Student abstracts

P1

IN VITRO CHARACTERISATION OF STAPHYLOCOCCAL PATHOGENICITY ISLAND REGULATORY ELEMENTS

Ibolya Leveles[1], Imre Zagyva[1], Ábris Ádám Bendes[1], Veronika Kádár[1], Judit Szabó[1], Veronika Pongrácz[1], Beáta G. Vértessy[1,2]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, H-1113, Hungary

[2]Department of Applied Biotechnology and Food Science, Faculty of Chemical and Biochemical Engineering, Budapest University of Technology and Economics, Budapest, H-1111, Hungary

The infections caused by *Staphylococcus aureus* represent a major healthcare problem, not only among hospitalized patients [1], but to the rest of the population, as well as it is a component of the normal human skin flora. The dormant *S. aureus* strain can be transformed to a pathogenic state by the transfer and integration of other already pathogenic *S. aureus* strain pathogenicity islands [2] mostly that of the superantigen-carrying pathogenicity island (SaPI) where intergenomial transfer is known to occur more frequent than the other islands [1]. The transcription of the SaPI genes is under control of a specific global repressor (Stl), in our case the Stl SaPIbov1 protein. It has been suggested that this repression is non-canonically deregulated by a protein-protein interaction of the dUTPase from the staphylococcal helper phage 11 [3].

In this study we report the successful expression and purification of wild type Stl SaPIbov1 and 11 dUTPase, as well as various mutants of the dUTPase. Functional and structural analysis (including activity measurements) has been carried out and the discussion of the results is presented. The structure of the wild type 11 dUTPase has been solved to a resolution of 2.98 Å. A crystallographic report of this experiment has been published [4] and the structural properties of the model are discussed in the poster. Further crystallization trials of the proteins alone and in the complexed form are in progress.

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P2

TOWARDS THE STRUCTURE OF LYMPHOCYTE RECEPTOR HLLT1

J. Bláha[1], O. Vaněk[1], P. Novák[1,2], T. Skálová[3], J. Dohnálek[3], K. Harlos[4], P. Pachl[5], K. Bezouška[1]

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

[2]Institute of Microbiology, AS CR, Vídeňská 1083, CZ-14220 Prague 4, Czech Republic.

[3]Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovského nám. 2/1888, CZ-16206 Praha 6, Czech Republic.

[4]Division of Structural Biology, University of Oxford and Oxford Protein Production Facility, The Henry Wellcome Building for Genomic Medicine, Headington, Oxford, United Kingdom.

[5]Institute of Organic Chemistry and Biochemistry, AS CR v.v.i., Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic

jahabla@gmail.com

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. 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. Recent research in their C-type lectin-like receptors repertoire has shown that ligands of some of these previously orphan receptors lie within their own family, describing a lectin-lectin interaction. This is the case of human inhibitory receptor NKRP1 (gene KLRB1) and its ligand LLT1 (gene CLEC2D) [1].

Previous studies have shown that overproduction of LLT1 in cancer cells [2] or lower production of NKRP1 in NK cells [3] is connected to cancerous manifestations.

Our previous efforts to study this system on a structural level via recombinant expression in *E. coli* have shown that the proteins aggregate to inclusion bodies and their refolding was inefficient. We decided to try mammalian expression system based on transient transfection of modified human embryonic kidney (HEK) cell lines.

Here we show a case study of recombinant expression in HEK293 cells based on an ongoing research of extracellular domain of LLT1. Five cysteins contained within this lectin domain tend to cause misfolding and formation of aggregates. Using a multiple alignment tool with similar proteins from this family we identified a conserved region in primary sequence where LLT1 lacks its sixth cystein residue. Utilizing a site directed mutagenesis approach we were able to stabilize this structure by addition of this "missing" residue. This led to significant improvement in yield and homogeneity of product that already enabled successful crystallization in two different crystalline forms and solution of the structure at 2.0 Å.

Supported by Grant Agency (P207/10/1040, 303/09/0477 and 305/09/H008) and Ministry of Education (1M0505) of the Czech Republic, by Charles University (UNCE 204025/2012), and by EC funding through the SPINE2–COMPLEXES (031220) and P-CUBE (227764) projects.

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P3

SELF-ASSEMBLY OF A -SHEET PROTEIN COVALENTLY MODIFIED AT A SURFACE CYSTEINE

G. Cattani and P.B. Crowley

School of Chemistry, National University of Ireland Galway, University Road, Galway, Ireland
g.cattani1@nuigalway.ie

Chemical modifications of proteins can be used to evaluate the contribution of non-native surface features to self-assembly [1].

Here, we show a mutant of the copper protein plastocyanin with a cysteine residue exposed to the solvent at the "hydrophobic patch". Although surface cysteines present significant challenges to protein production, this residue is the most convenient target for selective modification owing to the strongly nucleophilic side chain sulfhydryl [2]. Here we present a maleimide conjugated to the surface-exposed cysteine.

