Multidrug recognition and pumping by bacterial multidrug transporter, ~ A structural view.

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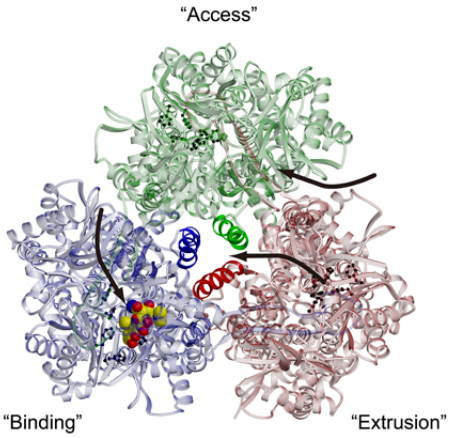
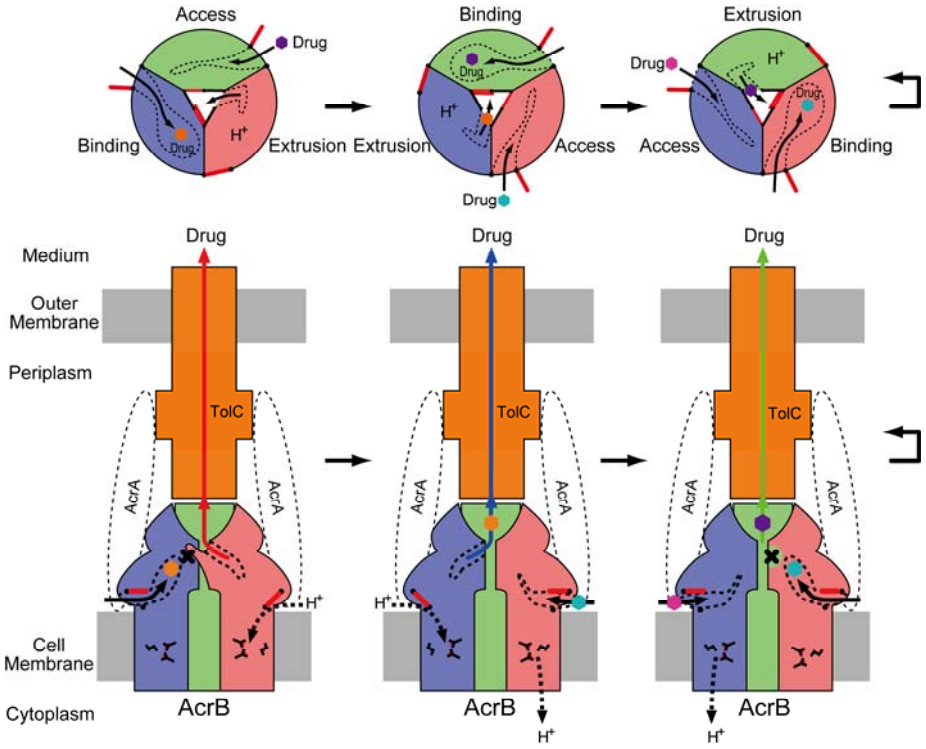
The emergence of bacterial multidrug resistance is an increasing problem in the treatment of such infectious diseases. The major cause for the multi-drug resistance of bacteria is a multi-drug efflux transporter, which exports drugs out of the cells. AcrB and its homologues are the major multi-drug efflux transporter in gram-negative bacteria, which confer intrinsic drug tolerance and multi-drug resistance when they are overproduced. AcrB exports a wide variety of antibiotics, antiseptics, anti-cancer chemotherapeutics and 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]. It is the first structure of not only a multi-drug efflux transporter but also a secondary active transporter, driven by proton-motive force.



Crystal structure of AcrB-drug complex. (Top View)

In 2006, we solve the crystal structures of AcrB with and without substrates in the new crystal form [2]. The crystal used in this study has lower crystallographic symmetry than that of crystal, which used in our previous study. 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. They are "access" in which substrate incorporates, "binding" to which substrate binds, and "extrusion" by which substrate extrudes. Bound substrate was found in the periplasmic domain of "binding" protomer. In the periplasmic part of each protomer, there is a substrate-binding pocket. In this pocket, there are many aromatic amino acid residues for binding of hydrophobic substrates by aromatic-aromatic interactions. Different side chains in this pocket form different binding sites to recognize different type of substrate. The mechanism, the multi-site binding, for multiple substrate recognition was also found in the soluble multi-drug binding transcription factor. The pocket is expanded in the "binding" state and allows substrate binding in this voluminous pocket. The expansion of binding pocket creates a possible exit between sub-domains forming the pocket. However, one functionally important alfa-helix belonging to the "extrusion" protomer inclines and fills this exit to make the pocket closed. At the same time, this inclination creates an opened space from the shrunk binding pocket in the "extrusion" protomer. So, this situation corresponds to just after extrusion of substrates from the pocket to the exit of the AcrB, which connects to the outer membrane channel, TolC. The remaining protomer "access" also has a shrunken binding pocket, but opened "vestibule" exposed to the periplasmic space. Substrates are incorporated from opened vestibule in the "access" state, and bind to the different locations in the voluminous aromatic pocket in the "binding" state. Then, in the "extrusion" state, the vestibule is closed and the exit is opened. At this state, the bound substrate is squeezed out into the TolC channel by shrinking the pocket. All these structural changes are coupled to proton translocation across the membrane. The protonation in the "extrusion" state and deprotonation in the "access" state of three functionally important charged residues (Asp407, Asp408 and Lys940) in the transmembrane domains would affect the accessibility or influence binding or extrusion of substrate that occurring in the periplasmic domains.

Based on three different conformations and transport states observed for the three protomers of AcrB, we propose that drugs are exported by a three-step functionally rotating mechanism in which drugs undergo ordered binding change [2]. Such an ordered binding change mechanism in trimer is similar in principal to the mechanism of the pseudo-trimeric F1ATPase, except that AcrB has no central stalk that undergoes mechanical rotation.



Schematic illustration of the proposed
"Functionally rotating ordered multidrug binding change mechanism" mediated by AcrB.

[1] S. Murakami, R. Nakashima, E. Yamashita, and A. Yamaguchi. "Crystal structure of bacterial multidrug efflux transporter AcrB." *Nature*, **419**, 587-593 (2002).

[2] S. Murakami, R. Nakashima, E. Yamashita, T. Matsumoto, and A. Yamaguchi. "Crystal structures of a multidrug transporter reveal a functionally rotating mechanism." *Nature*, **443**, 173-179 (2006).