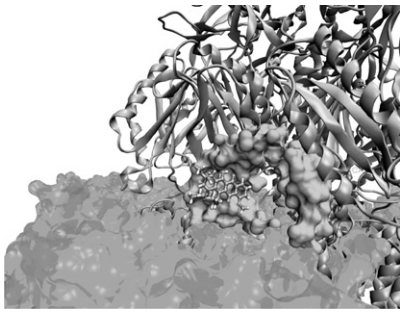
Bacteria use multidrug efflux pumps to extrude toxic substrates through their cell membranes. The RND transporters of the AcrAB-TolC (E.Coli) and MexAB-OprM (P.Aeruginosa) systems are able to export structurally and chemically different substrates, being responsible of multidrug resistance. While the energy conversion takes place in the transmembrane domain of AcrB and MexB, the energy is transducted towards the periplasmic part and used there to initiate what is believed to be a three-cyclic peristaltic pumping. Using different computational methods like adaptive bias force (ABF) and targeted molecular dynamics (TMD), we have investigated the mechanism of substrate uptake and pumping. With ABF we have studied the passage of antibiotics from the periplasm and protein-lipid interface into the inner pore of the pump, while TMD has been used to assess the effect of conformational changes on the extrusion of drugs (located into one of the proposed binding pockets). Finally, analysis of water distribution in the transmembrane region represents an important step to identify features of the energy transduction process. Comparison between the active pumps AcrB and MexB (which show different resistance patterns despite their homology) provide insights into the microscopic details of their functioning.



1963-Plat

Phenylalanine 508 Forms Intra-domain Contact Crucial To CFTR Folding And Dynamics

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Cystic fibrosis (CF) is the most prevalent genetically inherited lethal disease in the United States, with an especially high incidence rate among people of European descent and affects at least one in every 2500-4000 newborns. In 90% of CF patients, the disease is caused by the deletion of phenylalanine-508 (Phe508) from the first nucleotide-binding domain (NBD1) of the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ATP-binding cassette (ABC) transporter family. Prior experimental studies suggest that the Phe508 deletion cause the aberrant folding of NBD1 and the misassembly of various CFTR domains. To gain a fuller understanding of the role of Phe508 in channel function and of the impact of its deletion, we constructed a 3D structural model of CFTR using the high-resolution structure of another ABC transporter, Sav1866. The CFTR model predicts, and we validated experimentally, that Phe508 mediates an interaction between NBD1 and the fourth cytoplasmic loop in the second membrane-spanning domain (MSD2). This interface explains the observed sensitivity of CFTR assembly to many disease-associated mutations in CL4 as well as in NBD1. We also confirmed other intra-domain contacts between the cytoplasmic and membrane-spanning domains that were predicted by the CFTR model. Furthermore, our electrophysiological measurements and molecular dynamics simulations show that these interfaces regulate channel gating and are highly dynamic. Aside from advancing understanding of CFTR structure and function, this study identifies the region of CFTR that should be targeted in treating cystic fibrosis.

1964-Plat

Substrate-Dependent Conformational Changes of a Glutamate Transporter Homologue

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Glutamate transporters tightly control the extracellular concentration of glutamate in the brain, ensuring excitatory neurotransmission and cell integrity. These transporters utilize the energy of pre-existing ionic gradients to pump the transmitter against steep concentration gradients. Active transport is achieved by alternately exposing the ion and substrate binding sites to the extra- and the intracellular milieu and the catalytic cycle includes at least three conformational states: outward-facing, occluded and inward-facing. The crystal structures of a prokaryotic glutamate transporter homologue, GltPh, in complex with an inhibitor and a substrate showed the transporter in the outward-facing and occluded states, respectively, revealing how substrate and ions reach their binding sites from the extracellular medium. However, the conformational changes mediating the release of ligands into the cytoplasm are unknown. To gain insights into this process, we investigated ligand-dependent conformational transitions of the detergent-solubilized purified GltPh. Cysteine cross-linking experiments show that in the absence of ligands, helical hairpin 2 (HP2), which serves as an extracellular gate of the transporter, approaches TM2 bringing together residues that are more than 25 Å apart in the GltPh structures. The direction of HP2 movement is opposite of the observed in the outward-facing state, suggesting that the gate remains closed in the novel state of GltPh. We further probed the substrate-dependent solvent accessibility changes of key regions of the transporter by determining the kinetics of the fluorescence increase upon the reaction of fluorescein maleimide with single cysteine mutants. These experiments reveal a striking pattern: cytoplasmic residues become more solvent exposed and extracellular residues become less solvent-exposed upon substrate dissociation. Our results suggest that the ligand-free state is an inward-facing state of the catalytic cycle and demonstrate that the transporter can be conformationally constrained in this state by cysteine cross-linking.