This conjugate was characterized using analytical methods such as mass spectrometry, UV/Vis spectroscopy and PAGE techniques, and was crystallized in order to study the effect on self-assembled structures of plastocyanin [3].

In addition, the dimerization of the protein through cysteine oxidation was performed in order to investigate and evaluate crystallization opportunities [4]. Preliminary crystallization screens of the dimer look promising. Crystal optimization trials are in progress.

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P4

STRUCTURAL VIROLOGY OF THE MURID GAMMA-HERPESVIRUS 4

B. Correia[1], Colin McVey[2] and M. Miranda

[1]Macromolecular Crystallography Unit – Structural Genomics Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras-Lisboa, Portugal
[2]Viral Pathogenesis Unit, Instituto de Medicina Molecular , Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Capable of establishing lifelong latency in the host, Herpes viruses are a striking cause of human viral disease. The murid herpesvirus 4 has been widely explored as a model for research on the human gamma-herpesvirus pathogenesis due to its ability to infect mice via the nasal passages and also because it is genetically related to other malignancy-associated pathogens such as the human Epstain-Barr virus (EBV) and Kaposi's Sarcoma herpesvirus (KSHV) [1, 2]. KSHV has an etiologic role in Kaposi´s sarcoma, the leading AIDS malignancy disorder, and in certain lymphomas whilst EBV is also associated with lymphomas and nasopharyngeal carcinoma. As with EBV, MuHV-4 establishes a lifelong latency stage as a multicopy episome in the nucleus of host B-cells and only a small fraction of viral genes are expressed. Amongst this fraction of genes, an ORF protein, ORF73, was demonstrated to promote a deficit on the establishment of latency in vivo when mutant viruses failed to express them [3]. ORF73 is a nuclear protein with remarkable sequence homology with KSHV latency-associated nuclear antigen (LANA) and striking predicted structural homology with the C-terminal domain of the EBV nuclear antigen 1 (EBNA1), mutually crucial for viral latency by tethering the viral episome to host chromosomes, therefore ensuring replication and segregation of the viral genome to daughter cells [4, 5]. C-terminal LANA and EBNA1 were described to bind terminal repeat (TR) DNA of the viral genome upon dimerization, suggesting that C-terminal of ORF73 may also be involved in episome maintenance functions [6, 7]. Additionally, an unconventional SOCS-box motif in the C-terminal of ORF73 was also shown to be involved in the binding of several cellular and viral proteins to modulate their transcription and affect cellular growth through E3-ubiquitin ligase activity [8]. We aim to determine the X-ray crystallographic structure of ORF73 protein, bound and unbound to viral DNA, in order to highlight key residues and structural motifs that may exert functional relevance as therapeutic targets by manipulation of viral latency.

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P5

PROLYL ENDOPEPTIDASE FROM THE BLOOD FLUKE *SCHISTOSOMA MANSONI*

P. Fajtová [1,2], J. Váchová[1], A. Jílková[1], J. Vondrášek[1], J. Dvořák[3], M. Horn[1], M. Mareš[1]

[1]*Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nám. 2, 166 10 Prague, Czech Republic*
[2]*First Faculty of Medicine, Charles University of Prague, Kateřinská 32, 121 08, Prague, Czech Republic*
[3]*Institute of Molecular Genetics AS CR, Vídeňská 1083, 142 20, Prague, Czech Republic*

Prolyl endopeptidase SmPEP from the blood fluke *Schistosoma mansoni* is a potential drug target for the treatment of schistosomiasis. SmPEP was detected in the extract of adult worms by enzyme activity and immunoreactivity. Enzymatically active SmPEP was produced in the *E. coli* expression system and was chromatographically purified. Substrate specificity analysis revealed that SmPEP cleaves peptide substrates by endopeptidase activity, however, macromolecular protein substrates were not fragmented. The residue preferences in the positions P3 to P1´ were determined using synthetic fluorogenic peptide substrates. Specificity of SmPEP was analysed using a 3D model constructed by homology modeling. Furthermore, new specific inhibitors of SmPEP with reactive chloromethyl ketone or aldehyde warhead were developed and tested in an *in vitro* inhibition assay and *in vivo* against *S. mansoni*. A primary screening of crystallization conditions for recombinant SmPEP was performed.

