magnitude and location of the charge centers are independent of the nucleotide binding state of the NBDs. We propose that the repulsion between these charge centers is the main drive for the large separation between the NBDs in the absence of ATP. In particular, a conserved charged residue in the helical subdomain of the NBD is found to significantly contribute to the electrostatic repulsion between the NBD monomers. Removing the charge of this conserved residue during the MD simulations results in drastic changes of the NBD conformations, such that the NBDs are unable to complete their opening or closing motion in response to the bound nucleotide, hence a semi-open conformation is maintained in the mutant NBDs both in nucleotide-free and ATP-bound states.

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Functional Rotation of the Transporter AcrB: Insights into Drug Extrusion from Simulations

Attilio Vittorio Vargiu[1,2], Robert Schulz[3], Francesca Collu[1,2], Ulrich Kleinekathoefer[3], Paolo Ruggerone[1,2].
[1]Università di Cagliari, Cagliari, Italy, [2]CNR-SLACS, Cagliari, Italy, [3]Jacobs University Bremen, Bremen, Germany.

The tripartite complex AcrAB-TolC is the major efflux system in Escherichia coli. It extrudes out of the bacterium a wide spectrum of noxious compounds, including many novel antibiotics. Its active part, the homotrimeric transporter AcrB, is responsible for the selective binding of substrates and energy transduction. Based on the available crystal structures and biochemical data, the transport of substrates by AcrB has been proposed to take place via a functional rotation, in which each monomer neatly assumes a particular conformation. However, there is no molecular-level description of the conformational changes associated with such a rotation and of their connection to drug extrusion. To obtain insights thereon, we have performed extensive targeted molecular dynamics simulations mimicking the functional rotation of AcrB containing the antibiotic doxorubicin, one of the two substrates that were co-crystallized so far. The simulations, including almost half a million atoms, have been used to test several hypotheses concerning the structure-dynamics-function relationship of this transporter. Our results indicate that, upon induction of conformational changes, the substrate detaches from the binding pocket and approaches the gate to the central funnel. Furthermore, we provide strong evidence for the proposed peristaltic transport involving a zipper-like closure of the binding pocket, responsible for the displacement of the drug. A concerted opening of the channel between the binding pocket and the gate further favors the displacement of the drug. This microscopically well-funded information allows to identify the role of specific amino acids during the transitions and to shed light on the functioning of AcrB.

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Protein Dynamics in the Transport Cycle: NMR Study of the Multidrug Resistance Transporter, EmrE

Katherine Henzler-Wildman, Emma Morrison, Greg Dekoster, Sonal Bansal.
Washington University, St. Louis, MO, USA.

Bacterial antibiotic resistance is a growing public health concern. One mechanism of resistance arises through drug export by multidrug resistance transporters. To fully understand the function of these proteins requires multiple structures plus kinetic and thermodynamic data to characterize the transport cycle. NMR offers a unique tool to obtain all of this information. The small size of the small multidrug resistance transporter, EmrE, makes it ideal for such studies. EmrE is a secondary active transporter in *E. coli* that harnesses the H+ gradient to export a broad range of polyaromatic cations from the cell, thus conferring resistance to drugs of this type. Protein conformational change is required for proper transport, allowing alternating access to either side of the membrane in response to substrate binding. We have solubilized EmrE in isotropic bicelles and have found that two conformations are present under these conditions. These two states are interconverting slowly on the NMR timescale, allowing us to study this transporter in action with atomic resolution.

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Molecular Basis of the "Alternate Access Model" in the Cation-Substrate Symporter Mhp1 from Computer Simulations and X-Ray Crystallography

Oliver Beckstein[1,2], Tatsuro Shimamura[3,4], Simone Weyand[3,5], Nicholas G. Rutherford[6], Jonathan M. Hadden[6], David Sharples[6], So Iwata[3,7], Peter J.F. Henderson[6], Alexander D. Cameron[3,5], Mark S.P. Sansom[1].
[1]University of Oxford, Oxford, United Kingdom, [2]Johns Hopkins University, School of Medicine, Baltimore, MD, USA, [3]Imperial College, London, United Kingdom, [4]Japan Science and Technology Agency, Kyoto, Japan, [5]Diamond Light Source, Harwell Science and Innovation Campus, Didcot, United Kingdom, [6]University of Leeds, Leeds, United Kingdom, [7]RIKEN, Yokohama, Japan.

