Posters - Crystallization in Industry and Biomedicine

P13-1

CRYSTALLIZATION OF MOLECULAR CHAPERONE ESSENTIAL FOR PROTEIN DISAGGREGATION

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The process in which a newly synthesized polypeptide chain transforms itself into a perfectly folded protein depends both on the properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu. Uncovering the mechanism of protein folding and unfolding is one of the grand challenges of modern science. The three-dimensional arrangement of the polypeptide chain decide about the specific biological function of the protein in the cell. Only correctly folded proteins are fully functional, randomly arranged polypeptide chain doesn't have biological activity. The state of protein folding is controlled and regulated by the protein quality control system. The system is formed by chaperones involved in protein folding and the proteasomal degradation system. The proper functioning of the system is required because its dysfunction may lead to neurodegenerative diseases. The prion-related illnesses such as Creutzfeldt-Jakob disease, amyloid-related illnesses such as Alzheimer's disease as well as intracytoplasmic aggregation diseases such as Huntington's and Parkinson's disease those are neurodegenerative diseases whose pathogenesis is associated with protein aggregation of incorrectly folded proteins.

Many chaperones are heat shock proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress conditions. The project focuses on the protein Hsp104 which belongs to the Hsp100 family and the AAA+ superfamily. Hsp104 is important in the cell due to its ability to solubilize and refold proteins trapped in aggregates formed during heat stress [1]. It achieves this in cooperation with the Hsp70 chaperone system. The active form of the protein is a ring-shaped hexamer, which is thought to drive protein disaggregation by directly translocating substrates through its central channel. However, there is still no general consensus regarding the domain organization within the hexameric molecular machine. Substantial efforts have been made to elucidate the location of domain M, but the results are contradictory [2, 3]. We aim to learn the location and the orientation of the unique M domain by solving the crystal structure of Hsp104 using X-ray crystallography.

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P13-2

STRATEGIES FOR RECOMBINANT EXPRESSION OF DIFFICULT TARGETS FOR PROTEIN CRYSTALLOGRAPHY: piggyBac DNA TRANSPOSASE DEPLOYED

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As protein crystals are at the bases of protein crystallography, the sufficient amounts of pure protein are at the beginning of such crystals growth. And even though astrophysicists are today theorizing about the negative energy of empty space and implicating that whole universes could arise from nothing [1, 2], modern era of structural biology is still depending on heterologous recombinant protein expression as a primary source of well folded proteins.Thus although sometimes quite costly enterprise, as a primary source of material for biomedical applications and research, it is a necessary one. Unfortunately, there is no single recipe for expression of all protein constructs that would guarantee high yields. While some proteins are quite easily expressed in prokaryotic cells (either in soluble or

renaturable insoluble state), for many we need more „sophisticated" approaches and some we label as the „difficult targets ". Moreover, as the "easy targets" ale less abundant in these days the strategies for more "sophisticated" approaches are more and more in demand.

Here, on a *klr* and *clec* gene families [3] – a families of lymphocyte receptors that deserve the label of "difficult targets", we would like to showcase our efforts and different expression strategies that we employed in overcoming such difficulties. Our first efforts to study the ectodomains of these proteins on a structural level via recombinant expression in *E. coli* have shown that many aggregate to inclusion bodies and their refolding is inefficient. Although some perform well in transiently transfected human embryonic kidney 293 (HEK 293) cells, in some cases, an addition (restoring disulphide bond) or removal of cysteine residue is necessary for high yield of well folded product. For optimization of expression construct of *klrb1* we have used a high-throughput approach – combining 26 different constructs with 8 different affinity tags and tested the for expression both in prokaryotic, insect and mammalian cells without major yield improvement.

Finally, as a tool of last resort, we have tested stably transfected pools of HEK293S GnTI$^-$ [4] cells with either stochastic or controlled integration of expression cassette into the host genome. While the randomly integrated stable cell pools provided and excess of 10-fold improvement yield-wise, the TetOn inducible expression system employing the controlled integration via the piggyBac DNA transposase [5] is performing even better with roughly 20-fold improvement in yield, compared to transient transfection of HEK293S GnTI$^-$ cells.

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P13-3

XRAYLAB: A NOVEL X-RAY DIFFRACTION FACILITY FOR THE ISS

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The XRayLab is a novel X-ray diffraction (XRD) facility for the International Space Station (ISS) being developed by Space Applications Services, with the support [1] of the European Union (EU), to be used by industries, scientific and educational organizations, either on a commercial base or in partnership with national and international space agencies.

The primary function of the XRayLab is to perform in situ XRD measurements and this technique can be used for a wide range of applications, and in particular for protein crystallography in space.

The technological core of the XRayLab is an XRD instrument and an exchangeable sample dispenser/container (so-called 'XRayLab Card').

The in-orbit operations of the XRayLab will not rely on the astronauts (except for the Card exchanges). They will be monitored from ground and/or automatized to the maximum possible extent.