1965-Plat

A Role for Topologically-Inverted Structural Repeats in Secondary Active Transport by Membrane Proteins of the LeuT Fold

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Structures of secondary active transporters often reveal the presence of large structural repeats. In the case of LeuT from the Na^+-dependent neurotransmitter symporter (NSS) family, the two repeats, each composed of five transmembrane helices, have inverted topology with respect to the plane of the membrane. An asymmetry between these two repeats in the extracellular-facing conformation of LeuT is responsible for the formation of the extracellular pathway. Structural modeling was performed in order to exchange the conformations of the two repeats. This resulted in a ~25° net rotation of a four-helix bundle around an axis in the plane of the membrane, which remarkably, created a conformation of LeuT in an intracellular-facing state. That is, the substrate binding site became exposed on the cytoplasmic side, and closed on the extracellular side. The model therefore proposes a cytoplasmic pathway for proteins with the LeuT fold. We show that accessibility of residues in this cytoplasmic pathway is consistent with a substantial number of biochemical accessibility measurements on single-cysteine mutants at the cytoplasmic face of the homologous mammalian serotonin transporter (SERT). In addition, the inward-facing structure of the related sugar transporter vSGLT shows significant similarities (Faham *et al*, Science 2008). Comparison of the two states of LeuT suggests an alternating-access mechanism in which a bundle of four transmembrane helices 'rocks' within the structure of the remaining helices. More generally, these results suggest that topologically-inverted repeats in other secondary active transporters may provide useful clues in understanding their mechanisms.

1966-Plat

Detecting Transport-related Conformational Changes In The Glutamate Transporter Homologue, Glt_{Ph}.

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Glutamate transporters (EAATs) are essential in clearing glutamate from the synapse, allowing precise control over excitatory synaptic function. Much has been learned in recent years about how these proteins function, however little is known about how the structural basis of this function; how do protein conformational changes lead to transport activity? A homolog of these proteins, Glt_{Ph} from *Pyrococcus horikoshii*, is functionally similar to the mammalian proteins; co-transporting substrate and sodium ions whilst having an uncoupled chloride conductance. Importantly, however it has been crystallized and its structure determined, making it an excellent model for understanding how structure and function are related in the EAATs. In order for transport to proceed, conformational changes must occur that allow alternating access of the substrate binding site to both the internal and external solutions. Using fluorescein maleimide labeling and protease accessibility studies on single cysteine mutants of Glt_{Ph} we have identified regions of the protein involved in this process. Changes in accessibility are seen in the presence of both aspartate and TBOA, a non-transported competitive inhibitor. Surprisingly, our accessibility studies implicate heretofore unexpected areas of the Glt_{Ph} protein in the conformational changes associated with substrate and inhibitor binding. We are also further investigating functional aspects of Glt_{Ph} transport using radioligand flux assays.

1967-Plat

Crystal Structure Of Chloride Transporter From A Cyanobaterium

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The CLC family of chloride transporting proteins is constituted of chloride channels and chloride-proton antiporters. Of the transporter subclass CLC_ec1 from E.coli has been characterized extensively structurally and functionally,