A common structural motif is emerging for a wide class of substrate-cation symporters. The fold of the amino acid-sodium symporter LeuT is shared by proteins unrelated by sequence identity such as the galactose-sodium symporter vSGLT, the nucleobase-cation symporter Mhp1, the betaine-transporting osmoregulator BetP, and amino acid-proton transporters AdiC, and ApcT. The "alternating access" model explains transport as cycling between at least three distinct conformational states that connect a central binding site to either the extracellular or the intracellular compartment. The crystal structures solved so far can be broadly categorized in these three conformations, outward facing (LeuT, Mhp1, BetP, AdiC), occluded (Mhp1, BetP, ApcT), and inward facing (vSGLT). We are currently studying the crystal structure of Mhp1 hydantoin transporter from Microbacterium liquefaciens in the inward facing open state. Together with the previous structures [1] a full picture of the conformational change occurring during transport emerges. Dynamic importance (DIMS) molecular dynamics (MD) simulations allow us to connect these three states with continuous transition trajectories. The combination of structural and simulation data puts the alternate access model on a firm structural basis and will facilitate future detailed studies of the energetics of cation-substrate coupled transport.

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[1] Weyand et al. (2008) Science 322, 709-713.

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Symmetry in the Structure of the Glutamate Transporter GltPh Suggests Conformation of an Alternate State

Thomas J. Crisman[1], Shaogang Qu[2], Baruch Kanner[2], Lucy Forrest[1].
[1]Max Planck Institute for Biophysics, Frankfurt, Germany, [2]The Hebrew University of Jerusalem, Jerusalem, Israel.

Glutamate transporters regulate synaptic concentrations of L-glutamate to prevent excitotoxicity in nerve cells. Current crystal structures of GltPh, an archeal homologue of the Glutamate transporters, have an extracellular-facing binding site. The alternating access theory implies that a cytoplasm-facing state also exists. In order to model this state, we have identified two distinct sets of inverted-topology repeats, and used these repeats to model an inward-facing conformation of the protein. Specifically, we modeled the sequence of each repeat on the structure of its partner. In this model, a portion of the protein containing two transmembrane helices (TM7 and 8) and two helical hairpins (HP1 and HP2) is displaced relative to the crystal structure so that the binding site is exposed to the cytoplasm. In order to validate our model, pairs of cysteines were introduced into the neuronal glutamate transporter EAAC1 at positions that were greater than 27 Ångstroms apart in the outward-facing crystal structure, but closer to 10 Ångstroms apart in our model. Transport in these mutants was activated by pretreatment with the reducing agent dithithreitol. Once treated with the oxidizing agent copper(II)(1,10-phenantroline)3, however, activity ceased. Importantly, this inhibition was potentiated under conditions expected to promote the inward-facing conformation. This suggests that during the transport cycle these cysteines come within the range necessary to crosslink, as predicted by our inverted-topology repeat model of the cytoplasm-facing state. Previously, an alternative conformational state of the LeuT transporter was also modeled using inverted-topology repeats, suggesting that inverted-topology repeats may provide a general and elegant solution to the requirement for two symmetry-related states in a single protein.

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Opposite Movements of the External Gate in Glutamate Transporters upon Binding Different Cotransported Ligands Measured by EPR

Paul Focke[1], Pierre Moenne-Loccoz[1], Hans Koch[1], **Peter Larsson**[2].
[1]Oregon Health & Science University, Portland, OR, USA, [2]University of Miami, Miami, FL, USA.

Hairpin two (HP2) has been proposed as the extracellular gate of glutamate transporters. To test this hypothesis, we use double site-directed spin-labeling electron paramagnetic resonance spectroscopy on the bacterial transporter Glt_{Ph} to examine conformational changes in HP2. Surprisingly, the two co-ligands Na^+ and aspartate induce opposite movements of HP2. We find that Na^+ binding to the apo state of the transporter opens the extracellular gate, while the subsequent binding of aspartate closes the gate. In addition, using voltage clamp fluorometry on the mammalian excitatory amino acid transporter EAAT3, we confirm that the opposite conformational changes of HP2 induced by Na^+ and amino acid substrates also occur in mammalian amino acid transporters. Our findings are consistent with HP2 comprising the extracellular gate of glutamate transporters, and that Na^+ binding opens and stabilizes the extracellular gate thereby allowing for amino acid substrate binding.