH are amino acid hydroxylases that use -ketoglutarate and iron as cosubstrates for the activation of oxygen. This reaction produces ferryl species that act as oxidant to the target C-H bond where the hydroxylation will take place [4]. In order to determine why these enzymes are either gamma (C4) or delta (C5) specific, their crystal structures would reveal valuable structural information. The molecular structures of the enzymes will allow to understand catalytic properties, to study the enzymes' specificity towards several substrates in order to identify new products and also to suggest a mechanism for the hydroxylation reaction. Apart from direct crystallization attempts, a fusion-protein approach with GFP (green fluorescent protein) in N- and C-terminal position is being investigated. The product characterization of the amino acid hydroxylation reaction by AvH is done by HPLC, LC-MS and NMR spectroscopy with the aim of determine the specific activity and to identify whether the enzyme is gamma or delta specific. Complementary studies, such as ThermoFluor assays with the purpose to determine protein stability are ongoing.

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P15

FOREIGN PROTEIN PRESENTATION ON THE SURFACE OF FLEXIBLE FILAMENTOUS VIRUS-LIKE PARTICLES

A. Šink[1], N. Rojko[1], G. Anderluh[1,2] and M. Podobnik[1]

[1]Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
[2]Dep.of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

Virus-like particles (VLPs) are structures of self-assembled virus coat proteins with highly related or identical structure to their corresponding native viruses [1]. They appear in many different shapes and sizes, and due to their unique structural characteristics, they can be used in a number of fields of research like biology, biotechnology, medicine, biopharmacy, material science and electronics. We have focused on plant flexible filamentous virus-like particles for applications in biotechnology.

It has been shown before, that smaller peptides can be presented on the flexible filamentous VLP surface, while fusion of the green fluorescent protein to the viral coat protein disabled assembly into particles [2]. We expressed coat protein in fusion with melittin, enhanced yellow fluorescent protein (EYFP) and equinatoxin II, respectively. Our aim was to co-express the fusion protein with the wild type coat protein which would lead to incorporation of both forms of protein into VLP and would allow surface presentation of larger proteins. As a result we obtained different lengths of VLPs depending on the chosen method of co-expression. Fibrils in the samples with chimeric proteins did form, but they were in general shorter than wild type VLPs and the length of the fibril was inversely proportional to amount of expressed fusion protein. The conclusion was that the stoichiometry of chimeric and wild type protein is important for VLP assembly. We could also observe the

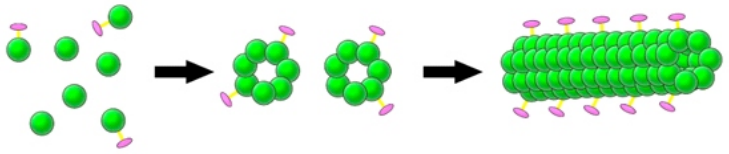

Figure 1. The prediction of assembly of co-expressed viral coat proteins.

fluorescence of EYFP, meaning that the fusion didn't affect protein folding and activity.

In the future we will focus on the determination of the structure of flexible filamentous virus and its corresponding VLPs since this is essential for further development of biotechnological applications based on flexible filamentous viruses and VLPs.

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P16

CRYSTAL STRUCTURE OF INORGANIC PYROPHOSPHATASE FROM *ACINETOBACTER BAUMANNII*

Crissy L. Tarver[1], Qunying Yuan[2], Marc Pusey[2], Joseph Ng[2]

[1]University of Alabama in Huntsville, 301 Sparkman Drive, Huntsville, AL, 35899
[2]iXpressGenes, Inc., 601 Genome Way, Huntsville, AL, 35806

