

edge (XANES) and extended (XAFS) at the Zn K-edge have been explored. We will present further insights into the divalent metal ions regulation of the hut operon in *Bacillus subtilis*, by combining both crystallography and X-ray absorption spectroscopic studies.

1) Kumarevel et al., *Nature* 434, 183-191 (2005), 2) Kumarevel et al., *Nucleic Acids Res.* 33, 5494-5502 (2005)

Keywords: X-ray absorption fine structure, protein crystallography with synchrotron radiation, protein refinement

MS.63.5

Acta Cryst. (2008). A64, C111

The structure of the Amyloid β -peptide high affinity copper II binding site in Alzheimer's disease

Victor A. Streltsov, Stephen J. Titmuss, Vidana Epa, Jose N. Varghese

CSIRO, Molecular and Health Technologies, 343 Royal Parade, Parkville, Victoria, 3052, Australia, E-mail: victor.streltsov@csiro.au

A major source of neurodegeneration observed in Alzheimer's disease (AD) is believed to be related to the toxicity from reactive oxygen species (ROS) produced in the brain by the Amyloid- β ($A\beta$) protein bound primarily to copper ions. The evidence for an oxidative stress role of $A\beta$ -Cu redox chemistry is still incomplete. Details of the copper binding site in $A\beta$ may be critical to the etiology of AD. Here we present the structure determined by combining X-ray absorption fine structure (XAFS) and Density Functional Theory analysis of truncated $A\beta$ (1-16) peptide complexed with Cu(II) in solution under a range of buffer conditions. PBS buffer salt (NaCl) concentration does not affect the copper binding mode. The XAFS spectra for truncated $A\beta$ (1-16)-Cu(II) and full length $A\beta$ (1-40/42)-Cu(II) peptides are similar. The novel six-coordinated (3N3O) geometry around copper in the $A\beta$ -Cu(II) complex includes three histidines, glutamic or/and aspartic acid and axial water. The structure of high affinity Cu²⁺ binding site is consistent with the hypothesis that the redox activity of the metal ion bound to $A\beta$ can lead to the formation of di-tyrosine linked dimers found in AD. X-ray absorption near-edge spectroscopy (XANES) has been used to probe the substrate mediated reduction of Cu(II) to Cu(I) in $A\beta$ -Cu(II) complexes by ascorbate and the neurotoxin 6-hydroxydopamine (6-OHDA), however dopamine and, in particular, cholesterol are incapable of reducing soluble monomeric $A\beta$ -Cu(II) complexes. The results are in agreement with assignment of the redox potentials for $A\beta$ -Cu(II), ascorbic acid and dopamine.

Keywords: beta-amyloids, Alzheimer's proteins, X-ray absorption

MS.64.1

Acta Cryst. (2008). A64, C111

Structural basis of a plant photosystem I sunlight conversion

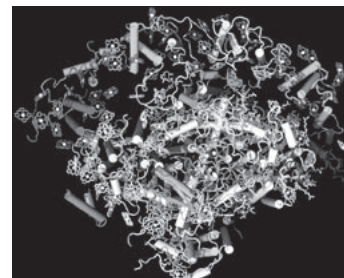
Alexey Amunts, Omri Drory, Nathan Nelson

Tel Aviv University, Biochemistry, Levanon st., Tel Aviv, Tel Aviv, 69978, Israel, E-mail: amunts@tauex.tau.ac.il

A plant Photosystem I (PSI) is a large membrane super-complex that drives photosynthesis. PSI captures sunlight through sophisticated pigment network and uses the energy to perform transmembrane electron transfer. It consists of the reaction center

complex (RC), where the charge separation reaction takes place and the light harvesting complex (LHCI), which serves as an additional antenna system. PSI performs a photochemical activity with the unprecedented quantum yield of close to 1.0, being the most efficient light capturing and energy conversion device. We determined the X-ray structure of intact PSI at 3.4 Å resolution [1]. The crystal structure provides a picture at near atomic detail of 17 protein subunits; 3038 amino acids were assigned, as well as 168 chlorophylls, 2 phyloquinones, 3 Fe₄S₄ clusters and 5 carotenoids. The remarkable feature of PSI is the unprecedented high content of non-protein components, approximately one third of the total mass of about 650 KDa consists of different co-factors. The structure reveals intriguing insights regarding unique interactions between the RC and the LHCI complexes.

