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Crystallization of a giant photosynthetic antenna complex – the phycobilisome

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The photosynthetic process is initiated by the absorption of light energy by pigment-protein complexes called light harvesting antennas that transfer the absorbed energy to the photochemical reaction centers with a quantum efficiency of near unity. In cyanobacteria and red algae the major antenna is called the Phycobilisome (PBS) which is one of the most efficient antenna systems that exists in nature. The PBS is an extremely large complex, with a molecular weight of 3-7MDa which is made up of pigmented proteins known as phycobiliproteins and unpigmented proteins known as linker proteins. Our research goal is to obtain high resolution crystal structures of the entire PBS complex and the two major PBS sub-complexes, (rods and cores), isolated from the cyanobacterium *Thermosynechococcus vulcanus* (*T. vulcanus*) and the novel *Acaryochloris marina* (*A. marina*) with its simplest of all PBS.

Intact PBS from *T.vulcanus* and *A .marina* were isolated in high phosphate buffer. The PBS from both organisms was crystallized in the presence of high phosphate concentration and special additives that mimicked the crowding of the PBS in vivo and provided suitable conditions for crystallization. *T. vulcanus* PBS Crystals and protein obtained from the dissolution of extensively washed crystals were analyzed by fluorescence spectroscopy, SDS-PAGE, mass spectrometry and confocal microscopy. The results of these experiments indicate that the crystals contain intact, functional PBS complex. Diffraction experiments have showed that the present crystals diffract to a resolution of ~ 3.5Å. In addition, preliminary TEM images indicate that the isolated *T. vulcanus* PBS forms dimers. This leads to a novel suggested model for the PBS arrangement in the crystal. *A. marina* PBS crystals were also analyzed by confocal microscopy. By utilizing the confocal microscope, the emission spectra of a single crystal at all different positions and depths were identical and presented an intact and functional PBS. Currently, additional experiments are being held in order to obtain well diffracting crystals.

In addition, during the process of *T. vulcanus* PBS isolation, fractions of the two subcomplexes (rods and core) were also obtained, and successfully crystallized. Large blue rod crystals, containing phycocyanin (one of the PBPs proteins located in the rod) and the three rod linker proteins were obtained. The structure was determined by molecular replacement to a resolution of 1.5 Å, with a monomer in the asymmetric unit [1]. Core crystals have also been obtained, however they require further improvement in order to perform structure determination.

[1] L. David at el, *J. Mol. Biol.* **2011** *405,* 201–213.

Keywords: photosynthesis, cyanobacteria, macromolecular complexes.

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Crystal direct : A new system for automated crystal harvesting

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The field of structural biology has gone through a revolution brought about by the systematic introduction of automation. The traditionally manual operations of identifying soluble protein fragments that can be produced in large amounts and the screening for crystallization conditions have been progressively replaced by high throughput approaches. Today these steps often occur at dedicated, fully-automated facilities that offer screening services to a number of research teams. Accompanying these developments, X-ray synchrotron beamlines have been progressively automated with the introduction of the sample changers and other automated systems. Despite this level of automation, crystallization and data collection remain poorly integrated and remains a bottleneck for very challenging projects, like those involving the study of membrane proteins or multi-protein complexes, that require the systematic evaluation of diffraction quality of large number of crystals. This is primarily due to the fact that recovering crystals from crystallization media and mounting them on the supports used for data collection still remains a manual, low-throughput operation.

We have developed a new method for protein crystallization and crystal recovery. This system is based on a new crystallization support directly compatible with data collection and that can be excised automatically by photo ablation to recover the crystalline material. The system is compatible with standard cryoprotection and other sample manipulations allowing X-ray diffraction measurements both at room temperature and under cryocooling conditions. Among the advantages of this new approach is the absence of mechanical stress for the crystals during the mounting process and that it facilitates handling of microcrystals, which can be challenging with standard methods. Moreover, this system is designed to enable full automation of the crystal mounting process allowing the operational integration of automated crystallization and data collection units. The Crystal Direct system could contribute significantly to the advancement of very challenging projects in structural biology.

Keywords: crystallization, automation, crystal mounting

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Humidity control can compensate the damage induced in protein crystals by alien solvents.

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The notion and observation that the diffraction pattern of protein crystals changes with the humidity of the media surrounding the crystals dates back to the first diffraction of protein crystals by Crowfoot and Bernal [1], and was a crucial insight for the development of the field. In recent years, the availability of devices that permit a fine control of the relative humidity of the crystals (Free Mounting Systems, FMS or Humidity Control, HC) [2], [3] has made it possible to improve the resolution of protein crystals whose diffraction properties were suboptimal. The current status of these developments in macromolecular crystallography have been reviewed recently [4], [5].