An inorganic pyrophosphate molecule (PPi) is hydrolyzed into two inorganic phosphate (Pi) molecules by inorganic pyrophosphatase (IPPase). IPPases are crucial for the growth and development of cells in archaea, eukaryotes, and prokaryotes because the hydrolysis of the product PPi is necessary to maintain the forward direction of metabolic reactions. IPPases are divided into two families, family I and family II. Family I include yeast and human IPPases, while family II is found in numerous bacterial and archaeal species. These families reveal no sequence homology, and family II IPPases prefer Mn^{2+} over Mg^{2+} as the divalent metal ion used for catalysis. *A. baumannii* is an opportunistic gram-negative bacillus that is aerobic and multidrug-resistant (MDR). This pathogen apparently has active site residues typical of both families. We are interested in exploring the use of IPPases as drug targets. In order to attempt structural-based drug target design, this enzyme was cloned, expressed, purified, and characterized. Three commercial and one in-house designed screen were used with the sitting drop vapor diffusion method to search for lead crystallization conditions. After 6 weeks the scored results were subjected to AED analysis and the results used to generate a new 96 condition screen. Eight lead conditions from the AED screen plate giving crystals after 1 week were selected and used to set up an optimization plate having increasing concentrations of glycerol (0, 10, 20, and 30 %) to prepare crystals for diffraction analysis. A protein crystal was retrieved from a well containing sodium cacodylate pH 6.5, 0.9 M monoammonium phosphate, 0.2 M Na/K tartrate, and 30% glycerol for x-ray diffraction. The crystal was retrieved on a 0.2mm loop and cryocooled in liquid nitrogen. Diffraction data was collected using the SBCbeamline BM19 at Argonne National Lab. The space group for the diffracted crystal was P2221, and the structure was determined to 2.1Å resolution. The structure was solved by molecular replacement using *E. coli* IPPase as a model.

P17

S/M PROTEINS AND THEIR ROLE IN SNARE ASSEMBLY AND MEMBRANE FUSION

Sabrina Kattelmann and Daniel Kümmel

Department of Biology/Chemistry, Structural Biology, University of Osnabrück, 49076 Osnabrück, Germany

In eukaryotic cells, fusion of membranes is an important process that is required for exchanging material between different compartments within the cell, maintenance of homeostasis and cell-cell communication. In order to maintain the cells physiology, membrane fusion is a critical aspect that needs to be temporally and spatially regulated. The key players in this process are part of a conserved fusion machinery in the endomembrane system that contains small Rab GTPases, SNAREs (soluble N-ethylmaleimide-sensitive-factor attachment receptors) and Sec1/Munc18 family (S/M) proteins. When the membranes are in close proximity, SNARE proteins drive fusion by forming a stable four-helix coiled-coil complex also called trans-complex. This complex of SNAREs is highly stable and it requires the hydrolysis of ATP by the ATPase NSF (N-ethylmaleimide-sensitive-factor) for disassembly after fusion [1]. S/M proteins contain a ~600 amino acids conserved sequence that forms a clasps-like structure. They can interact with monomeric SNAREs [2] and with SNARE complexes [3] to promote intracellular membrane fusion, however, mechanistic and structural insight into this process is incomplete. The aim of my research is to identify how S/M proteins interact with SNAREs to fulfill their function For this purpose we measure the affinity of the interaction of S/M proteins with monomeric SNAREs as well as with (sub)assemblies to identify the cognates substrate complex(es) as target for crystallization and structure determination. I have so far been successful in establishing a purification protocol for individual SNARE and SNARE subassembly and the crystallization of isolated S/M protein. My current focus is on the reconstitution and crystallization of the S/M-SNARE complexes for structural determination.

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P18

DEVELOPMENT OF A PROTOCOL FOR ENZYMATIC ASSAY, DOCKING AND CRYSTALLISATION OF ALDO-KETO REDUCTASE 1C3 (AKR1C3) WITH SPECIFIC INHIBITORS

J. J. Plavša[1], S. Bekić[2], M. Marinović[1], A. Ćelić[1], E. T. Petri[1]

[1]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, Serbia
[2]Dep. of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad

Aldo-keto reductase family 1 member C3 is an important enzyme in steroidogenesis and redox homeostasis in humans. This enzyme uses NADH and/or NADPH as cofactors to catalyze conversion of aldehydes and ketones to alcohols. Overexpression of AKR1C3 contributes to tumor development and inhibition of AKR1C3 activity represents a promising target for development of new therapies [1]. The PDB (Protein Data Bank) contains several crystal structures of AKR1C3 with different inhibitors, however there is a need for identification of more specific inhibitors, given the sequence similarity between AKR1C isoforms. To understand how specific inhibitors bind to AKR1C3, we are preparing recombinant AKR1C3 protein for crystallization in complex with new ligands.