[1] Amunts, A., Drory, O. & Nelson, N. (2007) *Nature*, 447, 58-63.



MS

Keywords: photosynthesis, membrane protein, electron transfer

MS.64.2

Acta Cryst. (2008). A64, C111

Inhibitor complexed structures of the Cyt bc1 from the photosynthetic bacterium *R. sphaeroides*

Di Xia¹, Lothar Esser¹, Chang-An Yu²

¹Center for Cancer Research, National Cancer Institute, NIH, Laboratory of Cell Biology, 37 Convent Dr., Building 37, Room 2122C, Bethesda, Maryland, 20892, USA, ²Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK, 74078, USA, E-mail: dixia@helix.nih.gov

The cytochrome bc₁ complex (bc₁) is a major contributor to the proton motive force across the membrane by coupling electron transfer to proton translocation. The crystal structures of wild type and mutant bc₁ complexes from the photosynthetic purple bacterium *Rhodospirillum rubrum* (*Rsbcl*), stabilized with the quinol oxidation (QP) site inhibitor stigmatellin alone or in combination of with the quinone reduction (QN) site inhibitor antimycin, were determined. The high quality electron density permitted assignments of a new metal-binding site to the cytochrome c₁ subunit and a number of lipid and detergent molecules. Structural differences between *Rsbcl* and its mitochondrial counterparts are mostly extra membranous and provide a basis for understanding the function of the predominantly longer sequences in the bacterial subunits. Functional implications for the bc₁ complex are derived from analyses of 10 independent molecules in various crystal forms and from comparisons with mitochondrial complexes.

Keywords: membrane protein crystallization, cytochrome bc₁ complex, mechanism of proton pumping

MS.64.3

Acta Cryst. (2008). A64, C111-112

Structure and mechanism of the DsbB-DsbA protein disulfide generation system in *E. coli*

Kenji Inaba

Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka, 812-8582, Japan, E-mail : inaba-k@bioreg.kyushu-u.ac.jp

DsbB is an *E. coli* membrane protein that oxidizes DsbA, the primary protein disulfide donor present in the periplasm. To understand how disulfide bonds are generated and introduced into secreted proteins, we determined the crystal structure of DsbB in a complex with DsbA and endogenous ubiquinone at 3.7 Å resolution. The first structure of DsbB revealed that DsbB contains the four-helix bundle scaffold in the transmembrane region and one short membrane-parallel α -helix in the long periplasmic loop. Strikingly, the disulfide-generating reaction center composed of Cys41, Cys44, Arg48 and ubiquinone is located near the N-terminus of the transmembrane helix 2, where oxidizing equivalents of ubiquinone are converted to a protein disulfide bond de novo. Whereas DsbB in the resting state contains a Cys104-Cys130 disulfide, Cys104 in the ternary complex is engaged in the intermolecular disulfide bond and captured by the hydrophobic groove of DsbA, resulting in its separation from Cys130. This DsbA-induced conformational change in DsbB seems to prevent the backward resolution of the complex and thereby promote the physiological electron flow from DsbA to DsbB. Recently, I examined functional roles of the membrane-parallel α -helix with strong amphiphilicity by systematic mutation analyses. Introduction of charged or helix-breaking residues into this region not only disrupted the peripheral membrane-association of this helix but also impaired DsbA oxidation activity of DsbB. On the basis of structural and biochemical data so far obtained, I propose the “cysteine relocation mechanism”, by which DsbB oxidizes the extremely oxidizing (reduction-prone) dithiol oxidase, DsbA, efficiently.