The objectives of this poster are to present the main characteristics of the XRayLab, and to stimulate scientists and industry to propose microgravity experiments to be performed using this microgravity facility.

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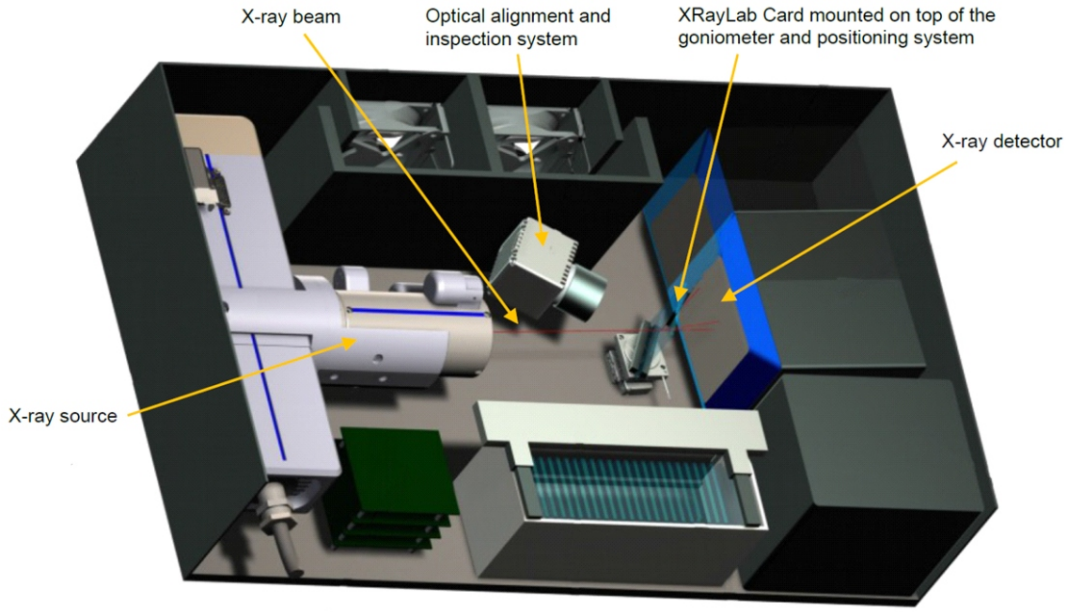

Figure 1. CAD rendering of the XRayLab facility

P13-4

STRUCTURAL INVESTIGATIONS OF THE INTERLEUKIN-5 RECEPTOR ECTODOMAIN

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Interleukin-5 (IL-5) is a hematopoietic cytokine produced mainly by T-lymphocytes controlling a multitude of functions in the immune system. It is mainly known as a key regulator of eosinophilic granulocytes (eosinophils). Here IL-5 controls almost any aspect of the eosinophil's life, from differentiation and maturation of eosinophil progenitor cells, migration into target tissues, to their proliferation and activation. Eosinophils are a part of the immune response for the defence of antigens and are usually activated during helminth infection, allergic diseases and asthma. Upon their activation pro-inflammatory mediators such as leukotriene, histamine and reactive oxygen species are released in a process called degranulation thereby leading to the known allergy symptoms. Signalling of IL-5 occurs in a sequential receptor binding mechanism. IL-5 binds first to its specific chain the IL-5 receptor (IL-5R) and then recruits the common beta chain (c, CD131) to form a ternary complex mainly activating the JAK/STAT signalling cascade. As IL-5 is the key regulator of eosinophils the cytokine has become a highly interesting target for pharmaceutical intervention. The molecular mechanism of IL-5 receptor activation is not fully known yet. We have previously determined the structure of IL-5 bound to the ectodomain of the IL-5 receptor IL-5R (binary complex) by crystallography [1], which provides insights into the first step of receptor activation. From a structure-function analysis we found hints that the novel wrench-like architecture of the IL-5 receptor is possibly preformed (Fig. 1). This notion has great impact for the design of small molecule-based IL-5 inhibitors as such molecules will target the structure of the unbound IL-5R , which must be known to successfully address the receptor. Our goal is therefore to

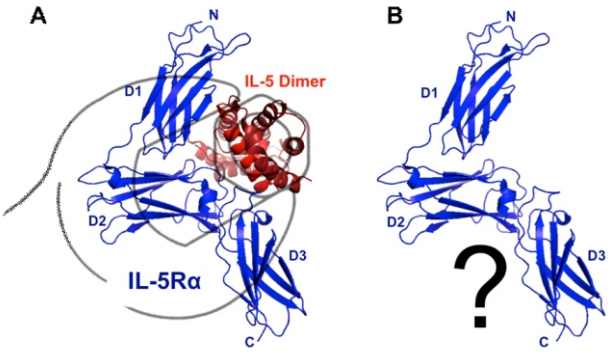

Figure 1. (A) The wrench-like architecture of the IL-5R ectodomain in complex with IL-5 [1]. (B) Possible fixed architecture of IL-5R ectodomain when not bound to the IL-5 ligand. (PDB: 3QT2)

determine the structure of the IL-5R ectodomain in its unbound conformation. We were able to optimize 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 IL-5R for structure determination by X-ray diffraction. First crystals have been obtained and optimization is in progress (Fig.2).