P6

CELLULASE CEL7A - THE MAJOR ENZYME OF THE TREE-KILLING FUNGUS *HETEROBASIDION ANNOSUM*

Majid Haddad Momeni[1], Henrik Hansson[1], Nils Egil Mikkelsen[1], Jesper Svedberg[1], Ake Engström[2], Mats Sandgren[1] and Jerry Stahlberg*[1]

[1]*Department of Molecular Biology, Swedish University of Agricultural Sciences, POB 590, SE-751 24 Uppsala, Sweden*
[2]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden. E-mail: jerry.stahlberg@molbio.slu.se*

Root-rot fungi of the *Heterobasidion annosum sensus lato* (s.l.) complex are the most damaging forest pathogens in the temperate region. They are apparently efficient wood degraders and may posess enzymes useful for lignocellulose processing. The genome of *H. irregulare* strain TC-32-1 has recently been sequenced. In this study the fungus was grown on spruce wood powder as carbon source, and the most abundant protein in the culture broth was unambiguously identified as the only glycoside hydrolase family 7 (GH7) enzyme in the genome. It consists of a single GH7 catalytic module of 440 amino acids, but it lacks a linker and carbohydrate-binding module common to GH7 cellulases [1]. The enzyme, Hir_Cel7A, was purified and crystallised, and the structure was solved by molecular replacement and refined at 1.6 Å resolution.

The structure confirms that Hir_Cel7A is a cellobiohydrolase rather than an endoglucanase, with a cellulose-binding tunnel that appears to be more closed than in *Phanerochaete chrysosporium* Cel7D [2] and more open than that of *Hypocrea jecorina* Cel7A, suggesting intermediate enzymatic properties. To further examine this new GH7 structure, molecular dynamics simulations were conducted for 250 ns to ascertain differences in ligand interactions, loop flexibility, and dynamic correlations between the *H. irregulare*, *H. jecorina*, and *P. chrysosporium* cellobiohydrolases .

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P7

STRUCTURAL AND FUNCTIONAL INVESTIGATIONS ON ENZYMES OF THE VITAMIN B1 METABOLISM OF *STAPHYLOCOCCUS AUREUS*

M. Künz[1], M. Perbandt[1], C. Wrenger[2], Ch. Betzel[1]

[1]Institute of Biochemistry and Molecular Biology, Hamburg, Germany, Laboratory for Structural Biology of Infection and Inflammation, c/o DESY Hamburg, Germany
[2]Department of Parasitology, Institute of Biomedical Science, University of Sao Paulo, Brazil

Staphylococcus aureus is a member of the growing number of multi-drug resistant pathogens which is in addition in many cases responsible for nosocomial acquired infections. It is involved in several severe diseases like pneumonia, fatal wound infections after surgery, endocarditis and septic rthritis [1, 2]. Due to this rising antibiotic resistance, there is an increasing requirement to develop novel antimicrobial substances.

The goal of structure based drug discovery investigations is to analyse the molecular 3D-structure of selected key enzymes of the *de novo* synthesis of Vitamin B1 and to identify potential drug candidates to treat infections of *Staphylococcus aureus.*

Three essential enzymes of this pathway are today in a main focus [1]:

- 4-amino-5 hydroxymethyl-2-methylpyrimidine (HMP) Kinase – *SaThiD*
- Thiamine Monophosphate (TMP) Synthase – *SaThiE*
- TMP dephosphorylating GTPase – *SaGTPase*

Beside structural investigations to support drug design investigations the mode of action of the target proteins will be examined and the directional inward transfer of inactive analogues to block the pathways is under investigation.

In parallel the Pyridoxine Kinase PdxK, a member of the Vitamin B6 cascade of *Staphylococcus aureus,* will be analysed as well in order to validate a joint enzymatic mechanistic [3].

So far the recombinant expression and purification of soluble proteins of the above mentioned enzymes were improved for going towards crystallisation trails and Dynamic Light Scattering experiments were performed to optimize crystallization conditions.

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P8

STRUCTURE AND DYNAMICS OF BACTERIAL S-LAYER PROTEINS

Jun Liu, Dominik Oberthuer and Christian Betzel

Institute of Biochemistry and Molecular Biology, University Hamburg 20146 Hamburg, Germany

The surface layer proteins (S-Layer protein) are the outmost of bacteria as an Armor to protect the bacteria from the external environment, other microorganisms, pressure and other factors, etc. . In my project, I try to characterize and crystallize selected surface layer functional A (SlfA) protein firstly, from *B. sphaericus* strain JG-A12 and JG-B53, respectively, is a typical S-Layer protein which was first isolated by J. Raff and his co-worker from a uranium mining waste pile [1-3]. These two proteins are made of 1228 and 1106 amino acids and has molecular weight approx. 130kDA and 116kDA, respectively. BLAST searching revealed that the highest protein structural sequence homology is only 28% in the protein data bank (PDB). To date, there's no full lenth structure of S-Layer protein was solved in PDB, only H. Jing ect. solved respectively the single domains of surface layer proteins from *Methanosarcina Mazei (M. Mazei)* [1] and predicted the complete structures by arrangement of these domains. It's worth noting that the S-Layer Homology(SLH) domain of the surface array protein(SAP) from *B. Anthracis* by J. Kern ect., recently. Many S-Layer proteins characteristically contain the SLH domains, two proteins which I have is also no exception. Base on that I plan to use the chemical and biological modification of S-Layer Proteins to enhance crystal retrievalllization properties [4]. For structure determination X-Ray crystallography and complementary analytical techniques (e.g. SAXS), furthermore will be applied, to analyse in detail the Structure-Function-Relationships. Finally, searching and designing compounds which inhibit S-layer Formation is the goal of the project, to prevent biofilm formation in pathogens.