Critical to any Structure-Based Drug Design (SBDD) effort and more so for Fragment-Based approaches (FBDD) is the availability of large numbers of target:ligand (target:fragment) complexes that can be used to validate the initial 'hits' or to optimize valuable lead compounds by medicinal chemistry efforts. Yet, it is a common observation that good-diffracting protein crystals deteriorate significantly and often also rapidly, upon soaking with concentrated solutions of the fragment or ligand compounds, typically dissolved in dimethyl-sulfoxide (DMSO).

In addition, Fragment-Based approaches for drug discovery and even conventional SBDD protocols quite often encounter difficulties in introducing ligands either by soaking or co-crystallization of low affinity compounds. Often, this is because the active sites of the targets of interests are ocuppied by salts, additives or other chemicals that preclude the succesful crystallization/soaking of target:ligand complexes.

We have explored the use of relative humidity control of protein crystals to overcome some of these issues. We have used crystals of PurE (EC.4.1.1.21), an enzyme from the purine biosynthesis pathway of *B. anthracis* as a test case. Our findings can be summarized as follows: i) using humidity control, it is possible to improve/optimize the diffraction quality of crystals soaked with ligands/inhibitors; ii) optimization of the relative humidity can compensate for the deterioration of the diffraction pattern that is observed upon desalting crystals grown in high salt; iii) combining de-salting protocols with PEG addition it is possible to achieve very high concentrations of weak ligands (5-10 mM range) in soaking solutions; and iv) fine control of the relative humidity of the crystals soaked in these solutions can compensate for the deterioration of crystal diffraction and restore 'high resolution' diffraction for SBDD and FBDD.

[1] J.D. Bernal, D.C. Crowfoot, *Nature* **1934,** *794*, 133-134. [2] Kiefersauer *et al. . J. Appl. Cryst.* **2000,** *33*, 1223-1230. [3] Sanchez-Weatherby *et al. Acta Cryst.* **2009,** *D65*, 1237-1246. [4] Böttcher *et al. Methods in Enzymology* **2011,** *493*, 61-89, [5] Russi *et al.* **2011** *J. Struct. Biol.* In press.

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Structural studies of an endotoxin biosynthesis enzyme from *neisseria meningitidis*

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Neisseria meningitidis is the causative agent of meningitis and septic shock. Septic shock results from the recognition of the neisserial endotoxin by the receptor complex, MD-2/TLR-4, that leads to high level induction of the pro-inflammatory cytokines. Endotoxin is a glycolipid that binds MD-2 via the lipid portion, lipid A, which can be modified with phosphoethanolamine (PEA).

The transfer of PEA to the lipid A is catalysed by the enzyme NmLptA. The addition of the PEA groups to lipid A appear to play important roles in moderating the ability of the bacterium to attach to host cells and increases resistance. Lastly, the potency of meningococcal lipid A to stimulate the inflammatory response via MD-2 is determined by the amount of PEA decorating the lipid A. As such, NmLptA may be suitable target for the future development of small molecule inhibitors for the treatment of meningococcal infections.

NmLptA is an integral membrane protein consisting of a transmembrane domain and a globular domain. In order to understand how NmLptA catalyses the addition of PEA to lipid A, we have solved the X-ray structure of the soluble domain of the enzyme by MAD phasing. The fold of the enzyme is strikingly similar to the alkaline phosphatase family, and the structure contains a zinc ion, which may be indicative of the location of the enzyme active site. In addition, a ligand covalently bound to Thr280 at the active site is apparent in the electron density map. These observations give important insight into NmLptA, and enable the identification of key catalytic residues essential for substrate binding and catalysis.

Keywords: protein crystallography, enzyme mechanism, structural biology

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Structure of respiratory complex I

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NADH-ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Complex I is implicated in many human neurodegenerative diseases, as well as in aging. We study bacterial complex I as a "minimal" model of human enzyme. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm. Previously, we have determined the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus*, revealing the arrangement of NADH, flavin and nine Fe-S clusters in an electron transfer chain [1,2].

The mechanism of coupling between the electron transfer and proton translocation in complex I is currently not established. Recently, we have crystallised the membrane domain of complex I from *E. coli* and determined, by X-ray crystallography, its α-helical structure [3]. We have also crystallised the entire complex I from *T. thermophilus* and determined its structure by molecular replacement with the previously determined structure of the hydrophilic domain and the α-helical structure of the *E. coli* membrane domain. The overall architecture of complex I, thus revealed, provides strong clues about the coupling mechanism. The conformational changes at the interface of the two main domains may drive the unusual long amphipathic α-helix in a piston-like motion, tilting nearby discontinuous trans-membrane helices in three similar antiporter-like subunits, resulting in proton translocation.

[1] L.A. Sazanov, P. Hinchliffe, *Science* **2006**, *311*, 1430-1436. [2] J.M. Berrisford, L.A. Sazanov, *J. Biol. Chem.* **2009**, *284*, 29773-29783. [3] R.G. Efremov, R. Baradaran, L.A. Sazanov, *Nature* **2010**, *465*, 441-445.

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Dissecting Enzyme Mechanisms

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Structure of lysine oxidase with a cysteine tryptophylquinone in the active site

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