Computational prediction of the potential binding affinity of new inhibitors is followed by induction and expression of recombinant AKR1C3 and purification of the protein. An optimized protocol for induction, expression and purification of recombinant AKR1C3 has been developed in our lab. Using docking results as a guide, we are optimizing AKR enzymatic assays with promising inhibitors, and developing a protocol for testing new substrates. Based on results of enzymatic assays we are planning preparation of crystallization trials in complex with various ligands for structure determination to guide design of more specific inhibitors.

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P19

CRYSTALLIZATION OF REPRESSORS DeoR AND C-GNTR FROM *BACILLUS SUBTILIS*

M. Nováková[1], J. Škerlová[1,2], M. Fábry[2], P. Řezáčová[1,2]

[1]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, Prague 6, Czech Republic*

[2]*Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, Prague 4, Czech Republic*

Bacterial repressors function as molecular switches: binding of an effector molecule modulates their ability to interact with DNA operator and the gene transcription is turned on or off. Understanding the structure-function relationship and molecular mechanisms by which individual repressors execute their regulatory functions greatly benefits from knowledge of their 3D structures. In this project, we selected two repressors as targets for structural characterization.

The DeoR protein from *Bacillus subtilis* negatively regulates the expression of catabolic genes for the utilization of deoxyribonucleosides and deoxyribose. The DeoR protein comprises an N-terminal DNA-binding domain (DB) and a C-terminal effector-binding domain (EBD). The structure of the EBD was determined in free form and in covalent complex with its effector [1]. Our aim is a determination of structure of full-length DeoR in complex with its DNA operator. The recombinant protein was expressed in host bacteria *E. coli* BL21(DE3). For the purification of the protein we used an immobilized nickel affinity chromatography, N-terminal His tag was removed by TEV protease cleavage. The yield of the protein preparation was 2.88 mg from 1L of bacterial culture. The complex of DeoR with its DNA operator (18bp) was prepared by mixing of the protein and DNA in ratio 1:1.1 and formation of the complex was proven by gel permeation chromatography. Crystallization screening followed by optimization yielded were needle-shaped monocrystals diffracting to resolution 7Å at a synchrotron radiation source. Optimization of crystallization conditions is now in progress.

The GntR protein from *B. subtilis* is a negative regulator which is responsible for gluconate metabolism in *B. subtilis.* Its structure has not been resolved yet. Our aim is to determine its EBD in complex with its effector gluconate.

For recombinant expression and protein purification was performed similarly to DeoR described above. The yield was 13.75 mg of purified protein from 1L of bacterial culture. Crystallization of GntR EBD in the presence of high molar excess of gluconate yielded hexagonal monocrystals with maximal dimension of 30 30 3 μm. Optimization of crystallization conditions is now in progress.

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P20

CRYSTALLOGENESIS STUDIES OF BACTERIAL AMINOACYL-TRNA SYNTHETASES AS POTENTIAL TARGETS FOR DRUG DESIGN

R. de Wijn, B. Lorber, C. Sauter

Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire (IBMC-CNRS), Université de Strasbourg, 15 rue René Descartes, F-67084 Strasbourg, France. r.dewijn@ibmc-cnrs.unistra.fr