Keywords: membrane proteins, redox enzymes, X-ray crystal structure determination

MS.64.4

Acta Cryst. (2008). A64, C112

Crystal structure of the plasma membrane proton pump

Bjorn P Pedersen¹, Morten Buch-Pedersen^{1,2}, J Preben Morth¹, Michael G Palmgren², Poul Nissen¹

¹Centre for Structural Biology, Department of Molecular Biology, Aarhus University, Gustav Wiedsvej 10c, Aarhus, DK, 8000, Denmark, ²PUMPKIN, Department of Molecular Biology, University of Aarhus, ³PUMPKIN, Department of Plant Biology, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg, DK, 1871, Denmark, E-mail : bjopp@bioxray.au.dk

A prerequisite for life is the ability to maintain electrochemical imbalances across biomembranes. In all eukaryotes the plasma membrane potential and secondary transport systems are energized by the activity of P-type ATPase membrane proteins: H⁺-ATPase (the proton pump) in plants and fungi, and Na⁺,K⁺-ATPase (the sodium-potassium pump) in animals. Electron microscopy has revealed the overall shape of proton pumps, however, an atomic structure has been lacking. We present the first structure of a P-type proton pump determined by X-ray crystallography¹. The structure was solved to a resolution of 3.6 Å based on heavy-atom derivatives and density modification by inter-crystal averaging. Ten transmembrane helices and three cytoplasmic domains define the functional unit of ATP-coupled proton transport across the plasma membrane, and the structure is locked in a functional state not previously observed in P-type ATPases. The transmembrane domain reveals a large cavity, which is likely to be filled with water, located near the middle of the membrane plane where it is lined by conserved hydrophilic

and charged residues. Proton transport against a high membrane potential is readily explained by this structural arrangement. We will also address some of the challenges of the low-resolution structure determination, such as generally high anisotropy, low phasing power of derivatives, and how to deal with poor electron density maps when building and refining a model.

1. Pedersen, B.P., Buch-Pedersen, M. J., Morth, J.P., Palmgren M.G. & Nissen P. Crystal structure of the plasma membrane proton pump. *Nature*. **450**, 1111-1114 (2007).

Keywords: membrane protein structure, membrane protein crystal structure determination, biological macromolecular crystallography

MS.64.5

Acta Cryst. (2008). A64, C112

Bacterial multi drug efflux transporter AcrB, - The pumping mechanism

Satoshi Murakami

Tokyo Institute of Technology, Graduate School of Bioscience and Biotechnology, Nagatsuta, Midori-ku, Yokohama, 226-8503, Japan, E-mail : murakami@bio.titech.ac.jp

The emergence of bacterial multi-drug resistance is an increasing problem in the treatment of infectious diseases. AcrB and its homologues are the major multi-drug efflux transporter in gram-negative bacteria, which confer intrinsic drug tolerance and multi-drug resistance when they are overproduced. AcrB exports a wide variety of toxic compounds including anionic, cationic, zwitterionic, and neutral compounds directly out of the cells bypassing the periplasm driven by proton motive force. To understand molecular mechanism of multidrug recognition and active transport by multidrug transporter, we performed X-ray crystallographic analysis of this transporter. In 2002, we successfully solved the crystal structure of AcrB at 3.5 angstrom resolution[1]. In 2006, we solve the crystal structures of AcrB with and without substrates in the new crystal form at 2.8 angstrom resolution[2]. The new crystal structure solved with new crystal form is asymmetric. The AcrB-drug complex consists of asymmetric three protomers, each of which has different conformation corresponding to one of the three functional states of the transport cycle. Bound substrate was found in the periplasmic domain of one of the three protomers. The voluminous binding pocket is aromatic and allows multi-site binding. The structures show that drugs are presumably exported by a three-step functionally rotating mechanism in which drugs undergo ordered binding change.

[1] Murakami, S. et al., *Nature*, 1997, 419, 587.

[2] Murakami, S. et al., *Nature*, 2006, 443, 173.

Keywords: membrane protein X-ray crystal structure determination, membrane transport, drug-protein interactions

MS.65.1

Acta Cryst. (2008). A64, C112-113

New Opportunities in Synchrotron Data Collection with the Pilatus Detectors

Christian Broennimann, Miroslav Kobas

DECTRIS Ltd., OSRA 007, Villigen-PSI, Villigen-PSI, 5232, Switzerland, E-mail : miroslav.kobas@dectris.com

A novel type of x-ray detector has been developed at the Paul Scherrer Institut at the Swiss Light Source (SLS). The PILATUS