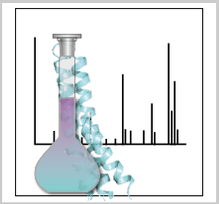
doi:10.2533/chimia.2012.802



Highlights of Analytical Chemistry in Switzerland

Division of Analytical Chemistry
A Division of the Swiss Chemical Society

X-Ray Structure of a Heterodimeric ABC Transporter Crystallized in its Inward-Facing Conformation

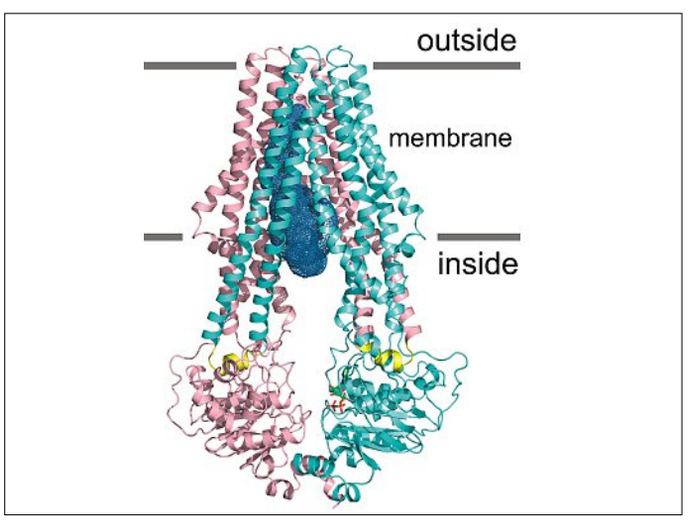
Michael Hohl, Markus G. Grütter*, and Markus A. Seeger*

*Correspondence: Prof. M. G. Grütter, Dr. M. A. Seeger
University of Zurich, Department of Biochemistry, CH-8057 Zürich
Tel.: +41 44 635 55 80, +41 44 635 55 52, E-mail: gruetter@bioc.uzh.ch, m.seeger@bioc.uzh.ch

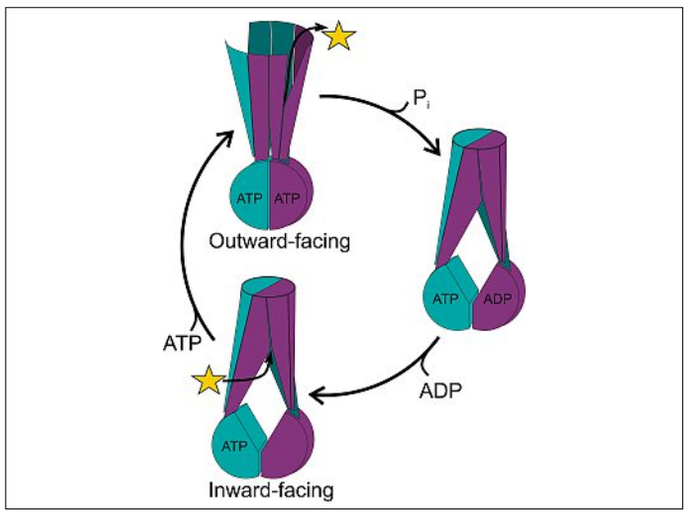
Keywords: ABC transporter · Alternating access model · Drug efflux · Membrane proteins · Structural biology

ATP-binding cassette transporters (ABC transporters) are found in all living organisms and use the energy from ATP binding and hydrolysis to shuttle a wide variety of substrates across cellular membranes. Many ABC transporters actively extrude drugs and thereby contribute to the development of multidrug resistance in cancers and pathogenic microorganisms which causes severe problems in chemotherapy and in the treatment of bacterial infections. In order to understand the molecular mechanism how ABC transporters recognize and transport their substrates, three-dimensional structures determined by X-ray crystallography are of critical importance. Unfortunately, most ABC transporters are reluctant to crystallize and currently only few structures are available for this clinically important class of membrane proteins.

The extrusion of drugs from the cell is accomplished based on an alternating access mechanism. The transmembrane domains of ABC transporters alternately form an inward- and an outward-facing substrate binding cavity which allows drug binding at the cytoplasmic side and its release to the external environment. This requires large conformational changes that are coupled to ATP binding and hydrolysis at highly conserved cytoplasmatic nucleotide binding domains. Until recently, structures of the inward-facing state of ABC transporters have been determined only at low resolution whereas the outward-facing state is well described by X-ray structures.



TM287/288 viewed along the membrane plane. The membrane boundary is indicated by gray lines. The two different polypeptide chains, which assemble to an asymmetric heterodimer, are colored cyan and pink. TM287/288 confines an inward-facing cavity shown as a blue mesh.



Alternating access mechanism of transport for heterodimeric ABC transporters. The transport cycle starts (lower left) with TM287/288 in its inward-facing conformation with one ATP bound. Upon binding of *e.g.* an antibiotic (yellow star) and a second molecule of ATP, the transporter adopts the outward-facing conformation. The substrate is released and ATP is hydrolyzed, which resets the transporter to its inward-facing state and a new transport cycle can begin.

We have succeeded in solving the X-ray structure of TM287/288, a heterodimeric ABC transporter which originates from the thermophilic bacterium *Thermotoga maritima,* at a resolution of 2.9 Å. The structure of TM287/288 reveals a large hydrophobic cavity formed by twelve transmembrane helices that is accessible for drugs from the inside of the cell and therefore represents the inward-facing state. Recombinantly expressed TM287/288 is capable of transporting the anticancer drug daunomycin.

An important feature of TM287/288 is its heterodimeric assembly. In the human body, more than 40 ABC transporters fulfill vital duties, and their malfunction due to inherited mutations may lead to severe diseases. Interestingly, about half of the human ABC transporters are heterodimers like TM287/288. Among these are CFTR, whose failure leads to cystic fibrosis, and SUR1, which is associated with neonatal diabetes. **In summary, we have solved the first X-ray structure of a heterodimeric ABC transporter homologous to medically important transporters connected to cystic fibrosis and multi-drug resistance.**

Received: July 10, 2012

Reference
M. Hohl, C. Briand, M. G. Grütter, M. A. Seeger, *Nat. Struct. Mol. Biol.* **2012**, *19,* 395.

Can you show us your analytical highlight?
Please contact: Dr. Veronika R. Meyer, EMPA St.Gallen, Lerchenfeldstrasse 5, 9014 St.Gallen
Phone: +41 58 765 77 87, Fax: +41 58 765 77 62, Mail to: veronika.meyer@empa.ch