Aminoacyl-tRNA synthetases (aaRSs) are ubiquitous enzymes that catalyze the first step of protein biosynthesis or translation. In the presence of ATP, they activate the amino acids as adenylates and subsequently bind the amino acid moity onto the 3' CCA end of transfer RNAs [1]. The resulting aminoacyl-tRNAs are then carried by the elongation factors to the ribosome to be incorporated into nascent polypeptide chains. AaRSs are a target of choice for drug design because they are essential enzymes having a high specificity for their substrates. Our study is focused on bacterial aspartyl-tRNA synthetases (AspRSs) that bind specifically L-aspartate. We have initiated the structural characterization of the binding mode of two families of inhibitors. One is a natural antibiotic produced by *E. coli* strains that targets the catalytic site of AspRSs (collaboration with Prof. S. Rebuffat, Museum National d'Histoire Naturelle, Paris) and the other a series of chemically synthesized peptides that were selected against an AspRS from the opportunistic human pathogen *Pseudomonas aeruginosa* (collaboration with Prof. Hiroaki Suga, University of Tokyo). We apply various crystallogenesis approaches to prepare crystals that are suitable for the X-ray diffraction analysis. They involve the optimization of crystal production either by cocrystallization or by soaking of native crys-

tals with ligands. In the case where the enzyme of one bacterial species does not yield exploitable crystals, the protein is either chemically methylated to change its surface properties and crystallizability, or a close structural homolog with a conserved active site is substituted to take advantage of genetic diversity. In the final step crystallization systematically takes place in an agarose gel with a low gelling temperature to improve crystal quality, stability during the soaking with inhibitors, and handling [2]. The rationale of our crystallogenesis strategy will be presented and illustrated with examples.

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P21

COMPARATIVE ANALYSIS OF dUTpase ENZYME - STL INHIBITOR PROTEIN INTERACTION ON A PROKARYOTIC AND A EUKARYOTIC ENZYME MODEL

Andras Benedek[1,2], Istvan Poloskei[2], Beata G. Vertessy[1,2]

[1]Institute of Enzymology, RCNS, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Applied Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary

The enzyme dUTPase plays a key role in maintaining sufficiently low cellular dUTP/dTTP ratio via effective hydrolysis of dUTP into dUMP and pyrophosphate. This enzymatic reaction is thought to be evolved in order to prevent hyperactivation of uracil excision repair process, which carries the risk of DNA double strand brakes or even cell death. The enzyme dUTPase is therefore a potential therapeutic target in certain infectious diseases but also in cancer treatment [1].

It has been recently shown that the *Staphylococcal 11* phage dUTPase inhibits DNA-binding ability of Stl, a pathogenicity island repressor protein of *Staphylococcus aureus* [2]. Subsequent quantitative characterization of this interaction revealed that Stl acts as a highly potent inhibitor of the *11* phage dUTPase, vice versa [3]. In addition, it also shows cross-species effects by inhibiting mycobacterial dUTPases both *in vitro* and *in vivo* [4]. In hope of identifying Stl as a broad-spectrum dUTPase inhibitor, we tested its in vitro effect on the eukaryotic *Drosophila melanogaster* and on the prokaryotic Escherichia coli dUTPase, respectively. Using steady-state activity assay we observed approximately 40% decrease in enzymatic activity of the *Drosophila* dUTPase but we did not detect any change in *E. coli* dUTPase activity. We further confirmed a strong interaction amongst the *D. melanogaster* dUTPase and Stl by native gel electrophoresis. Surprisingly, our native gels showed that Stl also forms a protein complex with the *E. coli* dUTPase despite of the fact that in this latter case no inhibitory effect of dUTPase activity upon Stl binding was detected.

In order to further investigate this intriguing cross-specific interaction and understand the exact structural background of inhibition, protein complex crystallization experiments are carried out among the *D. melanogaster* dUTPase-Stl and *E. coli* dUTPase-Stl interactive partners.

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P22

GLYCEROL ALTERS SUBSTRATE BINDING IN PTPLP:IP COMPLEXES

L. M. Bruder and S. C. Mosimann

Department of Chemistry and Biochemistry, University of Lethbridge, Alberta, Canada