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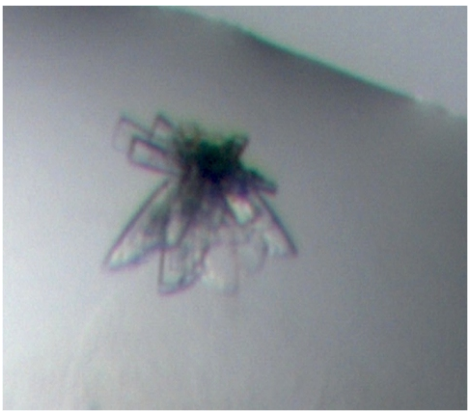

Figure 2. Cluster of crystals for IL-5R ectodomain protein in its unbound conformation.

P13-5

STRUCTURAL INSIGHTS INTO THE DUAL STRATEGY OF RECOGNITION BY PEPTIDOGLYCAN RECOGNITION PROTEIN: STRUCTURE OF THE TERNARY COMPLEX OF PGRP-S WITH LIPOPOLYSACCHARIDE AND STEARIC ACID

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Peptidoglycan recognition proteins (PGRPs) are part of the innate immune system. The 19 kDa Short PGRP (PGRP-S) is one of the four mammalian PGRPs. The concentration of PGRP-S in camel (CPGRP-S) has been shown to increase considerably during mastitis. The structure of CPGRP-S consists of four protein molecules designated as A, B, C and D forming stable intermolecular contacts, A-B and C-D. The A-B and C-D interfaces are located on the two opposite sides of the same monomer resulting in the formation of a linear chain with alternating A-B and C-D contacts. Two ligand binding sites, one at C-D contact and another at A-B contact have been observed. CPGRP-S binds to the components of bacterial cell wall molecules such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), and peptidoglycan (PGN) from both Gram-positive and Gram-negative bacteria. It also binds to fatty acids including mycolic acid of the *Mycobacterium tuberculosis* (*Mtb*). Previous structural studies of binary complexes of CPGRP-S with LPS and stearic acid (SA) have shown that LPS binds to CPGRP-S at C-D contact (Site-1) while SA binds to it at the A-B contact (Site-2). It shows that LPS and SA also bind simultaneously at sites, Site-1 and Site-2 respectively. In the structure of the ternary complex, LPS interacts with CPGRP-S through 13 hydrogen bonds and 159 van der Waals contacts (distances 4.2 Å) while SA forms 56 van der Waals contacts. The binding studies using surface plasmon resonance (SPR) have revealed that LPS bound to CPGRP-S in the presence of SA while SA interacts with CPGRP-S in the presence of LPS. The ELISA studies have shown that increased levels of productions of pro-inflammatory cytokines TNF- and IFN- due to LPS and SA reduced considerably upon adding CPGRP-S.

P13-6

CRYSTAL STRUCTURE OF INORGANIC PYROPHOSPHATASE FROM *ACINETOBACTER BAUMANNII*

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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 multi-drug-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 $P222_1$, and the structure was determined to 2.1C resolution. The structure was solved by molecular replacement using *E. coli* IPPase as a model.

P13-7

CRYSTALLIZATION AND DETERMINATION OF THE CRYSTAL STRUCTURE OF ANGIOPOIETIN-RELATED PROTEIN 4 FROM HUMAN

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Angiopoietin-related protein 4 (Angptl4) belongs to a family of eight proteins (Angptls) structurally similar to angiopoietins. They all possess an N-terminal coiled-coil domain, a disordered linker and a C-terminal fibrinogen-like domain. Angptl4 is involved in a variety of cellular processes, including angiogenesis and energy metabolism. Angptl4 plays a role in the lipid metabolism by inhibiting lipoprotein lipase. Recently, a C-terminal fibrinogen-like domain of Angptl4 was shown to interact and activate integrins ß1 and ß5 and their cognate extracellular matrix proteins to facilitate wound healing and enhance re-epithelialization. To clarify the role of Angptl4 in lipid metabolism and wound healing and identify protein determinants involved in interactions we determined the crystal structure of a fibrinogen-like domain of Angptl4 from human. The protein was produced using a system for the efficient production of disulfide bond containing proteins in the cytoplasm of *E. coli*, known as CyDisCo. A single crystal appeared in a crystallization screen and yielded in a 2.6 Å X-ray diffraction dataset. The crystal structure was solved by molecular replacement. Electrostatic surface potential analysis revealed the presence of an extensive positively charged patch on the exposed surface of one of the subdomains which is likely involved in binding of a ligand or interaction partner.