So far I have already identified best buffer conditions for the two S-Layer proteins from different strains (JG-B53 and JG-A12) confirmed by a monodispersity of the DLS measurments. Further I performed initial SAXS experiments for S-Layer protein from JG-B53. I will combine the

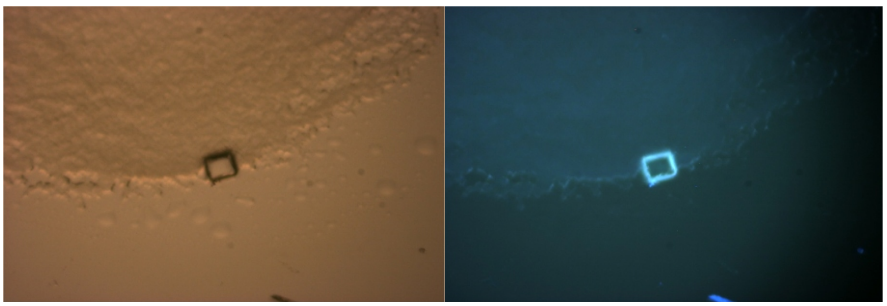

Figure 1. Crystalls of S-Layer protein obatained at 50%MPD, 20% 2-propanal, 50mM sodium chloride.

respective DLS and SAXS measurements to characterize different buffer or ions system which influenze the natural aggregation of these proteins. Further I have obtained already some small crystals for both S-Layer proteins from JG-B53 (Fig. 1) and JG-A12(not display here), however the size need to be improved. For further investigations I plan to truncate the proteins (maybe both) to small fragments to carry out the crystallization screening.

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P9

IDENTIFICATION OF MINIMAL REGULATORY L4-BINDING SITE ON S10-LIKE OPERON IN ARCHAEA

Mikhaylina, A. Sarckih, S. Tishchenko

Institute of Protein Research, RAS, Institutskaya 4, Pushchino, Moscow Region, RUSSIA

In bacteria the expression of many ribosomal (r) protein operons is regulated by a translational feedback mechanism. A well-studied example is the regulation of the S10 operon in *Escherichia coli* by r protein L4. Whereas other regulatory r proteins in *E. coli* work at the level of translation initiation [1], L4 inhibits both transcription and translation [2,3]. Up to now nothing was known about a regulatory function of archaeal r protein L4. In archaea, the transcriptional organization of the S10/spc operon region varies a lot [4]. The transcriptional unit from *Methanococcus jannaschii* ("MjaL3 operon") encodes 5 r proteins (L3, L4, L23, L2, S19) and corresponds to part of the *E. coli* S10 operon.

Recently we have shown that L4 of *M. jannaschii* (MjaL4) specifically inhibits the synthesis of *M. jannaschii* L3 (MjaL3) in a coupled transcription-translation system *in vitro*. As templates we used constructions carrying the MjaL3 gene flanked by different length 5'-untranslated leaders. It was shown that the inhibitory effect of MjaL4 on the syntheses of MjaL3 is fully maintained even when the MjaL3 untranslated leader is shortened to 25 nucleotides. This result indicates that the MjaL4 binding site on the MjaL3 mRNA is located within the first 25 nucleotides upstream of the AUG start codon, but we cannot excluded that it might be (partially) located in the coding sequence of MjaL3.

The main goal of my proposal is to define the minimal L4-binding mRNA fragment which is rather small but still retains the full affinity for MjaL4. This minimal RNA fragment will be required for the investigation of RNA-protein interaction in archaeal L4-mRNA complex and for screening crystallization conditions of complex.

We tried to localize exactly the regulatory MjaL4 minimal binding site on the mRNA by shortening the 5'-part of MjaL3 gene. Using gel-shift assay we tested the affinity of the resulting mRNA fragments for MjaL4. We showed that mRNA fragments carrying the sequences of the truncated versions untranslated leader less than 25-nt did not bind MjaL4. Thus, based of the presented data, we assume that the L4 binding site on the MjaL3 mRNA is located between 25-nt upstream of the AUG start codon and about 40-nt downstream. For the moment we have as a short fragment of MjaL3 mRNA which has the full affinity for MjaL4 carrying the sequence of the 25-nt untranslated leader and 40-nt truncated version of the MjaL3 gene.