Protein tyrosine phosphatase-like myo-inositol phosphatases (PTPLPs or phytases) follow an ordered, sequential dephosphorylation pathway that utilizes the abundant myo-inositol-1,2,3,4,5,6-hexakisphosphate (InsP6 or phytate; approximate charge of -6 to -9 at physiological pH) to produce less-phosphorylated myo-inositol phosphates (IPs) containing between one and five phosphoryl groups [1]. Ultimately, we aim to engineer PTPLPs by rational design to manipulate the substrate specificity and produce alternate IP products [2]. The engineered PTPLPs can then be used for large-scale production of IPs. To rationally design an enzyme, clear understanding of how the structure affects function is required. Therefore, the goal is to understand PTPLP substrate specificity at atomic resolution in order to identify and understand the structural determinants that govern substrate specificity. To this end, multiple crystal structures of different IP ligands in complex with IPases are necessary. This work focuses on two PTPLPs which have high activity towards InsP6 and divergent hydrolysis pathways: Phytase A from *Mitsuokella multacida* (PhyAmm; a tandem repeat) and *Selenomonas ruminantium* (PhyAsr) [1, 3].

Glycerol is commonly used to protect proteins while stored frozen, for stabilizing and enhancing protein solubility, in cryocrystallography, and is also used during protein crystallization [4-5]. PhyAmm can be crystallized in the presence or absence of glycerol at concentrations suitable for cryoprotection. The presence of glycerol during crystallization produces larger crystals with increased stability than those grown in the absence of glycerol. However, when soaked or co-crystallized with the highly-charged IP substrates, the resulting structures have the IPs bound to the active site in a catalytically incompetent manner, with inorganic phosphate bound to the phosphate-binding loop (P-loop) and the IP bound above the inorganic phosphate. When the concentration of glycerol is decreased before the PhyAmm crystal is soaked with an IP the structure results in the IP bound in a catalytically competent manner. In the case of PhyAsr, glycerol is used strictly as a cryoprotectant. When PhyAsr is soaked with an IP solution which contains glycerol, the IP binds to PhyAsr in a manner inconsistent with the known pathway or in a catalytically incompetent manner. When PhyAsr is first soaked with the IP and then glycerol added as the cryosolvent, an alternate substrate binding mode is observed.

This work demonstrates that glycerol can have a significant effect on protein-substrate interactions when involving high-charge density substrates. In the case of PhyAmm, the substrate is incapable of binding the P-loop, while the PhyAsr substrate binds the P-loop with alternate binding modes.

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P23

STRUCTURAL CHARACTERIZATION OF Anbu PROTEIN

Anna Piasecka[1] and Matthias Bochtler[1,2]

[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland

[2]International Institute of Molecular and Cell Biology, Trojdena 4, 02-109 Warsaw, Poland

Protein degradation plays an important role in cell homeostasis. Although this process has been under investigation for the past 20 years, there are still questions that remain unanswered. The architecture of a missing link between simple (HslV; two six-membered rings that form a single cavity) and advanced proteasomes (20S; four seven-membered rings that form three cavities) is one of them. An in silico study published by Valas and Bourne [1] proposed a prokaryotic protein called Anbu, with amino acid sequence resembling a subunit of 20S proteasome rather than HslV, to be the missing link.

Our biochemical data of *E. coli* overexpressed Anbu confirms that it forms large complex. The gel filtration, dynamic light scattering and analytical ultracentifugation experiments show a single peak and indicate high purity of the sample. Anbu protein crystallizes readily, but only in one crystallization buffer. Crystals of P2(1) symmetry (dimensions 95 Å 285 Å 197 Å , 91.8°) diffract to 2.8 Å.

Unfortunately, so far all phasing attempts have failed. For some heavy atom soaked crystals, SHELXC program indicates the presence of moderately strong anomalous signal, but the heavy atom substructure cannot be resolved. Molecular replacement attempts using various models of proteasomes were unsuccessful as well. This becomes less surprising in view of the SAXS analysis, which proved that the Anbu tertiary structure is very different from known proteasomes. Negative stain EM 2D class averages of Anbu show structures similar to both 20S and HslV proteasomes, suggesting that the sample might be non-homogeneous, which would however contradict all our prior results.

So far the work on Anbu project raised more questions than gave answers. Obtaining the structure of the Anbu complex might finally shed some light on the architecture and function of this molecule.

