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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  $\alpha$ -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  $\alpha$ -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

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### Crystal structure of the plasma membrane proton pump

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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<sup>+</sup>-ATPase (the proton pump) in plants and fungi, and Na<sup>+</sup>,K<sup>+</sup>-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 crystallography<sup>1</sup>. 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

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### Bacterial multi drug efflux transporter AcrB, - The pumping mechanism

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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

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### New Opportunities in Synchrotron Data Collection with the Pilatus Detectors

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

6M detector is a two-dimensional hybrid pixel array detector, which operates in single-photon counting mode (Broennimann et al., 2006). It consists of 2527 x 2463 pixels with a pixel size of 0.172 mm. This detector features several advantages compared to current state-of-the-art CCD and imaging plate detectors. The main features include: no readout noise, superior signal-to-noise ratio, a point spread function of one pixel, readout time of 5 ms, framing time of 100 ms, a dynamic range of 20bit, high detective quantum efficiency (100% at 8 keV, 80% at 12 keV, 50% at 16 keV) and the possibility to suppress fluorescence by an energy threshold. The short readout and fast framing time allow to take diffraction data in fine-phi-slicing mode with continuous rotation of the sample without opening and closing the shutter for each frame. Because of the specified properties, this detector is especially suited for the study of weak diffraction phenomena, time-resolved experiments and accurate measurements of Bragg intensities. Results from various x-ray experiments are presented, including crystallographic diffraction data, as well as results from diffuse scattering and x-ray absorption experiments. All data have been collected with the PILATUS 6M detector at the X06SA and X05LA beamline of the SLS. Broennimann, Ch., Eikenberry E. F., Henrich B., Horisberger R., Huelsen G., Pohl E., Schnitt B., Schulze-Briese C., Suzuka M., Tomizaki T., Toyokawa H., Wagner A. (2006). *J. Synchrotron Rad.* 13, 120-130. Kraft, P., Henrich B., Eikenberry E. F., Schlepuetz C. M., Kobas M., Graafsma H., Broennimann Ch. (2008). *J. Synchrotron Rad.*, submitted.

Keywords: macromolecular crystallography, area detectors, detector development

## MS.65.2

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### Remote access to the SSRL protein crystallography beam lines

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Protein crystallography users of the Stanford Synchrotron Radiation Laboratory (SSRL) have the option to conduct diffraction experiments from their home institutions by means of advanced software tools that enable network-based control of highly automated beam lines. Remote experimenters have access to the same tools as local users, and have the ability to mount, center, and screen pre-frozen samples, and to collect, analyze and backup diffraction data. Central to this remote access capability is the Stanford Auto Mounting (SAM) system which transports samples directly from a cassette or uni-puck storage container in a liquid nitrogen-filled dewar, to the beam line goniometer. SAM is seamlessly integrated into the Blu-Ice/DCS beam line control system. This efficient and reliable system gives researchers the ability to screen up to 288 crystals without human intervention and remount the best quality crystals for data collection. The technical details of the beam line automation and remote access developments and the impact on macromolecular crystallography experiments will be presented.

Keywords: remote access, macromolecular crystallography, automation

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### Exploiting the anisotropy of anomalous scattering boosts the phasing power of SAD/MAD experiments

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The anisotropy of anomalous scattering (AAS) in crystals of brominated nucleic acids and selenated proteins is shown to have significant effects on the diffraction data collected at an absorption edge. For conventionally collected single- or multi-wavelength anomalous diffraction data, the main manifestation of AAS is the breaking of the equivalence between symmetry-related reflections, inducing intensity differences that can be exploited to yield extra phase information. We present a new formalism for describing AAS which allows these effects to be incorporated into the general scheme of experimental phasing methods, using an extended Harker construction. This requires a paradigm shift in the data processing strategy, since the usual separation of the data merging and phasing steps is abandoned. The data are kept unmerged, down to the Harker construction where the symmetry-breaking is explicitly modelled and refined and becomes a source of supplementary phase information. These ideas have been implemented in the phasing program SHARP. Refinements on actual data show that the exploitation of anisotropy of anomalous scattering can deliver substantial extra phasing power compared to conventional approaches using the same raw data. Examples are given that show improvements in the phases which are typically of the same order of magnitude as those obtained in a conventional approach by adding a second wavelength data set to a SAD experiment. Such gains - which come without collecting new data - are highly significant, since radiation damage will frequently preclude the collection of a second wavelength data set. Finally, we outline further developments in synchrotron instrumentation and in the design of data collection strategies that could help to maximise those gains.

Keywords: anisotropy of anomalous scattering, SAD/MAD phasing, polarised dispersion

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### Microcrystal manipulation with laser tweezers

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Over the last years huge investments in several structural genomics initiatives have been undertaken to automate all steps from protein expression to structure solution. Robotic systems are used in almost all steps from protein expression to sample changers on synchrotron beamlines. The last purely manual step is the transfer of the crystal from the crystallization drop onto a support for the subsequent X-ray diffraction experiment. Crystal "fishing" is relatively easy for crystals with dimensions >25 microns, however difficult for smaller crystals. As microfocus synchrotron beamlines allow data collection of crystals with dimensions of only a few microns, new approaches have to be found to facilitate and automate this last manual step. Laser tweezers which are routinely used for cell sorting offer the