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P10

CRYSTALLIZATION AND STRUCTURAL STUDIES OF P1 STALK COMPONENTS OF ARCHAEAL GENUS *METHANOCOCCUS*

I. Mitroshin, O. Kravchenko, S. Nikonov, M. Garber

Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia

In all organisms studied, the large ribosomal subunit contains a highly flexible and functionally important lateral protuberance called the "stalk". The stalk is involved in the formation of the so-called "GTPase-associated site" and plays a key role in the interaction of the ribosome with translation factors and in the control of translation accuracy [1, 2]. Isolated ribosomal proteins of this stalk (L10-L12 in Bacteria, P0-P1 in Archaea and P0-P1/P2 in Eukarya) form in solution a stable complex which binds the large ribosomal RNA through the N-terminal domain of P0 protein (L10 in Bacteria). Archaeal ribosomal proteins P0 and P1 differ from their bacterial counterparts (L10 and L12) but are homologous to the eukaryotic ribosomal proteins P0 and P1/P2 [3].

The definition of the crystal structure of the stalk in the 50S ribosomal subunit and in the 70S ribosome from Archaea with high resolution is impeded by the great flexibility of this ribosomal protuberance. Efforts to solve the structures of the isolated components of the stalk were more successful. In 2010, the high-resolution structures were determined for the complex of P0 with the N-terminal domains of P1 from the archaeon *Pyrococcus horikoshii* [4] and for the two-domain N-terminal fragment of P0 from the archaeon *Methanococcus jannaschii* (the so-called MjaP0NTF) [5].

Our research is now focusing on the investigation of the P0 stalk of the archaeal genus *Methanococcus*. After determination of the structure of the MjaP0NTF protein [5] we could crystallize a complex of this fragment with a specific fragment of the 23S rRNA. The diffraction data set was collected at 3.2 Å resolution, however, our attempts to obtain better diffracting crystals of the complex were so far unsuccessful. Simultaneously we are searching conditions for crystallization of the full-length P0-P1 protein complex from the archaeon *M. thermolithotrophicus*. Due to the high level of flexibility of the protein P1 crystallization of such a complex is a difficult task. The crystal structures of the RNA-protein complex and of the P0-P1 complex undoubtedly will allow to refine the structure of the archaeal ribosome and to determine interactions between P0 (L10) protein and 23S rRNA in detail.

This work was supported by the Russian Academy of Sciences and the Russian Foundation for basic Research (No. 11-04-00327-a), grant of the Program for Molecular and Cellular Biology RAS.

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P11

A NEW RELATIVE OF THE DOUBLE BETA-BARREL LINEAGE: COAT PROTEIN STRUCTURES OF THE EXTREME THERMOPHILIC BACTERIOPHAGE P23-77

A. Pawlowski[1], I. Rissanen[1], J. M. Grimes[2,3], K. Harlos[2], D. I. Stuart[2,3], and J. K.H. Bamford[1]

[1]Department of Biological and Environmental Science, University of Jyväskylä, 40014 Jyväskylä, Finland
[2]Division of Structural Biology, Welcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, England
[3]Science Division, Diamond Light Source Ltd, Diamond House, Harwell Science and Innovation Campus, Didcot OX11 0DE, England

Analysis of viral structural elements such as major capsid proteins (MCPs) revealed highly conserved protein folds that can be used to group viruses into a reasonably small number of lineages [1]. The main characteristic of one of these lineages is a MCP fold that contains a vertical double beta-barrel. Members of the lineage infect hosts from all three domains of life suggesting that they have a common ancestor existing even before the separation of the current domains of life.

Recently, viruses from extreme environments have been discovered that appear to utilize unusual versions of the double beta-barrel fold. One of these viruses - P23-77, an icosahedral dsDNA bacteriophage infecting *Thermus thermophilus* – has two MCPs that are present in the capsid in approximately equal proportions. Both coat proteins were successfully expressed in *E. coli*, purified and crystallized [2]. The three dimensional structures of the isolated two MCPs and of a complex of the two - which represents an assembly intermediate - were solved. Both proteins contain a single beta-barrel fold and comparison with the EM reconstruction of the virion revealed a novel capsid organization completely different from the assembly mechanism of the double beta-barrel viruses.

The homology of the coat protein fold classifies thermophilic bacteriophage P23-77 as archetype of a new branch of the ubiquitous double-beta barrel lineage. The novel lineage comprises dsDNA viruses that use various building blocks of two MCPs with vertical single beta-barrel fold for capsid assembly.

Our findings emphasize the importance of high resolution structural analysis for the high-order classification of viruses and identification of ancient viral branches, linked to the most primordial prokaryotic populations and perhaps to the last universal common ancestor of cells.

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P12

STRUCTURAL CHARACTERIZATION OF THE DNA UPTAKE MACHINERY IN *STREPTOCOCCUS PNEUMONIAE*

Simone Pieretti, Albert Canals, Miquel Coll

Institute for Research in Biomedicine (IRB Barcelona), Parc Científic de Barcelona, C/ Baldiri Reixac 10, 08028 Barcelona, Spain

Horizontal gene transfer (HGT) [1] is a rapid evolutive mechanism by which entire genes are transferred among bacterial cells, thus enabling an almost immediate adaptation to new environmental conditions. HGT has obvious implications for human health as it is used by pathogenic microorganisms –even among different species– during the spread of virulence factors and antibiotic resistance. Three main HGT routes can be distinguished: conjugation (plasmid transfer through the direct interaction of two bacterial cells), transduction (bacteriophage-mediated DNA transfer) and transformation (uptake of naked DNA from the environment). Among them, natural transformation [2] [3] refers to the transport of environmental DNA to the cytosolic compartment, where it can meet several fates. If the incoming DNA is complementary to the resident chromosome, it can recombine; if it contains the information needed for independent replication, it can do so as a plasmid or a bacteriophage.