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P24

STRUCTURAL AND FUNCTIONAL INSIGHTS FOR Rab35 GTPASE AND ITS EFFECTORS: TOWARD A MECHANISM FOR CONTROL OF ACTIN DYNAMICS IN ENDOCYTOSIS AND CYTOKINESIS

Hussein Hammich[1,2], Gilles Malherbe[1], Florian Chardon[1], Maria-Elena Sirkia[1], David Stroebel[4], Stephane Fremont[3], Arnaud Echard[3], Olena Pylypenko[1] and Anne Houdusse[1,3]

[1]Institut Curie, PSL Research University, Subcellular Structure and cellular Dynamics, EPST, UMR 144, laboratory, Structural Motility team, 75005, Paris, France
[2]Sorbonne Universités, UPMC Univ Paris 06, 4 place Jussieu, 75252, France
[3]Membrane Traffic and Cell Division, Pasteur Institute, 25-28 rue du Docteur Roux, 75015 Paris, France
[4]Section Neurosciences, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris France

Rab35 is an essential regulator of a recycling pathway back to the plasma membrane, that is also required for the post-furrowing terminal steps during cytokinesis that are associated with F-actin depolymerisation [1]. Rab35 performs its role in the cell via recruitment and regulation of specific effector proteins. Recently the lab of our collaborator Arnaud Echard (Pasteur institute, Paris) has identified and is currently studying by cell biology approaches two novel Rab35 effectors - MICAL1 and MiniBAR proteins. MICAL1 may restrict actin levels before cytokinesis abscission, because it harbours a monooxygenase catalytical domain and has been shown to be an F-actin-disassembly factor [2]. But it is unknown whether MICAL1 has a function in cell division. Another effector of Rab35 currently uncharacterized was identified and named MiniBAR by our collaborators. They recently described that MiniBAR specifically binds to active Rab35, that it contains an unnoticed, putative BAR domain (known to sense membrane curvature) and that this domain, adjacent to the Rab35-binding domain, binds specifically to GTP-bound Rac1 - a well-known actin remodelling regulator. So, MiniBAR may function as a linker between the two small GTPases coordinating actin-remodelling processes in the cell. The aim of the project is to perform extensive structural/functional characterization of complexes between the Rab35 GTPase and its interacting effector proteins.

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P25

ACTINOPHAGE ENDOLYSIN Lyt µ1/6 PREPARED FOR CRYSTALLIZATION

M. Oravkinová[1], B. Vidová[1, 2], J. Farkašovská[2], A. Godány[1, 2]

[1]Department of Biology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, 917 01 Trnava, Slovak Republic

[2]Institute of Molecular Biology, Slovak Academy of Sciences, 845 51, Bratislava

Actinophage µ1/6, belonging to the phage family *Siphoviridae* in the order *Caudovirales*, has a narrow host range for industrially important *Streptomyces aureofaciens* strains producing tetracycline. For release of the progeny virions, actinophage µ1/6 accomplishes lysis of the host cell by the synergistic action of a two-component lysis system - holin and endolysin [1].

The protein of endolysin Lyt µ1/6 (gp50) has a modular structure organization consisting of C-terminal cell wall binding domain (CBD) and N-terminal catalytic domain (ECD) [2]. The sequence of ECD is similar with the sequences of bacteriophage endolysin catalytic PGRP domains (cd06583) that show amidase activity. The enzymatic function prediction of endolysin Lyt µ1/6 based on the homology protein structure is not yet possible because the absence of any actinophage endolysin protein structures. Therefore, the obtaining of crystal structure of this protein is very challenging.

The coding sequence of the gene for endolysin Lyt µ1/6 (GenBank AY321539.1) was optimized for expression in *E. coli* and synthesized (GenScript, USA) into the expression vector pET15b. The expression was carried out in E. coli Arctic Express strain by induction with IPTG. Cell disruption and protein extraction was provided by sonication in HEPES buffer (pH 8.0). The protein was purified using IMAC on Ni-NTA column. All samples were assayed for purity on 12% SDS-PAGE gels. Proteins were gel purified and prepared for crystallization. An understanding of endolysin Lyt µ1/6 structure can lead to its versions applicable in therapy with improved stability, activity, or host range.

