MS08 O1

Structure and molecular mechanism of bacterial multi-drug efflux transporter Satoshi Murakami, *Institute of Scientific and Industrial Research, Osaka University.*
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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. It cooperates with membrane fusion protein AcrA and outer membrane channel TolC. The X-ray crystal structure of AcrB was first solved by our group in 2002[1]. Now we solve the crystal structures of AcrB with and without substrates in the new crystal form[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.

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

MS08 O2

From structure to function of rhomboid intramembrane proteases Sin Urban **,** *Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine,*
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Rhomboid proteins are membrane-embedded enzymes with the unusual ability to cleave transmembrane protein segments within the membrane. This form of intramembrane proteolysis plays key roles in diverse cell communication events including EGF signalling during animal development, and quorum sensing during bacterial growth (1). In these contexts, rhomboid proteins act in the signal-sending cell to activate signal precursor proteins and initiate signalling. Recent biochemical advances have culminated in a pure enzyme reconstitution system for studying rhomboid activity (2), and the first high-resolution crystal structures of any intramembrane protease (3). This work revealed that intramembrane proteolysis is catalyzed within a 'sink', a cavity that opens to the outside of the cell and allows water to enter, but is protected laterally from membrane lipids by a ring of protein segments. Structure-function analysis has implicated one transmembrane helix that provides a rate-limiting movement to gate substrate entry laterally from the membrane (4). Functional studies have expanded the cellular role of rhomboid proteins to broad biological processes, including host-cell invasion by malaria parasites, which is the first implication of these enzymes as possible therapeutic targets in human disease (5).

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MS08 O3

Crystal structure of A3B3 subcomplex of V-ATPase from T. thermophilus Megan Meher[2], Koji Nagata[2], Momi Iwata[1,2], Hisayoshi Makyio[1,2], Masasuke Yoshida[1,3], Ken Yokoyama[1], So Iwata[1,2,4]. [1]ATP System Project, ERATO, Japan Science and Technology Corporation, [2]Imperial College London, [3]Tokyo Institute of Technology, [4] Diamond Light Source

The vacuole-type ATPases (V-ATPases) exist in various intracellular compartments of eukaryotic cells to regulate physiological processes by controlling the acidic environment. V-ATPase and the F-type ATP synthase (F-ATPase) are evolutionary related and share the rotary mechanism coupling ATP synthesis/hydrolysis and proton translocation across the membrane. However, reversible association/dissociation of the V_1 (soluble) and the V_o (membrane bound) domain is a unique activity regulation mechanism compared to F-ATPase. Subunit composition and structure in the stalk regions of V-ATPase, which connects the V_o and V_1 domains, are suggested to be significantly different from those in F-ATPase, thus, possibly responsible for the association/dissociation of the complex.
The crystal structure of the A_3B_3 subcomplex of *Thermus thermophilus* V-ATPase, homologous to eukaryotic V-ATPases, has been determined at 2.5Å resolution. The result shows that overall A_3B_3 structure is significantly different from a_3b_3 of F-ATPase, because of the projected domain in A subunit, a catalytic nucleotide binding subunit homologous to b subunit of F-ATPase. The domain includes the "non-homologous region", to F-ATPases but highly conserved in V-ATPases and suggested to be involved in the reversible association/dissociation by mutational studies. We will discuss the structure of the subcomplex in the context of the regulation of whole the membrane protein complex.
We will also update the progress of our "Membrane Protein Laboratory" at Diamond Light Source, which is a user facility dedicated to the crystallisation and crystallography of membrane proteins at the new UK synchrotron.