In this project we focus on the structural characterization of three proteins: CelA, CelB, ComFA in order to elucidate the molecular mechanism of bacterial transformation in *Streptococcus pneumoniae*, which is one of the most studied models for grampositive bacteria. In our laboratory, several constructs of these proteins were cloned using a high-throughput approach after amplification and purification of the relative genes. Taking advan-

tage of this starting point, we successfully expressed *S. p* CelA in soluble form in *E. coli* and we purified it to near homogeneity. Afterwards, we set up drops on 96-well plates by using commercial screenings at high protein concentration. In some conditions, we got some micro-crystals that were optimized playing with different pH and PEGs concentrations. This allowed us to obtain some needled-shape crystals. Unfortunately, these crystals have not been reproduced in bigger plates and they have not given any diffraction pattern by X-ray analysis.

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P13

STRUCTURAL CHARACTERIZATION OF A NOVEL EUKARYOTIC FAMILY OF PROTEINACEOUS RNASE P

Franziska Pinker[1, 2], Antony Gobert[2], Olivier Fuchsbauer[1], Philippe Giege[2], Claude Sauter[1]

[1]UPR 9002, CNRS, IBMC, Universite de Strasbourg, 15 rue Rene Descartes, 67084 Strasbourg, France
[2]UPR 2357, CNRS, IBMP, Universite de Strasbourg, 12 rue du General Zimmer, 67084 Strasbourg, France

The RNase P activity is ubiquitous and consists of the 5' maturation of pre-tRNAs. For a long time it has been thought that all known RNase P were ribozymes. However, the characterization of the human mitochondrial RNase P revealed a novel kind of RNase P composed of proteins only, called PRORP for • "Proteinaceous RNase P" [1]. Whereas in human mitochondria PRORP is formed by a complex of three subunits, RNase P activity in *Arabidopsis thaliana* is held by a single protein. There are three homologs in *A. thaliana*: PRORP1 in mitochondria and chloroplasts, PRORP2 and PRORP3 located in the nucleus. Each protein possesses a pentatricopeptide repeat domain (PPR) and a metallonuclease domain [2].

The aim of our project is to understand the recognition and cleavage of pre-tRNA molecules by PRORPs and to see if these proteins mimic the conventional ribonucleic RNase P or if they have evolved their own catalytic pathway. The His-tagged PRORPs are expressed in *E.coli* and purified using affinity and size exclusion chromatography. The sample purity and homogeneity is systematically verified by dynamic light scattering. Crystallization condition were searched using various commercial screens. Out of about 960 conditions, two promising hits were obtained by vapour diffusion. They were confirmed in batch and thin plate-like crystals led to preliminary diffraction data at 7 Å resolution. The optimization of crystal growth conditions and diffraction properties is in progress.

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P14

EXPRESSION AND PURIFICATION OF RECOMBINANT HCA7 FOR STRUCTURAL STUDIES WITH SELECTIVE INHIBITORS

K. Poncová[1], P. Mader[2], I. Sieglová[1,2], M. Fábry[2], and P. Řezáčová[1,2]

[1]Department of Structural Biology, Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
[2]Department of Structural Biology, Institute of Molecular Genetics of the ASCR, v. v. i.,Vídeňská 1083, 142 20 Prague, Czech Republic

The carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous zinc metalloenzymes present in prokaryotes and eukaryotes encoded by three distinct evolutionarily unrelated gene families: -CAs -CAs and -CAs. Human carbonic anhydrases belong to the class. These enzymes play a crucial role in many physiological functions as they catalyse the conversion between CO_2 and bicarbonate [1]. In humans, 15 CAs have been identified so far. They differ in subcellular localization, tissue distribution and also in kinetic parameters.

Human CA VII is an intracellular (cytosolic) isoform mainly expressed in the brain tissues such as cortex, hippocampus and thalamus where its activity is involved in generating neuronal excitation and seizures. CAVII is thus considered to be an important target for development of anticonvulsant agents and neuropathic pain killers [2].

Recently, a series of arylsulfonamides with significant inhibitory effect and selectivity toward CAVII has been reported [3]. To analyze inhibitor binding on structural level, we initiated structural studies.

The plasmid pNIC-28-Bsa4 containing the coding sequence of His-tagged CA VII under control of T7 promoter was obtained from Structural Genomics Consortium (SGC, Oxford). On the bases of previous structural studies of hCA VII [2] two cystein residues on the positions 183 and 217 were mutated to serines. The resulting gene was used for recombinant expression in *E.coli* BL21 (DE3) and the protein was further purified from cytosolic fraction using affinity Ni-column followed by gel filtration.