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P26

TOWARDS HIGH RESOLUTION CRYSTAL STRUCTURE OF CARDIAC SERCA2a

Alya Kotsubei[1,2], Claus Olesen[1], Ilse Vandecaetsbeek[2], Peter Vangheluwe[2], Poul Nissen[1]

[1]Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark

[2]Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium

alya@mbg.au.dk

The cardiac sarco(endo)plasmic reticulum Ca2+-ATPase isoform 2a is one of the key proteins of the heart. It is responsible for proper relaxation of heart muscle by pumping calcium ions back into the SR after their release during contraction. Any disruptions in expression or activity levels of SERCA2a can lead to poor contractility of the heart and thus eventually cause pathological conditions such as heart failure. In the current project we aim to gain molecular insights into SERCA2a regulation using a combination of structural and functional studies. High resolution crystal structures of SERCA2a as well as regulation of calcium affinity by small peptides are our main focuses.

P27

STRUCTURAL STUDIES OF THE 14-3-3 PROTEIN:PROTEIN KINASE ASK1 COMPLEX

Olivia Petrvalska[1,2], Dalibor Kosek[1,2], Zdenek Kukacka[3], Zdenek Tosner[1], Jaroslav Vecer[4], Petr Herman[4], Veronika Obsilova[2]*, Tomas Obsil[1,2]*

[1]Dep. of Physical and Macromolecular Chemistry, Fac. of Science, Charles Univ. in Prague, 12843 Prague,
[2]Institute of Physiology, The Czech Academy of Sciences, 14220 Prague,
[3]Institute of Microbiology, The Czech Academy of Sciences, 14220 Prague,
[4]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, 12116 Prague, Czech Republic

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. Since the increased activity of ASK1 has been linked to the development of several diseases including cancer, cardiovascular and neurodegenerative diseases, this enzyme is a promising drug target. The activity of ASK1 is regulated through homo-oligomerization and interaction with several proteins including the 14-3-3 protein which binds to the phosphorylated motif located at the C-terminus of the kinase domain of ASK1 and suppresses its catalytic activity through unknown mechanism.

We performed low-resolution structural analysis of the kinase domain of ASK1 (ASK1-CD) bound to 14-3-3 using chemical cross-linking, analytical ultracentrifugation and small angle X-ray scattering.

According to our results, the complex between 14-3-3 and pASK1-CD is dynamic and conformationally heterogeneous with both proteins sampling several mutual orientations. The low- resolution structural analysis together with results of phosphorus NMR and time-resolved tryptophan fluorescence measurements suggest that the 14-3-3 dimer interacts with regions from the C-lobe of the kinase domain of ASK1 and induces conformational change in its active site. Thus, these results suggest that the 14-3-3 binding suppresses the catalytic activity of ASK1 through direct structural modulation of its activation segment.

In addition, we also crystallized complex between 14-3-3z and synthetic phosphopeptide containing the 14-3-3 binding motif of ASK1 to obtain structural details of their interaction.

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THE EFFECT OF MACROMOLECULAR TRANSPORT ON MICROGRAVITY VERSUS GRAVITY PROTEIN CRYSTALLIZATION

A. Martirosyan[1], C. Schmidt[1], A. Meyer[2], C. Dierks[2], M. Perbandt[1], L. DeLucas[3] and C. Betzel[1]

[1]Univ. of Hamburg, Inst. für Biochemie und Molekularbiologie Martin-Luther-King Platz 6, 20146 Hamburg, Germany
[2]Xtal Concepts GmbH, Marlowring 19, 22525 Hamburg, Germany 3. University of Alabama at Birmingham, Center for Biophysical Sciences and Engineering, Birmingham, AL, USA

In macromolecular protein crystallization mass transport processes are a critical determinant in the character and quality of the protein crystals. Transport processes, particularly mass transport, are of high importance in the growth of crystalline materials from aqueous solutions as well [1-4]. Transport affects not only the molecules or ions that are incorporated into the growing crystal, but it also affects the rate of adsorption/incorporation of impurities [1]. As impurity incorporation can have a significant impact on the size, morphological development, and ultimate diffraction data of a crystal, differences in form or quality produced by diverse transport phenomena can be unexpectedly large [1-4]. Mass transport by convection can, of course, only occur if gravity is present. Only then solutions of higher density will fall and lighter fluids will rise, and only then convective currents can be established in a bulk solution [1]. Experience with many types of crystal growth confirmed, that the minimization of convective transport, which allows growth to occur principally by diffusion-based attachment of molecules to the surfaces, generally resulted in crystals of superior quality with improved optical and mechanical properties, reduced lattice defects , and greater size. Thus, it follows that crystals grown in a zero gravity, or a reduced gravity environment, might dem-

onstrate enhanced properties [1,5,6]. The purpose of this work is to evaluate the percentage incorporation of different molecular aggregates (protein impurities) into the crystalline lattice of growing crystals. Based on the percentage incorporation of larger aggregates in the crystals, the effect of molecular filtering based on differences in diffusion rates will indirectly be assessed. This will be accomplished by fluorescently tagging the aggregates. Once the crystals have returned to earth they will be subjected to Xray crystallography to determine the structure and hence understand the biology of the system. The target protein is the Glutathione-STransferase from the malarian parasite Plasmodium falciparum (PfGST). The PfGST dimer was crystallized in the presence of a small PfGST tetramer fraction. The tetramer was fluorescently tagged to screen for incorporation of tetrameric protein molecules into the crystalline lattice of the dimer crystals. Therefore the PfGST dimer was expressed and purified in the presence of S-pBrombenzylglutathione. S-pBrombenzylglutathione is needed to stabilize the formation of PfGST dimer crystals. The crystallization was successfully performed using the hanging-drop vapor-diffusion and the counter-diffusion method in capillaries. Preliminary results on 1 x gravity reveal that the fluorescently tagged PfGST tetramer incorporates into the crystalline lattice of PfGST dimer crystals.

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P29

CRYSTALLIZATION AND STRUCTURAL ANALYSIS OF THE FERRIC UPTAKE REGULATOR A (FurA) FROM *MYCOBACTERIUM AVIUM SUSP. PARATUBERCULOSIS* (MAP)

Christina Schmidt[1], Markus Perbandt[1], Jochen Mees[2], Ralph Goethe[2], Christian Betzel[1]

[1]Institut für Biochemie und Molekularbiologie, Martin-Luther-King Platz 6, 20146 Hamburg, Germany
[2]Institut für Mikrobiologie, Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, 30173 Hannover

Mycobacterium avium susp. paratuberculosis (MAP) is the causative pathogen of Johne's disease (paratuberculosis) a chronic transmural inflammation of the small intestine in ruminants. The disease causes high economical losses in the dairy industry worldwide [1]. MAP has also been discussed as a possible etiological agent in the development of human autoimmune associated diseases such as Crohn's disease [2].

MAP is able to resist the host immune response through changes in the metabolism and gene expression [3]. The MAP Ferric Uptake Regulator A (FurA) belongs to the family of Fur proteins that are mainly involved in ion homeostasis and stress response in bacteria. MAP FurA is involved in the oxidative stress response, regulating the expression of several associated genes in the presence of H_2O_2, besides negatively regulating its own expression. FurA can be functional as a homodimer if Fe^{2+} is bound, to repress gene expression via DNA binding of a distinctive sequence (Fur-Box) [4].

Moreover, the protein can regulate gene expression in its apo- form as well, probably inducing gene expression of genes involved in virulence [4]. However, FurA does not, unlike most of its homologues, participate in iron homeostasis [4].

The aim of this project is to solve the crystal structure of MAP FurA to gather information about possible inhibitors of this protein and its different mechanisms to regulate gene expression. The protein was expressed in *E. coli* BL21 DE3, purified with anion exchange chromatography and size exclusion chromatography. Dynamic light scattering measurements (DLS) were used to confirm the homodimer and the purity of the protein in solution. The folding of the protein was examined using circular dichroism spectroscopy. The protein was concentrated to 20 mg/ml and crystallization trials were set up using commercially available screens. Preliminary results outlining protein expression and purification as well as initial crystallography trials will be presented.

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