Screening for crystallization condition was performed with enzyme concentration 7 mg/ml and 1.1 molar excess of inhibitors. Successful conditions yielding initial crystals are in process of optimization now.

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P15

CO-CRYSTALLIZATION OF XANTINE OXIDOREDUCTASE WITH PURINE INHIBITORS

Šmídková Markéta

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague
smidkova@uochb.cas.cz

Xanthine oxidoreductase (XOR) is a key enzyme of purine catabolism, which is involved in the pathogenesis of hyperuricemia, gout, and oxidative stress-related cardiovascular diseases. Currently, allopurinol is a drug of the first choice in the treatment or prevention of these conditions. Although effective in many patients, some of them experience sensitivity to allopurinol, which is attributed to the fact that this drug is phosphoribosylated to the corresponding nucleotide [1]. Therefore, the search for new XOR inhibitors continues.

In our project, we investigate three different structural types of purine derivatives for their ability to inhibit XOR, cytotoxicity and degree of their phosphoribosylation. Moreover, their structure-activity relationships are also in the scope of our research.

Co-crystallization of XOR with inhibitors and X-ray analysis serve us for this purpose. Crystallization was carried out with commertially available XOR (Sigma-Aldrich, cat. no. X4500-100UN), which was purified into homogenity by affinity chromatography with folic acid. The attempt to repeat published the crystallization conditions [2] resulted in formation of pyrophosphate crystals. Therefore, pyrophosphate buffer was replaced with MES buffer. After preliminary screening of the crystallization conditions using JCSG kit, co-crystallization of XOR with all three inhibitors resulted in the growth of crystals by the help of the batch method and precipitant solution containing 0.1M HEPES (pH 6.5) and 10% PEG6000. However, crystals were small and they did not diffract well. It probably was the consequence of the fast growth of crystals. Currently, the optimization of the crystallization is in the process. In the first step, 30% glycerol was added into crystallization buffer in order to decelerate the speed of the crystals growth.

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Supported by the Research project of the IOCB #RVO: 61388963

P16

HELICASE DOMAIN OF GP43 PROTEIN FROM CORYNEPHAGE BFK20

Šoltészová Barbora, Halgašová Nora, Bukovská Gabriela

Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovak Republic, e-mail: barbora.solteszova@savba.sk

This research is focused on study of predicted helicase domain located on C terminus of gp43 protein, the member of the replication cassette of corynephage BFK20. By bioinformatics' analysis the gp43 was characterized as RepA like protein with the length of 978 amino acids. We have revealed the presence of primase-polymerase domain on N terminal region and the conservative F4 type helicase domain on C terminal region of gp43 protein. Recently we were successful with overexpression and isolation of gp43 protein as well as with the overexpression and purification of gp43N containing the primase/polymerase domain. Subsequently we decided to clone and express the helicase domain.

We have used two strategies for amplification of the helicase domain of *orf43* which have resulted in two PCR products named as gp43hel (52 kDa) and gp43helB (20 kDa). We have cloned the fragments into the expression vector pET28a+. The recombinant proteins in fusion with His-Tag at the C terminus of gp43hel and gp43helB were expressed in the host *Escherichia coli* BL21(DE3). We tested optimal conditions for protein expression (culture medium, temperature, IPTG, time of expression) to prepare soluble proteins. However, the expression of both recombinant proteins was not detectable. We have assumed the probable toxicity of this separate helicase domain. The expression of toxic proteins could be achieved by inoculation of the cell culture directly from the plate and lowering the temperature of protein induction and IPTG concentration. The leaking of uninduced protein in the overnight culture could be avoided by adding 1% of glucose to the LB medium. The amount of expressed gp43hel and gp43helB under different conditions will be determined by Western imunoblotting using His-tag antibodies.

This work was supported by VEGA grant 2/0110/11 from Slovak Academy of Sciences and APVV-0098-10 grant from Slovak Research and Development Agency.

P17

INTERACTION OF PLANT DEFENSE PROTEIN WITH INCEST DIGESTIVE ASPARTIC PEPTIDASE

J. Srp[1,2], M. Nussbaumerová[1], P. Řezáčová[1], I. Žebrakovská[1,2], P. Pachl[1], M. Šanda[1], M. Štajfová[1], M. Horn[1], M. Mareš[1]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 16610 Prague, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University, Albertov 6, 12843 Prague, Czech Republic

Potato leaves produce a wound-inducible 22 kDa protein PDI (Potato Cathepsin-D Inhibitor), which functions as a defense molecule directed against the herbivorous pest Colorado potato beetle (CPB), *Leptinotarsa decemlineata*. Our work is focused on the interaction of PDI with its target, a digestive cathepsin D-like protease LdCD located in the gut of CPB. For proteomic identification of LdCD, a selective activity-based probe was designed and synthesized, which specifically interacts with the LdCD active site. The labeled LdCD was visualized on SDS-PAGE and characterized by N-terminal microsequencing and mass spectrometry. Based on these data the cDNA sequence of LdCD was determined, and LdCD was cloned and produced as a recombinant protein in the *Pichia pastoris* expression system. LdCD was biochemically characterized and demonstrated to be specifically inhibited by PDI. Recently, we crystalized LdCD and solved its crystal structure at a resolutio of 1.95 Å. Crystallization of the LdCD-PDI complex is currently in progress.

P18

FIC PROTEINS AMPYLATE A BACTERIAL TARGET: CRYSTALLIZATION TRIALS OF THE FIC/ATP/TARGET COMPLEX

Frédéric V. Stanger[1,2*], Alexander Harms[2], Arnaud Goepfert[1,2], Christoph Dehio[2], Tilman Schirmer[1]

[1]Core Program Structural Biology and Biophysics, [2]Focal Area Infection Biology, Biozentrum, University of Basel
**frederic.stanger@unibas.ch*

The ubiquitous FIC domain present in almost 3000 proteins has recently been shown to catalyze a post-translational modification described as adenylylation (also called AMPylation), i.e. transfer of an AMP moiety from ATP onto specific target proteins. Translocation of the bacterial FIC effector proteins IbpA or VopS into human cells results in massive adenylylation of Rho-family GTPases, which disrupts interaction with their downstream effectors and thus causes collapse of the actin cytoskeleton and cell death [1].

Our group has recently found that FIC proteins are tightly regulated by an inhibition motif [2]. Mutation or deletion of this inhibition motif allowed us to find that FIC proteins also AMPylate a bacterial target (called X, unpublished data). The X protein, a 90 kDa protein, contains several domains. The AMPylation site has been mapped by mass spectrometry and confirmed by mutation of the residue.

The strategy used to produce and purify this protein is to tailor the construct. Constructs harbouring the AMPylated domain were over-expressed and purified (ei-

ther with an N-terminal or C-terminal HisTag). Pure protein was applied to a Thermal Shift Assay for buffer screening. The optimal buffer was used as size exclusion chromatography buffer for further experiments. An AMPylation assay, i.e. incubation of pure FIC protein and pure target protein with radioactive ATP (-^{32}P-ATP), has shown that tailored constructs of the X protein are AMPylated by FIC proteins. Binding assays by size exclusion chromatography (SEC) are currently under investigation. Crystallization trials using a 1:1 molar ratio of the partners are also on-going. I here show the preliminary results on my project investigating the FIC / bacterial target complex on the structural level.

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P19

STRUCTURAL STUDIES ON TRANSPORTIN-SR2, A HIV-1 INTEGRASE COFACTOR INVOLVED IN NUCLEAR IMPORT

Oliver Taltynov[1], Jonas Demeulemeester[1], Stéphanie De Houwer[1], Frauke Christ[1], Stephen Weeks[2], Sergei V. Strelkov[2] and Zeger Debyser[1]

[1]Laboratory of Molecular Virology and Gene Therapy, KU Leuven, Leuven, Flanders
[2]Laboratory for Biocrystallography, KU Leuven, Leuven, Flanders

To accomplish its life cycle human immunodeficiency virus type 1 (HIV-1) has to integrate its genome to the host chromosome. The key player in the integration process is viral enzyme integrase (IN). The karyopherin Transportin-SR2 (TRN-SR2) is cellular cofactor of HIV and a HIV-1 IN binding partner. We have demonstrated earlier that TRN-SR2 is involved in nuclear import of HIV-1 [1]. As the nuclear import is a bottleneck step for HIV-1 during infection of the cell, HIV-1 IN-TRN-SR2 interaction is an interesting potential target for development of novel anti-HIV drugs [2]. We used several biochemical and structural approaches to characterize TRN-SR2 and its function as an HIV-1 IN binding partner as well. Combination of several parallel approaches - X-ray crystallography, small angle X-ray scattering (SAXS), analytical size-exclusion chromatography and protein modeling allowed us to characterize TRN-SR2 from structural point of view. TRN-SR2 is highly flexible protein able to adopt different conformational states depending on particular binding partner. We characterized this flexibility by physical and hydrodynamical parameters.

Binding ability of TRN-SR2 for its cargos is regulated by interaction with Ran protein and the nucleotide state of this GTPase (RanGTP/GDP). We demonstrate dramatic conformational changes of TRN-SR2 in unbound state, in the complex with HIV-1 IN truncated core-C-terminal 2-domain mutant, and with RanQ69L.GTP. With the Ran protein TRN-SR2 adopts closer conformation than in the unbound state. The complex with the HIV-1 IN 2-domain mutant is a proof that HIV-1 IN N-terminal domain is not required for the complex between TRN-SR2 and HIV-1 IN.

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